WATER-SAVING POTATOES

E. B. Aliche

2018

WATER-SAVING POTATOES

Exploring and characterizing drought tolerance mechanisms

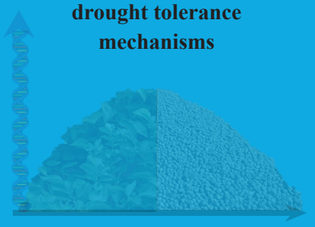
Ernest B. Aliche

INVITATION

You are cordially invited to the public defence of my PhD thesis entitled:

WATER-SAVING POTATOES

Exploring and characterizing drought tolerance mechanisms



Wednesday 13 June 2018,
at 13:30.
Aula of Wageningen University,
Generaal Foulkesweg 1,
6703 BG, Wageningen.

You are warmly invited to the reception: 15:30 - 17:00
Vredehorst Building, Tarthorst 1 (near Jumbo Supermarket), Wageningen.

PARANYMPHS
Johan Willemsen
johan.willemsen@wur.nl
&
James Ledo
jledo88@yahoo.com

Propositions

1. The importance of the physical properties of the plant transport system for drought tolerance is underestimated.

   (this thesis)

2. Fast rate of exponential growth is not always advantageous for final crop yield.

   (this thesis)

3. Qualitative data collection as done in the social sciences is a biased approach to facts, and cannot fully explore the variations that exist in nature.

4. Every scientist should learn from statisticians how to formulate hypotheses, set up the experimental design, and apply testing strategies.

5. The fact that weather is a common subject for lunch talk or coffee breaks, especially in the Netherlands, does not make climate change a hoax.

6. Societal change should emphasize educating our children much more than changing adults.

Propositions belonging to the thesis entitled

"Water-saving potatoes: Exploring and characterizing drought tolerance mechanisms"

Ernest Aliche

Wageningen, 13 June 2018

WATER-SAVING POTATOES

EXPLORING AND CHARACTERIZING DROUGHT TOLERANCE MECHANISMS

Ernest B. Aliche

Thesis committee

Promotor

Prof. Dr R.G.F. Visser

Professor of Plant Breeding

Wageningen University & Research

Co-promotor

Dr C.G. van der Linden

Group Leader, Plant Breeding

Wageningen University & Research

Other members

Prof. Dr A.J. Haverkort, Ömer Halisdemir University, Nigde Turkey

Prof. Dr P.C. Struik, Wageningen University & Research

Prof. Dr J.T.M. Elzenga, University of Groningen, The Netherlands

Dr W. Meijer, HZPC Holland B.V., Metslawier, The Netherlands

This research was conducted under the auspices of the Graduate School Experimental Plant Sciences.

WATER-SAVING POTATOES

EXPLORING AND CHARACTERIZING DROUGHT TOLERANCE MECHANISMS

Ernest B. Aliche

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus,
Prof. Dr A.P.J. Mol,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday 13 June 2018
at 1:30 p.m. in the Aula.

Ernest B. Aliche

WATER-SAVING POTATOES: Exploring and characterizing drought tolerance mechanisms,

260 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2018)
With references, with summary in English

ISBN 978-94-6343-267-2
DOI https://doi.org/10.18174/444999

This work is dedicated to Prof Anne van den Ban (1928 - 2016) for initiating the ABF scholarship scheme, which I benefitted from in my MSc program; and to the drought-impoverished farmers in Uganda, who represent the important but insufficiently-informed farmers that Prof Anne van den Ban invested his extension efforts to reach.

TABLE OF CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

THE POTATO CROP

Origin, domestication and globalization

Potato was used as far back as 12,000 years ago by the indigenous Americans (in South and Central America), who lived by hunting and gathering varieties of edible wild plants (Smith, 2011). Over 200 different species of potato were part of the vast collection of wild plants eaten by the inhabitants of the large land area of South and Central America. The first cultivation of the potato as a crop was in the Andes, in the region of Lake Titicaca bordering present-day Peru and Bolivia (Horton, 1987). The Andean farmers eventually domesticated about seven of the potato species in 10,000 BC, the most important being *Solanum tuberosum* and *Solanum tuberosum andigena* (Smith, 2011). Climate adaptation of the potato and other edible food crops facilitated the migration process of the Andean settlers to higher altitudes between 7,000 – 5,000 BC (Graves & Cabieses, 2001; Martins, 1976; Salaman, 1949).

Andean farmers propagated the potato from both seeds and tubers, which enhanced the diversity of the potato germplasm. However, the agricultural potential of the potato tuber began to be realized during the Incan civilization (100 – 153 BC) because the Inca employed terrace planting on steep slopes and a canal watering system at each terrace level (Berzok, 2003). The Incan techniques offered solutions to the fluctuating environmental conditions and poor soil of the Andean mountains (Waldron, 2015). Potato remained local to South America until the Spanish conquest of the region in 1500 AD and subsequent introduction of potato into Europe (Rodger, 2007). The initial cultivation of potato in Europe was in the early 1560s on the Canary Isles (Hawkes & Francisco-Ortega, 1993). The potato species introduced into Europe was *Solanum tuberosum* ssp. a*ndigena*, which tuberized only under short day conditions (Hawkes & Francisco-Ortega, 1992). Short days towards the end of the year in Spain must have facilitated the tuber formation of the crop. The spread of potato within Europe was rapid in the late 1500s/early 1600s. Through decades of breeding and selection in Europe, the short day-length requirement was selected against and in late 18th century all potato grown in Europe were long-day adapted *Solanum tuberosum* (Rajpal et al., 2016). From Europe, the potato spread to the rest of the world through the journeys of sailors, missionaries, colonialists and soldiers.

Evolutionary genetics and breeding of potato

It is widely speculated that at least four wild potato species (*S. acaule*, *S. sparsipilum*, *S. leptophyes* and *S. megistacrolobum*) are ancestral to the evolution of cultivated potato species (Rajpal et al., 2016). Open pollination among these wild species and unconscious human selection efforts resulted in the cultivated species we know today. The cultivated potato species comprise various ploidy levels ranging from diploid to hexaploid (NSF, 2016; Spooner, 1990). The most common cultivated species, *S. tuberosum*, consists of two sub species: *S. tuberosum* spp *andigena* and *S. tuberosum* spp *tuberosum*. One of the progenitors of the subspecies *andigena* is *S. stenotomum* while the other progenitor is disputed, either *S. sparsipilum* (Cribb & Hawkes, 1986) or *S. phureja* (Juzepczuk & Bukasov, 1929). *S. andigena* is tetraploid due to a chromosome doubling event in nature (Rajpal et al., 2016). Chloroplast DNA evidence and microsatellite data have unveiled the genetic differences between landraces of subspecies *andigena* and *tuberosum* (Hosaka & Hanneman, 1988; Raker & Spooner, 2002). But in Europe

it is generally accepted that *tuberosum* evolved from the short-day *andigena* (Spooner, 1990), despite the divergent views about the progenitors of *tuberosum* at the centre of origin of potato (Grun, 1990; Ugent et al., 1987). Geneticists uphold the Andean repertoire as the richest gene pool of potato because of the large diversity inherent in the germplasm as contributed by all the ploidy groups (CIP, 1980). However, tetraploids are the most predominantly cultivated on a commercial scale (Carney, 1980; Haan et al., 2010). The popularity of tetraploids over diploids in terms of commercial cultivation may partly be because the potato introduced into Europe, *andigena*, is a tetraploid species. Nevertheless, experimental evidences have shown that tetraploid potato has on average a higher tuber yield than the diploid species (Hutten et al., 1995; Maris, 1990). The commercially cultivated potato in Europe and in most of the world, *S. tuberosum* spp *tuberosum*, is autotetraploid (2n = 4x = 48) and is a highly heterozygous outbreeding species that can suffer intense inbreeding depression when self-pollinated (Haynes, 1993; PGSC, 2011). It exhibits tetrasomic inheritance and at a locus about four alleles are obtainable (Bradshaw, 2007), but on average, a tetraploid variety has 3.2 different alleles per locus, while over all tetraploids, a range of 10 – 25 different alleles are obtainable per locus. Tetrasomic inheritance implies, for instance, that a locus with dominant (*B*) and recessive (*b*) alleles will have quantitative allele dosage combinations as follows: nulliplex (*BBBB*), simplex (*BBBb*), duplex (*BBbb*), triplex (*Bbbb*), or quadruplex (*bbbb*) (Watanabe, 2015). A recessive allele at a locus where dominance gene action occurs can only influence the phenotype in the absence of any dominant allele, that is, in the quadruplex dosage (*bbbb*). The autotetraploid segregation pattern (tetrasomic inheritance) does not allow preferential pairing during the formation of bivalents or quadrivalents, which means that all allelic combinations are possible (Bourke et al., 2016; Bourke et al., 2015; Little, 1945). In potato, this generally implies that a large sample size is required during genetic analysis of inheritance of traits to increase the chances of finding preferred combinations. Also, the accumulation of a dominant allele of interest to its triplex or quadruplex dosages would require many generations of selfing, which introduces inbreeding depression. Furthermore, it is quite rigorous to identify such dominant alleles in triplex/quadruplex dosages because all allele dosages of the dominant gene show the same phenotype, except in additive gene models. On the other hand, attempts to combine two recessive genes would require extremely large number of F_2 progeny because the frequency of the double-recessive is 1 in 1296 plants (Muthoni et al., 2015). Therefore, genetic inheritance in potato is extremely complex (Stift et al., 2008). The genetics of quantitatively inherited traits like tolerance to environmental stresses are even more difficult because many loci are involved in the complex segregation ratios. Such genetic complexity negatively impacts potato breeding for these traits and elongates the duration of potato breeding programs.

Nonetheless, potato breeding currently thrives on crosses between parental lines and the possibilities of clonal propagation of interesting offspring through tubers. The earlier years of commercial potato breeding involved traditional breeding methods based on only phenotypic observations (Miller & Fontenot, 1965; Stevenson & Milstead, 1932), and is still conventional today in the selection for some traits. However, the construction of potato genetic maps has provided the possibility of molecular marker assisted selection (MAS) in potato (Barone, 2004; Li et al., 2013; Ottoman et al., 2009). MAS application at early stages of plant development to select against less interesting lines can shorten the number of years of a potato breeding

program. However, the use of MAS in potato breeding is still limited due to the following reasons: cost of marker development and commercialization, specificity of many markers only for the populations in which they have been developed, genetic linkage distance between some markers and the traits of interest, and the extent of polymorphism which the marker can account for in the traits of interest (Felcher & Douches, 2012).

Importance of potato

Potato is the world's 3rd most important food crop after rice and wheat with respect to human consumption (CIP, 2013). More than a billion people eat potato globally and it has been recommended by FAO as a food security crop (Andre et al., 2014; DeFauw et al., 2012). As a food source, the potato tuber is rich in nutrients like low-fat carbohydrates, vitamins B and C, and essential minerals (Manganese, Chromium, Selenium and Molybdenum) (CIP, 2013). Therefore, potato consumption can prevent malnutrition and nutrient deficiencies. In recent years, potato production in developing countries has exceeded the production in developed countries (Walker et al., 2011). Therefore, potato has the potential of reducing the food crises in the emerging world. One of the factors probably contributing to the increased production of potato in developing countries is that potato can easily be grown without much resource input.

In addition to its food uses, the potato can be utilized in other areas like starch production for industrial purposes (e.g. in paper and pharmaceutical industry) (Nwokocha et al., 2014). Potato waste can be fermented and used in bioethanol production (Izmirlioglu & Demirci, 2015). Furthermore, potato leaves are a good source of *solanesol*, which is a useful active ingredient in the synthesis of ubiquinone drugs (Yan et al., 2015). But the cultivation of potato for these non-food uses is not widespread, probably because of the technical requirements of processing the raw material and boosting production. Since the year 2012, potato global production has increased more than any time before (Fig.1). If this rising trend continues, potato will contribute more immensely to the feeding of the growing world population. However, factors such as environmental stress like drought could potentially stall the rising trend in potato production. Regional droughts reportedly had severe impacts on potato yield with hikes in prices and product unavailability in the market (Faulkner, 2012). Therefore, research efforts towards understanding the drought response of potato and ways of improving drought tolerance of this crop are essential.

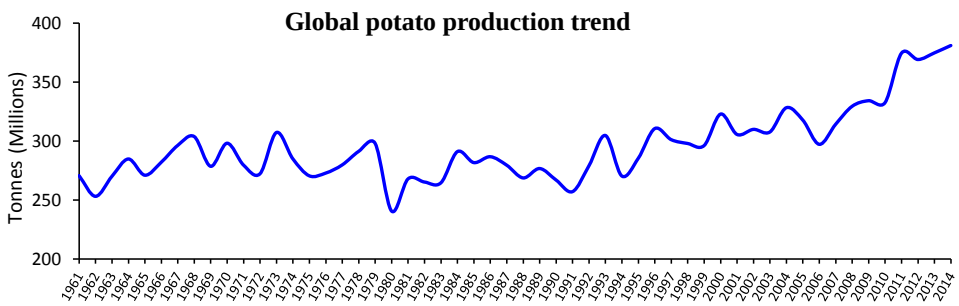


Figure 1: Trend of global potato production from 1961 till 2014 (FAOSTAT, 2014).

DROUGHT

Drought stress impact on crop cultivation

Drought, a climatic condition of prolonged water deficits, has been described in various ways. Based on its impact on the ecosystem, drought can be seen as an extended period of high evaporation and transpiration in instances of limited rainfall (Anderegg et al., 2015), causing a decrease in relative humidity (Herrmaan, 2008). The physiological perspective presents drought in the context of its stressful effects on living systems due to its interaction with the metabolism and development of various life forms (Anderegg et al., 2015). The occurrence of drought stress is not instant but progressive with increasing levels of dehydration (Herrmaan, 2008).

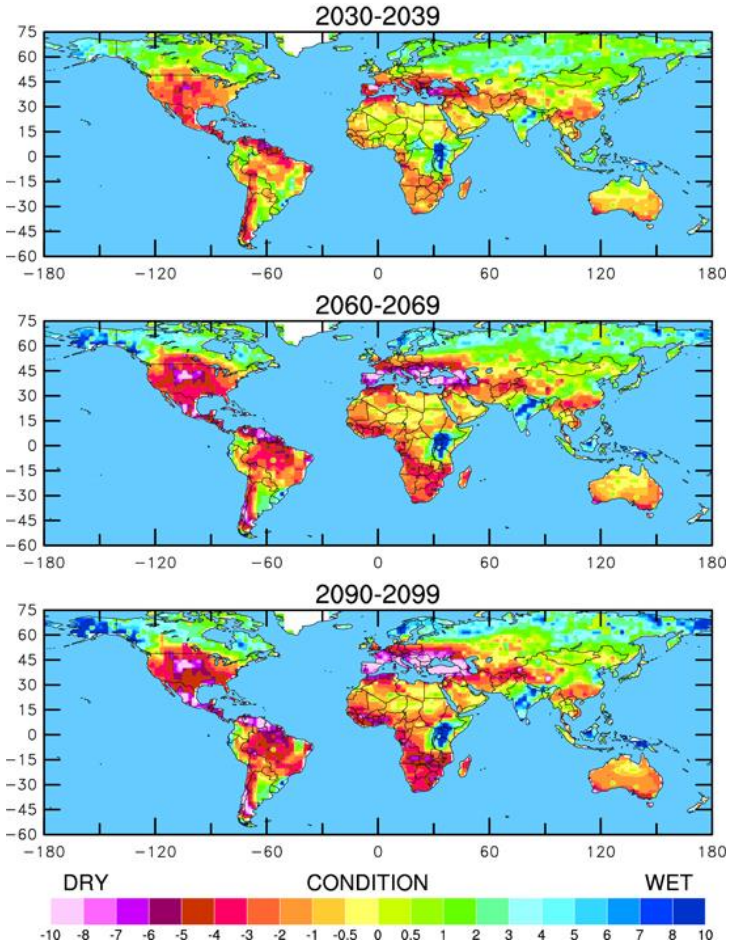


Figure 2: Potential for future drought over the decades indicated, based on projections of future greenhouse gas emissions. The maps were derived using the Palmer Drought Severity Index (PDSI) with the scale: Extreme drought likely (≤ -4), drought likely (-3 – 0.5), drought less likely (0.5 – 10) (Dai, 2011).

Therefore, the impact of drought on plants increases from mild to adverse effects depending on the duration of the drought. Drought stress has necessitated the adoption of irrigation practices in the cultivation of crops. According to FAOSTAT (2014), 20% of the world's cultivated land area is irrigated and this irrigated proportion contributes 40% of the total food production worldwide. Furthermore, drought predictions suggest that in the next 30-90 years many parts of the world will face severe drought scenarios resulting from reduced precipitation and/or increased evaporation (Dai, 2013) (Figure 2). This shows that drought presents a challenge to crop production.

Drought response of plants

Drought alters the molecular architecture in plants resulting in phenotypic changes. However, sometimes drought effects may not be readily observed in the phenotype even when molecular adaptation takes place, for instance, during a mild drought that lasts for only a short time. Nonetheless, it is recognized as drought stress when the molecular alterations affect the plant's physiological processes like transpiration, nutrient assimilation, gas exchange, among others (Passioura, 2007). Molecular alterations may be direct or indirect (Farooq et al., 2009). Direct effects refer to gene expression changes in response to drought perception, while indirect effects refer to gene expression changes that result from secondary stresses or injury responses due to the drought, for instance, oxidative stress. The differential expression of both the directly and indirectly drought-affected genes are believed to play important roles in drought tolerance (Kavar et al., 2008). Typically, expression of regulatory genes (e.g., transcription factors) is adapted earlier, while functional genes with protective or repair roles may be changed a bit later.

During drought, the stress is first perceived by the roots. Several receptor proteins on cell plasma membranes have been reported to be involved in the perception of drought stress. For example, receptor-like protein kinases (RPKs) are induced under drought and they trigger other downstream signalling events (Osakabe et al., 2013). Prompt drought stress perception is essential to enable the plants to prepare for a probably increasing severity of water scarcity and to preserve their cells from damage. A series of signalling cascades result from drought stress perception including the generation of second messengers like Ca^{2+}, phosphatidic acid, ROS and sugars (Bartels & Sunkar, 2005). Eventually, these second messengers trigger the synthesis of specific protein kinases, which by their phosphorylating functions trigger the induction of transcription factors (Farooq et al., 2009; Harb et al., 2010; Wang et al., 2016). Transcription factors induce the early responses to drought (Chaves et al., 2003), and drive the expression of further downstream functional drought-response genes (Harb et al., 2010). Some of these downstream functional genes include aquaporin(s) that facilitate the exchange of water across membranes (Farooq et al., 2009; Javot & Maurel, 2002); LEA (late embryogenesis abundant) genes that have the ability to stabilize other proteins and membranes during dry conditions (Hand et al., 2011); and heat shock proteins that act as molecular chaperones involved in ATP-dependent folding, refolding and unfolding of proteins to ensure protein stability (Farooq et al., 2009; Kregel, 2002; Park & Seo, 2015). These regulatory and functional molecular elements form cascades of pathway interactions that influence plant physiology, leading to the production of membrane stabilizers, osmolytes, osmoprotectants and antioxidants, which determine the

plant's drought response at the cellular level. Drought stress signalling has been categorized into ABA-dependent and ABA-independent pathways (Yoshida et al., 2014). In the ABA-dependent pathway, drought stress triggers ABA synthesis thereby inducing transcription factors like ABA-binding factor (ABF) that trigger the transcription of genes through binding to stress-responsive gene promoter elements like the ABA-responsive element (ABRE). In the ABA-independent pathway, other transcription factors like the dehydration-responsive element-binding (DREB) proteins induce the transcription of genes by binding to stress-responsive elements like Dehydration-responsive element (DRE) (Lata & Prasad, 2011; Yoshida et al., 2014). Interestingly, a cross-talk exists between the ABA-dependent and ABA-independent pathways, which keeps the system balanced (Yoshida et al., 2014).

One of the early drought responses, stomatal closure, is an adaptive water conservatory mechanism that also helps to maintain the turgor pressure of the guard cells (Chaves et al., 2003). A delay or defect in stomatal closure under drought causes excessive transpiration and turgor loss of leaf cells (Cominelli et al., 2010), leading to wilting beginning from the older leaves (Waseem et al., 2011). However, the adaptive water conservation through a prompt stomatal closure also results in reduction of CO_2 uptake from the atmosphere. The plant needs to balance the photons of light energy it assimilates during drought in order to avoid the accumulation of ROS (Xu et al., 2010). The CO_2 pressure drop in the stomatal cavity of the leaves reduces the regeneration of the electron acceptor, $NADP^+$, through the Calvin cycle. This poor $NADP^+$ regeneration in the electron transport chain triggers electron leakage to O_2 leading to the formation of reactive oxygen species (ROS) (Sharma et al., 2012). The plant adopts various strategies like photorespiration, leaf movements (paraheliotropism) and thermal dissipation to protect their photosystems against damage (Cornic & Massacci, 1996). The consequence of these adjustments during drought is a drastic reduction in photosynthesis and photosynthesis efficiency (Li et al., 2017). This reduction in photosynthesis may be expressed through reduced leaf growth in cases of early drought stress, or accelerated leaf senescence when the stress occurs late in the season (Bassam et al., 1990), also depending on the stress severity. In addition to the effects on leaves, drought stress may among others reduce plant height (Boutraa et al., 2010), enhance flower abortion (Sivakumar & Srividhya, 2016) and induce root elongation (Asch et al., 2005), depending on the plant's strategy of drought response.

Various mechanisms are integrated in the plant drought response. These mechanisms have been classified into several categories (Levitt, 1980): drought escape, drought avoidance, drought tolerance, drought resistance, drought abandon and drought recovery (upon re-watering) (Belhassen, 2013; Harb et al., 2010; Xu et al., 2010). A plant may *escape* drought by completing its life cycle earlier before the drought becomes severe. This can be observed in terms of early flowering or quick differentiation into a propagating tissue. Drought *avoidance* is the maintenance of a relatively high tissue water potential despite deficits in soil water (Harb et al., 2010). This means the plant is able to optimize its water uptake from the soil, for instance, through longer rooting system. Alternatively, the plant may reduce water loss from its shoot through reduced stomatal conductance and formation of waxy cuticles that cover the lenticels. Drought *tolerance* is the ability of the plant to maintain its cellular and molecular structures

amidst drought conditions that cause deficits in its tissue water potential. This may involve osmotic adjustment and cell wall elasticity to keep the turgor in plant tissues. Drought *resistance* is the ability of the plant to alter its metabolic pathways in order to synthesize essential molecules that help it control the secondary effects of drought at the molecular level. For instance, enhancement of its antioxidant metabolism to scavenge ROS is a drought resistance mechanism (Xu et al., 2010). Drought *abandon* describes the abandonment of some plant parts, like shedding of older leaves under drought in order to reduce the metabolic load of the plant. Drought *recovery* is a post-drought plant response that facilitates the restoration of the plant to its normal homeostasis during re-watering. During drought, a plant may exhibit a combination of two or more of the above drought response mechanisms at the same or different time points. Some mechanisms may be common within a plant species. Also, genotypic differences within a species may be observed. An understanding of the variations in drought response mechanisms within a species (e.g., potato) would enhance the chances of exploiting the most optimal mechanisms for crop improvement.

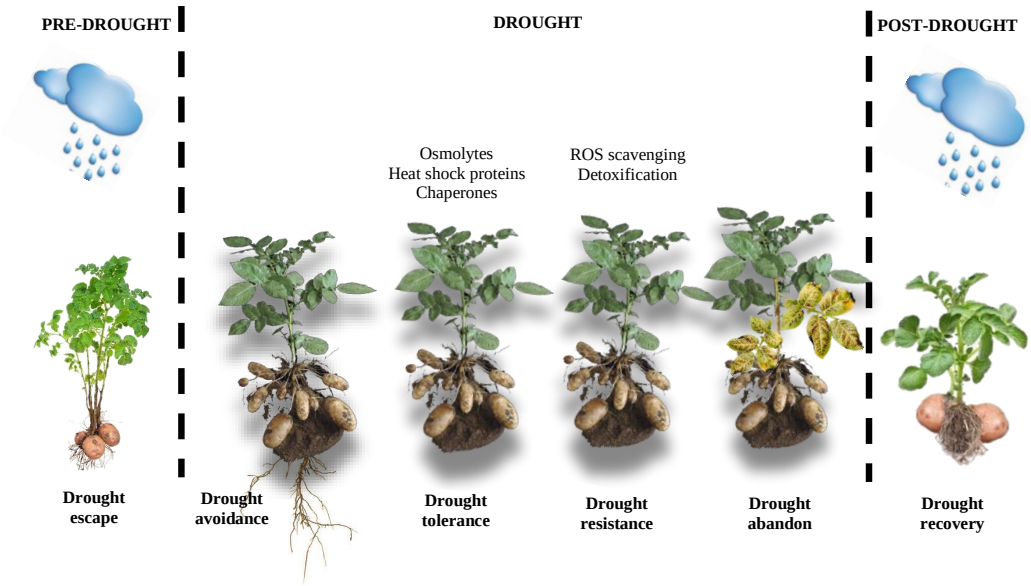


Figure 3: Illustration of the various drought response mechanisms of plants.

DROUGHT STRESS EFFECTS ON POTATO

Right from the domestication in the Andes, potato cultivation has been dependent on water availability, in the case of the Andean region, rainfall (MacQuarrie, 2015; Mayer & Shea, 1979; Onern, 1976). Potato has a high water use efficiency (Bacon, 2009; Tanner, 1981): it is estimated that the crop produces 5600 calories of dietary energy for every 1000 litres of water input, while maize, wheat and rice produce 3860, 2300 and 2000 calories, respectively given the same water application (FAOSTAT, 2008). However, under sub-optimal water availability

potato tuber production is severely affected (Loon, 1981). There may be different reasons for the drought sensitivity of potato and these include its shallow (and weak) root system that cannot penetrate a plough surface (Loon, 1981), high transpiration rate (Manhas & Sukumaran, 1988) and poor leaf expansion (Weisz et al., 1994). The impact of drought stress on the plant depends on the severity and timing of the stress in the growing season. At the planting stage, drought delays emergence and root establishment. The proliferation of potato stems from the mother tuber at these initial stages of growth is hampered (Lahlou et al., 2003). The effect of drought at the early stages of plant growth may also affect plant height, leaf expansion, flower budding and stolon initiation (Ojala et al., 1990). On the other hand, at later stages of plant development drought stress may cause leaf senescence and flower abortion, and may affect tuber bulking (Kuppinger et al., 2014). Drought may also cause the potato plant to invest in its root properties including root length and root-to-shoot ratio, as a means of enhancing its access to the limited soil water (Jefferies, 1993). Investment in roots or other tissues during drought may be at the expense of its investment in tuber yield (Jefferies, 1993). Therefore, the tuber number, tuber weight and plant biomass are reduced under drought (Fasan & Haverkort, 1991; Lahlou et al., 2003). It is therefore essential to understand the mechanism by which such investments in other tissues, like in canopy growth, affects tuber yield.

Potato physiological and morphological adaptations to drought stress

Potato, like any plant, closes its stomata at the perception of drought (Liu et al., 2005). Additionally, depending on the regulation of stomatal closure, reductions in leaf water potential (LWP) and relative water content (RWC) may occur, and these could rapidly reduce photosynthetic rates prior to the observation of wilting phenotype (Haverkort et al., 1991; Heuer & Nadler, 1998; Moorby et al., 1975). Furthermore, reductions in leaf area index and canopy expansion rate have been reported under drought (Jefferies & Mackerron, 1993). Interestingly, it has been shown that physiological processes associated with leaf expansion may be involved in potato drought sensitivity, in addition to its limited soil water extraction under drought (Weisz et al., 1994). However, various aspects of canopy expansion that may be related to drought tolerance are yet elusive, and these are addressed in this thesis.

Molecular basis of the response to drought stress of potato

Drought stress triggers cascades of molecular alterations, which are the basis for the observed genotypic variations in the phenotype (Evers et al., 2010). Transgenic studies have contributed in revealing some of the genes involved in this complex network of drought response interactions, like the aquaporin *StPIP1* that functions as water transport channel (Wang et al., 2017); *STANN1* annexin for maintenance of cell redox homeostasis (Szalonek et al., 2015); non-specific Lipid Transfer Protein-1 (*StnsLTP1*) that enhances cell membrane integrity and increases antioxidation (Gangadhar et al., 2016). However, single-gene studies may not appropriately represent the molecular signature of drought stress response because of the quantitative nature of drought response, which is controlled by many contributory loci. Therefore, improving the potato crop for drought tolerance would benefit from studies that involve a large genotypic background in order to explore the variation potential in the potato

germplasm. Examples of such studies include genetic studies, transcriptomic studies in a number of contrasting genotypes and genome-wide association studies.

In genetic studies using mapping populations, several QTLs linked to plant shoot, root and tuber yield traits under drought have been unveiled (Anithakumari, 2011; Tessema, 2017). A remarkable finding from the genetic study of Anithakumari (2011) is an eQTL hotspot on potato chromosome *V* in the vicinity of the yield/maturity locus, which is associated with myriads of regulatory networks under drought. A further investigation of this eQTL hotspot using systems genetics approach identified *NFY-C4* as candidate master-regulator at this hotspot regulating myriads of drought response cascades in potato (van Muijen et al., 2016). Secondly, a transcriptomic study of two potato genotypes with microarrays in a growth chamber reported the upregulation of genes involved in stress sensing and signalling (calmodulins and calreticulin that function in calcium signalling), cell wall modification (pectin methylesterase inhibitor and lipid transfer protein), cell rescue and detoxification (thioredoxins, metallothioneins, glutaredoxins, ascorbate peroxidase, heat shock proteins and dehydrins), protective compounds formation (amines, asparagine synthetase, UDP-4-glucose epimerase, galactinol synthase and raffinose synthase), degradation of damaged proteins (*RD19A* cysteine protease) and stabilization of electron transfer in Photosystem II (PsbR and PsbW). The microarray findings suggest that the potato plant may undergo some molecular adjustments under drought to reduce excess light collection and optimize the turnover from photosystem reaction subunits. Also, there may be a partial diversion in carbon flux from starch/cellulose biosynthesis toward raffinose metabolism (Legay et al., 2011). Furthermore, an RNA-seq study of a potato genotype at tuber bulking stage revealed an upregulation of heat shock proteins, dehydrins (*TAS14*), protein phosphatase 2C (*PP2C*), aquaporin, starch biosynthesis genes (phytochrome B and granule-bound starch synthase 1) and bidirectional sugar transporters (Gong et al., 2015). The down-regulated genes in that study were lipid transfer proteins, peroxidases, LOX, gibberellin 2-beta-dioxygenase and gibberellin 20-oxidase. These transcriptomic studies have contributed to our understanding of the molecular response of potato to drought. However, these studies often involve one or two genotypes, which limits our understanding of the causal variations for the phenotypic differences among genotypes.

Reports of Genome-Wide Association Studies (GWAS) on potato drought stress response are scarce. A first-attempt association study involving a relatively limited set of potato cultivars grown in pots in the greenhouse unveiled loci on Chromosomes *VI* and *IX* associated with stolon initiation and tuber initiation, respectively under drought (Tessema 2017). This shows the potential of GWAS to contribute to our knowledge of the molecular markers and possibly, candidate genes that are involved in potato molecular drought response. There is thus a need to take further steps in association studies of potato drought response, for instance, under field conditions.

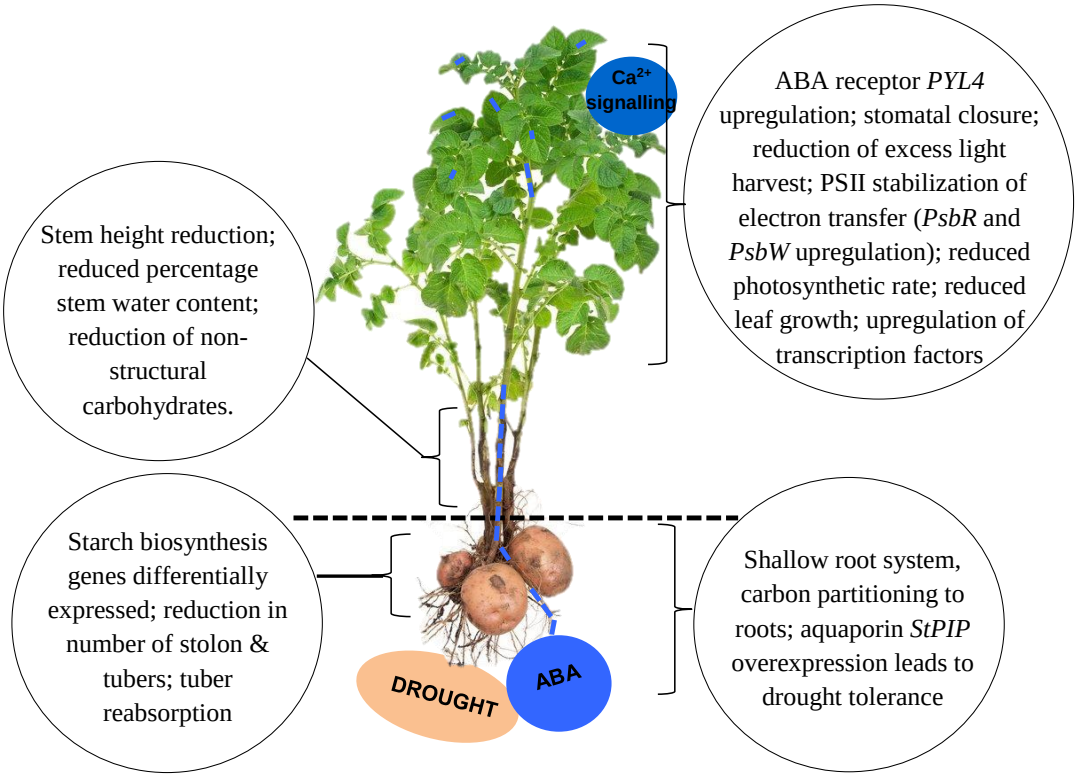


Figure 4: Overview of molecular, physiological and morphological responses of potato to drought stress in different plant tissues.

Several other studies have also reported the roles of a number of drought response genes in potato including a *DREB* gene that facilitated proline osmoprotectant accumulation (Bouaziz et al., 2013); LEA proteins like dehydrins (Charfeddine et al., 2015); heat shock proteins (Sprenger et al., 2016) and several more. The various molecular, physiological and morphological responses of potato to drought are summarized in Figure 4.

Drought stress at different developmental stages of potato triggers different molecular factors in response to the drought. At the seedling stage of growth, superoxide dismutase has been shown as more important an antioxidant protective enzyme than catalase and peroxidase (Li et al., 2017). At tuberization and tuber bulking stages, photosynthesis- and carbohydrate biosynthesis-related genes were reportedly critical (Evers et al., 2010); and in another study, different mechanisms were employed by various genotypes resulting in either tolerance or sensitivity (Boguszewska-Mańkowska et al., 2018). These reports suggest that studies that target different phenological stages in diverse genotypic backgrounds may be the way forward in unveiling the drought tolerance potential of the potato. This approach will lead to precision in targeting specific genes for drought-prone regions with known drought patterns that coincide with given stages of plant growth.

Potential for drought tolerance breeding in potato

Breeding essentially requires the existence of robust variation, which enhances the selection of the best combining progenitors of breeding programs. The existence of natural variation for drought tolerance has been demonstrated in potato (Anithakumari, 2011). This variation for drought tolerance has been exploited through experimental crosses in diploid potato background to generate mapping populations that gave insights on loci of interest for drought tolerance in potato (Anithakumari, 2011). Such interesting loci are potential tools for drought tolerance breeding in potato. However, this rich potential is not yet fully harnessed in drought tolerance improvement of potato, partly because drought tolerance is a quantitative trait involving several loci that contribute to tolerance. Additionally, sourcing for drought tolerance from wild relatives of cultivated potato that may have evolved in the harsh environments of the centre of origin may also introgress unwanted traits due to linkage drag and tetrasomic inheritance. Breeders rather prefer drought tolerance breeding using plant material that has already been improved for other traits. Therefore, additional knowledge on the extent of drought tolerance in commercially bred potato cultivars ascertain the potential of commercial cultivars as progenitors of potato drought tolerance breeding programmes.

THESIS: WATER-SAVING POTATOES

Thesis Background

This thesis project aims to fill the knowledge gap about the feasibility of breeding for drought tolerance using modern potato cultivars as starting material. Four Dutch potato breeding companies, Averis Seeds B.V., C. Meijer, HZPC Holland B.V. and KWS POTATO, collaborated with Plant Breeding, Wageningen University and Research, on this Topsector T&U project “Water-saving potatoes”. Breeding programs for improved drought tolerance in potato would benefit from

- knowing the level of genetic variation for drought tolerance response in modern potato cultivars,
- the traits that can be utilized to select for drought tolerance,
- physiological and molecular mechanisms that play key roles in drought tolerance and
- optimal selection conditions for potato drought tolerance breeding.

Breeding programmes in potato can take as long as 13-15 years, due to its tetraploid inheritance and self-incompatibility. Breeding for drought tolerance most likely requires combining multiple contributing loci, and still needs to retain elite material properties, which makes introgression breeding from wild progenitors even more challenging. Therefore, finding sources among commercial cultivars for introduction of improved drought tolerance would be a clear advantage. To avoid a continuation of the trend where old cultivars of over 100 years dominate potato cultivated areas, newly bred varieties have to meet the needs of the current markets. Drought tolerance in potato has become a current need due to the rise in potato consumption in the emerging worlds where drought stress is a serious threat. Also, the effect of climate change is leading to erratic droughts in areas where this was previously not the case, like the Netherlands.

Goal and aims

The main goal of this thesis was to create a platform that will facilitate the breeding of improved drought tolerance in potato. The realization of this goal involves an evaluation of a representative set of European potato cultivars to ascertain the inherent level of drought tolerance and the needed improvement. Also, this goal requires an understanding of the mechanisms of drought tolerance response in the European potato germplasm. The aims of this thesis project are thus:

1. to provide breeding tools for the breeding of drought-tolerant potato cultivar(s). These breeding tools are genotypes with high yielding capacity under drought, which can serve as genitors in breeding programs
2. to provide reliably measurable traits that can be used to efficiently select for drought-tolerant genotypes in a selection scheme
3. to define molecular tools like molecular markers and possibly the implicated candidate genes responsible for drought tolerance in potato. This may boost the possibility of using marker assisted selection in potato breeding programmes
4. to zoom in on the interacting pathways involved in drought responses and how these affect carbon partitioning, which is a strong determinant of tuber yield.

Approach and Techniques

Different aspects of the potato drought response are linked to one another. Therefore, it is often not feasible to gain a full understanding of one aspect of drought response without involving other parts. Therefore, a multi-disciplinary approach is required for a complete understanding of drought tolerance in potato. In this thesis, we adopted various techniques using a multi-disciplinary approach to investigate the genetic, molecular, biochemical, physiological and morphological aspects of drought tolerance in potato. The techniques used include: canopy growth modelling, association mapping, phenotyping and transcriptomics.

Growth modelling: crop growth models have been employed in the study of various aspects of potato growth (Goeser et al., 2012; Griffin et al., 1992; Kooman & Haverkort, 1995; MacKerron & Waister, 1985). Through modelling, the various factors that could possibly influence crop growth are integrated into mathematical equations for a better understanding of their effects. In this thesis, we used models to simulate potato canopy growth in the field (Chapter two). The data points for the canopy growth model were generated by taking pictures of the canopy ground cover from emergence until harvest. The green pixels of the pictures were extracted in MATLAB and used to infer canopy development throughout the growing season. Additionally, we used models to describe potato tuber size distribution in order to extract parameters that describe drought effects on tuber size (Chapter three). The data for modelling tuber size distribution were obtained by grading potato tuber sizes after harvest.

Association mapping: this technique is useful in dissecting complex traits by establishing causal relationships between genotypes and phenotypes in a given representative set of genotypes. Association mapping harnesses the several generations of recombination that took place in natural populations, which often result in tight linkage of causal polymorphisms in a linkage

disequilibrium (LD) (Abdurakhmonov & Abdukarimov, 2008; González-Martínez & Grivet, 2009). Association mapping of a number of traits with molecular markers has been reported in potato (Berdugo-Cely et al., 2017; D'hoop, 2009; Vos, 2016), however, literature reports on association mapping of drought tolerance in potato are limited. Therefore, we applied this technique to discover SNP markers in different genomic regions of the potato that are associated with drought tolerance traits (Chapter three).

Phenotyping: precision in phenotyping is core to research and breeding. The identification of an actual causal molecular factor is dependent on precision of phenotyping. In this thesis, we used several phenotyping techniques in the field, greenhouse and in growth chambers. These observations were aimed at distinguishing drought tolerance and sensitivity among the genotypes we studied. Phenotypic measurements in this thesis may be categorized into high throughput phenotyping and deep phenotyping. High throughput phenotyping was done using camera picture images, hand-held machines and weighing machines. Deep phenotyping includes the imaging techniques used in this thesis, like microscopy and magnetic resonance imaging (MRI). Microscopic techniques have been used to study the architecture of the potato tuber (Bordoloi et al., 2012)(Bordoloi et al., 1967), but studies on the potato stem using this technique are quite limited. In this thesis, we used microscopic techniques to study the effects of drought on the morphology of stem transport tissues. Furthermore, we used MRI to study the transport of water via the xylem vessels and assimilates via the phloem conduits of the stem *in vivo*. MRI is a state-of-the-art imaging technique that is non-destructive and non-invasive, and useful for studying the dynamics of plant water relations (Van As & Windt, 2008) (Chapter four).

Transcriptomics: Myriads of regulatory and functional genes that make up the transcriptome profile of plants are regulated in expression during environmental stress conditions. Analyses of such transcriptome profiles aid our understanding of the various molecular pathways and genes that are associated with such environmental cues. Transcriptomic techniques have been used in other studies to investigate the drought response of potato, but often with a limited number of genotype(s). In this thesis, we used transcriptomics (RNA-seq) to study the potato drought response in five different contrasting genotypes at two different time points in the growing season and in two different tissues of the plant (Chapter six).

Objectives and scope of thesis

In this thesis, we carried out experiments to uncover various mechanisms of the drought stress response of commercial potato cultivars. We conducted field trials in different locations representing different drought stress regimes in order to study the impact of drought on canopy growth, and how this affects eventual tuber yield. We evaluated Genotype by Environment interactions in the field trials as well. We also conducted greenhouse trials in pots and laboratory experiments to further investigate the physiological and molecular aspects of the drought response in cultivated potato. Our objectives were to understand what mechanisms, pathways, molecular markers and possibly candidate genes are involved in and would potentially improve drought tolerance in potato.

Chapter two reports on the results from the field trials we conducted in multiple locations within three years. Our objectives were to investigate the effects of drought stress on canopy growth and tuber yield, and Genotype-by-Environment interactions. The different locations represented varying levels of drought severity and different years in the same location presented different environmental conditions as well. We explored the variations in drought timing between two years at the same location, and unveiled the impact of early and late drought on potato canopy growth and tuber yield. We also found a strong effect of foliage maturity on the drought response of the cultivars.

Chapter three describes the impact of drought stress in the field on tuber size distribution using relevant models. Modelling aided our extraction of parameters of tuber size distribution that distinguished drought tolerant from sensitive genotypes. We also showed the relationship between marketable tuber yield and total tuber yield with respect to drought response of the genotypes. Furthermore, we used our cultivar set and a 14K SNP array for association mapping, and found marker association with tuber size distribution parameters and marketable tuber yield.

Chapter four is a greenhouse study of a subset of genotypes selected from the field trials and grown in pots for an in-depth investigation of carbon partitioning under drought conditions. In this chapter we report the findings from our physiological, biochemical and gene expression studies, which pointed to genes that are essential for assimilate partitioning toward tuber yield during drought stress.

In Chapter five we investigated the impact of drought on transport through the vascular tissues, and used a magnetic resonance imaging (MRI) technique to visualize the transport of water and assimilates through the vascular tissues of the potato stem. We also did a microscopic investigation of the stem cross-sections and evaluated the impact of drought on the xylem vessels. We found morphological modifications in vascular tissues that may contribute to drought tolerance. We also investigated the effect of drought stress on day-night rhythms of water and assimilate transport.

Chapter six describes the findings from a transcriptomic study we carried out using RNA sequencing on tissues of five cultivars with contrasting drought responses. The plants were grown in a rain-out tunnel in the field. We investigated the drought response of these cultivars at two time points coinciding with tuber initiation and tuber bulking stages. In this chapter we report the relationship between differentially expressed genes under drought and the observed phenotypic response during the growing season. We found various molecular pathways that are involved in the drought tolerance response and cross-talks between pathways.

Lastly, in the General Discussion (Chapter 7) I discuss the various perspectives of our findings in the different chapters, and their relevance for the aims of this thesis. The additions that this thesis provides to the breeder's toolbox are highlighted and recommendations are made regarding the key points of attention for drought tolerance breeding in potato.

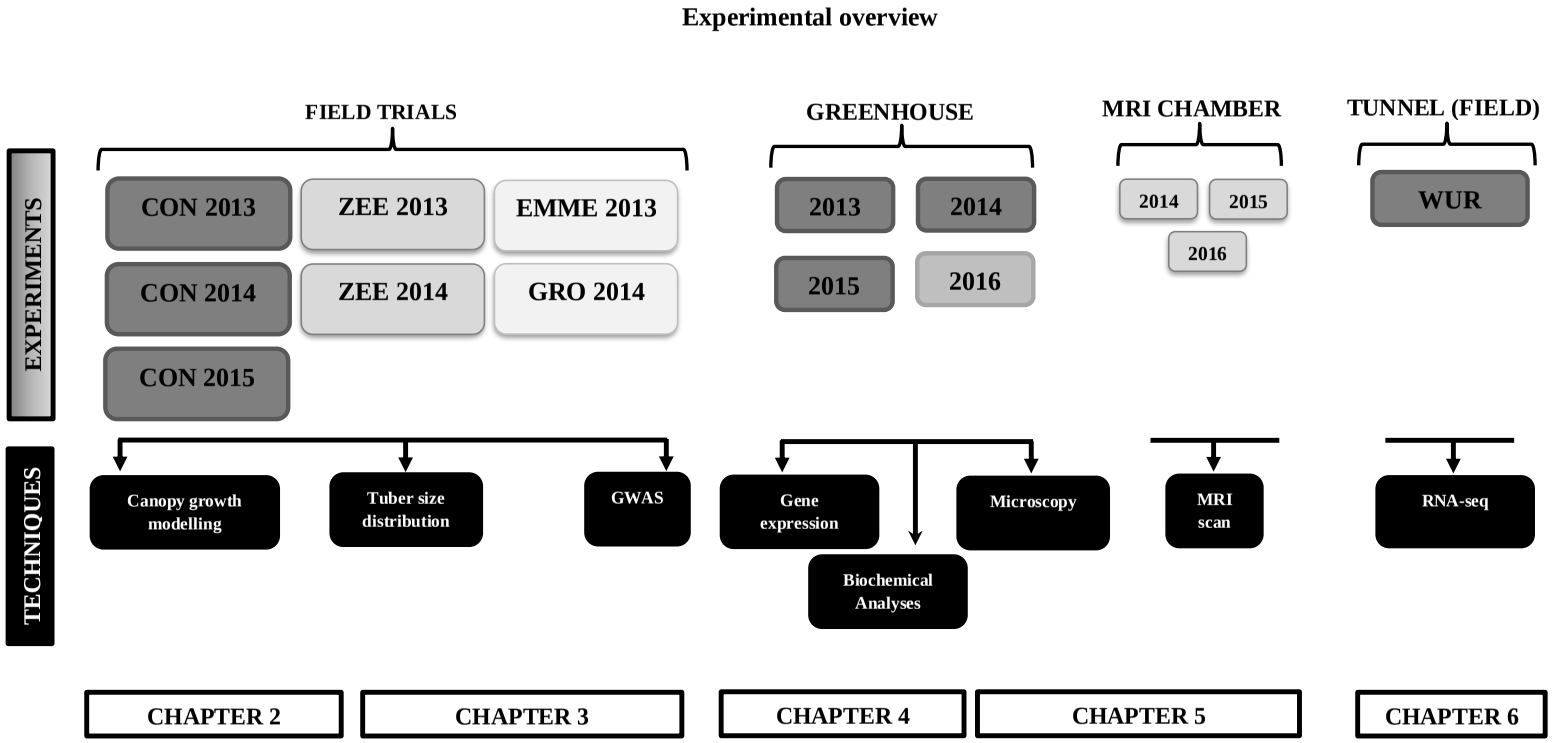


Figure 5: An overview of the experimental set up used in this thesis. Darker background shades of experiments indicate higher drought stress severity in the respective locations and years: CON – Connantre (France), ZEE – Zeeland (The Netherlands), EMME – Emmeloord (The Netherlands), GRO – Grolloo (The Netherlands), WUR – Unifarm, Wageningen University & Research (The Netherlands).

REFERENCES

Abdurakhmonov, I. Y., & Abdukarimov, A. (2008). Application of Association Mapping to Understanding the Genetic Diversity of Plant Germplasm Resources. *International Journal of Plant Genomics, 2008*, 18. doi: 10.1155/2008/574927

Anderegg, W. R. L., Hicke, J. A., Fisher, R. A., Allen, C. D., Aukema, J., Bentz, B., Hood, S., Lichstein, J. W., Macalady, A. K., McDowell, N., Pan, Y., Raffa, K., Sala, A., Shaw, J. D., Stephenson, N. L., Tague, C., & Zeppel, M. C. (2015). Tree mortality from drought, insects, and their interactions in a changing climate. *New Phytologist, 208*(3), 674-683. doi: 10.1111/nph.13477

Andre, D., Peter, K., & Oscar, O. (2014). Potatoes for Sustainable Global Food Security. *Potato Research, 57*(3), 185-199.

Anithakumari, A. M. (2011). *Genetic dissection of drought tolerance in potato.* (Ph.D.), Wageningen University, Wageningen. Retrieved from http://edepot.wur.nl/165211

Asch, F., Dingkuhn, M., Sow, A., & Audebert, A. (2005). Drought-induced changes in rooting patterns and assimilate partitioning between root and shoot in upland rice. *Field Crops Research, 93*(2–3), 223-236. doi: https://doi.org/10.1016/j.fcr.2004.10.002

Bacon, M. (2009). *Water use efficiency in plant biology*: John Wiley & Sons.

Barone, A. (2004). Molecular marker-assisted selection for potato breeding. *American Journal of Potato Research, 81*(2), 111-117. doi: 10.1007/bf02853608

Bartels, D., & Sunkar, R. (2005). Drought and Salt Tolerance in Plants. *Critical Reviews in Plant Sciences, 24*(1), 23-58. doi: 10.1080/07352680590910410

Bassam, N., Dambroth, M., Loughman, B. C., Spitters, C. J. T., & Schapendonk, A. H. C. M. (1990). Evaluation of breeding strategies for drought tolerance in potato by means of crop growth simulation *Genetic Aspects of Plant Mineral Nutrition* (Vol. 42, pp. 151-161): Springer Netherlands.

Belhassen, E. (2013). *Drought Tolerance in Higher Plants: Genetical, Physiological and Molecular Biological Analysis*: Springer Netherlands.

Berdugo-Cely, J., Valbuena, R. I., Sánchez-Betancourt, E., Barrero, L. S., & Yockteng, R. (2017). Genetic diversity and association mapping in the Colombian Central Collection of Solanum tuberosum L. Andigenum group using SNPs markers. *PLoS ONE, 12*(3), e0173039.

Berzok, L. M. (Producer). (2003, Retrieved July 26, 2016 from Encyclopedia.com). "Potato.". *Encyclopedia of Food and Culture*. Retrieved from http://www.encyclopedia.com/doc/1G2-3403400489.html

Boguszewska-Mańkowska, D., Pieczyński, M., Wyrzykowska, A., Kalaji, H. M., Sieczko, L., Szweykowska-Kulińska, Z., & Zagdańska, B. (2018). Divergent strategies displayed by potato (Solanum tuberosum L.) cultivars to cope with soil drought. *Journal of Agronomy and Crop Science, 204*(1), 13-30. doi: 10.1111/jac.12245

Bordoloi, A., Kaur, L., & Singh, J. (2012). Parenchyma cell microstructure and textural characteristics of raw and cooked potatoes. *Food Chemistry, 133*(4), 1092-1100. doi: http://dx.doi.org/10.1016/j.foodchem.2011.11.044

Bouaziz, D., Pirrello, J., Charfeddine, M., Hammami, A., Jbir, R., Dhieb, A., Bouzayen, M., & Gargouri-Bouzid, R. (2013). Overexpression of StDREB1 transcription factor increases

tolerance to salt in transgenic potato plants. *Mol Biotechnol, 54*(3), 803-817. doi: 10.1007/s12033-012-9628-2

Bourke, P. M., Voorrips, R. E., Kranenburg, T., Jansen, J., Visser, R. G., & Maliepaard, C. (2016). Integrating haplotype-specific linkage maps in tetraploid species using SNP markers. *Theor Appl Genet, 129*(11), 2211-2226. doi: 10.1007/s00122-016-2768-1

Bourke, P. M., Voorrips, R. E., Visser, R. G., & Maliepaard, C. (2015). The Double-Reduction Landscape in Tetraploid Potato as Revealed by a High-Density Linkage Map. *Genetics, 201*(3), 853-863. doi: 10.1534/genetics.115.181008

Boutraa, T., Akhkha, A., Al-Shoaibi, A. A., & Alhejeli, A. M. (2010). Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (Triticum durum) grown in Saudi Arabia. *Journal of Taibah University for Science, 3*, 39-48. doi: https://doi.org/10.1016/S1658-3655(12)60019-3

Carney, H. J. (1980). *Diversity, distribution and peasant selection of indigenous potato varieties in the Mantaro Valley, Peru: a biocultural evolutionary process*: International Potato Center.

Charfeddine, S., Saidi, M. N., Charfeddine, M., & Gargouri-Bouzid, R. (2015). Genome-wide identification and expression profiling of the late embryogenesis abundant genes in potato with emphasis on dehydrins. *Mol Biol Rep, 42*(7), 1163-1174. doi: 10.1007/s11033-015-3853-2

Chaves, M. M., Maroco, J., #227, P., o., Pereira, J., #227, & S., o. (2003). Understanding plant responses to drought — from genes to the whole plant. *Functional Plant Biology, 30*(3), 239-264. doi: http://dx.doi.org/10.1071/FP02076

CIP. (1980). *The Dynamics of Andean Potato Agriculture*: International Potato Center.

CIP (Producer). (2013, Monday, 15th August 2016). Potato Facts and Figures. Retrieved from http://cipotato.org/potato/facts/

Cominelli, E., Galbiati, M., & Tonelli, C. (2010). Transcription factors controlling stomatal movements and drought tolerance. *Transcription, 1*(1), 41-45. doi: 10.4161/trns.1.1.12064

Cornic, G., & Massacci, A. (1996). Leaf Photosynthesis Under Drought Stress. In N. R. Baker (Ed.), *Photosynthesis and the Environment* (pp. 347-366). Dordrecht: Springer Netherlands.

Cribb, P. J., & Hawkes, J. G. (Eds.). (1986). *Experimental evidence for the origin of Solanum tuberosum subspecies andigenum*. New York: Columbia University Press.

D'hoop, B. B. (2009). *Association mapping in tetraploid potato.* (PhD PhD), Wageningen University, Wageningen. Retrieved from http://edepot.wur.nl/1616

Dai, A. (2011). Drought under global warming: a review. *Wiley Interdisciplinary Reviews: Climate Change, 2*(1), 45-65. doi: 10.1002/wcc.81

Dai, A. (2013). Increasing drought under global warming in observations and models. *3*(1), 52-58.

DeFauw, S. L., He, Z., Larkin, R. P., & Mansour, S. A. (2012). Sustainable Potato Production and Global Food Security. In Z. He, R. Larkin, & W. Honeycutt (Eds.), *Sustainable Potato Production: Global Case Studies* (pp. 3-19). Dordrecht: Springer Netherlands.

Evers, D., Lefèvre, I., Legay, S., Lamoureux, D., Hausman, J.-F., Rosales, R. O. G., Marca, L. R. T., Hoffmann, L., Bonierbale, M., & Schafleitner, R. (2010). Identification of

drought-responsive compounds in potato through a combined transcriptomic and targeted metabolite approach. *Journal of Experimental Botany, 61*(9), 2327-2343. doi: 10.1093/jxb/erq060

FAOSTAT. (2008). Potato and water resources. Retrieved from http://www.fao.org/potato-2008/en/potato/water.html

FAOSTAT. (2014). Food Supply - Crops Primary Equivalent - Potatoes. Retrieved 17th Feb. 2017 http://www.fao.org/faostat/en/#data/CC

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development, 29*(1), 185-212. doi: 10.1051/agro:2008021

Fasan, T., & Haverkort, A. J. (1991). The influence of cyst nematodes and drought on potato growth. 1. Effects on plant growth under semi-controlled conditions. *Netherlands Journal of Plant Pathology, 97*(3), 151-161. doi: 10.1007/bf01995964

Faulkner, G. (2012). Essential trends in World Potato Markets *World Potato Markets* (pp. 23): Agri Markets Ltd.

Felcher, K. J., & Douches, D. (2012). Marker-Assisted Selection for PVY Resistance in Potato. *Plant Breeding and Genomics.* Retrieved August 11th, 2016

Gangadhar, B. H., Sajeesh, K., Venkatesh, J., Baskar, V., Abhinandan, K., Yu, J. W., Prasad, R., & Mishra, R. K. (2016). Enhanced Tolerance of Transgenic Potato Plants Over-Expressing Non-specific Lipid Transfer Protein-1 (StnsLTP1) against Multiple Abiotic Stresses. *Frontiers in Plant Science, 7*, 1228. doi: 10.3389/fpls.2016.01228

Goeser, N. J., Mitchell, P. D., Esker, P. D., Curwen, D., Weis, G., & Bussan, A. J. (2012). Modeling Long-Term Trends in Russet Burbank Potato Growth and Development in Wisconsin. *Agronomy*. doi: 10.3390/agronomy2010014

González-Martínez, S. C., & Grivet, D. (2009). Association mapping in plants. *Annals of Botany, 104*(6), ix-x.

Graves, C., & Cabieses, F. (2001). *The Potato Treasure of the Andes: From Agriculture to Culture*: International Potato Center.

Griffin, T. S., Johnson, S., & Ritchie, T. (1992). A Simulation Model for Potato Growth and Development: SUBSTOR-Potato Version 2.0.

Grun, P. (1990). The evolution of cultivated potatoes. *Economic Botany, 44*(3), 39-55. doi: 10.1007/bf02860474

Haan, S. d., Núñez, J., Bonierbale, M., & Ghislain, M. (2010). Multilevel Agrobiodiversity and Conservation of Andean Potatoes in Central Peru. *Mountain Research and Development, 30*(3), 222-231. doi: 10.1659/mrd-journal-d-10-00020.1

Hand, S. C., Menze, M. A., Toner, M., Boswell, L., & Moore, D. (2011). LEA proteins during water stress: not just for plants anymore. *Annu Rev Physiol, 73*, 115-134. doi: 10.1146/annurev-physiol-012110-142203

Harb, A., Krishnan, A., Ambavaram, M. M. R., & Pereira, A. (2010). Molecular and Physiological Analysis of Drought Stress in Arabidopsis Reveals Early Responses Leading to Acclimation in Plant Growth. *Plant Physiology, 154*(3), 1254-1271. doi: 10.1104/pp.110.161752

Haverkort, A. J., Fasan, T., & van de Waart, M. (1991). The influence of cyst nematodes and drought on potato growth. 2. Effects on plant water relations under semi-controlled

conditions. *Netherlands Journal of Plant Pathology, 97*(3), 162-170. doi: 10.1007/bf01995965

Hawkes, J. G., & Francisco-Ortega, J. (1992). The potato in Spain during the late 16th century. *Economic Botany, 46*(1), 86-97. doi: 10.1007/bf02985257

Hawkes, J. G., & Francisco-Ortega, J. (1993). The early history of the potato in Europe. *Euphytica, 70*(1), 1-7. doi: 10.1007/bf00029633

Haynes, K. G. (1993). Some aspects of inbreeding in haploids of tetraploidSolanum tuberosum L. *American Potato Journal, 70*(4), 339-344. doi: 10.1007/bf02851427

Herrmaan, S. M. (2008). Drought. In T. Gale (Ed.), *International Encyclopedia of the Social Sciences*: Encyclopedia.com.

Heuer, B., & Nadler, A. (1998). Physiological response of potato plants to soil salinity and water deficit. *Plant Science, 137*(1), 43-51. doi: https://doi.org/10.1016/S0168-9452(98)00133-2

Horton, D. E. (1987). *Potatoes: Production, Marketing, and Programs for Developing Countries*: Westview Press.

Hosaka, K., & Hanneman, R. E., Jr. (1988). Origin of chloroplast DNA diversity in the Andean potatoes. *Theor Appl Genet, 76*(3), 333-340. doi: 10.1007/bf00265332

Hutten, R. C. B., Schippers, M. G. M., Hermsen, J. G. T., & Jacobsen, E. (1995). Comparative performance of diploid and tetraploid progenies from 2x.2x crosses in potato. *Euphytica, 81*(2), 187-192. doi: 10.1007/bf00025432

Izmirlioglu, G., & Demirci, A. (2015). Enhanced Bio-Ethanol Production from Industrial Potato Waste by Statistical Medium Optimization. *Int J Mol Sci, 16*(10), 24490-24505. doi: 10.3390/ijms161024490

Javot, H., & Maurel, C. (2002). The Role of Aquaporins in Root Water Uptake. *Annals of Botany, 90*(3), 301-313. doi: 10.1093/aob/mcf199

Jefferies, R. A. (1993). Cultivar responses to water stress in potato: effects of shoot and roots. *New Phytologist, 123*(3), 491-498. doi: 10.1111/j.1469-8137.1993.tb03761.x

Jefferies, R. A., & Mackerron, D. K. L. (1993). Responses of potato genotypes to drought. II. Leaf area index, growth and yield. *Annals of Applied Biology, 122*(1), 105-112. doi: 10.1111/j.1744-7348.1993.tb04018.x

Juzepczuk, S. W., & Bukasov, S. M. (1929). *A contribution to the question of the origin of the potato.* . Paper presented at the Proc. U.S.S.R. Congr. Plant Anim. Breed, Russia.

Kavar, T., Maras, M., Kidrič, M., Šuštar-Vozlič, J., & Meglič, V. (2008). Identification of genes involved in the response of leaves of Phaseolus vulgaris to drought stress. *Molecular Breeding, 21*(2), 159-172. doi: 10.1007/s11032-007-9116-8

Kooman, P. L., & Haverkort, A. J. (Eds.). (1995). *Modelling development and growth of the potato crop influenced by temperature and daylength: LINTUL-POTATO*. Netherlands: Kluwer Academic Publishers.

Kregel, K. C. (2002). Invited Review: Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *Journal of Applied Physiology, 92*(5), 2177-2186. doi: 10.1152/japplphysiol.01267.2001

Kuppinger, L., Auber, J., Khan, M. A., Farfan, E., Bonierbale, M., & Asch, F. (2014). *Effect of Drought Stress on Crop Development, Growth and Chlorophyll Fluorescence in five Potato Clones*

Lahlou, O., Ouattar, S., & Ledent, J. (2003). The effect of drought and cultivar on growth parameters, yield and yield components of potato. *Agronomie, 23* 257-268. doi: 10.1051/agro:2002089

Lata, C., & Prasad, M. (2011). Role of DREBs in regulation of abiotic stress responses in plants. *Journal of Experimental Botany*. doi: 10.1093/jxb/err210

Legay, S., Lefevre, I., Lamoureux, D., Barreda, C., Luz, R. T., Gutierrez, R., Quiroz, R., Hoffmann, L., Hausman, J. F., Bonierbale, M., Evers, D., & Schafleitner, R. (2011). Carbohydrate metabolism and cell protection mechanisms differentiate drought tolerance and sensitivity in advanced potato clones (Solanum tuberosum L.). *Funct Integr Genomics, 11*(2), 275-291. doi: 10.1007/s10142-010-0206-z

Levitt, J. (1980). *Responses of Plants to Environmental Stresses: Water, Radiation, Salt, and other Stresses* (Vol. 2). New York: Academic Press.

Li, J., Cang, Z., Jiao, F., Bai, X., Zhang, D., & Zhai, R. (2017). Influence of drought stress on photosynthetic characteristics and protective enzymes of potato at seedling stage. *Journal of the Saudi Society of Agricultural Sciences*. doi: http://dx.doi.org/10.1016/j.jssas.2015.03.001

Li, L., Tacke, E., Hofferbert, H. R., Lubeck, J., Strahwald, J., Draffehn, A. M., Walkemeier, B., & Gebhardt, C. (2013). Validation of candidate gene markers for marker-assisted selection of potato cultivars with improved tuber quality. *Theor Appl Genet, 126*(4), 1039-1052. doi: 10.1007/s00122-012-2035-z

Little, T. M. (1945). Gene segregation in autotetraploids. *The Botanical Review, 11*(1), 60-85. doi: 10.1007/bf02861164

Liu, F., Jensen, C. R., Shahanzari, A., Andersen, M. N., & Jacobsen, S.-E. (2005). ABA regulated stomatal control and photosynthetic water use efficiency of potato (Solanum tuberosum L.) during progressive soil drying. *Plant Science, 168*(3), 831-836. doi: http://dx.doi.org/10.1016/j.plantsci.2004.10.016

Loon, C. D. (1981). The effect of water stress on potato growth, development, and yield. [American Potato Journal]. *58*(1), 51-69. doi: 10.1007/bf02855380

MacKerron, D. K. L., & Waister, P. D. (1985). A simple model of potato growth and yield. Part I. Model development and sensitivity analysis. *Agricultural and Forest Meteorology, 34*(2), 241-252. doi: http://dx.doi.org/10.1016/0168-1923(85)90024-3

MacQuarrie, K. (2015). *Life and Death in the Andes: On the Trail of Bandits, Heroes, and Revolutionaries*: Simon & Schuster.

Manhas, J. S., & Sukumaran, N. P. (1988). Diurnal changes in net photosynthetic rate in potato in two environments. *Potato Research, 31*(3), 375-378. doi: 10.1007/bf02357871

Maris, B. (1990). Comparison of diploid and tetraploid potato families derived from Solanum phureja x dihaploid S. tuberosum hybrids and their vegetatively doubled counterparts. *Euphytica, 46*(1), 15-33. doi: 10.1007/bf00057615

Martins, F. R. (1976). *New Archaeological Techniques for the stud of Ancient Root Crops in Peru.* (Ph.D), University of Birmingham, Birmingham.

Mayer, E., & Shea, R. C. (1979). *Land-use in the Andes: Ecology and Agriculture in the Mantaro Valley of Peru with Special Reference to Potatoes*: International Potato Center, Social Science Unit.

Miller, J. C., & Fontenot, J. F. (1965). Methods and techniques for breeding the Irish potato. *American Potato Journal, 42*(6), 174-176. doi: 10.1007/bf02934035

Moorby, J., Munns, R., & Walcott, J. (1975). Effect of Water Deficit on Photosynthesis and Tuber Metabolism in Potatoes. *Functional Plant Biology, 2*(3), 323-333. doi: https://doi.org/10.1071/PP9750323

Muthoni, J., Kabira, J., Shimelis, H., & Melis, R. (2015). Tetrasomic inheritance in cultivated potato and implications in conventional breeding. *Australian Journal of Crop Science, 9*, 185-190.

NSF, P. G. P. (2016). Potato Biology: polyploidy. from NSF

Nwokocha, L. M., Aviara, N. A., Senan, C., & Williams, P. A. (2014). A comparative study of properties of starches from Irish potato (Solanum tuberosum) and sweet potato (Ipomea batatas) grown in Nigeria. *Starch - Stärke, 66*(7-8), 714-723. doi: 10.1002/star.201300237

Ojala, J. C., Stark, J. C., & Kleinkopf, G. E. (1990). Influence of irrigation and nitrogen management on potato yield and quality. *American Potato Journal, 67*(1), 29-43. doi: 10.1007/bf02986910

Onern. (1976). Inventario y Evaluacion de los Recursos Naturales de la SAIS In T. Amaru (Ed.). Lima, Peru: Nacional de Evaluacion de Recursos Naturales.

Osakabe, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., & Tran, L.-S. P. (2013). Sensing the environment: key roles of membrane-localized kinases in plant perception and response to abiotic stress. *Journal of Experimental Botany, 64*(2), 445-458. doi: 10.1093/jxb/ers354

Ottoman, R. J., Hane, D. C., Brown, C. R., Yilma, S., James, S. R., Mosley, A. R., Crosslin, J. M., & Vales, M. I. (2009). Validation and Implementation of Marker-Assisted Selection (MAS) for PVY Resistance (Ry adg gene) in a Tetraploid Potato Breeding Program. *American Journal of Potato Research, 86*(4), 304-314. doi: 10.1007/s12230-009-9084-0

Park, C.-J., & Seo, Y.-S. (2015). Heat Shock Proteins: A Review of the Molecular Chaperones for Plant Immunity. *The Plant Pathology Journal, 31*(4), 323-333. doi: 10.5423/ppj.rw.08.2015.0150

Passioura, J. (2007). The drought environment: physical, biological and agricultural perspectives. *J Exp Bot, 58*(2), 113-117. doi: 10.1093/jxb/erl212

PGSC. (2011). Genome sequence and analysis of the tuber crop potato. *475*(7355), 189-195.

Rajpal, V. R., Rao, S. R., & Raina, S. N. (2016). *Gene Pool Diversity and Crop Improvement*: Springer International Publishing.

Raker, C. M., & Spooner, D. M. (2002). Chilean Tetraploid Cultivated Potato, Solanum tuberosum, is Distinct from the Andean Populations: Microsatellite Data. *Crop Sci., 42*, 1451–1458.

Rodger, E. (2007). *The Biography of Potatoes*: Crabtree Publishing Company.

Salaman, R. N. (1949). The history and social influence of the Potato. Cambridge: Press Syndicate of the University of Cambridge.

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of Botany, 2012*, 26. doi: 10.1155/2012/217037

Sivakumar, R., & Srividhya, S. (2016). *Impact of drought on flowering, yield and quality parameters in diverse genotypes of tomato (Solanum lycopersicum L.)* (Vol. 30).

Smith, A. F. (2011). *Potato: A Global History*. London: Reaktion Books Ltd.

Spooner, D. M. (1990). The potato: Evolution, biodiversity and genetic resources. J.G. Hawkes. *American Potato Journal, 67*(10), 733-735. doi: 10.1007/bf03044023

Sprenger, H., Kurowsky, C., Horn, R., Erban, A., Seddig, S., Rudack, K., Fischer, A., Walther, D., Zuther, E., Kohl, K., Hincha, D. K., & Kopka, J. (2016). The drought response of potato reference cultivars with contrasting tolerance. *Plant Cell Environ*. doi: 10.1111/pce.12780

Stevenson, F. J., & Milstead, E. H. (1932). Potato breeding technique. *American Potato Journal, 9*(7), 111-116. doi: 10.1007/bf02880125

Stift, M., Berenos, C., Kuperus, P., & van Tienderen, P. H. (2008). Segregation Models for Disomic, Tetrasomic and Intermediate Inheritance in Tetraploids: A General Procedure Applied to Rorippa (Yellow Cress) Microsatellite Data. *Genetics, 179*(4), 2113-2123. doi: 10.1534/genetics.107.085027

Szalonek, M., Sierpien, B., Rymaszewski, W., Gieczewska, K., Garstka, M., Lichocka, M., Sass, L., Paul, K., Vass, I., Vankova, R., Dobrev, P., Szczesny, P., Marczewski, W., Krusiewicz, D., Strzelczyk-Zyta, D., Hennig, J., & Konopka-Postupolska, D. (2015). Potato Annexin STANN1 Promotes Drought Tolerance and Mitigates Light Stress in Transgenic <italic>Solanum tuberosum</italic> L. Plants. *PLoS ONE, 10*(7), e0132683.

Tanner, C. B. (1981). Transpiration Efficiency of Potato1. *Agronomy Journal, 73*(1), 59-64. doi: 10.2134/agronj1981.00021962007300010014x

Tessema, B. B. (2017). *Genetic studies towards elucidation of drought tolerance of potato.* Wageningen University, Wageningen. Retrieved from http://edepot.wur.nl/413763

Ugent, D., Dillehay, T., & Ramirez, C. (1987). Potato remains from a late pleistocene settlement in southcentral Chile. *Economic Botany, 41*(1), 17-27. doi: 10.1007/bf02859340

Van As, H., & Windt, C. W. (2008). Magnetic Resonance Imaging of Plants: Water Balance and Water Transport in Relation to Photosynthetic Activity. In T. J. Aartsma & J. Matysik (Eds.), *Biophysical Techniques in Photosynthesis* (pp. 55-75). Dordrecht: Springer Netherlands.

van Muijen, D., Anithakumari, A. M., Maliepaard, C., Visser, R. G. F., & van der Linden, C. G. (2016). Systems genetics reveals key genetic elements of drought induced gene regulation in diploid potato. *Plant, Cell & Environment, 39*(9), 1895-1908. doi: 10.1111/pce.12744

Vos, P. (2016). *Development and application of a 20K SNP array in potato.* (Includes bibliographical references.-With summary in English), Wageningen University, Wageningen. Retrieved from http://edepot.wur.nl/392278C1 - NN08200,6504

Waldron, M. (2015). *Geography Matters in the Inca Empire*: Raintree.

Walker, T., Thiele, G., Suarez, V., & Crissmann, C. (2011). *Hindsight and foresight about potato production and consumption*.

Wang, H., Wang, H., Shao, H., & Tang, X. (2016). Recent Advances in Utilizing Transcription Factors to Improve Plant Abiotic Stress Tolerance by Transgenic Technology. *Frontiers in Plant Science, 7*(67). doi: 10.3389/fpls.2016.00067

Wang, L., Liu, Y., Feng, S., Yang, J., Li, D., & Zhang, J. (2017). Roles of Plasmalemma Aquaporin Gene StPIP1 in Enhancing Drought Tolerance in Potato. *Front Plant Sci, 8*, 616. doi: 10.3389/fpls.2017.00616

Waseem, M., Ali, A., M.Tahir, Nadeem, M. A., Ayub, M., Tanveer, A., Ahmad, R., & Hussain, M. (2011). Mechanism of drought tolerance in plant and its management through different methods. *Continental J. Agricultural Science, 5*(1), 10 - 25.

Watanabe, K. (2015). Potato genetics, genomics, and applications. *Breeding Science, 65*(1), 53-68. doi: 10.1270/jsbbs.65.53

Weisz, R., Kaminski, J., & Smilowitz, Z. (1994). Water deficit effects on potato leaf growth and transpiration: Utilizing fraction extractable soil water for comparison with other crops. *American Potato Journal, 71*(12), 829-840. doi: 10.1007/bf02849378

Xu, Z., Zhou, G., & Shimizu, H. (2010). Plant responses to drought and rewatering. *Plant Signaling & Behavior, 5*(6), 649-654.

Yan, N., Liu, Y., Gong, D., Du, Y., Zhang, H., & Zhang, Z. (2015). Solanesol: a review of its resources, derivatives, bioactivities, medicinal applications, and biosynthesis. *Phytochemistry Reviews, 14*(3), 403-417. doi: 10.1007/s11101-015-9393-5

Yoshida, T., Mogami, J., & Yamaguchi-Shinozaki, K. (2014). ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. *Curr Opin Plant Biol, 21*, 133-139. doi: https://doi.org/10.1016/j.pbi.2014.07.009

DROUGHT RESPONSE IN FIELD GROWN POTATOES AND THE INTERACTIONS BETWEEN CANOPY GROWTH AND YIELD

Ernest B. Aliche[a, b], Marian Oortwijn[a], Tom P. J. M. Theeuwen[a, b], Christian W. B. Bachem[a], Richard G. F. Visser[a], C. Gerard van der Linden[a]

[a]*Laboratory of Plant Breeding, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands*

[b]*Graduate School Experimental Plant Sciences, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands*

Corresponding author: ***C. Gerard van der Linden*** +31(0)317 480 850, gerard.vanderlinden@wur.nl

Published in Agricultural Water Management Journal as:

Ernest B. Aliche, Marian Oortwijn, Tom P.J.M. Theeuwen, Christian W.B. Bachem, Richard G.F. Visser, C. Gerard van der Linden, Drought response in field grown potatoes and the interactions between canopy growth and yield, Agricultural Water Management, Volume 206, Pages 20-30, ISSN 0378-3774, https://doi.org/10.1016/j.agwat.2018.04.013.

ABSTRACT

Potato is an important food crop with high yields. However, when exposed to drought it suffers major yield losses. Considering its global importance and the increasing incidence of drought due to climate change, research toward drought tolerance in potato remains imperative. We have studied a set of 103 commercial cultivars representing the genetic diversity in the European potato market. The cultivars were grown in different field locations in three subsequent years (2013 – 2015). Our aim was to understand how different field drought regimes affect canopy growth in potato, and how these effects translate to tuber yield. The field environmental conditions were monitored and pictures of canopy ground cover during the growing season were taken. Canopy growth parameters were extracted by an iterative method using the beta sigmoid growth function to model canopy growth. At harvest, tuber yield was scored and tuber size was graded. The GGE (Genotype and Genotype-by-Environment) bi-plot and Finlay Wilkinson's Regression were used to investigate Genotype-by-Environment interactions. We observed that the timing of the drought occurrence differentially affected canopy growth and tuber yield. Under drought stress, fast attainment of exponential growth and maximum canopy cover had negative effects on tuber formation and tuber bulking. Growth rate, maximum canopy cover and area under the canopy curve (photosynthetic capacity over the growth season) were more important for tuber bulking than they were for tuber formation under drought stress. Cultivars with high yield were identified as potential material for improvement to drought tolerance. These findings will contribute to the breeding of drought-tolerant potato amidst the threats of climate change.

Keywords: Irrigation; Rainfall; Stress; AUC; Maturity.

INTRODUCTION

Climate change negatively impacts agricultural production, especially in marginal regions with limited inputs like fresh water. The negative effects of water limitation on crop yield are critical for drought-sensitive crops of high importance for food production and security, like potato. Potato is the world's 3rd most important food crop, and its production in the developing world has increased in the last two decades, demonstrating its important contribution to food security (Acton, 2013). The global production of potato is estimated at 377 million tonnes in about 19 million hectares (FAOSTAT, 2016). When compared to grain-producing crops, a hectare of potato can yield about two- to four-fold more calories (CIP, 2013). Potato is known for its efficiency in water usage (Shahnazari *et al.,* 2007; Vreugdenhil *et al*., 2007). In comparison with other major crops, potato produces the highest amount of calories per unit water input and it is seven times more efficient than some cereals, like wheat, maize, etc. (CIP, 2013; FAO, 2008). However, potato is generally drought-sensitive (Schafleitner *et al.*, 2008), with losses in yield that can reach 79% reduction if water requirements are not met (Binod *et al.,* 2015).

The Palmer Drought Severity Index predicts a widespread drought in many regions of the globe including Europe in the next 30 – 90 years arising from reduced rainfall and/or increased evaporation (Dai, 2013). The drought sensitivity of potato may be attributed to the stress effects

on its foliage characteristics (Deblonde and Ledent, 2001; Schittenhelm *et al*., 2006; Soltys-Kalina *et al*., 2016; Romero *et al*., 2017;) and its shallow root system (van Loon, 1981; Yamaguchi and Tanaka, 1990; Iwama *et al.*, 1993; Opena and Porter, 1999; Stalham *et al.* 2007; Zarzynska et al., 2017) that make water uptake inefficient (Luisa *et al.*, 1997). In comparison with many other crops, leaf stomatal closure occurs in potato at relatively low soil moisture deficits perceived by the roots (Sadras and Milroy, 1996). This leads to a significant drop in transpiration even before significant reduction in leaf water potential occurs (Liu *et al.*, 2005). Stomatal closure at relatively high leaf water potential (-0.4 MPa and -0.6 MPa) may already limit photosynthesis, with reduced production of assimilates and canopy growth, and a resultant drop in tuber yield and quality (Luisa *et al*., 1997). Therefore, the drought response in potato and possibly, tolerance, may be closely linked to a bias-free quantification of the progress of canopy growth. (Bojacá *et al.*, 2011).

Many techniques have been developed to facilitate the monitoring of canopy growth. These include the grid system that measures ground area covered; near-infrared reflectance, which measures interception of solar radiation; picture image capture of canopy cover and image analysis; and remote sensing using satellite data (Bojacá *et al.*, 2011; Bouman *et al.*, 1992; Korva, 1996; Prashar & Jones, 2014; Sivarajan, 2011). In addition to monitoring canopy growth as described above, accurate quantification, extraction and interpretation of canopy growth parameters will give deeper insight into the traits of interest for crop improvement (Chen *et al*., 2014). Potato canopy growth has been described by several authors using growth models of good fit to show the progress of canopy from emergence towards senescence (Khan, 2012; Ospina *et al.*, 2014). Under drought conditions several growth measurements in field grown potato have been reported, which have enhanced our understanding on how to manage different drought regimes in the field (Jefferies & Mackerron, 1993; Mackerron *et al.*, 1988; Ouiam *et al.*, 2003; Shiri *et al.*, 2009; Steyn *et al.*, 2007). The modelling of potato growth under drought, however, still requires more research to understand canopy cover dynamics. Moreover, due to the difficulties in managing field experiments, potato field drought reports are often based on only a few genotypes. This challenges the generalization of conclusions from such field reports.

Percentage ground cover by canopy is known as a good measure of intercepted solar radiation in potato, which is also reflected in dry matter production (Haverkort *et al.*, 1991; Lemaga & Caesar, 1990; Vreugdenhil *et al.*, 2011). Interception of solar radiation is reduced under drought conditions depending on the severity of the stress, due to reduced leaf expansion and reduction in total number of leaves (Harris, 2012). Potato canopy growth has been described in three phases including the build-up phase, maximum canopy cover phase and decline or senescence phase (Khan, 2012). The build-up phase includes the period from emergence till full canopy cover, and this often coincides with tuber initiation stage of the plant (Haverkort & Mackerron, 1995). The maximum canopy cover and decline phases are periods during which the tubers have to be filled with assimilates (bulking). The duration of these phases depends on the tuber growth rate and foliage maturity class of the potato genotype (Haverkort & Mackerron, 1995). Potato genotypes that invest a major part of their life cycle in canopy growth (late maturity genotypes) can intercept about 700 MJ/m^2 (Zaag, 1992), while early maturity potato genotypes start investing photosynthetic assimilates in their tubers much earlier, and thus complete their

life cycle early (Kooman & Rabbinge, 1996). These differences in genotype and maturity type imply different effects of canopy cover on yield. Our study is the first to investigate these canopy cover effects on potato yield using an extensive set of genotypes representing different foliage maturity types under field drought conditions in different environments. Potato yield is the resultant of the number of tubers formed and the volume (weight and size) of the tubers. Deblonde and Ledent (2001) reported that tuber number was reduced under drought, which was compensated by a higher tuber dry weight. Some reports indicate that drought causes more reduction in tuber weight than tuber number (Binod *et al.*, 2015), but this may be highly dependent on genotypic differences and timing of the drought. Partitioning of assimilates to tubers for tuber formation as well as bulking and the interaction between these processes may be important for drought tolerance improvement of potato.

In this study we have evaluated the growth and yield of 103 potato cultivars in three different locations in three years. The aim was to investigate the genotypic variation of the drought response in cultivated potato with respect to canopy growth and yield under field conditions. Our objectives were to understand (i) how the timing of drought in the growing season affects potato growth and yield in the field (ii) which canopy growth characteristics are critical for potato tuber yield under drought in the field (iii) the stability of drought tolerance of potato cultivars across locations and in different years (iv) which aspects of yield are adversely affected in the field during drought.

MATERIALS AND METHODS

Field Location and Planting

A selection of 103 commercial potato cultivars with different genetic backgrounds and foliage maturity classes (early, intermediate and late) were used in this study (see Chapter 3 - Supplementary Table 1). The cultivars are part of the European potato gene pool used by D' Hoop *et al.* (2010) for genome-wide association studies. Field trials were conducted in partnership with four potato breeding companies (Averis seeds B. V., C. Meijer, HZPC Holland BV and KWS POTATO). Tubers used for the trials of each year were multiplied in the previous year at a single breeding station ensuring uniformity of seed tuber conditions. A split-plot design was used for each of the trials in three consecutive years (2013, 2014 and 2015), with irrigation levels assigned in the main plots as blocks and genotypes assigned in subplots. The fields were located in Connantre, France (48.7258°N, 3.9219°E) from 2013 - 2015; and in the Netherlands, Zeeland (51.5667°N, 3.7500°E) in 2013 and 2014; Emmeloord (52.7097°N, 5.7508°E) in 2013; and Grolloo (52.9305°N, 6.6943°E) in 2014. The field structure in each location and year included two blocks, irrigated (WR) and non-irrigated (DR) treatments. In each block, the cultivars were randomized as sub-plots within the blocks. Each subplot (experimental unit) had eight plants of a single cultivar in two rows (four plants per row). The spacing between plants in a row was 30cm, and 70cm between rows. Border plants were planted in between subplots of each row. The rows were set on ridges. The tubers were planted in April 2013 at Connantre, Zeeland and Emmeloord; April 2014 at Connantre and Zeeland, and May 2014 at Grolloo; and April 2015 at Connantre. The plants remained in the field until harvest at

the beginning of Fall in the respective locations and years. Environmental conditions of rainfall, temperature (aerial and soil), radiation, relative humidity, wind speed and wind direction were monitored at the Connantre field in 2014 and 2015 using facilities provided by Dacom B.V. Environmental data from nearby weather stations were used for the other trials. The control blocks were irrigated weekly during periods of the drought (less rainfall) (e.g., Fig.6d).

Phenotyping and Data collection

Potato tubers germinated within three weeks of planting. The emergence date was recorded as days after planting when more than half of the plants per plot had germinated. Canopy ground cover was monitored by taking pictures of each plot weekly with a SONY DSC-W610 digital camera, to infer canopy growth. The camera was mounted on a rectangular frame at a specific height from the frame throughout the trial, and the frame was positioned just above the canopy. The dimension of the rectangular frame was set to capture the inner two plants of each plot. *Plant height* was scored within a month from emergence using the highest apex of each plot. At harvest various yield traits were measured including *tuber fresh weight* (TBW), *tuber number* (TBN), *underwater weight* (UWW), *dry matter percentage* (DMP, only in Connantre) and tuber quality by visual impression. A sample of 5.05kg of harvested tubers per plot was used to measure UWW. The 5.05kg was lowered in water and the weight under water measured according to EU-direction https://webgate.ec.europa.eu/agriportal/angebleu/pdf.download?docNum=32009r0571&lg=EN. UWW is used to infer dry matter and starch content of tubers (Haase, 2003). A smart grader system was employed to grade the tubers into size classes as follows: 0-40mm, 40-50mm, 50-60mm, 60-70mm and >70mm. This enabled us to score *tuber number* and *tuber fresh weight* per size class.

Data Processing

We transformed the calendar days after emergence of the plants into thermal days in Beta Thermal Time (BTT(td)) according to Khan (2012); Ospina *et al.* (2014); and Hurtado-Lopez *et al.* (2015), to account for differences in the effects of temperature on crop development in various years and locations. We accounted for this non-linear relationship between temperature (T) and growth rate ($g(T)$) according to the equation, $g(T) = [((T_c-T)/(T_c-T_o))*((T-T_b)/(T_o-T_b))^{((To-Tb)/(Tc-To))}]^{ct}$ described in Yin *et al* (1995), using base Temperature (T_b=5.5ºC), optimal temperature (T_o=23.4ºC), ceiling temperature (T_c=34.6ºC), temperature response curvature coefficient (c_t=1.6) and daily mean temperature (T). Thus, the BTT(td) for a given day is the accumulated $g(T)$ from emergence up until that day.

We processed the canopy pictures in MATLAB^R software R2013a version with DIPimage toolbox, using an algorithm as in Ospina *et al.* (2014). The percentage canopy cover output from MATLAB was used to fit a canopy growth model according to the sigmoid phase of the beta function for determinate growth as described by Khan (2012). This model was fitted using the iterative non-linear least-square regression method implemented in the PROC NLIN package of the SAS software (SAS Institute Inc., 2015). From the fitted model canopy growth parameters were extracted including exponential growth rate ($\boldsymbol{C_{m1}}$), time to reach exponential

growth rate (t_{m1}), maximum canopy cover (V_{max}), time to reach maximum canopy cover (t_1) and area under canopy cover (A_1).

Statistical Analysis

The data from the yield traits was analysed using GENSTAT 17th Edition. Our aim was to test for the significance of multi-factorial effects on drought tolerance in our dataset. These factors include: genotype, location and year effects, and their interaction effects. We used a threshold level of significance of 0.05. The model is as follows:

$$t_{ijk} = \mu + G_i + E_j + Y_k + GE_{ij} + GY_{ik} + EY_{jk} + GEY_{ijk} + \varepsilon_{ijk} \qquad (1)$$

where, t_{ijk} is the mean phenotypic trait value of the i^{th} genotype in the j^{th} location and k^{th} year; μ is the overall mean; G_i is the i^{th} genotypic effect; E_j is the j^{th} location effect; Y_k is the k^{th} year effect; GE_{ij} is a two-way interaction between the genotypic and location factors; GY_{ik} is a two-way interaction between the genotypic and year factors; EY_{jk} is a two-way interaction between the location and year factors, GEY_{ijk} is a three-way interaction between the genotypic, location and year factors; and ε_{ijk} is the residual or random error effect.

Multivariate bi-plots were used to observe trait interactions and their contributions to the principal components. For this, the traits were visualized as vectors, showing their respective effects on the variations observed in the dataset. Furthermore, the Spearman's correlation coefficients between each of the traits were computed in RStudio 3.2.3 to show specific trait-to-trait relationships.

Performance and Stability Analysis

Finlay Wilkinson's Regression (FWR) was used to assess the quality of the different environments with respect to drought impact on the plants. We used Tuber fresh weight (TBW, also referred to as yield or tuber yield) to implement FWR by subtracting the mean tuber weight of each environment from the overall mean tuber weight of all environments to derive *Environmental indices* (Finlay & Wilkinson, 1963). For this, we used each year-location-treatment combination as a separate environment, summing up to 14 different environments. This revealed the relative quality of each environment (*Environmental indices*) and the level of drought effects in the various locations, giving insights on the locations with higher priority for this study. Quality in this context describes the extent of the effects of drought stress within a location as well as the effect of other environmental differences between locations on the tuber yield. The difference between mean yield under non-irrigated and irrigated conditions of a location per year is a measure of the drought stress effect in that particular field trial. The FWR was also applied to individual cultivars to observe the responsiveness and stability in yield to drought of the various cultivars in the different locations.

Furthermore, based on the outcome of the Analyses of Variance genotype by environment interactions were investigated using GGE bi-plots. GGE bi-plots display the partitioning of the genotype main effect (G) plus GxE interaction effect (GE), with genotypes as entries to be tested in multi-environments (testers). It gives information on which environment is most representative of others (mega-environment), the best test environment and genotypes that are

superior to others in terms of high and stable performance in a mega-environment (Yan & Tinker, 2006). It uses the singular value decomposition (SVD) and partitioning to decompose G+GE effects into principal components, represented with bi-plots (Gedif & Yigzaw, 2014).

RESULTS

Drought effects on yield traits

In all field locations in the three years of study of 103 commercial cultivars, drought had the most severe effect on tuber fresh weight (TBW), with the strongest reduction (54%) in Connantre (2015) (Fig.1). Less reduction in tuber number (TBN) than in TBW was observed. The strongest reduction (14%) in TBN occurred in Connantre (2014). Drought stress did not reduce underwater weight (UWW). We analysed the effects of the environment (location and year variations) on the drought response of the cultivars using tuber fresh weight.

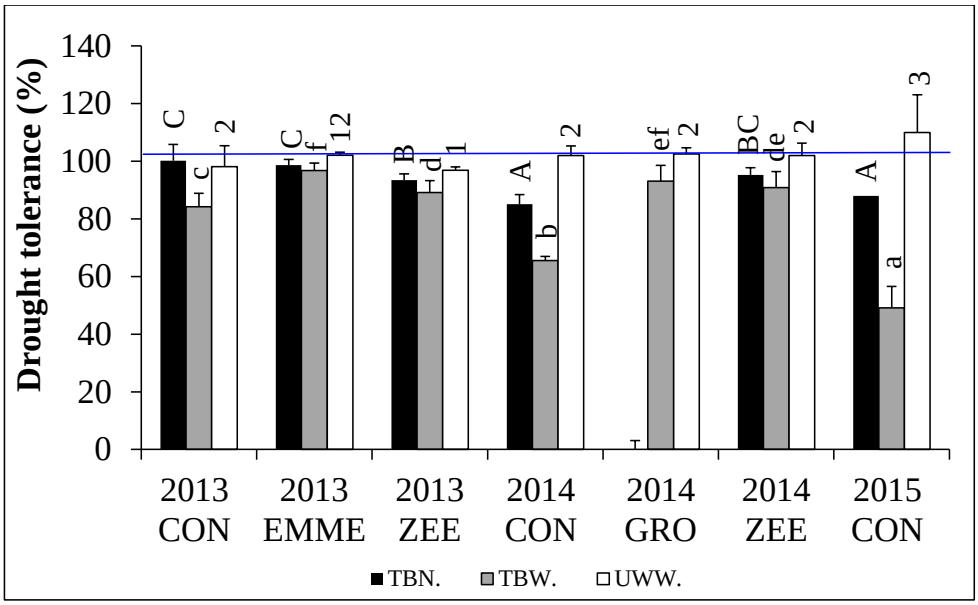


Figure 1: Drought tolerance (Mean percentage of yield trait values observed under drought stress) in all locations for all cultivars. This was computed as: *(Trait value at No-irrigation/Trait value at Irrigation) * 100*. The yield traits are TBN (tuber number), TBW (tuber fresh weight) and UWW (underwater weight). The locations are CON (Connantre), EMME (Emmeloord), ZEE (Zeeland) and GRO (Grolloo). In Grolloo, TBN was not scored. Error bars are standard deviations and sample size is 103 cultivars per location. Bars with different upper case letters, lower case letters, or numbers are significantly different.

Multi-Environment characteristics

Environmental quality and cultivars' responsiveness

According to the FWR environmental indices, the weakest effect of drought stress was observed in Emmeloord (2013) and Grolloo (2014) (Fig.2). In fact, the weather data indicated that these

trials only had few days between successive rainfalls, so these were likely to experience no water limitation (data not shown). Locations of intermediate quality were Connantre (2013) and Zeeland (2013 & 2014). The highest level of drought stress relative to the irrigated field was observed in the two Connantre trials (2014 & 2015).

The FWR also gave information on the responsiveness and stability of the individual cultivars used in this study. The regression equation for each genotype was fitted on the FWR. The slope and intercept of the regression lines were extracted for each genotype. The slope gives information on how stable or responsive (with respect to tuber yield) a genotype is across all locations. Responsive and unstable genotypes have a steep slope >1, with high yields in irrigated and less-stressed environments, but highly reduced yield in more stressed fields. A less steep slope is attributed to a more stable genotype that is less responsive to changing conditions. The intercept is a measure of the relative performance of the genotype across all environments of irrigated and non-irrigated treatments. Thus, the FWR outcome for each genotype presents its tuber yield average across all environments (both irrigated and non-irrigated).

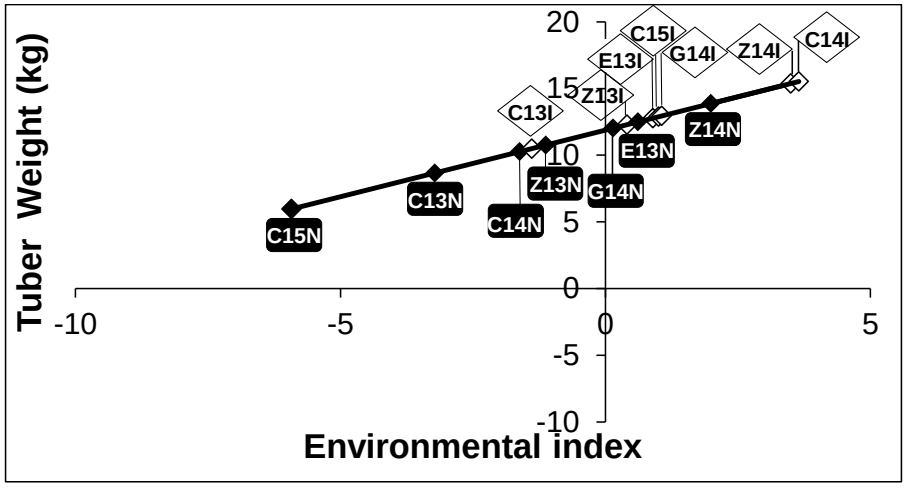


Figure 2: Finlay Wilkinson's Regression of tuber fresh weight per subplot, showing the environmental indices of different field environments where the 103 potato cultivars were grown. Black diamonds on the Regression line represent non-irrigated (stress) environments while white diamonds are irrigated environments. The codes: C=Connantre; Z=Zeeland; E=Emmeloord; G=Grolloo; 13=2013; 14=2014; 15=2015; I=irrigated; N=non-irrigated.

A comparison of three genotypes (Lady Lenora, Mondial and Adora) with contrasting features is shown in Figure 3a. Lady Lenora had the lowest slope and was therefore the genotype with the most stable tuber yield. Mondial had the highest intercept, with a large difference in tuber yield between irrigated and non-irrigated treatments, and Adora had the lowest relative tuber yield (the lowest intercept). A scatter plot of all intercepts vs. slopes from the FWR of all genotypes shows that there was a positive correlation between intercept (relative performance) and slope (responsiveness across environments towards less stress and instability) (Fig.3b). The

figure was divided in 4 quadrants, and different maturity types were not equally distributed over the quadrants. Quadrant ***I*** contains 13 genotypes with slope < 1 and intercept > 0 that were quite stable across all locations (both irrigated and non-irrigated) and higher than overall average in tuber yield. The genotypes in this quadrant include five late maturity types (50% of all **late** maturity types), three intermediate maturity types (7% of all intermediate maturity types) and five early maturity types (10% of all early maturity types). Quadrant ***II*** contains 38 genotypes with slope > 1 and intercept > 0 that have a low stability across locations, but with higher performance than the overall average. These include three late maturity types (30% of all late maturity types), 27 intermediate maturity types (61% of all **intermediate** maturity types) and eight early maturity types (17% of all early maturity types). Quadrant ***III*** contains 41 genotypes that have slope < 1 and intercept < 0 and are quite stable across locations, but are lower than overall average in yield. These include two late maturity types (20% of all late maturity types), 13 intermediate maturity types (32% of all intermediate maturity types) and 27 early maturity types (57% of all **early** maturity types). Quadrant ***IV*** has slope > 1 and intercept < 0, contains 9 unstable and lower-than-average yielding genotypes with no late maturity type, two intermediate maturity types (4.5% of all intermediate types) and seven early maturity types (15% of all early maturity types).

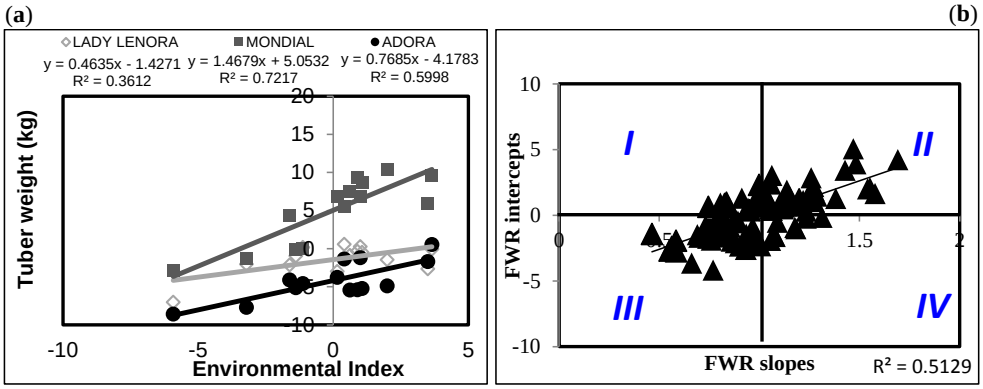


Figure 3: (**a**) Finlay Wilkinson's Regression (FWR) of relative tuber yield of three genotypes (tuber fresh weight of the genotype minus mean tuber fresh weight of all genotypes) for each environment versus environmental index of each environment. The environmental index axis represents the quality of each environment and is the same as the x-axis of Figure 2, (**b**) Scatter plot of all intercepts and slopes from the Finlay Wilkinson's Regression (FWR) of the 103 genotypes, showing a positive correlation between slope and intercept. The genotypes are divided over four quadrants (***I- IV***)**: I** (stably high-yielding across all environments), **II** (high-yielding but sensitive in drought-stressed environments), **III** (low-yielding across all environments), **IV** (low-yielding under irrigation and drought)

<u>Genotype by Environment interaction</u>

In order to incorporate the genotypic effect of drought tolerance as a factor and still account for the contribution of location and year to the variation in our dataset, drought tolerance expressed as percentage ((*Tuber weight under stress / Tuber weight under irrigation*) * *100%*) was used

for an ANOVA. High percentages imply drought tolerance. The ANOVA linear model was fitted according to equation (1). Significant effects of genotype, location and year were observed (Table 1). Also, we observed significant interactions between location and year, and between genotype and location. Location and Genotype by Location interaction had the highest contribution to the non-random total variation (Sum of Squares), 37.92% and 22.41%, respectively. Therefore, we further investigated Genotype by Environment (GxE) interaction. This was done using GGE bi-plot analysis (Gedif & Yigzaw, 2014; Yan & Tinker, 2006).

Table 1: Analysis of variance (ANOVA) of mean percentage tuber weight reduction under stress

Factors (Sources of Variation)	Sum of Squares	F.pr*	Percentage of Variation
Genotype	58915.4	0.044	7.74
Location	288791.3	<.001	37.92
Year	123294.1	<.001	16.19
Genotype x Location	170697.5	0.007	22.41
Genotype x Year	58272.6	1.000	7.65
Location x Year	21177.0	<.001	2.78
Genotype x Location x Year	40406.9	0.720	5.31

*F-probability (at α=0.05 level of significance)

Genotypic drought response in mega-environments

The first two principal components (PC1 and PC2) of the GGE bi-plots using all year-location combinations accounted for 42% of the GGE variation (Fig.4a and b). The different years of both the Connantre and Zeeland trials show a similar effect on drought tolerance (Fig.4a), but the level of variation differed between years for each location, confirming that year effect was significant (Table 1). The GGE bi-plot did not capture most of the variation in the dataset due to the huge differences in environmental quality as seen from the FWR (Fig.2). Therefore, we used the mega-environment option to structure the dataset into groups of similar drought severity. This resulted in five mega-environments (Fig.4b). We used the mega-environments with highest consensus (Connantre (2014 & 2015) and Zeeland (2013 & 2014)) to re-compute the GGE plot (Yan & Tinker, 2006). These year-location combinations also showed the strongest yield quality difference between drought and irrigated fields in the FWR (Fig.2).

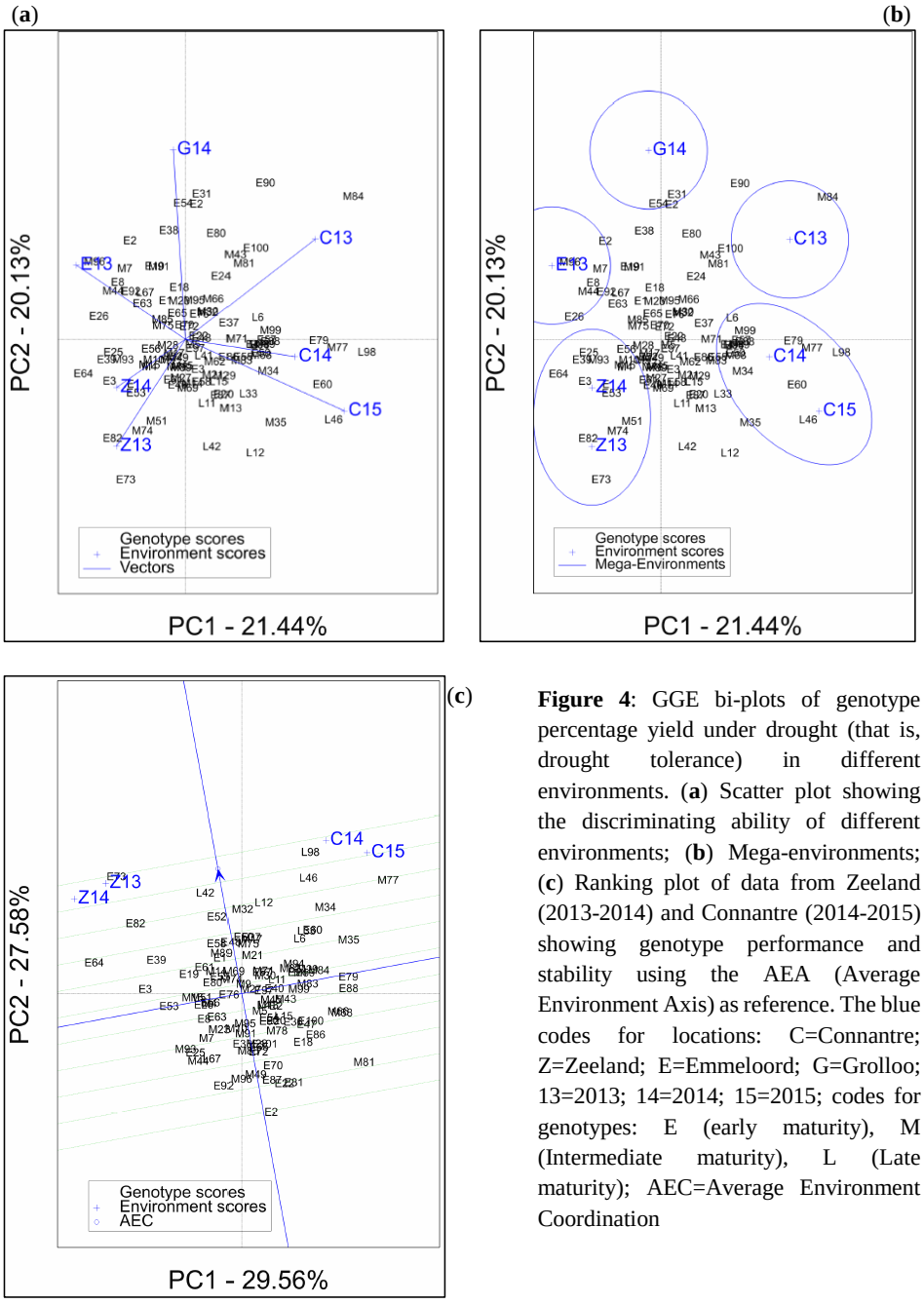


Figure 4: GGE bi-plots of genotype percentage yield under drought (that is, drought tolerance) in different environments. (**a**) Scatter plot showing the discriminating ability of different environments; (**b**) Mega-environments; (**c**) Ranking plot of data from Zeeland (2013-2014) and Connantre (2014-2015) showing genotype performance and stability using the AEA (Average Environment Axis) as reference. The blue codes for locations: C=Connantre; Z=Zeeland; E=Emmeloord; G=Grolloo; 13=2013; 14=2014; 15=2015; codes for genotypes: E (early maturity), M (Intermediate maturity), L (Late maturity); AEC=Average Environment Coordination

GGE bi-plot analysis of this subset resulted in 57.15% variation explained between these two locations and years based on PC1 and PC2 (Fig.4c). The selection of genotypes out of our genotype set for cultivation in North-Western Europe (and similar climates) with respect to yield maintenance under drought can be based on these two mega-environments. We used the

GGE ranking bi-plot to evaluate the genotype mean drought tolerance and stability for these two mega environments (Fig.4c). In the ranking bi-plot (Fig.4c), the Average Environment Axis (AEA) arrow direction indicates higher drought tolerance across environments, and genotypes close to AEA line are more stable in their drought response across environments. Genotypes with high drought tolerance in Zeeland (2013/2014) and Connantre (2014/2015) included Musica (E73), Valiant (L98) and Karnico (L42) (Fig.4c). In the FWR based on yield, Valiant and Karnico clustered in Quadrant *I* (Fig.3b), and were identified as cultivars with stable and high yield. The GGE bi-plots presented Karnico as a better choice than Valiant with respect to stability in drought tolerance, based on its close proximity to the AEA line (Fig.4c). Therefore, with the GGE bi-plots we were able to better identify stable best-performing drought tolerant genotypes among genotypes of high yield performance. An example of a genotype that was drought sensitive across environments is Adora (E2) (Fig.4c). Adora was identified as a stable low-yielding cultivar, clustering in Quadrant *III* of the FWR (Fig.3b).
In summary, genotype and environment had a significant contribution to the variation in our dataset, and employing both FWR and GGE enabled us to explore these.

Drought effects on tuber growth

Tuber fresh weight is the yield trait that was the most drought-affected in our trials. To learn more about which aspect of tuber yield was most affected by drought, the data on tuber size grading was used as a measure of the extent to which drought stress affected tuber formation and bulking. Figure 5a-d shows the differences in tuber number distribution over the different size classes in response to drought particularly in the Connantre trials. The other location-year combinations, with only minor or no water limitation, had a similar pattern of tuber size distribution under irrigated conditions with no effect of drought (Fig.5d). In Connantre, each year had a unique pattern of tuber size distribution. In 2013, drought caused a small shift to smaller tubers (0-40mm) at the expense of 50-60mm size class (Fig.5a). In 2014, the 40-50mm size class was more abundant under stress while the larger size classes had reductions in tuber number (Fig.5b). The 2015 tuber size distributions showed a more severe effect (Fig.5c). The smallest size class was highly represented, while the largest size class was absent. These differences in drought response between the years are likely caused by different timing and severity of drought in each year.
Similarly, in terms of tuber weight per size class, there was no reduction in the various size classes in Emmeloord. Reductions in higher size classes were observed in Zeeland, and even more severe reductions in Connantre especially in 2014 and 2015 (Supplementary Fig.SF1), again indicative of the higher level of drought stress in these years at this location. Interestingly, foliage maturity type differences among the cultivars had little effect on tuber size distribution in our dataset. So drought similarly affected tuber number and tuber weight distribution across various tuber size classes, with less tubers of large sizes in more severe drought conditions.

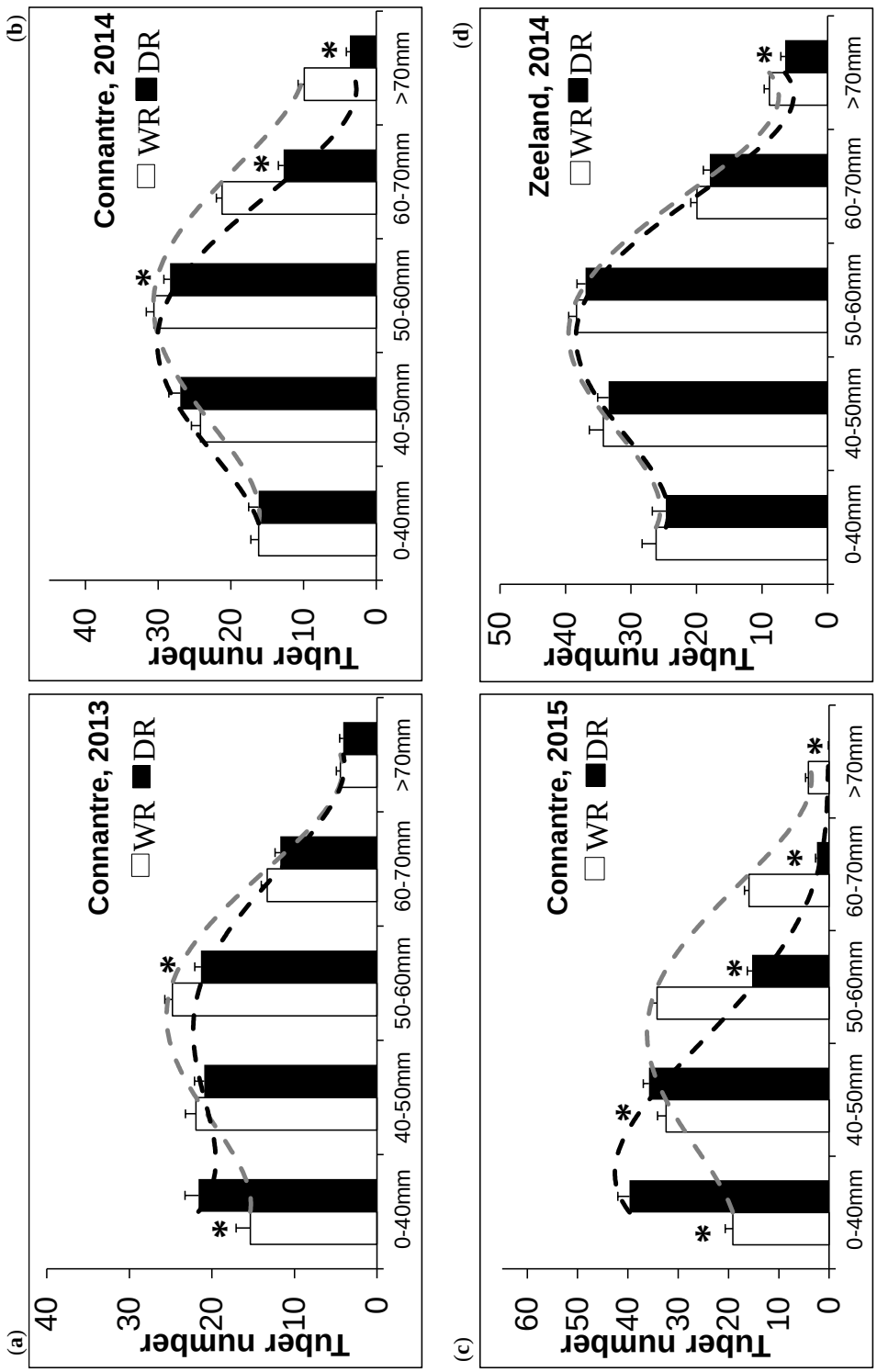


Figure 5: (**a-c**) Mean number of tubers in each subplot per size class of 103 genotypes in Connantre (2013-2015) and (**d**) Zeeland (2014), under irrigated (WR) and non-irrigated (DR) conditions. Error bars represent standard errors of the mean of each dataset. Significant differences between WR and DR in each size class are indicated with '*' at $p \geq 0.05$

Drought stress at Connantre

The yield in Connantre in 2014 & 2015 was strongly reduced by drought, but the drought had a differential effect on the tuber number and tuber fresh weight (Fig.1). The differences between the two years were further examined, using the extensive weather data for these year-location combinations. This included rainfall, temperature, relative humidity and radiation (Supplementary Fig.SF2). The most obvious difference is that the total rainfall and mean relative humidity in 2015 during the potato growing season (May – August) were less than in 2014 (Supplementary Fig.SF2), with only minor differences in mean temperature and radiation, confirming that the drought severity was different between the two years. The higher drought stress in 2015 was reflected in more severely reduced tuber fresh weight in 2015 compared to 2014 (Fig.1). However, total tuber number was affected more in 2014 than in 2015. There were differences in drought timing between the two years. In 2015, drought set in later in the growing season and may have affected tuber bulking more than tuber formation, while the early drought in 2014 may have delayed the formation of new tubers. In order to better understand how the rainfall pattern may have affected the eventual yield, the canopy data was analysed alongside available rainfall/irrigation information (Fig.6).

Drought Impact on Canopy Growth

Two clearly different patterns of canopy growth were observed in 2014 and 2015 at Connantre (Fig.6). Canopy cover was reduced at an early stage in 2014 (Fig.6a), coinciding with drought at an early stage of the growth cycle as indicated in the rainfall information for 2014 (Fig.6c). Little rain had fallen during early stages of growth (from emergence till 30 thermal days after emergence). Ninety percent of the total amount of rainfall occurred after day 30. This early drought most likely caused the delay in increase of canopy growth rate (Fig.6a). Later on in the season when the rainfall became more frequent and water availability increased, the canopy growth rate increased until maximum canopy cover (Fig. 6a, c). The irrigated genotypes reached maximum canopy cover earlier than the stressed genotypes. In 2015, canopy cover was reduced at later growth stages under non-irrigated conditions (Fig.6b). The rainfall information for 2015 (Fig.6d) showed that 62.6% of rain fell before 32 thermal days. As a result of this, the canopy growth was not reduced until this stage. However, after this a drought spell affected the plants from 34 till 58 thermal days. The low water availability in this period most likely resulted in reduction in canopy growth, reaching a maximum canopy cover that is only about half of the maximum canopy cover under irrigated conditions. Genotypes in both irrigated and non-irrigated treatments of 2015 reached their maximum canopy cover at the same time and began to senesce at about the same time (Fig.6b).

The canopy growth of the 103 cultivars was studied to explore the variation among the commercial cultivars. For this, a canopy growth curve was fitted using the beta function proposed by Khan (2012) with emphasis on the sigmoid phase and the maximum canopy cover phase of growth according to available data. This iterative estimation approach resulted in a good-fit derivation of the following growth parameters: exponential growth rate (C_{m1}), time to reach exponential growth rate (t_{m1}), maximum canopy cover (V_{max}), time to reach maximum canopy cover (t_1) and area under canopy cover curve (A_1).

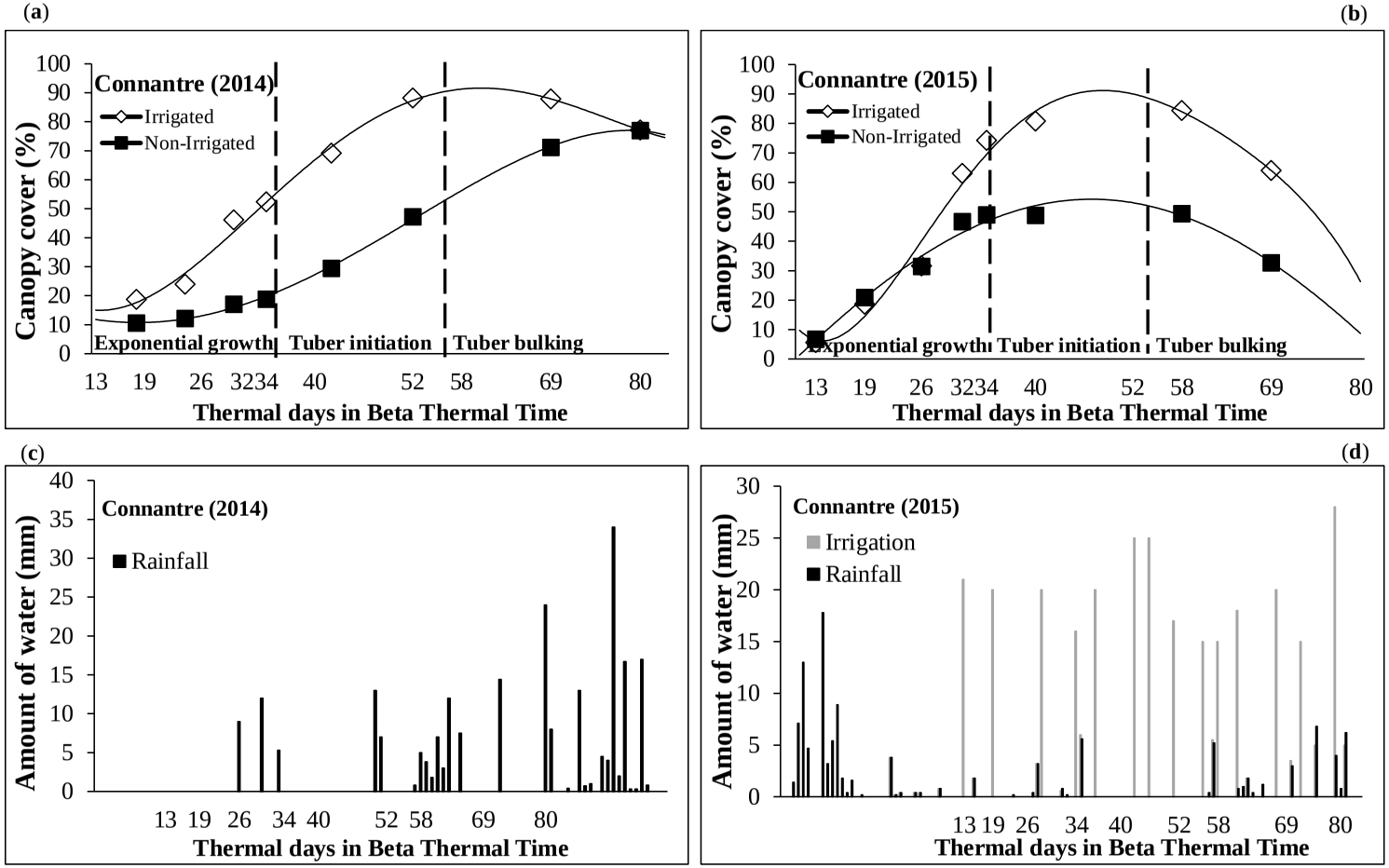


Figure 6: Interaction between rainfall distribution/drought stress and canopy growth in thermal days after emergence (td) in Connantre. (**a**) Canopy growth in 2014. (**b**) Canopy growth in 2015. (**c**) Rainfall in 2014. (**d**) Rainfall and irrigation in 2015.

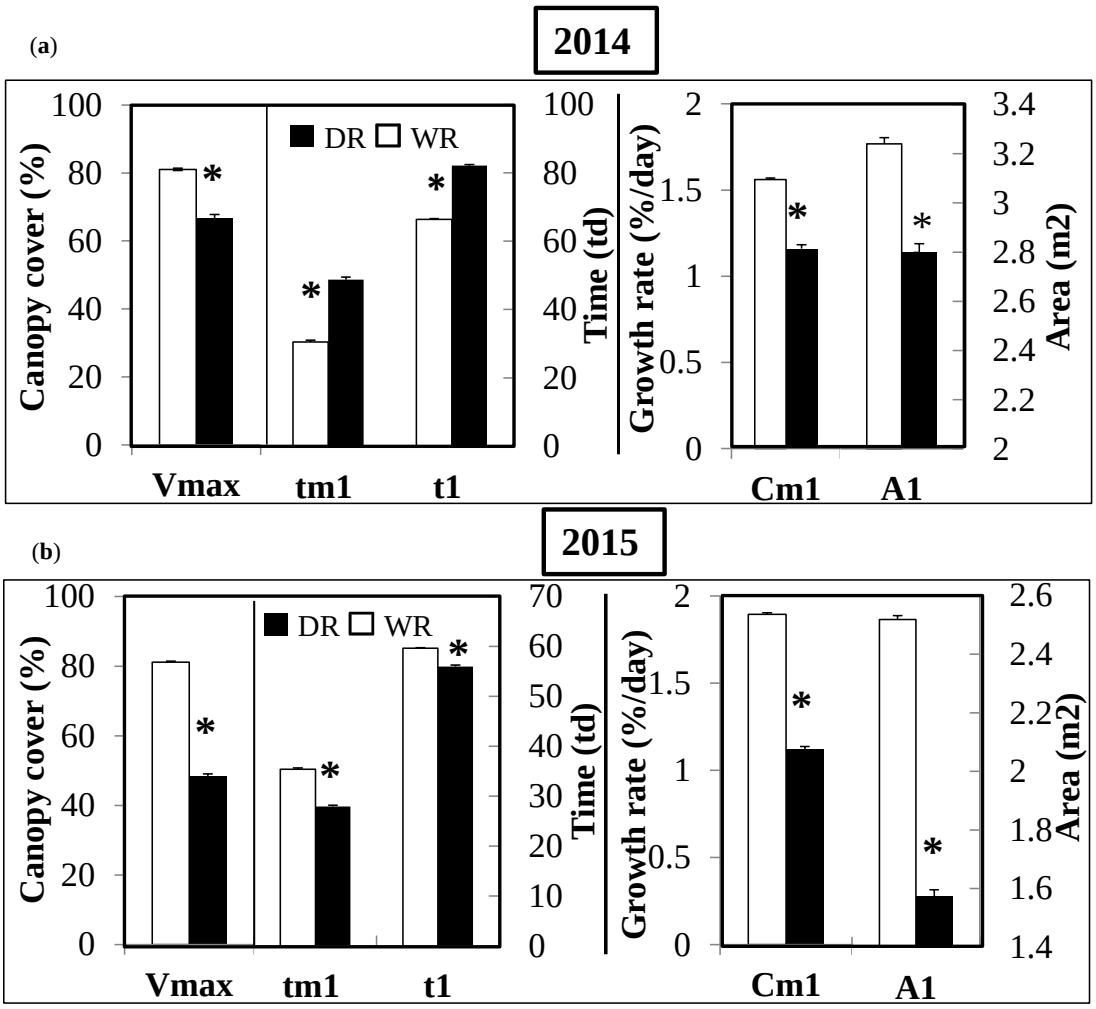


Figure 7: Comparison of drought effects on canopy growth parameters in Connantre between (**a**)2014 and (**b**)2015. V_{max}: maximum canopy cover (in %), t_1**:** time to reach maximum canopy cover (td), t_{m1}: time to reach exponential growth (td), C_{m1}**:** exponential growth rate (in % per day), A_1**:** area under canopy cover (in m^2). Error bars represent the standard error of the mean of all cultivars. * is significant differences between irrigated (WR) and non-irrigated (DR) treatments

Drought stress in both 2014 and 2015 at Connantre reduced C_{m1}, A_1 and V_{max}. However, t_{m1} and t_1 were longer under drought stress in 2014, whereas in 2015 drought stress did not delay t_{m1} and t_1 (Fig.7). For each of these growth parameters, a higher standard deviation under stress than control showed that there is considerable variation in canopy growth response to drought among the cultivars. It took fewer days (t_1) to reach maximum cover (V_{max}) in 2015 than in 2014. However, the canopy area (A_1) was larger in 2014, which reflects the effect of the difference in timing of the drought in the growing season.

Bi-plots of the growth parameters under irrigated conditions showed an even distribution of cultivars without distinctive groupings, whereas under non-irrigated conditions the maturity groups could be distinguished (Supplementary Fig.SF3). The bi-plot of the irrigated treatment showed C_{m1}, A_1 and V_{max} contributing similarly to the variation in the dataset along the first principal component axis, although V_{max} tended to have a slightly higher impact than A_1 and C_{m1}. Parameters t_{m1} and t_1 had similar effects along PC1 (Supplementary Fig.SF3). In irrigated conditions, cultivars that had longer t_{m1} and t_1 had less C_{m1}, A_1 and V_{max}, and vice versa. According to the bi-plots, the biomass factors (C_{m1}, A_1 and V_{max}) appeared to be more important drivers for the variation of canopy growth along PC1 than the time factors of canopy growth (t_{m1} and t_1). Along PC2 under non-irrigated conditions t_{m1} and t_1 were critical in distinguishing early and late maturity classes. Most late maturity types had longer t_{m1} and t_1 under drought than early maturity types. Thus, the tolerant and sensitive late maturity types did not differ much in their t_{m1} and t_1 (Supplementary Table S2). On the other hand, early maturity types responded differentially to t_{m1} and t_1, and tolerant versus sensitive early maturity types differed significantly in their t_{m1} and t_1 (Supplementary Table S2).

Correlations between Canopy Growth parameters and Yield

Spearman's correlations were computed between all canopy growth and yield traits from Connantre 2014 and 2015 under irrigated and non-irrigated conditions (Fig.8). Foliage maturity was negatively correlated with most traits in both years except time to reach exponential growth in 2014 (t_{m1}**14**) under irrigated treatment. Foliage maturity was scored on a scale of 1 (very late maturing) to 9 (very early) according to the scoring scheme of CBSG (Centre for Biosystems Genomics), the Netherlands (D'Hoop B *et al.*, 2010).

Tuber fresh weight (TBW) was more positively correlated with the canopy growth parameters under non-irrigated conditions than irrigated. However, in 2015 there was no difference between irrigated and non-irrigated treatments in tuber fresh weight correlation with C_{m1}. In this year (2015), both irrigated and non-irrigated treatments showed a similar pattern of canopy progress at the exponential phase of canopy growth (see also Fig.6b). The positive correlations of t_{m1} and t_1 with tuber weight under stress were much stronger in 2015 than 2014.

Generally, reaching maximum growth rate (t_{m1}) took much longer time under non-irrigated conditions than under well-watered conditions. This is reflected in the higher positive correlation coefficients between C_{m1} and t_{m1} under drought in both years compared to irrigation (Fig.8). Under irrigated conditions, negative correlations existed between tuber number and t_{m1}. However, under stress this link between canopy and tuber number was not existent anymore both in 2014 and 2015.

The underwater weight correlated more positively with V_{max}, C_{m1} and A_1 under stress than in normal conditions only in 2015. In summary, the Connantre trials (2014 and 2015) provided a platform to investigate early versus late drought effects on canopy development and tuber yield.

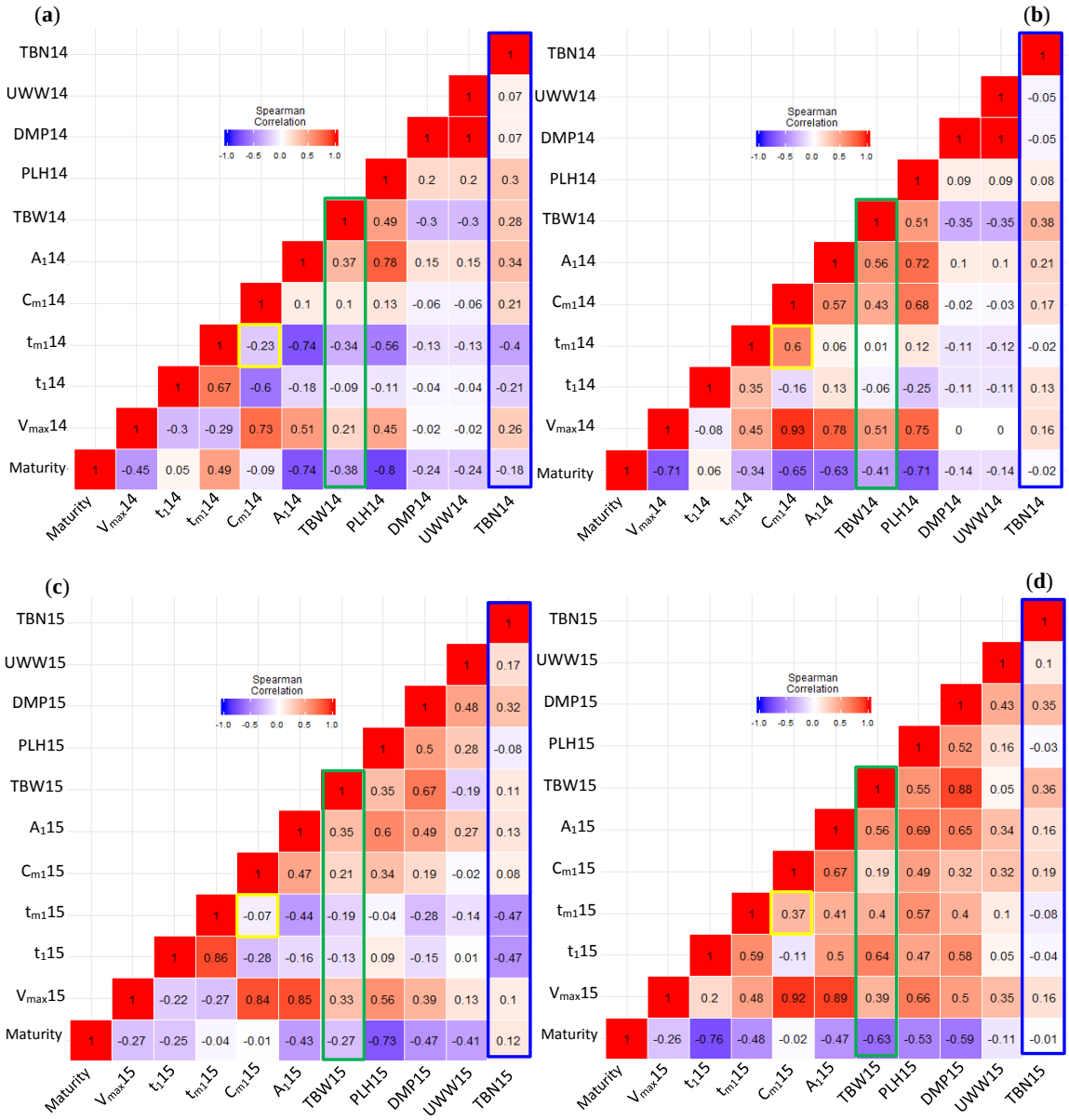


Figure 8: Correlation heatmap of canopy growth and tuber yield traits in Connantre. (**a**) Irrigated treatment in 2014, (**b**) Non-irrigated treatment in 2014, (**c**) Irrigated treatment in 2015 and (**d**) Non-irrigated treatment in 2015. The traits include: Maturity (on a scale of late [1] to early [9]), V_{max} (maximum canopy cover), t_1 (time to reach maximum canopy cover), t_{m1} (time to reach exponential growth rate), C_{m1} (Exponential growth rate), A_1 (area under the canopy curve), TBW (tuber weight), PLH (plant height), DMP (dry matter percentage), UWW (underwater weight) and TBN (tuber number). The numbers after each trait represent the years (14 = 2014 and 15 = 2015).

DISCUSSION

The development of potato canopy growth during drought has not yet been studied in detail. Logically, it can easily be assumed that fast establishment of exponential growth rate and maximum canopy cover are advantageous for productivity. Our findings however suggest a deviation from this assumption under stress conditions, and can justify the statement in Struik and Wiersema (1999), that 'fastest overall development is not necessarily associated with the highest yields'. We discuss these findings and give the practical implications for drought tolerance breeding in potato.

Drought effects on canopy growth and yield

The field trials at Connantre in 2014 and 2015 had the highest yield contrast between irrigated and non-irrigated blocks, and were used for a comparative analysis of the drought response. Early drought in the growing season of 2014 reduced the progress of canopy growth and coincided with tuber initiation (Fig.6a), leading to a reduction in tuber number and tuber yield. The early drought reduction effects on tuber number in our study is contrary to the report of no effect or increase in tuber number in Haverkort *et al.* (1990). The contrast between these two studies may be due to the different sets of genotypes studied, but also possible differences in drought severity. In 2015 the drought in the Connantre trial set in later and reduced maximum canopy cover (Fig.6b) and tuber bulking, but also tuber number. In Martin *et al.* (1992) both early and late drought reduced tuber number in Russet Burbank, and in particular, a more severe early drought reduced tuber number more than mild early drought and late drought. In our study, the late drought in 2015 exposed the potato cultivars to a more severe stress than the early drought in 2014 (Supplementary Fig.SF2). According to Haverkort and Goudriaan (1994), late droughts occurring during the tuber bulking phase of plant development have more effect on tuber yield because of increased crop transpiration, reduced formation of new leaves and likely premature leaf shedding at this stage. The plants in our 2015 experiment may have been penalized for the strong growth with optimal water availability at the early canopy-expanding stages before the drought started. Nevertheless, the canopy growth parameters, maximum canopy cover (V_{max}), exponential growth rate (C_{m1}) and area under canopy cover curve (A_1) were all reduced by drought in both years. Maximum canopy cover was more severely reduced in 2015 than in 2014 indicating that the late drought was more devastating for maximum light interception (Fig.7). Maximum canopy cover percentage is a determinant factor for the amount of light interception, which affects the photosynthetic capacity of plants and tuber bulking in potato (Barreda *et al.*, 1996; Li, 2012; Navarre & Pavek, 2014; Steyn *et al.*, 2007). Our results showed that tuber weight was more severely reduced in 2015 than 2014 at Connantre (Fig.1). This may be attributed to reduced tuber bulking resulting from reduced photosynthetic capacity of the canopy in 2015 under drought (Fig.6b). Li *et al.* (2016) have demonstrated in potted plants of *cv.* Atlantic that limited water resource can reduce potato yield by affecting the net photosynthetic rates of the source (canopy) tissues. In our Connantre 2014 trial there was a delay in time to reach exponential growth (t_{m1}) and time to reach maximum canopy cover under drought (t_1), while in 2015 these were shorter, compared to control conditions (Fig.7). This hastened effect on canopy growth rate and time to establish full canopy cover did, however, not

seem to contribute to tuber bulking as much as the reduced photosynthetic capacity of the canopy in 2015. At the build-up phase of canopy growth, the critical yield-determining parameter is tuber initiation, which may be reflected in tuber number at harvest. However, there were no significant correlations of tuber number with the canopy growth traits under drought stress. It should be noted that tuber number was only scored at the end of the growing season. Tuber formation may have been arrested when the early drought occurred, and reinitiated later in the growth season. This effect of drought on tuberization would thus not be reflected in tuber number at the time of harvest.

The strong correlation of higher tuber weight with delayed attainment of exponential growth and maximum canopy cover (t_{m1} and t_1) under stress in 2015 (Fig.8d) suggests that the maintenance of tuber yield under drought would require the plants to balance investment in exponential growth and maximum canopy cover with tuber bulking. Previous research has demonstrated that partitioning of assimilates to tubers can influence foliage earliness and longevity (Kooman & Rabbinge, 1996). Also, Marcelis (1996) illustrated in a model that when resources are limited, the sink organ with a lower K_m value (higher affinity for assimilates) attracts more assimilates. The delay in attainment of canopy exponential growth as seen in Connantre 2014 (Fig.7) may therefore be a trade-off due to an adaptive mechanism under drought to facilitate (or resulting from) continued formation and bulking of tubers.

The canopy growth parameters may be categorized into two groups: biomass-based growth parameters (maximum canopy cover (V_{max}), exponential growth rate (C_{m1}) and area under the canopy curve (A_1)) and time-based growth parameters (time to reach exponential growth rate (t_{m1}) and time to reach maximum canopy cover (t_1)). Under irrigated conditions, the biomass-based growth parameters and the time-based growth parameters of the cultivars were differentially affected (Supplementary Fig.SF3). This indicates that under favourable conditions of growth, commercial potato cultivars may have varying capacities for canopy biomass production. Also, the variations in time-based growth parameters under normal conditions may reflect the differences in foliage maturity types found in our dataset. In fact, we observed a negative correlation of maturity with most of the traits in both treatments in the Connantre trials (Fig.8). The correlations suggest that cultivars of the late maturity type tended to have higher canopy growth area (A_1), maximum canopy cover (V_{max}) and yield than early maturity types. Similarly, in another study on cultivated potato nitrogen (N) use, Ospina *et al.* (2014) reported that late maturing potato cultivars had higher canopy growth area (A_{UC}) and tuber yield than early maturing ones, though the effect of maturity became weaker when N availability was limited. Under drought, all growth parameters contributed similarly along PC1 to drought response variation among the cultivars (Supplementary Fig.SF3). In the late drought scenario (Connantre 2015), the time-based growth parameters (t_{m1} and t_1) were significantly higher in late maturing cultivars than the early maturing ones under drought. This suggests that the late drought delayed the canopy progress more in late maturing cultivars. This delay seemed to be advantageous for yield of the late maturing cultivars according to tuber fresh weight correlations with maturity (Fig.8c, d). Under low nitrogen availability in the experiments of Ospina *et al* (2014), delayed attainment of maximum canopy growth also correlated positively with an increased area under the canopy curve and tuber yield. The late maturity types in that

study were delayed in attainment of maximum canopy cover (t_1) compared to the early maturity types.

The timing of the stress seems to differentially affect maturity classes because in our early drought trial of 2014, there were no correlations between maturity and time to attain maximum canopy cover (Fig.8a and b). The early drought spanned critical points in potato phenology at which the early maturing cultivars already had maximum canopy cover, while the canopy of the late cultivars was still expanding. Zaag (1992) suggested that both foliage maturity type and water availability determine the length of the crop growth cycle. Our results give insight on how these factors (maturity, drought, length of growth cycle) interact in determining tuber yield. It should be noted that the lower yield of the early maturity types compared to the late maturity types may also be caused by differences in water use. The transpirational demands of the fully expanded canopy in early maturity types may have depleted the limited soil water more quickly, with a stronger effect on tuber bulking and thus yield than the late maturing types.

Genotype by Environment interaction

Cultivars grown under rain-fed conditions comparable to environments in this study would ideally have a combination of high yield potential with yield stability under drought stress across locations. We identified high yielding cultivars with relatively higher stability across different environments. These include Liseta, Karnico, Orchestra, Lady Olympia, Altus, Labadia, Lady Sara, Hermes, Kondor, Avano, Valiant, Fontane and Kuras (quadrant ***I*** of Fig. 3b). Cultivars in this quadrant represent the three maturity types used in the study, but the late maturity types had a higher relative representation in this quadrant. Based on the representation of late maturing cultivars in quadrant ***I*** of Fig.3b and their relative ability to balance drought effects on their canopy growth and tuber yield, late maturity may be an advantageous characteristic for cultivation under water-limited conditions. Yet, a careful consideration of the timing of drought or drought severity in the location of interest is essential for the choice of selection environments in drought tolerance breeding programs. These environmental factors are likely to fluctuate even more in the coming years due to climate change (Dai, 2011).

Tuberization under drought

Our results clearly show that canopy development is affected by drought, and that the reduced light interception, assimilate production and transport are likely to affect tuber yield. Another important factor to consider is the effect of drought on tuberization. Reduced tuber formation will have a strong effect on tuber yield as well. Several studies reported on drought stress effects on tuberization, as inferred from tuber number (Hirut et al., 2017; Stalham et al., 2007; Ouiam *et al.*, 2003; Schafleitner et al., 2007) but there is no agreement on the direction of the effect (positive or negative). Therefore, there is need to further understand how drought interferes with tuberization (Gong *et al.*, 2015). In this study, we attempted to address the effect of drought on tuberization by quantifying the effect on tuber size distribution. Yield reduction under drought was the result of lower number of tubers as well as a reduced average weight of the tubers produced (Fig.5). The extent of these effects appeared to be dependent on the drought stress level perceived by the plants and/or the timing of the drought, among other environmental

factors (Fig.2). The drought stress that reduces tuber bulking may not necessarily affect differentiation of stolons into young tubers. In Connantre (2015) the number of small tubers increased under drought at the expense of larger-sized tubers (Fig.5c), and this was also reflected in the tuber weight distribution over tuber size classes (Supplementary Fig.SF1). There may be two possible explanations for this observation: the bulking of the tubers was affected but tuberization was not arrested, or tuberization was arrested by the drought early in the season and the higher number of small tubers were formed late in the growing season with little time left for bulking. It was previously reported that drought stress limits tuber size due to late stolon and tuber formation (Struik & Van Voorst, 1986). A separation of the tuber size data into the different maturity classes also shows some slight modifications in response as a result of maturity differences (Data not shown). However, our results do not allow clear distinction between effects on tuberization and tuber bulking. A dedicated experiment with intermediate assessments of tuber formation and yield may be more informative, and a dedicated molecular investigation is recommended for further understanding of the underlying mechanisms of drought interference with tuberization.

CONCLUSIONS

Field cultivated potato plants are often vulnerable to drought stress during the growing season, which heavily impacts canopy development and eventual tuber yield. In order to minimize the reductions in tuber yield during drought, potato plants need to balance their canopy growth with tuber growth. Our findings show that one of the ways the plants could do this is by delaying their attainment of fast exponential canopy growth rate and maximum canopy cover. Generally, late maturity genotypes were more capable of moderating their canopy development to favour tuber growth under drought. Breeding for drought tolerance would benefit from incorporating these findings as further work is required in understanding the genetic basis of canopy growth rate modulation under drought.

ACKNOWLEDGMENTS

We thank the following for the contribution of their experience, ideas, time in the field trials of this study: Guus Heselmans, Jan de Haas, Maurice Schehr, Remi Ducreux, Jeroen van Soesbergen, Remko Koeman, Abco de Buck, Emmet Dalton, Nick de Vetten, Johan Hopman, Nico Rookmaker and Hellen Lensing.

REFERENCES

Acton, Q. A., 2013. Issues in Food and Health: 2013 Edition: ScholarlyEditions.

Barreda, C., Gavilan, C., Quiroz, R., 1996. Modelling potato growth and development with parameters derived from remotely sensed data. Lima: International Society for Tropical Root Crops (ISTRC). 15th Triennial ISTRC Symposium

Binod, P. L., Bhim, B. K., Duryodhan, C., Bishnu, P. P., Sung, J. S., On-Sook, H., Yul, R. K., 2015. Growth and yield characters of potato genotypes grown in drought and irrigated conditions of Nepal. Int J Appl Sci Biotechnol,, Vol 3, 513-519. doi: DOI: 10.3126/ijasbt.v3i3.13347

Bojacá, C., García, S., Schrevens, E., 2011. Analysis of Potato Canopy Coverage as Assessed Through Digital Imagery by Nonlinear Mixed Effects Models. Potato Research, 54, 237-252. doi: 10.1007/s11540-011-9189-y

Bouman, B. A. M., Uenk, D., Haverkort, A. J., 1992. The estimation of ground cover of potato by reflectance measurements. Potato Research, 35, 111-125. doi: 10.1007/bf02357604

Chen, D., Neumann, K., Friedel, S., Kilian, B., Chen, M., Altmann, T., Klukas, C., 2014. Dissecting the phenotypic components of crop plant growth and drought responses based on high-throughput image analysis. Plant Cell, 26, 4636-4655. doi: 10.1105/tpc.114.129601

CIP, 2013. Agricultural research for development: Potato facts and figures. http://cipotato.org/potato/facts (accessed 2nd May, 2013).

D'Hoop B, B., Paulo, M. J., Kowitwanich, K., Sengers, M., Visser, R. G., van Eck, H. J., van Eeuwijk, F. A., 2010. Population structure and linkage disequilibrium unravelled in tetraploid potato. Theor Appl Genet, 121, 1151-1170. doi: 10.1007/s00122-010-1379-5

Dai, A., 2011. Drought under global warming: a review. Wiley Interdisciplinary Reviews: Climate Change, 2, 45-65. doi: 10.1002/wcc.81

Dai, A., 2013. Increasing dorught under global warming in observations and models. Nature Climate Change, 3, 52-58. doi: DOI: 10.1038/nclimate1633.

Deblonde, P. M. K., Ledent, J. F., 2001. Effects of moderate drought conditions on green leaf number, stem height, leaf length and tuber yield of potato cultivars. European Journal of Agronomy, 14, 31-41. doi: http://dx.doi.org/10.1016/S1161-0301(00)00081-2

FAO, 2008. Potato and water resources. Hidden Treasure: International year of the Potato. http://www.potato2008.org/en/potato/water.html (accessed 5th May, 2013).

FAOSTAT, 2016. http://www.fao.org/faostat/en/#data/QC (accessed 28th January, 2018)

Finlay, K. W., Wilkinson, G. N., 1963. The analysis of adaptation in a plant breeding programme. Aust. J. Agric. Res., 14, 742-754.

Gedif, M., Yigzaw, D., 2014. Genotype by Environment Interaction Analysis for Tuber Yield of Potato (Solanum tuberosum L.) Using a GGE Biplot Method in Amhara Region, Ethiopia. Agricultural Sciences, 5, 239-249. DOI: 10.4236/as.2014.54027

Gong, L., Zhang, H., Gan, X., Zhang, L., Chen, Y., Nie, F., Song, Y., 2015. Transcriptome Profiling of the Potato (Solanum tuberosum L.) Plant under Drought Stress and Water-Stimulus Conditions. PLoS ONE, 10, e0128041. doi: 10.1371/journal.pone.0128041

Haase, N. U. (2003). Estimation of dry matter and starch concentration in potatoes by determination of under-water weight and near infrared spectroscopy. Potato Research 46(3): 117-127.

Harris, P. M., 2012. The Potato Crop: The scientific basis for improvement: Springer Netherlands.

Haverkort, A. J., Mackerron, D. K. L., 1995. Potato ecology and modelling of crops grown under conditions limiting growth. Dordrecht: Kluwer Academic Publishers.

Haverkort, A. J. and J. Goudriaan (1994). Perspectives of improved tolerance of drought in crops. Aspects of Applied Biology 38.

Haverkort, A. J., Uenk, D., Veroude, H., Van De Waart, M., 1991. Relationships between ground cover, intercepted solar radiation, leaf area index and infrared reflectance of potato crops. Potato Research, 34, 113-121. doi: 10.1007/bf02358105

Haverkort, A. J., Van De Waart, M., Bodlaender, K. B. A. (1990). The effect of early drought stress on numbers of tubers and stolons of potato in controlled and field conditions. Potato Research 33(1): 89-96

Hirut B, Shimelis H, Fentahun M, Bonierbale M, Gastelo M, Asfaw A (2017). Combining ability of highland tropic adapted potato for tuber yield and yield components under drought. PLoS ONE 12(7): e0181541.

Hurtado-Lopez, P. X., Tessema, B. B., Schnabel, S. K., Maliepaard, C., Van der Linden, C. G., Eilers, P. H. C., Jansen, J., van Eeuwijk, F. A., Visser, R. G. F. (2015). Understanding the genetic basis of potato development using a multi-trait QTL analysis. Euphytica 204(1): 229-241.

Iwama, K., Hukushima, T., Yoshimura, T., & Nakaseko, K. (1993). Influence of Planting Density on Root Growth and Yield in Potato. Japanese journal of crop science, 62(4), 628-635. doi: 10.1626/jcs.62.628

Jefferies, R. A., Mackerron, D. K. L., 1993. Responses of potato genotypes to drought. II. Leaf area index, growth and yield. Annals of Applied Biology, 122, 105-112. doi: 10.1111/j.1744-7348.1993.tb04018.x

Khan, M. S., 2012. Assessing Genetic Variation in Growth and Development of Potato: (Ph.D.), Wageningen University.

Kooman, P. L., Rabbinge, R., 1996. An Analysis of the Relation between Dry Matter Allocation to the Tuber and Earliness of a Potato Crop. Annals of Botany, 77, 235 - 242. DOI: https://doi.org/10.1006/anbo.1996.0027

Korva, J. T., 1996. Grids in groud cover measurements. Potato Research, 39, 533-540. doi: 10.1007/bf02358472

Lemaga, B., Caesar, K., 1990. Relationships between numbers of main stems and yield components of potato (Solanum tuberosum L. cv. Erntestolz) as influenced by different daylengths. Potato Research, 33, 257-267. doi: 10.1007/bf02358455

Li W, Xiong B, Wang S, Deng X, Yin L, Li, H. (2016). Regulation Effects of Water and Nitrogen on the Source-Sink Relationship in Potato during the Tuber Bulking Stage. PLoS ONE 11(1): e0146877.

Li, P., 2012. Potato Physiology: Elsevier Science.

Liu, F., Jensen, C. R., Shahanzari, A., Andersen, M. N., Jacobsen, S. E., 2005. ABA regulated stomatal control and photosynthetic water use efficiency of potato (Solanum tuberosum

L.) during progressive soil drying. Plant Science, 168, 831-836. doi: https://doi.org/10.1016/j.plantsci.2004.10.016

Luisa, D. C. G., Delle, V., Gianquintoi, G., Giovanardi, R., Peressotti, A., 1997. Yield, water use efficiency and nitrogen uptake in potato: influence of drought stress. Potato Research, 40, 19-34.

Mackerron, D. K. L., Marshall, B., Jefferies, R. A., 1988. The distributions of tuber sizes in droughted and irrigated crops of potato. II. Relation between size and weight of tubers and the variability of tuber-size distributions. Potato Research, 31, 279-288. doi: 10.1007/bf02365536

Marcelis, L., 1996. Sink strength as a determinant of dry matter partitioning in the whole plant. Journal of Experimental Botany, 47(suppl 1), 1281.

Martin, R. J., Jamieson, P. D., Wilson, D. R., Francis, G. S. (1992). Effects of soil moisture deficits on yield and quality of 'Russet Burbank' potatoes. New Zealand Journal of Crop and Horticultural Science 20(1): 1-9.

Navarre, R., Pavek, M. J., 2014. The Potato: Botany, Production and Uses: CABI.

Opena, G. B. and G. A. Porter (1999). Soil Management and Supplemental Irrigation Effects on Potato: II. Root Growth. Agronomy Journal 91: 426-431.

Ospina, C. A., Lammerts van Bueren, E. T., Allefs, J. J. H. M., Engel, B., van der Putten, P. E. L., van der Linden, C. G., Struik, P. C., 2014. Diversity of crop development traits and nitrogen use efficiency among potato cultivars grown under contrasting nitrogen regimes. Euphytica, 199, 13-29. doi: 10.1007/s10681-014-1203-4

Ouiam, L., Said, O., Ledent, J. F., 2003. The effect of drought and cultivar on growth parameters, yield and yield components of potato. Agronomie 23 257-268. doi: 10.1051/agro:2002089

Prashar, A., Jones, H., 2014. Infra-Red Thermography as a High-Throughput Tool for Field Phenotyping. Agronomy, 4, 397. doi.org/10.1371/journal.pone.0065816

Romero, A. P., et al. (2017). Physiological Assessment of Water Stress in Potato Using Spectral Information. Frontiers in Plant Science 8(1608).

Sadras, V. O. and S. P. Milroy (1996). Soil-water thresholds for the responses of leaf expansion and gas exchange: A review. Field Crops Research 47(2): 253-266.Schafleitner, R., Gutierrez, R., Legay, S., Evers, D., Bonierbale, M., 2008. Drought stress tolerance traits of potato. http://www.istrc.org/sites/default/files/files/symposium/Internal/2009_Peru/s5_schafleitner.pdf (accessed March 9, 2013)

Schafleitner, R., Gutierrez, R., Espino, R., Gaudin, A., Perez, J., Martinez, M., Dominguez, A., Tincopa, L., Alvarado, C., Numberto, G., Bonierbale, M. (2007). Field Screening for Variation of Drought Tolerance in Solanum tuberosum L. by Agronomical, Physiological and Genetic Analysis. 50(1): 71-85.

Schittenhelm, S., Sourell, H., Lopmeier, F. J. (2006). Drought resistance of potato cultivars with contrasting canopy architecture. European Journal of Agronomy 24(3): 193-202.

Shiri, J. M., Tobeh, A. A., Jamaati, S. S., Hassanzadeh, M. A., Zabihi, M. R., 2009. Effects of Water Stress on Water Demand, Growth and Tuber Grade of Potato (Solanum

tuberosum L.) Crop. Research Journal of Environmental Sciences, , 3, 476-485. doi: 10.3923/rjes.2009.476.485

Sivarajan, S., 2011. Estimating Yield of Irrigated Potatoes Using Aerial and Satellite Remote Sensing. (Ph.D.), Utah State University, Utah. http://digitalcommons.usu.edu/etd/1049 (Paper 1049)

Shahnazari, A., Liu, F., Andersen, M. N., Jacobsen, S., Jensen, C. R. (2007). Effects of partial root-zone drying on yield, tuber size and water use efficiency in potato under field conditions. Field Crops Research 100(1): 117-124.

Soltys-Kalina, D., Plich, J., Strzelczyk, D., Sliwka, J., Marczewski, W. (2016). The effect of drought stress on the leaf relative water content and tuber yield of a half-sib family of 'Katahdin'-derived potato cultivars. Breeding Science 66(2): 328-331.

Stalham, M. A., et al. (2007). Effects of soil compaction in potato (Solanum tuberosum) crops. The Journal of Agricultural Science **145**(4): 295-312.

Steyn, J. M., Kagabo, D. M., Annandale, J. G., 2007. Potato growth and yield responses to irrigation regimes in constrasting seasons of a subtropical region. Paper presented at the African Crop Science Conference Proceedings, Egypt.

Struik, P. C. and S. G. Wiersema (1999). Seed Potato Technology, Wageningen Academic Publishers.

Struik, P. C., Van Voorst, G., 1986. Effects of droght on the initiation, yeild, and size distribution of tubers ofSolanum tuberosum L. cv. Bintje. Potato Research, 29, 487-500. doi: 10.1007/bf02357913

van Loon, C. D., 1981. The effect of water stress on potato growth, development, and yield. American Potato Journal, 58, 51-69. doi: 10.1007/bf02855380

Vreugdenhil, D., Bradshaw, J., Gebhardt, C., Govers, F., Taylor, M. A., MacKerron, D. K. L., Ross, H. A., 2011. Potato Biology and Biotechnology: Advances and Perspectives: Elsevier Science.

Vreugdenhil, D., Bradshaw, J., Gebhardt, C., Govers, F., Taylor, M. A., MacKerron, D. K. L., Ross, H. A. Eds. (2007). Water Availability and Potato Crop Performance. Potato Biology and Biotechnology: Advances and Perspectives. Amsterdam, Elsevier.

Yamaguchi, J. and A. Tanaka (1990). Quantitative observation on the root system of various crops growing in the field. Soil Science and Plant Nutrition 36(3): 483-493.

Yan, W., Tinker, N. A., 2006. Biplot analysis of multi-environment trial data: Principles and applications. Canadian Journal of Plant Science, 86, 623-645. doi: 10.4141/p05-169

Yin, X., Kropff, M. J., McLaren, G., Visperas, R. M. (1995). A nonlinear model for crop development as a function of temperature. Agricultural and Forest Meteorology 77(1): 1-16.

Zaag, D. E., 1992. Potatoes and their cultivation in the Netherlands (pp. 47). The Hague: NIVAA (Netherlands Potato Consultative Institute).

Zarzyńska K., Boguszewska-Mańkowska D., Nosalewicz A. (2017). Differences in size and architecture of the potato cultivars root system and their tolerance to drought stress. Plant Soil Environ **63**: 159-164.

MAPPING TUBER SIZE DISTRIBUTION AND MARKETABLE TUBER YIELD UNDER DROUGHT IN POTATOES

Ernest B Aliche[1,2], Marian Oortwijn[1], Tom P. J. M. Theeuwen[1,2], Christian W. B. Bachem[1], Herman J. van Eck[1], Richard G. F. Visser[1], C. Gerard van der Linden[1]

[1]Plant Breeding, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen.

[2]Graduate School Experimental Plant Sciences, Droevendaalsesteeg 1, 6708 PB, Wageningen.

To be submitted...

ABSTRACT

Drought sensitivity of potato is the reason for a reduction of potato tuber yield during drought stress conditions. Alongside the reduction in total tuber yield, marketable yield is also affected. An investigation of drought effects on tuber yield attributes and tuber size distribution will facilitate our understanding of how to reduce the huge yield losses resulting from drought. We have carried out an evaluation of tuber yield and tuber size distribution of a set of 103 European commercial potato cultivars under irrigated and non-irrigated conditions. The results from two locations, Connantre, France (2013 – 2015) and Nieuw-Namen in Zeeland, The Netherlands (2013 – 2014), were analysed. We used the Normal Distribution and the Gamma Distribution models to describe the tuber size distribution of tuber fresh weight and tuber number, respectively. We extracted the tuber size distribution parameters and calculated the coefficient of variability and marketable fraction for each cultivar in the dataset. Correlation and biplot analyses were used to evaluate the interactions among these parameters/traits, and with tuber yield. Finally, we used a 14K Infinium SNP marker array to find associations between the obtained parameter/traits and genes in the potato genome. Our findings show that late foliage maturity facilitates a wider spread of tuber size distribution in favour of larger-sized tubers. Wide-spread tuber size distribution and high coefficient of variability in tuber number positively contributed to marketable tuber size. Drought effects on total yield were quite representative of its impact on marketable yield, however, absolute values of total tuber number may not be indicative of marketable number of tubers. The formation of fewer tubers is more advantageous for tuber bulking than numerous tubers. The timing of drought and the tuberization stage of the plant affected by the drought influence tuber size variability. Tuber number and tuber fresh weight were highly heritable yield traits. We also found significant marker-trait associations between a region on Chromosome 3 of the potato genome and the spread of tuber number distribution, size class with maximum tuber number, marketable fraction of tuber number and marketable fraction of tuber weight. A keen consideration of these findings during selection in breeding trials and a further investigation of the associated genomic region will facilitate the advancement of drought tolerance breeding of potato.

INTRODUCTION

Potato is a perennial herb that is cultivated today as an annual crop (Zarka et al., 2009). It is grown for its underground storage organ, the tuber. Potato is consumed by more than a billion people globally, and more than 230 million tonnes is consumed yearly (Devaux et al., 2014). Therefore, it is recognized as a food security crop by the Food and Agriculture Organization (FAOSTAT, 2014). Additionally, potato tubers are utilized for industrial production of starch and other uses (Kraak, 1992; Stearns et al., 1994). Potato yield under optimal conditions of growth is over 47 tonnes/ha (FAOSTAT, 2014), up to about 50-60 tonnes/ha in The Netherlands. However, under sub-optimal conditions like water limitation, yield is drastically reduced (Trebejo & Midmore, 1990).

Climate change makes it increasingly difficult to predict the occurrence and scale of drought periods (Lal, 2014). The most devastating effects of drought stress on potato occur when water limitation coincides with the tuberization stage of potato (Daryanto et al., 2016). Tuberization

in potato involves the differentiation of stolon tips into young tubers (tuber initiation) and the bulking of the young tubers (Catchpole & Hillman, 1969; Dutt et al., 2017; Minhas et al., 2004; O'Brien et al., 1998; Ozgen et al., 2003). Drought may affect tuberization by reducing the number of tubers that are initiated (Mackerron et al., 1988). Also, drought may reduce the filling of the tubers with assimilates in the tuber bulking phase of plant growth (Lahlou et al., 2003). In both cases, the result is reduced tuber yield. Most potato drought research efforts have focussed on understanding the reduction in total tuber yield. However, the implication on marketable tuber yield requires research attention as well.

Marketable tuber yield consists of the fraction of total yield that meets the size, shape, weight and quality requirements of the intended market (Love & Thompson-Johns, 1999). It has been demonstrated that marketable tuber yield is dependent on mean tuber size, that is, both total tuber weight and total number of tubers (Harris, 2012). Therefore, it was recommended to cultivate potato cultivars that produce fewer tubers in drought-prone areas (Mackerron et al., 1988). The lower number of tubers are more likely to be bulked when photo-assimilates are limited during drought, thus increasing the average size of the tubers. However, the bulking of the tubers also depends on the time of tuber initiation in the growing season and the maturity type of the potato (Zaag, 1992). Drought is known to delay tuber initiation (Walworth & Carling, 2002). When the drought persists to later stages of the growing season, tubers that are formed towards the end of the growth cycle may hardly be bulked. In this way, drought reduces the marketable fraction of potato tuber yield (Cantore et al., 2014; Luitel et al., 2015; Nouri et al., 2016; Vayda, 1994).

Potato cultivars with different genetic backgrounds respond differently to drought in terms of their tuberization. Some cultivars have a fixed tuber initiation period, while others may initiate tubers several times during the growing season (Celis-Gamboa et al., 2003; Walworth & Carling, 2002). These genotypic variations and the corresponding unique drought responses complicate the understanding of potato tuber yield marketability under drought. Consequently, modelling techniques are used to study potato tuber size distribution in order to gain more insight. For instance, potato tuber size distribution has been modelled using the truncated Gaussian or normal distribution model (Mackerron et al., 1988; Ospina et al., 2014; Sands & Regel, 1983), log-normal model (Glasbey et al., 1988; Marshall et al., 1993), Weibull model (Nemecek T et al., 1996) and the gamma distribution model (MAFF, 2000). These models have been used to extract important parameters that describe the features of the tuber size distribution. Some of the insights gained from the modelling approach include the spread and skewness of tuber size distribution, which can provide information on the marketable proportion of tuber yield. However, not much research has been conducted towards understanding the genetic basis of the model parameters that describe total and marketable tuber size distribution. Celis-Gamboa (2002) suggested that tuber size distribution in potato is under quantitative inheritance (Celis-Gamboa, 2002). Also, the factors that influence tuber size distribution and marketable yield including stolon branching, the duration of the stolon tip swelling period and tuber resorption, are genotype-dependent (Celis-Gamboa et al., 2003; Pasare et al., 2013). These observations indicate the need to further investigate the role of

genetic factors in determining tuber size distribution and marketable yield and how these are influenced by stress conditions.

In this study we have used the best fitting models to extract tuber size distribution parameters in order to evaluate their effects on marketable yield in a set of 103 potato cultivars. Furthermore, we have used a 14K SNP array from the potato genome to search for genetic loci that may be associated with total yield, marketable yield and any of the tuber size distribution parameters.

MATERIALS AND METHODS

Planting and data collection

A set of 103 commercial cultivars representing a significant part of the European potato gene pool was used in this study. This set consists of different genetic backgrounds, maturity classes and market niches (Supplementary Table 1). The maturity classes comprise 10 late, 44 intermediate and 47 early maturing cultivars. The plants were grown at Connantre, France in three years (2013-2015) and at Zeeland (Nieuw-Namen), The Netherlands in two years (2013-2014). Plants in the control block were irrigated during the dry period of the growing season, while irrigation was withheld from the stress block. Each block contained the 103 cultivars randomized within plots. Each plot had eight plants and there were two plots for each genotype within a block. The environmental conditions were monitored in the Connantre trial in 2014 and 2015. At the end of the growing season, tuber fresh weight and tuber number were measured. Also, a Smart Grader was used to grade the tubers into the various tuber size classes: 0-40mm, 40-50mm, 50-60mm, 60-70mm and >70mm. Tuber fresh weight and tuber number per size class were scored.

Processing tuber size data

The data of tuber fresh weight per size class and tuber number per size class formed unique tuber size distributions. The tuber size distribution for tuber fresh weight per size class was modelled using a Gaussian normal distribution equation in DataFit (version 9.1.32) (Ospina et al., 2014):

$$TBW = MX * \exp(- (mcl - B)^2/A)$$

Where TBW is the tuber fresh weight, MX is the maximum fresh weight observed among the size classes, mcl is the mid-point of each size class boundaries, B is the average size of the class at which MX occurs and A is the dispersion parameter showing the spread of the distribution across the size classes. An illustration of the model parameters is shown in the graphical representation in Figure 1. For clarity of nomenclature in the results analyses, MX is used as *TBW MX*, B as *TBW mcs* and A as *TBW spread*.

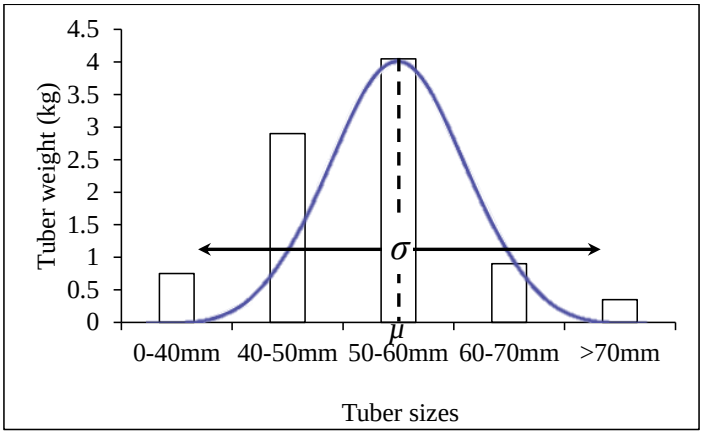


Figure 1: Graphical representation of a normal distribution graph showing the spread (σ) and mean (μ) of the distribution. In our dataset, σ represents the dispersion parameter (A) and μ represents the maximum tuber weight (MX)

The Gaussian normal distribution model did not appropriately describe the distribution of tuber number across the tuber size classes. The gamma distribution model was demonstrated to give a better fit (MAFF, 2000). The gamma model was fitted in NCSS (version 11), which predicts the model parameter estimates of the distribution using a maximum likelihood estimate (MLE) approach. The gamma distribution model is given as:

$$TBN = (w^{\alpha-1} \exp^{-\beta w}) / (\beta^{\alpha}_{1} \{\int w^{\alpha-1} \exp^{-w} \Delta w\})$$

Where, TBN is the tuber number; *w* is the tuber size class ranging from 1 to 5, representing the five size classes (0-40mm, 40-50mm, 50-60mm, 60-70mm and >70mm, respectively); α is the shape of the curve; and β the is the rate. The mean (μ) of the distribution and the standard deviation (σ) are determined by the equation:

$$\mu = \alpha/\beta; \text{ and } \sigma = (\sqrt{\alpha})/\beta$$

Where, μ is the size class with the maximum tuber number and σ describes the spread of the distribution. In the results description, μ is represented as *TBN ms* and σ as *TBN spread*.

Calculations and statistical analyses

The marketable fractions of tuber fresh weight and tuber number were calculated by dividing the tuber fresh weight and tuber number of size classes ≥50mm by the total tuber fresh weight and tuber number, respectively. The ≥50mm size threshold refers to the longitudinal length of a tuber. The coefficients of variation for both tuber fresh weight and tuber number were computed as $((\sigma/\mu)*100)$, that is, ((*TBN spread*/*TBN ms*)*100) in the case of tuber number, for instance. The calculated traits and parameters from tuber size distributions of tuber fresh weight and tuber number were statistically correlated with overall tuber fresh weight and tuber number. This was implemented in R-Studio 3.2.3. Principal Component (PCA) bi-plots were used to

investigate the contribution of the various traits/parameters to the variation in the dataset. Tuber size difference between maturity classes was studied using an ANOVA in GENSTAT 17th Edition. Statistical analyses were effected at 0.05 level of significance.

Association Mapping

The set of commercial cultivars grown in the field was used as a panel for mapping marker-trait associations. A dataset of 14,402 Infinium SNP markers was used in mapping the association of the traits and parameters to physical positions on the potato genome. This marker dataset was described fully (Vos et al., 2015). SNP markers were available for 97 and 95 cultivars in the Connantre trial of 2014 and 2015, respectively. The assignment of allele dosage classes to the markers was done using the freely available R package fitTetra algorithms (Voorrips et al., 2011). Numeric score of dosage classes was applied on the markers from 0 to 4 representing nulliplex (aaaa), simplex (Aaaa), duplex (AAaa), triplex (AAAa) and quadruplex (AAAA) marker dosages respectively. The Q+K linear mixed model for GWAS approach was used to map the marker-trait associations (Yu et al., 2006). The GWAS model was implemented as follows:

$$\boldsymbol{t} = \boldsymbol{X\beta} + \boldsymbol{Z_S\zeta} + \boldsymbol{Z_Q v} + \boldsymbol{Z_u} + \boldsymbol{\varepsilon}$$

where, $\boldsymbol{t}$ is phenotypic trait; $\boldsymbol{X\beta}$ accounts for covariates like environmental effects; $\boldsymbol{Z_S\zeta}$ describes the SNP effects using the genetic model to map genotype to phenotype; $\boldsymbol{Z_Q v}$ accounts for subpopulations of the given population size in the association panel; $\boldsymbol{Z_u}$ considers polygenic effects and its covariance matrix is proportional to the kinship (K) matrix; and the residual ($\boldsymbol{\varepsilon}$) is based on the model assumptions of independence, normality and equality of variance (*iid – independent and identically distributed*). The variance of the random effects is given as follows, $Var[\boldsymbol{\varepsilon}]=I\sigma^2_{\varepsilon}$ and $Var[\boldsymbol{u}]=\sigma^2_g K$.

Kinship (relatedness of the association panel) was calculated using the realized relationship matrix (Rosyara et al., 2015). The population structure groups of the association panel were accounted for by using the structure grouping information (D'Hoop B et al., 2010). Different gene models were imposed on the marker associations including: general, additive, simplex dominant and duplex dominant. The quantile-quantile plot was used to check the association of the markers on a log scale to avoid spurious associations. Manhattan plots were used to visualize trait-associated loci on the physical map of potato. The entire association mapping procedure was implement in R Studio version 3.2.3, using the GWASpoly package for autotetraploids (Rosyara et al., 2015).

RESULTS

The foliage development and tuber yield at Zeeland, which is a coastal region of the Netherlands, were much less affected by drought than the Connantre trials, indicating that the trial in Zeeland only suffered minor water limitation stress. The foliage of the plants in the irrigated block of the Connantre (2013) trial at some time points in the growing season turned

yellow, which may be indicative of a lack of nitrogen. We speculate that the irrigation may have resulted in the leaching of nutrients to lower soil depths beyond the reach of the plant roots. Environmental information was collected in the Connantre trials of 2014 and 2015. The environmental data showed that at the Connantre 2014 trial, total rainfall was 258.2mm and the drought occurred during the early stages of plant growth. The total rainfall in Connantre 2015 was only 42mm and the drought occurred at a later stage of plant development (*See* Chapter 2 – Fig.6). Therefore, the Connantre 2014 and Connantre 2015 trials are described as early and late drought respectively.

Size distribution of tuber fresh weight and tuber number

The tuber size with maximum fresh weight (*TBW mcs*) and the spread of the tuber size distribution (*TBW spread*) were significantly negatively affected by drought in Connantre (2014 and 2015) and in Zeeland 2014 (Fig.2). The size class with the maximum tuber number (*TBN ms*) was significantly lower under drought in Connantre (2013 – 2015) but not in Zeeland. The spread of the distribution of tuber number in the various sizes (*TBN spread*) was only significantly reduced in Connantre 2014 and 2015 (Fig.3).

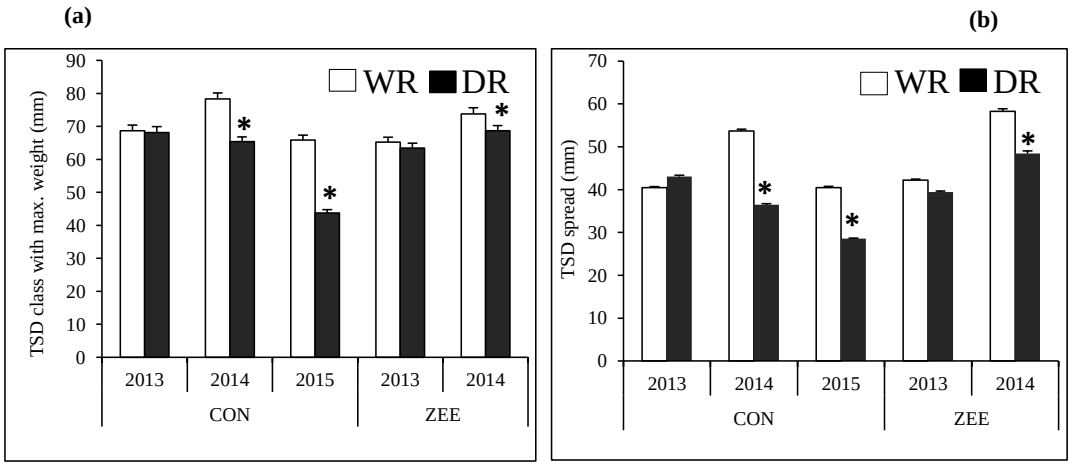


Figure 2: (**a**) Tuber size with the maximum tuber fresh weight (*TBW mcs*) under irrigated (WR) and non-irrigated (DR) conditions, (**b**) Spread of the tuber size distribution (*TBW spread*) under irrigated (WR) and non-irrigated (DR) conditions, at CON (Connantre), ZEE (Zeeland) in the years, 2013 – 2015. Error bars are standard errors of the mean values of 103 cultivars. Significant differences between WR and DR are given by asterisks at 0.05 level of significance. TSD is tuber size distribution.

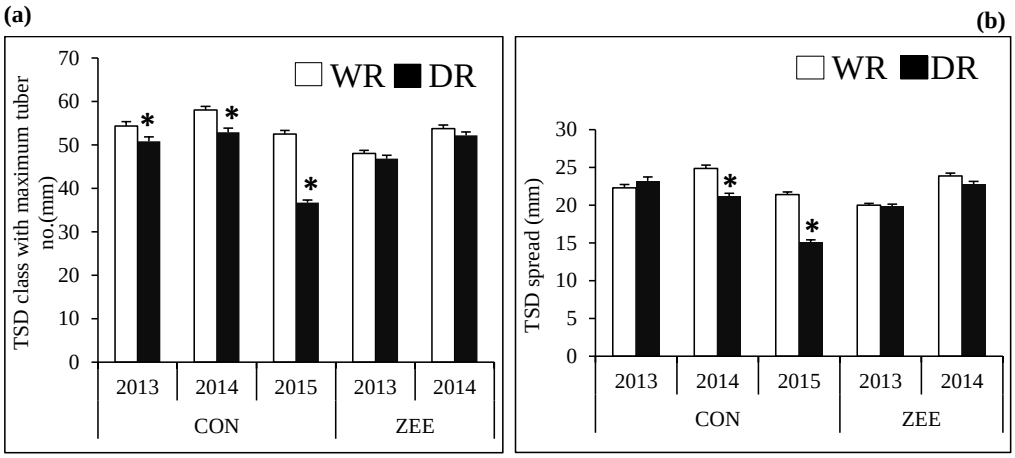


Figure 3: (**a**) Tuber size with the maximum tuber number (*TBN ms*) under irrigated (WR) and non-irrigated (DR) conditions, (**b**) Spread of tuber size distribution curve (*TBN spread*) under irrigated (WR) and non-irrigated (DR) conditions, at CON (Connantre), ZEE (Zeeland) in the years, 2013 – 2015. Error bars are standard errors of the mean values of 103 cultivars. Significant differences between WR and DR are given by asterisks at 0.05 level of significance. TSD is tuber size distribution.

Marketable tuber size fraction

A tuber size threshold of ≥50mm along the longitudinal plane of the tuber was used to determine marketable fraction of tuber yield under irrigated and non-irrigated conditions. This size threshold was applied to both tuber fresh weight (TBW) and tuber number (TBN).

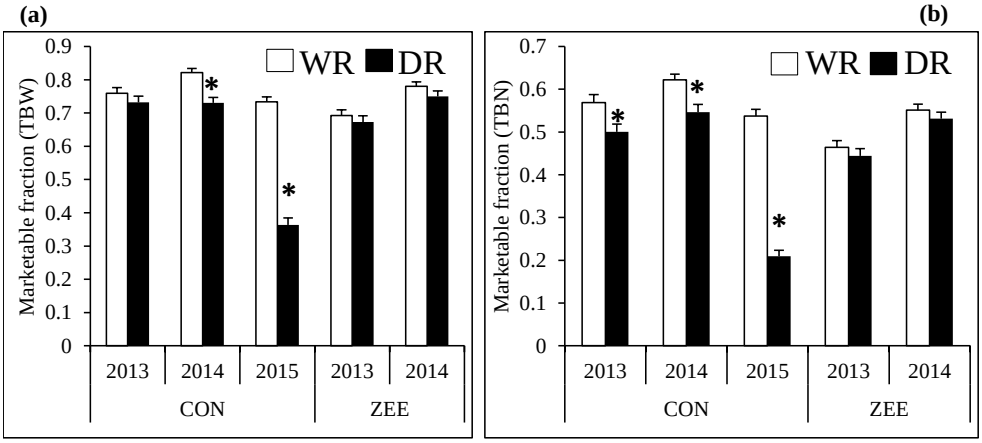


Figure 4: (**a**) Marketable fraction of tuber fresh weight (*TBW mf*) under irrigated (WR) and non-irrigated (DR) conditions, (**b**) Marketable fraction of tuber number (*TBN mf*) under irrigated (WR) and non-irrigated (DR) conditions, at CON (Connantre) and ZEE (Zeeland) in the years, 2013 – 2015. Error bars are standard errors of the mean values of 103 cultivars. Significant differences between WR and DR are given by asterisks at 0.05 level of significance

Marketable fraction was expressed as the sum of TBW or TBN for all tuber sizes ≥50mm divided by the total sum of TBW or TBN, respectively for each cultivar. Drought stress significantly reduced the fresh weight of the marketable tuber size fraction in Connantre (2014 and 2015). Also, the number of marketable tubers was reduced in the three trial years at Connantre (Fig.4). The reduction in marketable yield was most severe in Connantre 2015. However, there was no significant reduction of marketable yield in the Zeeland trials.

Correlation of tuber size distribution parameters

Generally, foliage maturity correlated negatively with most tuber size distribution parameters in all trials under irrigated as well as non-irrigated conditions (Data not shown). Foliage maturity was scored on a scale of 1 (very late maturing) to 9 (very early) according to the scoring scheme of CBSG (Centre for Biosystems Genomics), the Netherlands (D'Hoop B et al., 2010), which means that late maturing cultivars had higher values for the parameters (Data not shown). However, in the late drought trial (Connantre 2015), foliage maturity was more negatively correlated with tuber size distribution parameters under stress than under irrigated conditions (Fig.5). Also, marketable fractions of tuber yield (*TBN mf* and *TBW mf*) were higher in late maturing cultivars than in early maturity types, especially under drought. The coefficient of variation (CV) was not correlated with foliage maturity under irrigated conditions (Fig.5a). But in non-irrigated conditions the CV of tuber number (*TBN CV*) was negatively correlated with foliage maturity while CV of tuber fresh weight (*TBW CV*) was positively correlated with foliage maturity (Fig.5b). Interestingly, *TBN CV* and *TBW CV* were positively correlated under irrigation, but negatively correlated under drought stress. In both treatments, high number of tubers (*TBN*) and high CV of tuber fresh weight (*TBW CV*) were correlated, but only under drought did high tuber fresh weight (*TBW*) correlate with high CV in tuber number (*TBN CV*) (Fig.5). *TBN CV* correlated positively with TBN in irrigated treatment, but negatively in non-irrigated treatment. Generally, *TBW CV* correlated negatively with the tuber size distribution parameters and marketable fraction under irrigated and non-irrigated conditions. *TBN CV*, however, was positively correlated with these parameters and marketable fraction under stress, but not under irrigation. The total tuber number (*TBN*) correlated negatively with the tuber size with maximum tuber yield (*TBN ms* and *TBW mcs*), spread of tuber size distribution (*TBN spread* and *TBW spread*) and marketable fractions (*TBN mf* and *TBW mf*). That is, high total number of tubers tended to skew size distribution towards smaller size classes, narrower distribution curves and loss of tuber marketability. These negative correlations of total tuber number were observed under irrigated as well as non-irrigated conditions. The marketable fractions of tuber yield (*TBN mf* and *TBW mf*) correlated positively with spread of tuber size distribution (*TBN spread* and *TBW spread*).

(a)

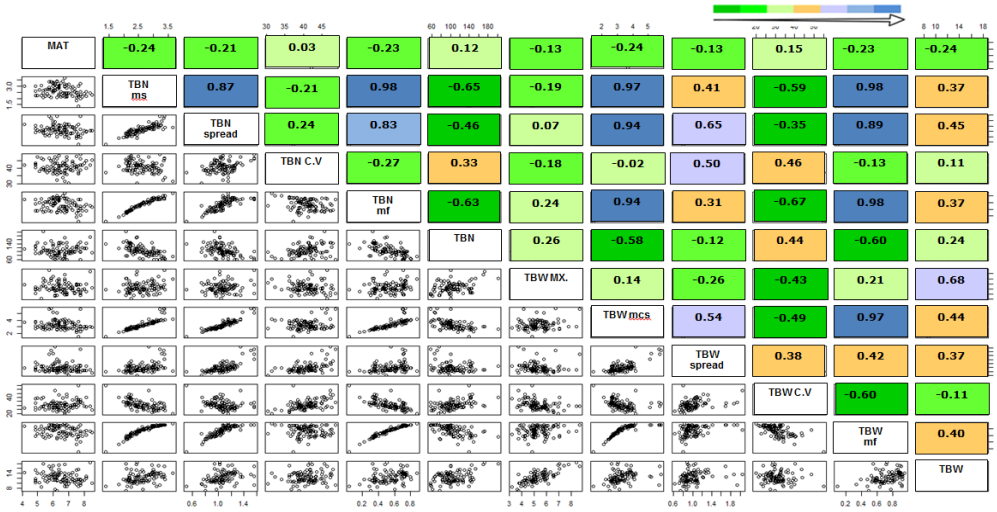


Figure 5a: Correlation of tuber distribution parameters and yield traits under irrigated condition at Connantre 2015 trial. MAT (maturity on a scale of late (1) to early (9)), TBN ms (tuber number mean size), TBN spread (tuber number size distribution spread), TBN CV (Coefficient of variability in tuber number), TBN mf (tuber number marketable fraction), TBN (tuber number), TBW MX. (maximum tuber fresh weight among size classes), TBW mcs (size class with the maximum tuber fresh weight), TBW spread (tuber fresh weight size distribution spread), TBW CV (Coefficient of variability in tuber fresh weight), TBW mf (tuber fresh weight marketable fraction), TBW (tuber fresh weight).

(b)

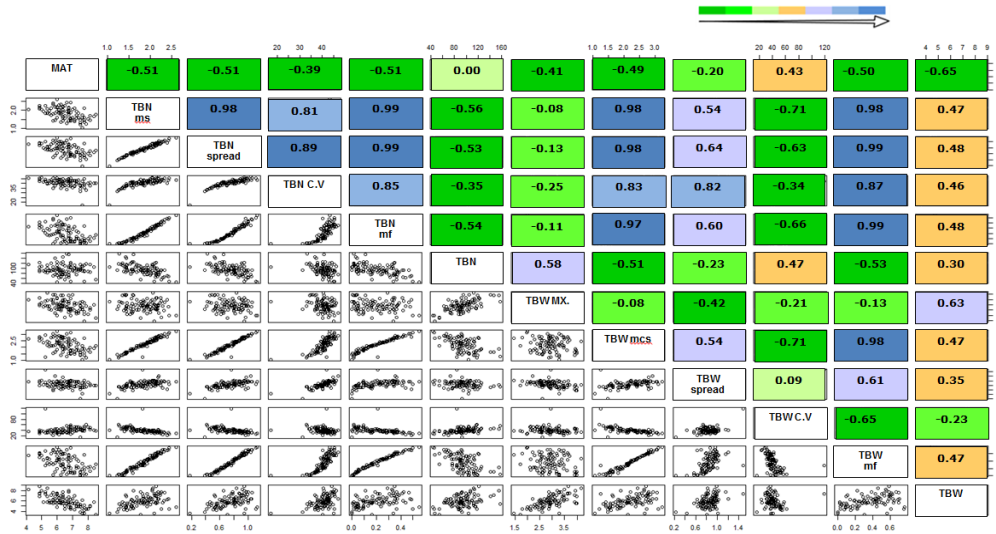


Figure 5b: Correlation of tuber distribution parameters and yield traits under non-irrigated condition at Connantre 2015 trial. MAT (maturity on a scale of late (1) to early (9)), TBN ms (tuber number mean size), TBN spread (tuber number size distribution spread), TBN CV (Coefficient of variability in tuber number), TBN mf (tuber number marketable fraction), TBN (tuber number), TBW MX. (maximum tuber fresh weight among size classes), TBW mcs (size class with the maximum tuber fresh weight), TBW spread (tuber fresh weight size distribution spread), TBW CV (Coefficient of variability in tuber fresh weight), TBW mf (tuber fresh weight marketable fraction), TBW (tuber fresh weight).

Bi-plots of principal components analyses were used to further investigate the effects of foliage maturity and cultivar-specific differences. Only the late drought (Connantre 2015) showed an observable contrast between irrigated and non-irrigated conditions in terms of maturity type influences (Fig.6a and b). Under irrigated conditions, cultivars of the different maturity classes were evenly distributed and strongly overlapped with no distinction of maturity groups (Fig.6a). However, in non-irrigated condition there was an apparent maturity grouping along the PC1 axis, with a less even distribution especially in the late maturity types (Fig.6b). Most of the tuber size distribution parameters made similar contribution to the variation in the dataset under the respective conditions except *TBW MX* (maximum tuber fresh weight among size classes). The parameter with the lowest contribution to the variation among the cultivars is the spread of tuber size distribution for fresh weight (*TBW spread*). Under non-irrigated conditions, the later maturity types tended to have higher total tuber weight (*TBW*) and higher values of tuber size distribution parameters than early maturity types. However, a few cultivars escaped the trend of their maturity class. Some of these 'outlying cultivars' were selected from the bi-plots for a closer study of their tuber size distributions. The selection was based on the position of the outliers on the bi-plots and also on the uniqueness of the cultivar's tuber size distribution. These include: Jazzy, Kuroda, Hansa, Terragold, Valiant and Avano (tagged number 1-6, respectively in the bi-plots of Fig.6).

The better-performing cultivars in each maturity class among the six are Kuroda (early type), Terragold (intermediate type) and Avano (late type). These three cultivars had their maximum tuber fresh weight or tuber number in the 50-60mm size class under stress (Fig.7). Jazzy (early type), Hansa (intermediate type) and Valiant (late type) had their maximum tuber weight or tuber number in lower classes under stress. Hansa and Jazzy produced the highest number of tubers in the dataset in all trials under irrigated and non-irrigated conditions, but most of their tubers were within 0-40mm (non-marketable) size class. Under drought conditions, Jazzy had no marketable tuber while only 3% of the total number of Hansa was marketable (Table 1). In the Connantre 2014 trial (early drought), the performance of these six cultivars in terms of their tuber size distribution parameters, marketable yield and total yield, followed a trend that was similar to the Connantre 2015 trial. However, in general drought had a more severe effect on the cultivars in the Connantre 2015 trial (Table 1).

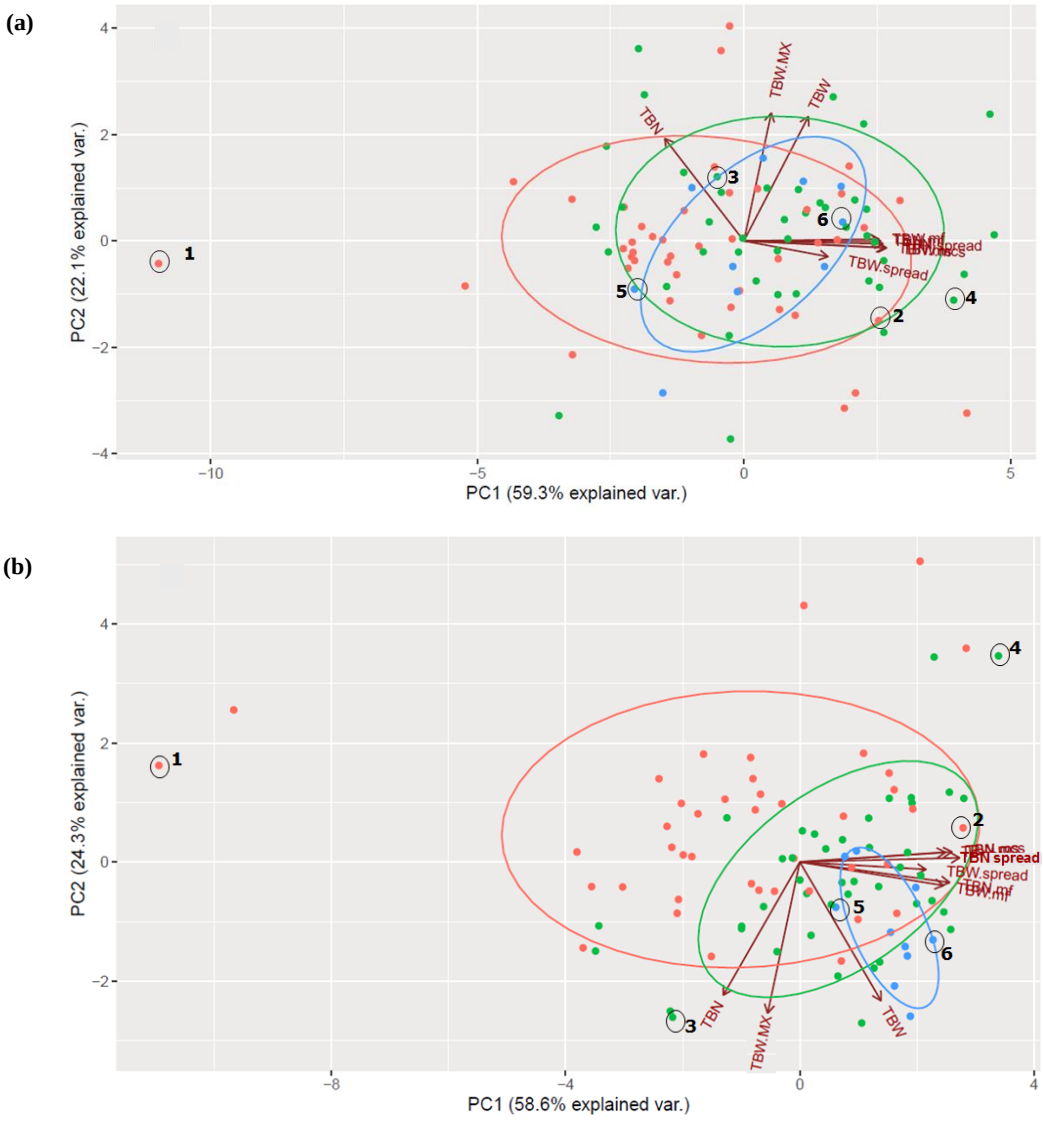


Figure 6: PCA bi-plots of tuber size distribution parameters and yield traits at the Connantre 2015 trial under (**a**) irrigated condition and (**b**) non-irrigated condition. The dots represent individual cultivars according to their maturity classes: Early (red), Intermediate (green) and late (blue). The vectors represent tuber size distribution parameters and yield traits: *TBN ms* (tuber number mean size), *TBN spread* (tuber number size distribution spread), *TBN mf* (tuber number marketable fraction), *TBN* (tuber number), *TBW MX*. (maximum tuber fresh weight among size classes), *TBW mcs* (size class with the maximum tuber fresh weight), *TBW spread* (tuber fresh weight size distribution spread), *TBW mf* (tuber fresh weight marketable fraction), *TBW* (tuber fresh weight). Dots enclosed in black circles and tagged with numbers 1-6 are cultivars from the three maturity classes with contrasting results: Jazzy (1), Kuroda (2), Hansa (3), Terragold (4), Valiant (5) and Avano (6).

Table 1: Parameters of tuber size distribution for six cultivars in the Connantre 2014 and 2015 trials

CULTIVARS	TRT*	YEAR	MAT	TBN ms**	TBN spread	TBN mf***	TBN	TBW MX[a](kg)	TBW mcs[b]	TBW spread	TBW mf[c]	TBW (kg)
AVANO	WR	2014	Late	63.66	31.48	0.67	88	8.18	115.86	35.63	0.91	16.30
AVANO	DR	2014	Late	54.88	25.78	0.61	80	4.59	71.63	18.71	0.84	11.05
AVANO	WR	2015	Late	60.63	23.88	0.71	96	5.47	73.70	20.84	0.87	13.90
AVANO	DR	2015	Late	45.16	20.05	0.42	93	3.37	58.45	18.49	0.67	7.65
VALIANT	WR	2014	Late	48.12	21.62	0.44	117	3.77	58.56	24.79	0.66	10.35
VALIANT	DR	2014	Late	46.30	18.20	0.43	100	4.30	53.34	15.98	0.59	8.95
VALIANT	WR	2015	Late	44.24	19.06	0.35	128	4.11	53.78	20.56	0.58	10.35
VALIANT	DR	2015	Late	41.91	17.57	0.30	105	3.05	49.86	19.61	0.50	7.35
TERRAGOLD	WR	2014	Int.	65.63	26.70	0.75	107	6.59	86.08	25.60	0.90	17.75
TERRAGOLD	DR	2014	Int.	63.46	28.38	0.70	64	3.73	88.21	29.03	0.89	10.55
TERRAGOLD	WR	2015	Int.	65.56	27.57	0.71	67	5.19	104.09	34.17	0.91	12.60
TERRAGOLD	DR	2015	Int.	52.18	22.64	0.55	44	1.99	64.37	19.16	0.77	4.75
HANSA	WR	2014	Int.	40.51	17.74	0.28	157	5.85	50.84	20.50	0.53	14.75
HANSA	DR	2014	Int.	36.47	15.42	0.19	159	5.83	44.33	17.25	0.37	12.35
HANSA	WR	2015	Int.	44.82	21.50	0.38	141	4.99	60.81	26.43	0.67	15.70
HANSA	DR	2015	Int.	28.56	10.45	0.03	146	3.90	32.31	15.53	0.11	7.10
KURODA	WR	2014	Early	72.74	24.21	0.86	84	7.51	107.68	33.96	0.96	16.50
KURODA	DR	2014	Early	77.31	21.04	0.92	52	5.20	87.47	17.33	0.97	10.50
KURODA	WR	2015	Early	67.94	23.07	0.80	63	4.88	81.91	19.69	0.92	11.45
KURODA	DR	2015	Early	48.33	20.87	0.48	66	2.80	60.76	19.00	0.70	6.40
JAZZY	WR	2014	Early	30.33	12.61	0.09	152	5.01	34.17	18.14	0.20	10.60
JAZZY	DR	2014	Early	28.29	10.72	0.05	144	3.90	29.56	17.36	0.11	7.45
JAZZY	WR	2015	Early	27.57	10.27	0.04	140	4.85	29.35	16.11	0.09	8.80
JAZZY	DR	2015	Early	20.86	3.60	0.00	104	3.27	21.07	8.66	0.00	3.55

*Treatment (WR – Irrigated, DR – Non-irrigated)

**TBN ms: tuber size where overall average tuber number occurred

***TBN mf: marketable fraction of tuber number

[a]TBW MX: maximum tuber fresh weight among the tuber size classes

[b]TBW mcs: tuber size where maximum tuber fresh weight occurred

[c]TBW mf: marketable fraction of tuber fresh weight

MAT: Maturity (Int. – intermediate)

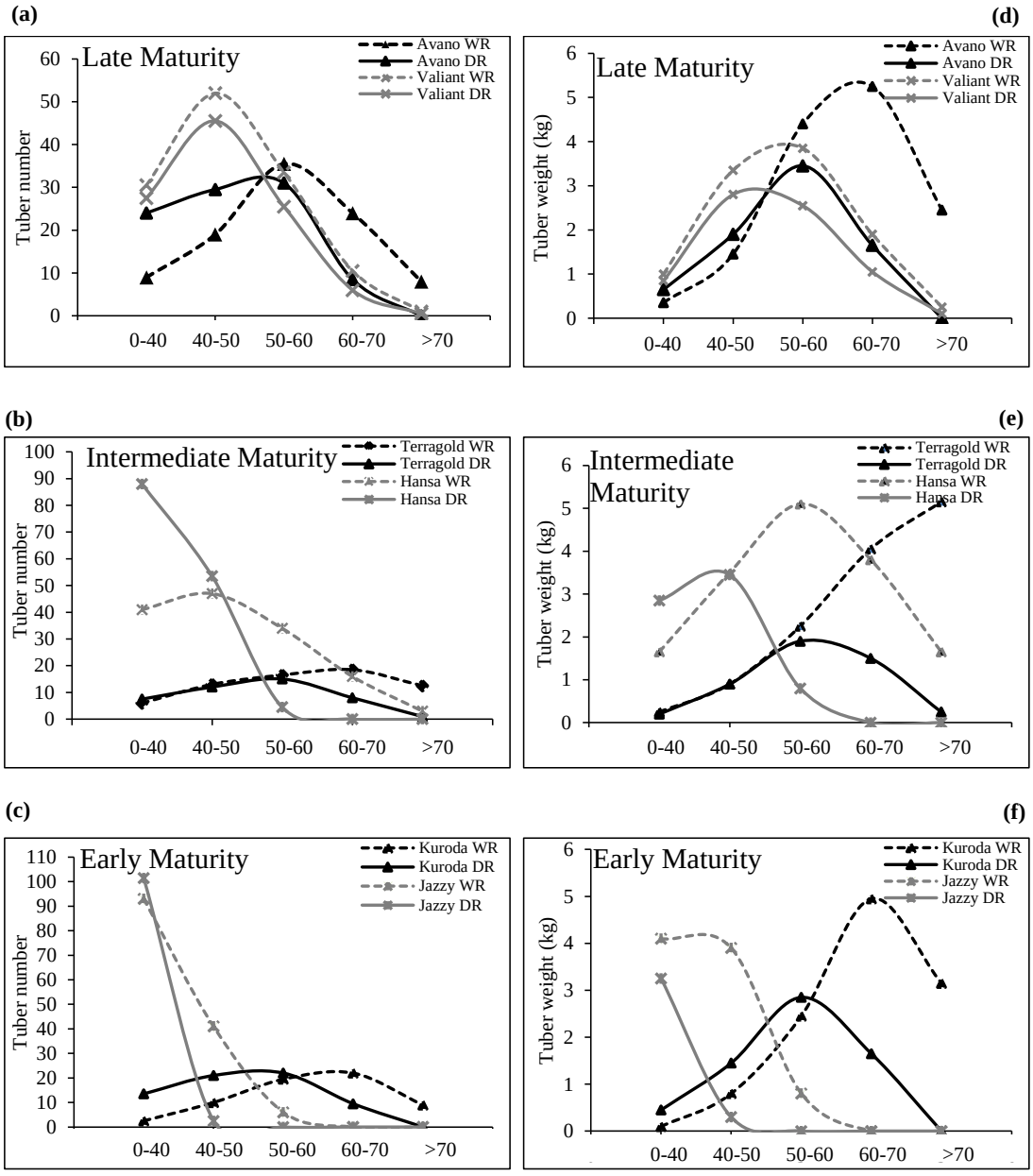


Figure 7: (**a-c**) Number of tubers in the size classes (0-40mm, 40-50mm, 50-60mm, 60-70mm and >70mm) for selected cultivars of late, intermediate and early maturity types, respectively. (**d-f**) Fresh weight of tubers in the size classes (0-40mm, 40-50mm, 50-60mm, 60-70mm and >70mm) for selected cultivars of late, intermediate and early maturity types respectively. In each maturity class, the tuber size distribution of two cultivars with contrasting drought response are compared under irrigated (WR) and non-irrigated (DR) conditions.

Heritability of yield traits in all locations

The tuber size distribution parameters were derived from the means of the size-graded tuber yield traits (total tuber number and total tuber weight) for each cultivar. In order to evaluate the breeding value of the parameters, we calculated the broad-sense heritability of the yield traits from which the parameters were derived (Table 2). Tuber number had high heritability ($H^2 \geq 0.5$) in all locations under irrigated (WR) and non-irrigated (DR) conditions. Tuber fresh weight also had high heritability ($H^2 \geq 0.5$) except in Zeeland 2013 (irrigated treatment) and Zeeland 2014 (non-irrigated treatment). Among the locations, Connantre 2015 had the lowest environmental noise as shown by the environmental variance for TBW under DR, which was lowest in this trial. Therefore, we further studied the performance of the individual cultivars in Connantre 2015 in order to evaluate the genetic variation in the dataset.

Profiling of tuber size distribution and marketable yield of all cultivars

The overall means of the traits and size distribution parameters for all cultivars (*TBN ms*, *TBN spread*, *TBN mf*, *TBW mcs*, *TBW spread* and *TBW mf*) were obtained under irrigated (WR) and non-irrigated (DR) conditions in the Connantre 2015 trial. Also, drought tolerance (DT) values of each cultivar for these parameters (Parameter in DR/Parameter in WR) were obtained. These data were used to profile each cultivar in order to assess their tuber size distribution and marketability under irrigated and drought conditions, and to assess the impact of drought. The performances of the cultivars are rated based on their parameter values that are above overall average (Supplementary Table 2). Based on these, drought response grades (DRG) are assigned for each cultivar in the dataset. The DRG shows the number of size parameters for which a cultivar has above-average value in its drought tolerance. This cultivar profile list can serve as a guide on tuber size properties of the cultivars and the aspects of tuber size distribution that are peculiar to particular cultivars. Three cultivars had above average values for all parameters in WR, DR and DT. These three cultivars are Avano, Eurostar and Labadia (Supplementary Table 2).

Table 2: heritability of yield traits in Connantre and Zeeland under irrigated and non-irrigated conditions

YEAR	LOCATION	TRAIT	TRT*	MEAN	MIN.[a]	MAX[b]	*F.pr*	V_g**	V_e***	H^2
2013	CONNANTRE	TBW	WR	10.49	4.003	17.05	<.001	4.408	1.98	0.69
		TBW	DR	8.666	3.118	13.78	<.001	2.9785	1.573	0.65
		TBN	WR	79.82	29	223	<.001	566.9	139.4	0.80
		TBN	DR	79.68	32	188	<.001	476.2	296.6	0.62
2014	CONNANTRE	TBW	WR	15.53	7.2	26.96	<.001	9.0095	3.902	0.70
		TBW	DR	10.27	5.44	18.34	<.001	3.3985	1.366	0.71
		TBN	WR	101.8	38	181	<.001	405.6	298.8	0.58
		TBN	DR	87.26	34	182	<.001	603.1	107.3	0.85
2015	CONNANTRE	TBW	WR	12.88	7.4	19.7	<.001	3.701	1.869	0.66
		TBW	DR	5.961	2.3	10.5	<.001	1.247	0.6315	0.66
		TBN	WR	105.8	52	229	<.001	469.2	529.7	0.47
		TBN	DR	93.3	27	168	<.001	472.3	148	0.76
2013	ZEELAND	TBW	WR	12.29	5.52	20.53	<.001	2.2325	4.715	0.32
		TBW	DR	10.76	4.77	15.55	<.001	2.0485	2.251	0.48
		TBN	WR	126.4	56	265	<.001	1135.1	348.6	0.77
		TBN	DR	117.8	40	317	<.001	957.9	386.7	0.71
2014	ZEELAND	TBW	WR	15.38	7.11	26.62	<.001	7.36	4.109	0.64
		TBW	DR	12.26	3.83	24.02	0.004	2.65	10.2	0.21
		TBN	WR	127.5	66	328	<.001	897.75	489.2	0.65
		TBN	DR	118.6	40	317	<.001	1507.4	516	0.74

*Treatment (WR – Irrigated, DR – Non-irrigated)

[a]Minimum trait value, [b]Maximum trait value

**Genotypic variance, ***Environmental variance

Association mapping

An Infinium array of 14,402 SNP markers was used for discovering associations of the tuber distribution parameters to physical positions on the potato genome for the Connantre 2014 and 2015 trials. Quantile-quantile plots confirmed the absence of spurious associations of the markers on a log scale. We observed significant associations of tuber fresh weight under irrigated conditions with three SNP markers in close proximity to the *StCDF*/Maturity locus on Chromosome 5, which was found to be strongly associated with tuber yield in other studies (Kloosterman et al., 2013; Schönhals et al., 2016) (Supplementary Fig.SF1). Under drought conditions in the Connantre 2015 trial we found significant association of a SNP marker with *TBN spread*, *TBN ms*, *TBN mf* and *TBW mf* (Fig.8). This marker, PotVar0030768, is found at position 55,657,256bp on the scaffold PGSC0003DMB000000062 of Chromosome 3 of the potato genome.

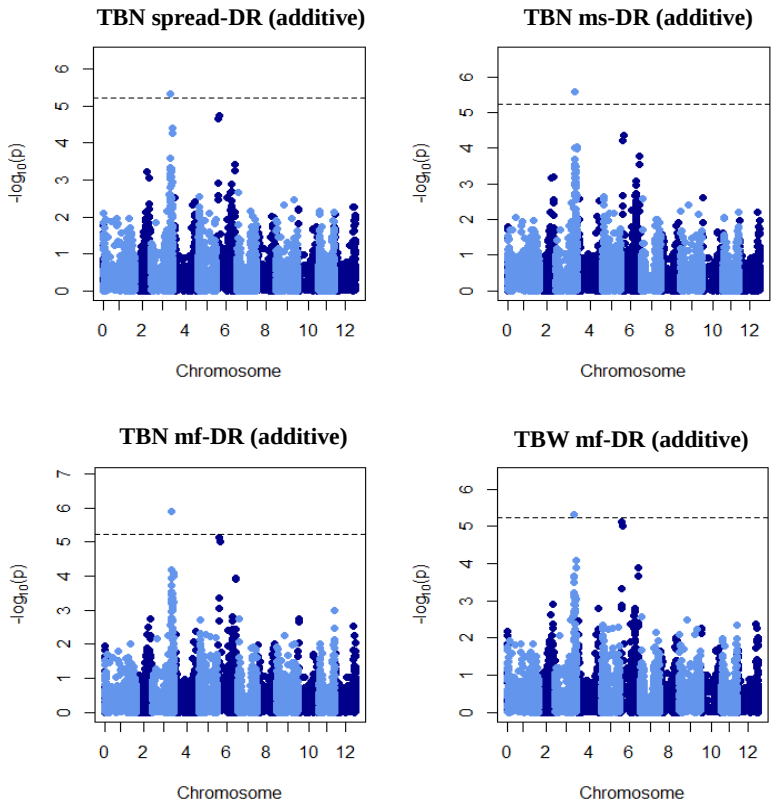


Figure 8: Manhattan plots showing significant association of SNP marker, PotVar0030768, on Chromosome 3 of the potato genome with *TBN spread* (size distribution spread of tuber number), *TBN ms* (size class where maximum tuber number occurred), *TBN mf* (marketable fractions of tuber number) and *TBW mf* (marketable fractions of tuber fresh weight) under drought (DR) condition.

The SNP marker PotVar0030768 has an A/G polymorphism with an additive allele dosage effect. An evaluation of the effect of its additivity on the parameters, *TBN spread*, *TBN ms*, *TBN mf* and *TBW mf*, is illustrated in Figure 9. For every allele dosage increase from nulliplex through quadruplex, there is an increase in parameter value. Among the cultivars with contrasting drought response illustrated in Figure 7 above, the following dosages of this SNP marker were observed for the favourable allele: quadruplex (Kuroda), triplex (Avano and Terragold), simplex (Valiant), nulliplex (Hansa). These allele dosages fitted our expectation based on the variation among these widely contrasting cultivars for the traits (*TBN spread*, *TBN ms*, *TBN mf* and *TBW mf*). No marker information for Jazzy was available. Among the entire set of cultivars, the allelic distribution (number of cultivars in the respective allele dosage classes) is as follows: quadruplex (5), triplex (20), duplex (38), simplex (20) and nulliplex (13).

The Ensembl Plant database was blasted for the super-scaffold of this significant SNP marker, PGSC0003DMB000000062. The blast result showed many genes including a gene (PGSC0003DMG400019503) encoding a pentatricopeptide repeat-containing protein (PPR) located at 2.9kb downstream of the PotVar0030768 marker.

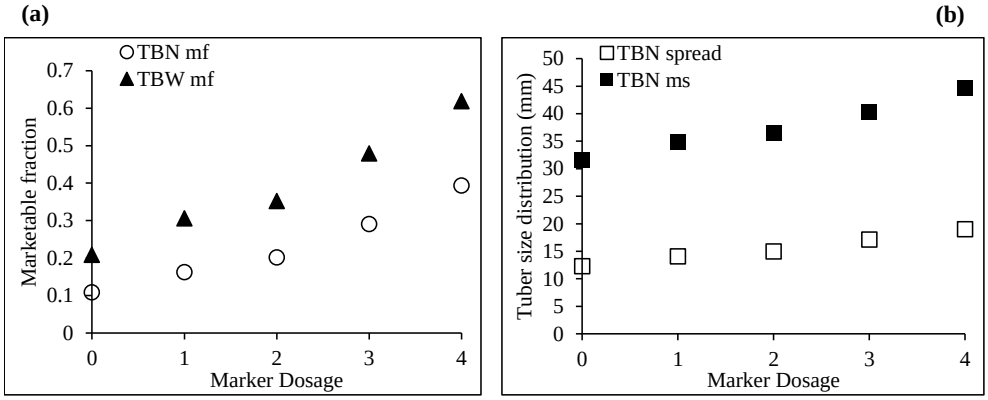


Figure 9: Illustration of the additive effect of allele dosages of the SNP marker, PotVar0030768, on the mean values of the parameters from 95 cultivars under drought: (**a**) *TBN mf* (marketable fractions of tuber number) and *TBW mf* (marketable fractions of tuber fresh weight, (**b**) *TBN spread* (size distribution spread of tuber number) and *TBN ms* (size class where maximum tuber number occurred)

DISCUSSION AND CONCLUSIONS

Differences in drought scenarios

The potato crop is known to be generally sensitive to drought stress, leading to severe reduction in tuber yield (Loon, 1981). The severity of drought stress, however, may vary between different environments and thus differently impact yield. Information on the environmental conditions in a given region and the type of drought frequently encountered will facilitate targeted and effective drought tolerance breeding for such a region. In this study, we evaluated the performance of 103

commercial potato cultivars in several locations under non-irrigated (drought) and irrigated conditions. Generally, Zeeland, a coastal Westland area of The Netherlands, experiences less drought than Connantre (Northern France). However, different years in these locations presented unique patterns of drought that affected the cultivars differentially. Interestingly, available environmental data enabled us to understand the drought patterns in the Connantre 2014 and 2015 trials in detail (See Chapter 2). The two main aspects of the drought patterns were the timing/duration of the drought and the total amount of water available to the crops in the field during the crop cycle. The Connantre 2014 trial was exposed to early drought (delayed rainfall), and there was also a higher total amount of rainfall than in Connantre 2015 (late drought). Therefore, the stronger effects of drought on traits in the Connantre 2015 trial were a combination of both the timing of the drought and the smaller amount of rainfall. For this kind of locations with highly fluctuating drought patterns between years, the monitoring of environmental information during trials would generate meta-data that facilitates the precise modelling of the crop drought response (Bassam et al., 1990; Kooman & Haverkort, 1995; Rey et al., 2016).

Tuber size distribution (TSD) parameters

Grading of potato tubers after harvest is a way of assessing the value of the total yield produced by the crop. With the aid of the Normal distribution and Gamma distribution models we have used graded tuber size data to interpret the distribution of tuber fresh weight and tuber number, respectively. From these size distributions, we extracted parameters that gave information about the respective distributions. Tuber size distribution (TSD) parameters are especially important in the description of the yield of the crop and which aspects are differently affected by drought between cultivars. For instance, the distribution parameters (*TBW mcs* and *TBN ms*) describe the balance between tuber initiation and the bulking of the formed tubers. Cultivars that make more tubers than they can bulk during the growing season would have a distribution that is skewed towards the smaller sized tubers. In our dataset, Jazzy and Hansa produced lots of tubers, but were not able to bulk them by the end of the growing season. Also during the drought stress conditions, the distributions were skewed further towards smaller tubers and higher number of tubers than under non-water limiting condition, depending on the severity of the drought. Interestingly, we observed that the skewed distribution towards smaller and more tubers in early maturing cultivars compared to the late types was more pronounced under drought than under irrigated conditions at the Connantre 2015 trial (data not shown). The late drought in Connantre 2015 coincided with the tuber bulking stage of the plant growth. During this drought period the early maturity types had a relatively shorter time to bulk their tubers than the later maturity types. The longer crop growth cycle is advantageous for a longer duration of light interception and photosynthesis, which seems to translate to tuber bulking even more under drought. Ishimaru *et al.* (2008) reported a field trial comparison of a potato cultivar (cv. May Queen) and its transgenic lines (*Ag1203*) overexpressing sucrose-phosphate synthase. The *Ag1203* lines had the same photosynthetic rate as the wild type May Queen. However, the *Ag1203* lines had delayed senescence, which increased their period of photosynthetic activity. They also had an improved translocation of photosynthates to the tubers, resulting in a higher yield (Ishimaru et al., 2008). In our study, this would imply that the later maturity types may have gained the advantage of a delayed senescence to produce more photosynthates that could partly be used for tuber bulking. However, generally the turnover of photosynthetic products is severely reduced by drought (Ashraf & Harris, 2013; Li et al., 2017). The transport of the limited amount of

photosynthates to growing tubers may be a contributory factor for drought tolerance in potato (see also Chapters 4 and 5). We did observe that within the same maturity class, some cultivars bulked a large fraction of their tubers much more than others, indicative of genetic variation in the effective use of photosynthates for tuber bulking under drought stress. Therefore, the photosynthetic duration is maturity-dependent, but the effective use of photosynthates for tubers bulking may be highly genotype-dependent within the maturity class.

The spread of the distribution (*TBN spread* and *TBW spread*) is another important tuber size distribution parameter that describes the degree of variation in the sizes of potato tubers at harvest (Wurr et al., 1993). Often, a wide spread of tuber size would imply that the larger-sized tubers are also present. Marshall and Thompson (1986) reported a linear relationship between spread of distribution and class size with the maximum tuber yield. A wide distribution suggests that the plant translocated assimilates to most of its tuber size classes, and tubers are still being formed, whereas narrow spread would imply that a narrow range of tuber sizes were prioritized during bulking, or tubers were still formed, but hardly bulked. In our drought trials, spread of TSD was reduced for both tuber number (*TBN*) and tuber fresh weight (*TBW*) in Connantre 2014 and 2015 (Figs.2 and 3). Among these trials, however, foliage maturity only affected *TBN spread* and *TBN CV* under drought in the Connantre 2015 trial to the advantage of late maturity types (Fig.5b). This suggests that a longer growth cycle (delayed senescence) facilitated the partitioning of assimilates to a larger range of tuber size classes. In earlier studies, coefficient of variation (CV) has also been used to describe the relative variation in TSD. TSD CV is defined as ((spread of distribution/class size containing the highest tuber yield) * 100) (Wurr et al., 1993). The findings from these earlier studies are that a drought treatment did not affect TSD CV (Marshall & Thompson, 1986; Wurr et al., 1993). However, in our study we observed a differential effect of drought on TSD CV depending on the drought pattern and possibly the drought severity. In the Connantre 2014 trial, the drought significantly reduced *TBN CV* but not *TBW CV*. On the other hand, in Connantre 2015 the drought significantly reduced *TBW CV* but not *TBN CV*. The Connantre 2014 trial had an early drought while Connantre 2015 trial had a late drought. The timing of the drought coincided with different tuberization stages of plant growth. The early drought of Connantre 2014 coincided with the tuber initiation stage of plant growth, and this may have affected the number of tubers formed. This drought effect on tuber initiation may be the reason for the observed drought effect on *TBN CV* in this trial. The late drought of Connantre 2015 coincided with the tuber bulking stage, with more severe effects on tuber fresh weight, resulting in a stronger effect on *TBW CV*. Moreover, there were similar levels of variation between *TBN CV* and *TBW CV* under irrigation, but these (*TBN CV* and *TBW CV*) differed widely under stress (Fig.5). The reason for the disparity in findings between our study and earlier studies may be due to the limited number of genotypes on which the conclusions from these earlier studies were based. For instance, MacKerron *et al.* (1988) used six genotypes, and they concluded that drought only affects CV of tuber number when it equally affects tuber number (Mackerron et al., 1988). In our study, however, the drought stress in both the Connantre 2014 and 2015 trials significantly reduced tuber number (See Chapter 2 – Fig.1), but only in the Connantre 2014 trial was *TBN CV* significantly reduced (data not shown). Also, in the Connantre 2015 trial *TBN CV* associated negatively with *TBN* under drought (Fig.5). That is, the formation of more tubers did not cause an increased variability of tuber number among size classes. Therefore, tuber size variability under drought may not only be related to tuber number. Moreover, Wurr et al. (1993) reported not being able to demonstrate that total tuber number affected CV, and they

suggested a complex influence. Our findings indicate that the drought pattern, severity and the tuberization stage of the plant affected by the drought may be more directly responsible for the impact on CV than the supposed relationship between drought effects on tuber number and CV. Furthermore, it has been shown that stolon characteristics, date of tuber initiation and position along the stolon, sugar metabolising enzymes, hormones, mineral compositions and turgor potential contribute to tuber size variability in potato (Struik et al., 1991).

Effects of tuber size distribution (TSD) parameters on total and marketable yield

Potato tuber yield can be described as total yield, which considers all tubers formed, or marketable yield, which only accounts for the proportion of total yield that can be marketed. Under normal (irrigation) conditions, not all the tubers at the end of the growing season are marketable. The marketable proportion must meet the specific requirements of the intended market, including tuber size. Under drought conditions there is a severer reduction in the marketable proportion of yield (Abbas & Ranjan, 2015). Therefore, we investigated the relationship of TSD parameters with total yield and marketable yield.

The two total yield components in our study, *TBN* and *TBW*, had differential correlations with their TSD parameters. The TSD parameters of TBN, *TBN ms* and *TBN spread*, correlated negatively with *TBN*, while those of TBW, *TBW mcs* and *TBW spread*, correlated positively with *TBW* (Fig.5). Nonetheless, *TBN* and *TBW* were not negatively correlated (Fig.5). The negative correlations of *TBN* with its TSD parameters indicate that the formation of many tubers reduces spread of the distribution to a range of small-sized tubers, implying a reduction in individual tuber bulking. In an earlier study using the potato cultivar *Ostara* grown on a nutrient medium, it was shown that the removal of individual tubers with known growth rates from the potato plant increased the growth rate of the remaining tubers within four days (Engels & Marschner, 1987). Probably a competition for assimilates among the tuber sinks may be responsible for this observation. In our study, the high number of small tubers may be associated with such competition for limited assimilates under stress. Therefore, potato cultivars that produce relatively less tubers but are able to bulk them under drought may be preferred. Furthermore, it was demonstrated that some potato genotypes maintain a single tuber initiation period while some others have multiple tuber initiation periods (Walworth & Carling, 2002). In our cultivar set, probably the effect of multiple tuber initiation periods coupled with delays in tuber initiation under drought may have played a role in the proliferation of small-sized tubers in some cultivars. The young tubers formed at the end of the growing season would not have enough time to be bulked. On the other hand, the positive correlation of *TBW* with *TBW mcs* and *TBW spread* indicates a higher tendency to bulk larger-sized tubers, even under drought. Marcelis (1996) already showed that sink tissues with higher sink strength would attract more assimilates (Marcelis, 1996). In our study, when tubers of the large size classes are bulked, *TBW spread* widened since there were always some small tubers on the left hand side of the distribution.

Interestingly, unlike the differential correlations between the total yield traits (*TBN* and *TBW*) and their TSD parameters, the marketable fractions of tuber yield (*TBN mf* and *TBW mf*) were positively correlated with all TSD parameters under stress and control conditions (Figs.5a and b). Although the late drought stress (Connantre 2015) did not generally interfere with the correlations of marketable fractions of tuber yield and the TSD parameters, the correlations with spread of TSD was quite remarkable. There were more positive correlations of *TBN mf* and *TBW mf* with *TBN*

spread and *TBW spread*, respectively under the late drought than under irrigation (Figs.5a and b). This drought effect on correlations between marketable fraction and spread of TSD was not observed in the Connantre 2014 trial or the other trials (data not shown). Moreover, from the Connantre 2015 trial we noticed that a high *TBN CV* was remarkably associated with high values of the TSD parameters and marketable fraction, but only under drought (Fig.5b). Therefore, depending on the pattern and severity of drought, spread of tuber size distribution may be an indicator of how the drought affects marketable fraction. A wider spread would indicate a higher marketability.

Marketable yield has received far less considerations from scientific reports than total yield. This may be because of the rigorous process of scoring marketable yield from total yield. In our study, under irrigated conditions in Connantre 2015 about 73% of *TBW* and 54% of *TBN* were marketable (Fig.4). In Connantre 2014 under irrigated conditions, 82% of *TBW* and 62% of *TBN* were marketable. However, in the late drought (Connantre 2015), 36% of *TBW* and 21% of *TBN* were marketable, while in the early drought condition (Connantre 2014), 73% of *TBW* and 54% of *TBN* were marketable (Fig.4). The marketable percentages for Connantre 2013 trial were within these limits, and there was no drought effect on marketable yield in Zeeland. The late drought led to a more severe reduction in marketable yield than the early drought. Moreover, in the late drought the plants also had a much lower amount of water available in the growth season. The overall most severe drought effect on *TBW* was 54% reduction (Connantre, 2015) and on *TBN* was 14% reduction (Connantre 2014) (see Chapter 2). Interestingly, the most severe effect of drought on *TBW mf* is 51% reduction (Connantre, 2015) and on *TBN mf* is 13% reduction (Connantre 2014). This suggests that drought impact on total yield may be representative of its impact on marketable yield. However, *TBN mf* was always negatively correlated with *TBN* under irrigated and non-irrigated conditions. Therefore, reduction in *TBN* due to drought may be used to infer reduction in *TBN mf*; and high absolute *TBN* values would be indicative of low marketable number of tubers. Furthermore, some genotypes may not show this relationship between marketable fraction and total yield. A comparison between two late maturity types, Avano and Valiant, showed that Valiant was more drought tolerant than Avano in terms of *TBW* in the Connantre 2015 trial (Table 1). But the *TBW* of Valiant under drought (7.35kg) only had 50% marketable fraction, while the 7.65kg yield of Avano under drought had 67% marketable fraction. In another study that compared total yield and marketable fraction, the effect of nutrient (Nitrogen) deficiency on marketable tuber number and total tuber number was demonstrated (Zelalem A. et al., 2009). The nitrogen limitation (0kg N/ha versus 207 kg N/ha) resulted in a 24% and 48% reduction in total tuber number and marketable tuber number, respectively. This report suggests a more adverse effect of nutrient deficiency on marketable tuber number over total tuber number. The differences between this report and the findings from our study may be due to differences in the kind of stress, stress perception and stress severity. It is known that reduced nitrogen favours tuber initiation in potato (Vecchio et al., 2004), which can potentially lead to an increased total number of tubers, whereas reduced water availability retards tuber initiation (Walworth & Carling, 2002).

Maturity effects on TSD parameters, marketable fraction and total yield under drought vs control

The cultivars in this study were classified into three groups based on their foliage maturity types: early, intermediate and late maturity types. This classification enabled us to investigate the role of

maturity differences and the genotype variation within the maturity groups. Using a biplot analysis we observed that the effect of drought on the TSD parameters was quite dependent on maturity grouping in the Connantre 2015 trial (Fig.6), probably due to the pattern and/or severity of the drought in this trial. The late maturing cultivars and some intermediate maturing cultivars had higher values of the TSD parameters than most of the early maturity types. Interestingly, the level of variation within each maturity class was highest within the early maturity class and lowest within the late maturity class, as shown by the convex hulls of the maturity groups (Fig.6b). The late maturity types had less variation under drought than under irrigation (Fig.6a and b). Therefore, late foliage maturity facilitated the attainment of high values for the TSD parameters (and yield). This advantage of late maturity towards high TSD parameter values is also indicated from the correlation coefficients under drought (Fig.5). The late maturing cultivars had more large-sized tubers and a wider spread of tuber size distribution than the early maturing cultivars, especially under drought (Fig.5a and b)

Marker-parameter associations

One of the aims of studying tuber yield and yield distribution parameters is to understand the extent of genetic control on the variation in these phenotypic characteristics under environmental stress conditions like drought. We have investigated a relatively large set of 103 cultivars that showed significant genotypic effects on the variation in tuber yield parameters in our dataset.

Based on the existence of relatively high heritability for the tuber yield traits in our dataset, we have used a 14K SNP marker array to search for associations between regions of the potato genome and the TSD parameters in 95 of the cultivars. Under drought in the Connantre 2015 trial we found significant marker-trait associations of the TSD parameters, *TBN spread* and *TBN ms*, and marketable yield (*TBN mf* and *TBW mf*), with a region on chromosome 3 of the potato genome. Increases in the allelic dosage in this region improved parameter value and marketable fraction, indicating additive effects. Amongst other genes, a pentatricopeptide repeat-containing protein (PPR) is present in this region. PPR proteins were recently discovered (Schmitz-Linneweber & Small), and are known to recognize RNA editing sites and bind to the upstream sequences of such editing sites through their repeat elements (Ichinose & Sugita, 2017; Schmitz-Linneweber & Small). In literature, this gene family is shown to be involved in the tolerance to abiotic stresses including drought in Arabidopsis (Jiang et al., 2015; Liu et al., 2010; Lv et al., 2014; Sharma & Pandey, 2015; Yuan & Liu, 2012; Zhu et al., 2012; Zsigmond et al., 2008). The role of PPRs has not been reported in potato. In other plant systems, PPRs have been reported in the restoration of fertility to cytoplasmic male sterile lines in Petunia (Bentolila et al., 2002) and *Brassica napus* (Brown et al., 2003). However, their role in stress tolerance has not been demonstrated in any other plant systems apart from Arabidopsis. Further work is therefore necessary to understand the role of this gene in crop systems. Also, this significantly associated SNP locus will need more dedicated investigation to understand its functional involvement in yield, and how it can be integrated into the breeding for stress tolerance in crops

ACKNOWLEDGMENTS

We thank the following for their contribution of the SNP marker data used in this study: Peter Vos and Johan Willemsen

REFERENCES

Abbas, H., & Ranjan, R. S. (2015). Effect of soil moisture deficit on marketable yield and quality of potatoes. *Canadian Biosystems Engineering, 57*, 125-137.

Ashraf, M., & Harris, P. J. C. (2013). Photosynthesis under stressful environments: An overview. *Photosynthetica, 51*(2), 163-190. doi: 10.1007/s11099-013-0021-6

Bassam, N., Dambroth, M., Loughman, B. C., Spitters, C. J. T., & Schapendonk, A. H. C. M. (1990). Evaluation of breeding strategies for drought tolerance in potato by means of crop growth simulation *Genetic Aspects of Plant Mineral Nutrition* (Vol. 42, pp. 151-161): Springer Netherlands.

Bentolila, S., Alfonso, A. A., & Hanson, M. R. (2002). A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. *Proc Natl Acad Sci U S A, 99*(16), 10887-10892. doi: 10.1073/pnas.102301599

Brown, G. G., Formanova, N., Jin, H., Wargachuk, R., Dendy, C., Patil, P., Laforest, M., Zhang, J., Cheung, W. Y., & Landry, B. S. (2003). The radish Rfo restorer gene of Ogura cytoplasmic male sterility encodes a protein with multiple pentatricopeptide repeats. *Plant J, 35*(2), 262-272.

Cantore, V., Wassar, F., Yamac, S. S., Sellami, M. H., Albrizo, R., Stellacci, A. M., & Todorovic, M. (2014). Yield and water use efficiency of early potato grown under different irrigation regimes. *International Journal of Plant Production, 8*(3), 1735-8043.

Catchpole, A. H., & Hillman, J. (1969). Effect of Ethylene on Tuber Initiation in Solanum tuberosum L. *223*(5213), 1387-1387.

Celis-Gamboa, B. C. (2002). *The life cycle of the potato (Solanum tuberosum L.): from crop physiology to genetics.* s.n.], [S.l. Retrieved from http://library.wur.nl/WebQuery/wurpubs/122845

Celis-Gamboa, C., Struik, P. C., Jacobsen, E., & Visser, R. G. F. (2003). Temporal dynamics of tuber formation and related processes in a crossing population of potato (Solanum tuberosum). *Annals of Applied Biology, 143*(2), 175-186. doi: 10.1111/j.1744-7348.2003.tb00284.x

D'Hoop B, B., Paulo, M. J., Kowitwanich, K., Sengers, M., Visser, R. G., van Eck, H. J., & van Eeuwijk, F. A. (2010). Population structure and linkage disequilibrium unravelled in tetraploid potato. *Theor Appl Genet, 121*(6), 1151-1170. doi: 10.1007/s00122-010-1379-5

Daryanto, S., Wang, L., & Jacinthe, P.-A. (2016). Drought effects on root and tuber production: A meta-analysis. *Agricultural Water Management, 176*, 122-131. doi: http://dx.doi.org/10.1016/j.agwat.2016.05.019

Devaux, A., Kromann, P., & Ortiz, O. (2014). Potatoes for Sustainable Global Food Security. *Potato Research, 57*(3), 185-199. doi: 10.1007/s11540-014-9265-1

Dutt, S., Manjul, A. S., Raigond, P., Singh, B., Siddappa, S., Bhardwaj, V., Kawar, P. G., Patil, V. U., & Kardile, H. B. (2017). Key players associated with tuberization in potato: potential candidates for genetic engineering. *Critical Reviews in Biotechnology*, 1-19. doi: 10.1080/07388551.2016.1274876

Engels, C., & Marschner, H. (1987). Effects of reducing leaf area and tuber number on the growth rates of tubers on individual potato plants. *Potato Research, 30*(2), 177-186. doi: 10.1007/bf02357661

FAOSTAT. (2014). Food Supply - Crops Primary Equivalent - Potatoes. Retrieved 17th Feb. 2017 http://www.fao.org/faostat/en/#data/CC

Glasbey, C. A., McRae, D. C., & Fleming, J. (1988). The size distribution of potato tubers and its application to grading schemes. *Annals of Applied Biology, 113*(3), 579-587. doi: 10.1111/j.1744-7348.1988.tb03335.x

Harris, P. M. (2012). *The Potato Crop: The scientific basis for improvement*: Springer Netherlands.

Ichinose, M., & Sugita, M. (2017). RNA Editing and Its Molecular Mechanism in Plant Organelles. *Genes, 8*(1), 5.

Ishimaru, K., Hirotsu, N., Kashiwagi, T., Madoka, Y., Nagasuga, K., Ono, K., & Ohsugi, R. (2008). Overexpression of a Maize SPS Gene Improves Yield Characters of Potato under Field Conditions. *Plant Production Science, 11*(1), 104-107. doi: 10.1626/pps.11.104

Jiang, S.-C., Mei, C., Liang, S., Yu, Y.-T., Lu, K., Wu, Z., Wang, X.-F., & Zhang, D.-P. (2015). Crucial roles of the pentatricopeptide repeat protein SOAR1 in Arabidopsis response to drought, salt and cold stresses. *Plant Mol Biol, 88*(4-5), 369-385. doi: 10.1007/s11103-015-0327-9

Kloosterman, B., Abelenda, J. A., Gomez, M. d. M. C., Oortwijn, M., de Boer, J. M., Kowitwanich, K., Horvath, B. M., van Eck, H. J., Smaczniak, C., Prat, S., Visser, R. G. F., & Bachem, C. W. B. (2013). Naturally occurring allele diversity allows potato cultivation in northern latitudes. *Nature 495*(7440), 246-250.

Kooman, P. L., & Haverkort, A. J. (Eds.). (1995). *Modelling development and growth of the potato crop influenced by temperature and daylength: LINTUL-POTATO*. Netherlands: Kluwer Academic Publishers.

Kraak, A. (1992). Industrial applications of potato starch products. *Industrial Crops and Products, 1*(2), 107-112. doi: http://dx.doi.org/10.1016/0926-6690(92)90007-I

Lahlou, O., Ouattar, S., & Ledent, J. (2003). The effect of drought and cultivar on growth parameters, yield and yield components of potato. *Agronomie, 23* 257-268. doi: 10.1051/agro:2002089

Lal, R. (2014). Climate Strategic Soil Management. *Challenges, 5*(1), 43.

Li, J., Cang, Z., Jiao, F., Bai, X., Zhang, D., & Zhai, R. (2017). Influence of drought stress on photosynthetic characteristics and protective enzymes of potato at seedling stage. *Journal of the Saudi Society of Agricultural Sciences, 16*(1), 82-88. doi: http://dx.doi.org/10.1016/j.jssas.2015.03.001

Liu, Y., He, J., Chen, Z., Ren, X., Hong, X., & Gong, Z. (2010). ABA overly-sensitive 5 (ABO5), encoding a pentatricopeptide repeat protein required for cis-splicing of mitochondrial nad2 intron 3, is involved in the abscisic acid response in Arabidopsis. *Plant J, 63*(5), 749-765. doi: 10.1111/j.1365-313X.2010.04280.x

Loon, C. D. (1981). The effect of water stress on potato growth, development, and yield. [American Potato Journal]. *58*(1), 51-69. doi: 10.1007/bf02855380

Love, S. L., & Thompson-Johns, A. (1999). Seed Piece Spacing Influences Yield, Tuber Size Distribution, Stem and Tuber Density, and Net Returns of Three Processing Potato Cultivars. *HORTSCIENCE, 34*(4), 629–633.

Luitel, B. P., Khatri, B. B., Choudhary, D., Paudel, B. P., Jung-Sook, S., Hur, O., Baek , H. J., Cheol, K. H., & Yul, R. K. (2015). Growth and yield characters of potato genotypes grown in

drought and irrigated conditions of Nepal. *Int J Appl Sci Biotechnol,, Vol 3* ((3)), 513-519. doi: DOI: 10.3126/ijasbt.v3i3.13347

Lv, H.-X., Huang, C., Guo, G.-Q., & Yang, Z.-N. (2014). Roles of the nuclear-encoded chloroplast SMR domain-containing PPR protein SVR7 in photosynthesis and oxidative stress tolerance in Arabidopsis. *Journal of Plant Biology, 57*(5), 291-301. doi: 10.1007/s12374-014-0041-1

Mackerron, D. K. L., Marshall, B., & Jefferies, R. A. (1988). The distributions of tuber sizes in droughted and irrigated crops of potato. II. Relation between size and weight of tubers and the variability of tuber-size distributions. *Potato Research, 31*(2), 279-288. doi: 10.1007/bf02365536

MAFF. (2000). A Predicitve Model of Potato Size Distribution and Procedures to Optimize its Operation (pp. 23). London: Mylnefield Research Service Ltd Invergowrie, Dundee.

Marcelis, L. (1996). Sink strength as a determinant of dry matter partitioning in the whole plant. *Journal of Experimental Botany, 47*(suppl 1), 1281.

Marshall, B., Holwerda, H. T., & Struik, P. C. (1993). Synchronisation of tuber growth in potato (Solanum tuberosum): a statistical model. *Field Crops Research, 32*, 343-357.

Marshall, B., & Thompson, R. (1986). Tuber-size distribution. *Potato Research, 29*, 261-262.

Minhas, J. S., Rai, V. K., & Saini, H. S. (2004). Carbohydrate metabolism during tuber initiation in potato: A transient surge in invertase activity marks the stolon to tuber transition. *Potato Research, 47*(3), 113. doi: 10.1007/bf02735978

Nemecek T, Derron JO, O, R., & Fischlin A. (1996). Adaptation of a crop-growth model and its extension by a tuber size function for use in seed potato forecasting system. . *Agricultural Systems, 52*, 419-437.

Nouri, A., Nezami, A., Kafi, M., & Hassanpanah, D. (2016). Growth and yield response of potato genotypes to deficit irrigation. *International Journal of Plant Production, 10*(2), 139-157. doi: 10.22069/ijpp.2016.2785

O'Brien, P. J., Allen, E. J., & Firman, D. M. (1998). REVIEW A review of some studies into tuber initiation in potato (Solanum tuberosum) crops. *The Journal of Agricultural Science, 130*(3), 251-270.

Ospina, C. A., Lammerts van Bueren, E. T., Allefs, J. J. H. M., Engel, B., van der Putten, P. E. L., van der Linden, C. G., & Struik, P. C. (2014). Diversity of crop development traits and nitrogen use efficiency among potato cultivars grown under contrasting nitrogen regimes. *Euphytica, 199*(1-2), 13-29. doi: 10.1007/s10681-014-1203-4

Ozgen, S., Palta, J. P., & Kleinhenz, M. D. (2003). *Influence of supplemental calcium fertilization on potato tuber size and tuber number.* Paper presented at the Acta Horticulturae.

Pasare, S. A., Ducreux, L. J. M., Morris, W. L., Campbell, R., Sharma, S. K., Roumeliotis, E., Kohlen, W., van der Krol, S., Bramley, P. M., Roberts, A. G., Fraser, P. D., & Taylor, M. A. (2013). The role of the potato (Solanum tuberosum) CCD8 gene in stolon and tuber development. *New Phytologist, 198*(4), 1108-1120. doi: 10.1111/nph.12217

Rey, D., Holman, I. P., Daccache, A., Morris, J., Weatherhead, E. K., & Knox, J. W. (2016). Modelling and mapping the economic value of supplemental irrigation in a humid climate. *Agricultural Water Management, 173*, 13-22. doi: http://dx.doi.org/10.1016/j.agwat.2016.04.017

Rosyara, U. R., De Jong, W. S., Douches, D. S., & Endelman, J. B. (2015). Software for genome-wide association studies in autopolyploids and its application to potato. *Plant Genome*.

Sands, P. J., & Regel, P. A. (1983). A model of the development and bulking of potatoes (Solanum tuberosum L.) V. A simple model for predicting graded yields. *Field Crops Research, 6*, 25-40.

Schmitz-Linneweber, C., & Small, I. Pentatricopeptide repeat proteins: a socket set for organelle gene expression. *Trends Plant Sci, 13*(12), 663-670. doi: 10.1016/j.tplants.2008.10.001

Schönhals, E. M., Ortega, F., Barandalla, L., Aragones, A., Ruiz de Galarreta, J. I., Liao, J. C., Sanetomo, R., Walkemeier, B., Tacke, E., Ritter, E., & Gebhardt, C. (2016). Identification and reproducibility of diagnostic DNA markers for tuber starch and yield optimization in a novel association mapping population of potato (Solanum tuberosum L.). *Theor Appl Genet, 129*, 767-785. doi: 10.1007/s00122-016-2665-7

Sharma, M., & Pandey, G. K. (2015). Expansion and Function of Repeat Domain Proteins During Stress and Development in Plants. *Frontiers in Plant Science, 6*, 1218. doi: 10.3389/fpls.2015.01218

Stearns, L. D., Petry, T. A., & Krause, M. A. (1994). Potential Food and Nonfood Utilization of Potatoes and Related Byproducts in North Dakota (pp. 60). North Dakota Department of Agricultural Economics-Agricultural Experiment Station, North Dakota University.

Struik, P. C., Vreugdenhil, D., Haverkort, A. J., Bus, C. B., & Dankert, R. (1991). Possible mechanisms of size hierarchy among tubers on one stem of a potato (Solanum tuberosum L.) plant. *Potato Research, 34*(2), 187-203. doi: 10.1007/bf02358041

Trebejo, I., & Midmore, D. J. (1990). Effect of water stress on potato growth, yield and water use in a hot and a cool tropical climate. *The Journal of Agricultural Science, 114*(3), 321-334. doi: 10.1017/s0021859600072713

Vayda, M. E. (Ed.). (1994). *Environmental Stress and Its Impact on Tuber Yield*. Wallingford, UK: CAB International: .

Vecchio, V., Andrenelli, L., & Benedettelli, S. (2004). Effect of nitrogen interruption on in vitro tuberization and potato microtuber storage. *Advances in Horticultural Science, 18*(2), 63-67.

Voorrips, R. E., Gort, G., & Vosman, B. (2011). Genotype calling in tetraploid species from bi-allelic marker data using mixture models. *BMC Bioinformatics, 12*(1), 1-11. doi: 10.1186/1471-2105-12-172

Vos, P. G., Uitdewilligen, J. G. A. M. L., Voorrips, R. E., Visser, R. G. F., & van Eck, H. J. (2015). Development and analysis of a 20K SNP array for potato (Solanum tuberosum): an insight into the breeding history. *Theor Appl Genet, 128*(12), 2387-2401. doi: 10.1007/s00122-015-2593-y

Walworth, J. L., & Carling, D. E. (2002). Tuber initiation and development in irrigated and non-irrigated potatoes. *American Journal of Potato Research, 79*(6), 387-395. doi: 10.1007/bf02871683

Wurr, D. C. E., Fellows, J. R., Lynn, J. R., & Allen, E. J. (1993). The impact of some agronomic factors on the variability of potato tuber size distribution. *Potato Research, 36*(3), 237-245. doi: 10.1007/bf02360532

Yu, J., Pressoir, G., Briggs, W. H., Vroh Bi, I., Yamasaki, M., Doebley, J. F., McMullen, M. D., Gaut, B. S., Nielsen, D. M., Holland, J. B., Kresovich, S., & Buckler, E. S. (2006). A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nat Genet, 38*(2), 203-208. doi: 10.1038/ng1702

Yuan, H., & Liu, D. (2012). Functional disruption of the pentatricopeptide protein SLG1 affects mitochondrial RNA editing, plant development, and responses to abiotic stresses in Arabidopsis. *Plant J, 70*(3), 432-444. doi: 10.1111/j.1365-313X.2011.04883.x

Zaag, D. E. v. d. (1992). Potatoes and their cultivation in the Netherlands (pp. 47). The Hague: NIVAA (Netherlands Potato Consultative Institute).

Zarka, K. A., Kells, D. C., Douches, D. S., & Buell, C. R. (2009). A Guide to Growing Potatoes In Your Home Garden.

Zelalem A., T., T., & D., N. (2009). Response of potato (Solanum tuberosum L.) to different rates of nitrogen and phosphorus fertilization on vertisols at Debre Berhan, in the central highlands of Ethiopia. *African Journal of Plant Science, 3*(2), 016-024.

Zhu, Q., Dugardeyn, J., Zhang, C., Takenaka, M., Kuhn, K., Craddock, C., Smalle, J., Karampelias, M., Denecke, J., Peters, J., Gerats, T., Brennicke, A., Eastmond, P., Meyer, E. H., & Van Der Straeten, D. (2012). SLO2, a mitochondrial pentatricopeptide repeat protein affecting several RNA editing sites, is required for energy metabolism. *Plant J, 71*(5), 836-849. doi: 10.1111/j.1365-313X.2012.05036.x

Zsigmond, L., Rigo, G., Szarka, A., Szekely, G., Otvos, K., Darula, Z., Medzihradszky, K. F., Koncz, C., Koncz, Z., & Szabados, L. (2008). Arabidopsis PPR40 connects abiotic stress responses to mitochondrial electron transport. *Plant Physiol, 146*(4), 1721-1737. doi: 10.1104/pp.107.111260

CARBON PARTITIONING MECHANISMS IN POTATO UNDER DROUGHT STRESS

Ernest B Aliche[1,2], Tom P. J. M. Theeuwen[1,2], Marian Oortwijn[1], Richard G. F. Visser[1,2], C. Gerard van der Linden[1,2]

[1]Plant Breeding, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen.

[2]Graduate School Experimental Plant Sciences, Droevendaalsesteeg 1, 6708 PB, Wageningen

To be submitted...

ABSTRACT

Potato (Solanum tuberosum) is an important crop species consumed all over the world, but it is generally sensitive to drought conditions. In view of the huge yield losses resulting from drought stress, the drive for improved drought tolerance in potato has gained global research and agricultural interest. One of the major physiological processes affected by drought stress is carbon partitioning: the plant's choice of where to allocate its photoassimilates under stress is strongly affecting yield in crops. Carbon partitioning and its relation to yield involve many processes including photosynthesis, sucrose metabolism, transport of metabolites and starch biosynthesis. These processes were studied in the greenhouse from 2013 – 2015 using potato cultivars with contrasting drought responses. Our results indicate that one of the most severe effects of drought stress on potato is the arrest of stolon differentiation and formation of tubers. Our phenotypic studies also point to some physiological traits like stomatal conductance and chlorophyll fluorescence that affect carbon assimilation, partitioning and eventual tuber yield. Multidisciplinary studies of photoassimilate metabolism and transport were done using gene expression analyses and biochemical assays to measure the role of genes involved in sucrose metabolism in various source and sink tissues in combination with phenotypic assessments. The results highlight the various tissues prioritized by the plant for assimilate transport during drought stress, and give indications of what distinguishes drought tolerance and sensitivity of cultivated potato. Some of the key genes studied (like Sucrose synthase, Sucrose transporter and Granule-bound starch synthase) may be inclusive breeding targets for drought tolerance in potato.

INTRODUCTION

Potato is a food security crop grown for its tubers as a staple food source. The potato tuber is a low-fat source of carbohydrates, and is formed from differentiation of the stolon tissue (CIP, 2013). The bulking of the growing potato tuber takes place alongside other growth or developmental processes in the plant such as flowering, initiation of new tubers, leaf expansion and foliage development. To facilitate growth and development, plant tissues exchange carbon molecules in the form of sugars throughout the growing season. This involves the transport of photo-assimilates from source tissues (mature leaves) to the sink tissues of the plant (young leaves, flowers and underground tissues). This transport of photo-assimilates to various sink tissues is known as carbon partitioning and is determined by source-sink relationships.

Carbon partitioning encompasses molecular interactions and physiological mechanisms involved in the distribution and utilization of photosynthetic assimilates (Braun & Slewinski, 2009; Gifford & Evans, 1981; Minchin & Thorpe, 1996; Moorby, 1994; Osorio et al., 2014; Sharkey, 2015). Carbon partitioning and photosynthesis are highly connected. Photosynthesis produces the assimilates that are partitioned, and carbon partitioning feeds back on the rate of photosynthesis (Araya et al., 2006; Azcon-Bieto, 1983; Blechschmidt-Schneider et al., 1989; Thorne & Koller, 1974). Therefore, carbohydrates from photosynthesis need to be optimally transported to ensure the continuity of the plant's anabolic and catabolic processes.

In plants, transport between source and sink tissues is mainly facilitated by the translocation of sucrose molecules (Lemoine, 2000; Liu et al., 2012) or raffinose-family oligosaccharides (RFOs) (Hannah et al., 2006). There is evidence suggesting that hexoses (glucose and fructose) are transported as well (van Bel & Hess, 2008). The various components of carbon partitioning have been elaborately summarized in studies on the starch biosynthesis pathway (Nazarian-Firouzabadi & Visser, 2017; Ross & Davies, 1992). Starch production for storage and remobilization into sucrose occurs during carbon partitioning (Baur-hoch et al., 1990; Geiger & Servaites, 1994; Paul & Arthur, 1996; Sun et al., 2011; Zeeman et al., 2004), and this interacts with photosynthetic sucrose synthesis and export to sink tissues (Stitt & Sonnewald, 1995). Carbon partitioning is a dynamic process that needs to be tightly regulated in order to adapt to the energy demands of the different tissues of the plant. The regulatory component in the starch biosynthesis pathway includes key enzymes like sucrose phosphate synthase (*SPS*) and sucrose phosphate phosphatase (*SPP*) that convert the 3-carbon sugars formed after assimilation of CO_2 into sucrose in the cytoplasm of leaf cells (Huber & Huber, 1996; Maloney et al., 2015; Tobias et al., 1999; Wang Li et al., 2013). It also includes invertases and sucrose synthases (*SUSY*) that hydrolyse a part of the sucrose to meet the needs of leaf metabolism (Koch, 2004; Ricardo & Aprees, 1970; Roitsch & González, 2004; Sturm & Tang, 1999; Winter & Huber, 2000; Zrenner et al., 1995). Ultimately, the non-hydrolysed sucrose can be loaded into the phloem for export to sink tissues.

Sucrose (sugar) transport from source to sink tissues can occur symplastically through plasmodesmatal networks or apoplastically (Atwell et al., 1999; De Schepper et al., 2013; Dickinson et al., 1991; Giaquinta, 1977; Turgeon & Medville, 2004). Apoplastic phloem loading is an active transport process that is facilitated by sucrose transporters like SWEETs and SUTs (Chen et al., 2012; Riesmeier et al., 1993b; Truernit, 2001). The ATP required for this active transport is made available through sucrose breakdown by *SUSY* in source leaves (Martin et al., 1993). In potato, both symplastic and apoplastic sucrose transport have been reported (Schulz et al., 1998). Sucrose is imported into the sieve elements-companion cells (SE-CC) complex, and it flows from the companion cells into the sieve tube elements, via the lateral sieve area and specialized plasmodesmatal connections, and to sink tissues for metabolism or storage as starch (Leisner & Turgeon, 1993). Sink tissues receive sugars depending on their sink strength (affinity for assimilates) (Marcelis, 1996), which seems to partly drive carbon partitioning, but is highly affected by environmental stresses (Roitsch, 1999). Drought stress is one of such environmental stresses that interferes with carbon partitioning by affecting photosynthesis, xylem and phloem transport, and by inducing sugar synthesis for osmotic adjustment (DaCosta & Huang, 2006; Lemoine et al., 2013; McDowell, 2011; Nicolas et al., 1985; Onillon et al., 1995; Rambal et al., 2014; Xu et al., 2007).

Experimental evidence suggests that during the initial stages of drought stress, plants prioritize carbon partitioning of assimilates towards the root (DaCosta & Huang, 2006; Nicolas et al., 1985), possibly as an adaptive mechanism to access the limited soil water (Brunner et al., 2015; Comas et al., 2013). However, a prioritization of other tissues (Gargallo-Garriga et al., 2014) may be at the expense of tuber yield under stress (Bacon, 2009; Tanner, 1981). Drought stress triggers several molecular and physiological responses in the crop related to carbon partitioning.

For instance, drought stress of about 40% of field irrigation capacity was reported to trigger the accumulation of soluble sugars in sink leaves of *S. tuberosum* cv. Marfuna (Farhad et al., 2011). In another study, moderate drought was shown to cause a 17% reduction in tuber number which was, however, not associated with lower tuber yields because dry weight per tuber was maintained under the drought (Deblonde & Ledent, 2001). Under a more severe drought, about 79% reduction in tuber yield was reported, alongside reductions in other growth characteristics like canopy cover and stem height (Luitel et al., 2015). More insight in the regulation of carbon partitioning under drought is essential, but indeed complex because carbohydrates (as sugars) are also believed to function in stress signalling (Lalonde et al., 1999; Rolland et al., 2002; Rosa et al., 2009).
In this study, we have evaluated potato genotypes with contrasting drought responses in order to gain insights in the mechanisms and molecular factors that influence carbon partitioning during drought. Our findings suggest that assimilate transport within mature leaves and export from mature leaves may constitute a major bottleneck in carbon partitioning.

MATERIALS AND METHODS

Planting and drought application

Several potato cultivars were used to study the effect of drought on carbon partitioning in three consecutive years (2013 – 2015) in the greenhouse at Unifarm, Wageningen University & Research. The cultivars grown in each year were selected based on their contrasting drought responses in field and greenhouse trials of previous years. The cultivars used in the final (2015) trial included Biogold, Mozart, Hansa, Mondial, Eos and Festien. The cultivars grown in each trial were propagated through mother tubers. The tubers were planted in potting soil medium in pots of 19cm diameter in spring (March/April) of each trial year. A staggered planting approach was adopted to account for the differences in foliage maturity among the cultivars and synchronize the phenological timing of the plants as much as possible during the drought treatment. The late maturity types were planted before the earlier maturing cultivars. We allowed a space of one week between the planting of tubers from different maturity classes in the sequence: late (Eos and Festien) – intermediate (Hansa and Mondial) – early (Mozart and Biogold). A split-plot experimental design was used for each trial. In the 2015 trial,16 biological replicates per treatment for each cultivar were used to facilitate intermittent destructive sampling for the study of belowground tissues. The germinated seedlings were allowed to establish in the greenhouse environment for at least two weeks from emergence before the application of drought stress. Mild drought was applied by reducing the amount of water given to the plants to obtain a soil water content of 20±4% v/v of soil. This was monitored using a Grodan Water Content Meter with a maximum reading of 60% v/v at full water capacity in control plants.

Phenotyping and tissue sampling

We monitored drought effects on plant phenotypes. After two weeks (14days) of mild stress we measured several vegetative, physiological and yield traits. These measurements involved a destructive harvest of four replicates per treatment (stress and control) for each cultivar. These

destructive measurements were repeated after four (28days) and seven weeks (49days) of mild stress. The following tissue samples were also collected from the plants at each harvest: source (mature) leaves (5^{th}-6^{th} fully expanded leaf from the plant apex), sink (young) leaves (3^{rd} leaf from plant apex), roots and stolons. The plant tissues were collected in 1.5ml Eppendorf tubes or wrapped in aluminium foil, immediately snap-frozen in liquid nitrogen and stored in -80^{o}C freezers until laboratory analyses. At the end of the growing season, the remaining four replicates of each cultivar per treatment were harvested and final yield was measured for each cultivar. The details of the measured traits are briefly described below.

Morphological and growth traits included plant height, number of leaves, number of stems, shoot fresh and dry weight, leaf area and root dry weight. Prior to drought application, plant height, number of leaves and number of stems were measured. These initial measurements were subtracted from the measurements at the intermittent sampling time points in order to evaluate increase in plant height, number of leaves and number of stems: $\Delta H = H_X - H_0$, where H_X is height (or number of leaves, number of stems) after a period of stress and H_0 is height (or number of leaves, number of stems) at the beginning of stress application. Shoot Fresh Weight (SFW) was measured directly after harvest, and Shoot Dry Weight (SDW) and Root Dry Weight (RDW) after drying the tissues overnight in an oven at 105^{o}C. We also determined leaf area in mm^2 using a LI-COR 3100 area meter.

Physiological traits included stomatal conductance, chlorophyll content, relative water content and chlorophyll fluorescence. Physiological traits were scored for both source leaves and sink leaves, except for relative water content, which was only scored on sink leaves. Stomatal conductance was scored on the abaxial surface of the leaf using the hand-held Decagon Devices SC-1 Porometer. The flow rate of gases through the stomatal pores was measured in $mmol/m^2s$. Chlorophyll content was scored with the Minolta SPAD 502 Chlorophyll Meter. It measures the relative concentration of chlorophyll molecules per unit area of the leaf surface (Ling et al., 2011). This also gives an impression of the progress of senescence as plants mature (Li et al., 2014). Relative water content (RWC) was determined using the uppermost fully expanded leaf according to Anithakumari *et al.* (2011). For this, the FW (Fresh Weight) was determined immediately after excision. The leaves were then placed in de-ionized water overnight and the TW (Turgid Weight) was measured (Anithakumari, 2011). The leaves were subsequently dried overnight in an oven at 105^{o}C and the DW (Dry Weight) was determined. RWC was calculated using the formula: $RWC\ (\%) = ([FW\text{-}DW]/[TW\text{-}DW]) * 100$ (Smart & Bingham, 1974). Chlorophyll fluorescence was scored using the handheld Chlorophyll fluorometer OS-30P (Opti-Science, Inc. USA). The measured parameter F_v/F_m describes the potential quantum efficiency of the PSII (Maxwell & Johnson, 2000), where F_v is variable fluorescence and F_m is maximal fluorescence. The leaves were dark-adapted for 30 minutes prior to measurements (Anithakumari, 2011). The other physiological traits measured include senescence and flowering. These traits were monitored weekly and scored using a qualitative scoring scale of 1-7 to represent no senescence – full senescence, or no flowering - full flowering.

Yield traits were the underground traits associated with tuber formation and bulking. The number of stolons and tubers were counted manually and the weights were measured. Tuber

dry weight was also measured after drying the tubers overnight at 105ºC. Underwater weight of the tubers was measured and used as a determinant for dry matter content (Haase, 2003).

Molecular and biochemical analyses

The collected tissues samples were ground using the Qiagen Tissue Lyser II machine (for leaf and root tissues) and mortar and pestle (for stolon tissues). Total RNA was isolated from the ground samples using the Qiagen RNeasy protocol. The RNA quantity and quality were measured using the Isogen Nanodrop Spectrophotometer ND-1000 and agarose gel electrophoresis, respectively. The RNA (500ng) was used for cDNA synthesis following a DNase treatment using the iScript reaction protocol for the reverse transcription of the mRNA into cDNA with the profile: 25°C for 5min, 42°C for 30min, 85°C for 5min, 4°C for 5min and 85°C for 5min in the Bio-Rad C1000TM Thermal Cycler PCR machine. The cDNA was diluted to a concentration of 5ng/µl and used for gene expression studies. Based on pilot trials of many housekeeping genes under drought, the adenine phosphoribosyl transferase (*APRT*) gene was selected as reference gene due to its relative expression stability under drought and control conditions (Nicot et al., 2005). The expression of 36 genes associated with the starch biosynthesis pathway were studied using the Quantitative PCR (QPCR) method in the Bio-Rad CFX384TM Real Time System. The following profile was used for the gene expression studies: 95°C for 3 minutes, 39 cycles of (95°C for 15 seconds, 60°C for 1 minute, 95°C for 10 seconds, 65°C for 5 seconds) and a melting curve determination at 95°C. The CT values for each gene of interest were normalized against CT values for *APRT* to obtain gene expression (Livak & Schmittgen, 2001).

The concentrations of starch and sucrose were determined in the source and sink leaves of two cultivars, Biogold and Mondial. These two cultivars were selected based on their phenotypic contrasts in drought response. The ground tissues were dissolved in 280µl of 80% ethanol and incubated for an hour at 80ºC. The samples were subsequently centrifuged at 13,000rpm for 5 minutes in IEC Micromax Eppendorf Centrifuge 5417 and the supernatant was collected into a fresh Eppendorf tube. The extraction step was repeated by re-dissolving the residue in 100µl of 80% ethanol. The supernatants from both extraction steps were pooled and stored at -20ºC for later use in the analyses of sugars. The pellet residue from the above extraction step was dissolved in 2ml of dimethyl sulfoxide (DMSO) and 0.5ml of 8M HCl and incubated for 30 minutes at 60ºC. After cooling to room temperature, each sample was divided into aliquots of 250µl; and 5N NaOH and 600µl of 0.1M Citrate buffer (pH 4) were added and the samples were stored at -20ºC for later use in the analyses of starch. The sugar and starch contents were analysed using the Boehringer Mannheim/R-Biopharm kit for sucrose/starch analysis with 10x dilution in micro titre plates (Velterop & Vos, 2001).

Statistical analysis of data

The data generated from the phenotypic measurements, molecular and biochemical analyses were analysed in GENSTAT (17TH Edition) and R-Studio (version 3.3.2) using the ANOVA suites of the software packages. Factorial effects of drought, genotype and interaction between genotype and drought were investigated with the ANOVAs.

RESULTS

Tuber yield and shoot biomass under irrigation and drought

In the 2015 greenhouse drought trial, a number of traits were measured at 14, 28 and 49 days after initiation of the drought stress (14DOD, 28DOD and 49DOD, respectively) and at final harvest (77DOD). Figure 1 shows that at 28DOD, the effect of drought on tuber formation and yield was obvious in all genotypes, but also distinct responses of the different cultivars were observed (Supplementary Figs.SF1 and SF2), whereas the other time points were either too early (14DOD) or late (49DOD) to notice the initial distinctive responses to the drought stress. Therefore, our drought analyses were mainly focused on the data obtained at 28DOD. Tuber weight, tuber number and shoot weight were severely reduced under drought (Fig.1). No tubers were formed under stress by Festien, Mondial, Hansa and Mozart. Only Biogold and to a much lesser extent Eos still produced tubers under the drought treatment.

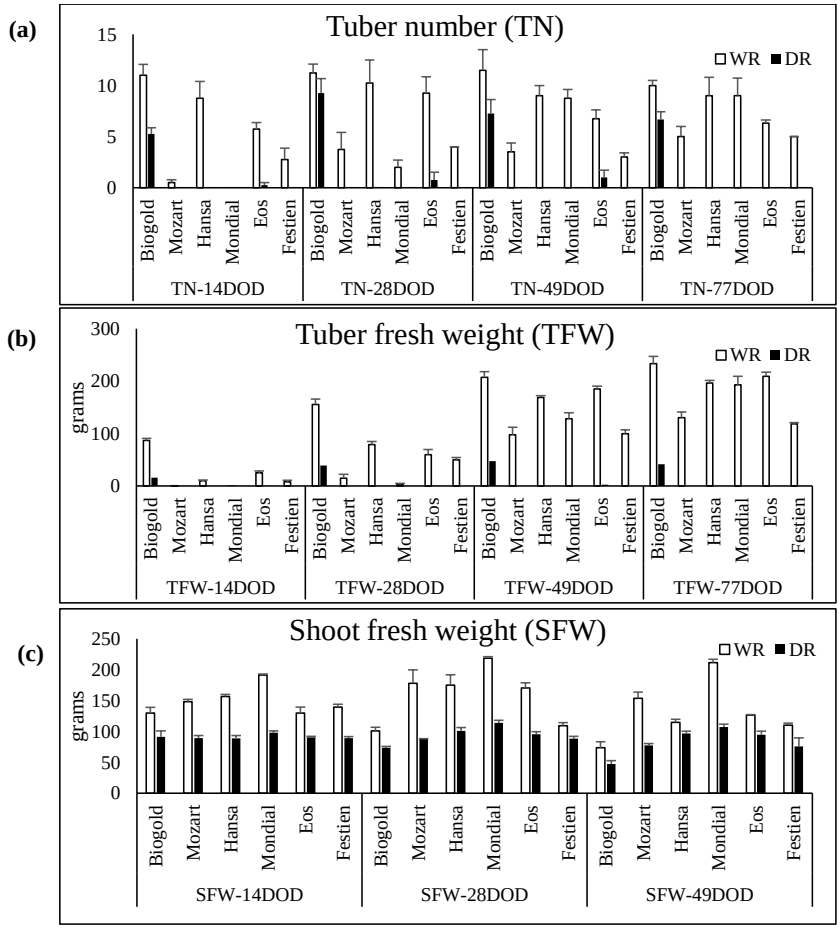


Figure 1: (**a**) Number of tubers per plant, (**b**) Tuber weight per plant, (**c**) Shoot fresh weight per plant, under irrigated (WR) and non-irrigated (DR) conditions at various time points in the growing season. Error bars represent standard errors of the mean (n=4 plant replicates). DOD is days of drought.

Trait associations under drought

The contribution of the various traits to the overall variation in the dataset was investigated using a principal component analysis (PCA) at 28DOD (Fig.2). According to the PCA biplot, 61.3% of the variation was explained by PC1 and PC2, with clear separation of irrigated vs. non-irrigated data points along the main axis (Fig.2). All the morphological/growth traits and yield traits had higher values under irrigated conditions than under drought. Chlorophyll content in source and sink leaves had significantly higher values under drought (Fig.2). Stomatal conductance was significantly reduced under drought relative to irrigated conditions (Supplementary Fig.SF1). Chlorophyll fluorescence was not significantly different between irrigated and non-irrigated treatment (Supplementary Fig.SF1). Additionally, we observed leaf rolling under drought.

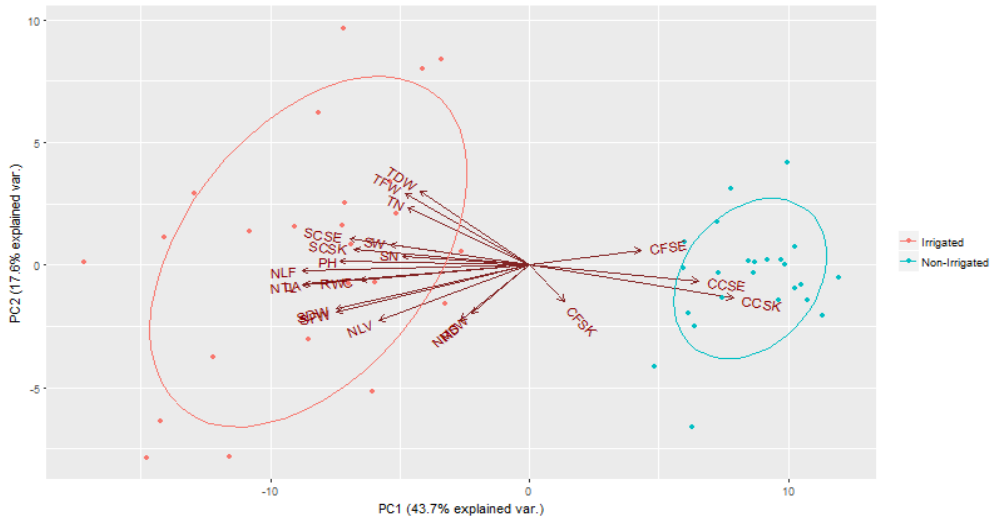


Figure 2: PCA biplot showing the clustering of irrigated (red) and non-irrigated (drought-stressed) plants (blue), and the contribution of various traits to the variation in the dataset at 28DOD. The traits are PH (Plant Height), NMS (Number of Stems), NLV (Number of leaves), NLF (Number of leaflets), NTL (Total number of leaves, that is, leaves plus leaflets), SCSE (Stomatal conductance of Source leaves), SCSK (Stomatal Conductance of Sink leaves), CCSE (Chlorophyll Content of Source leaves), CCSK (Chlorophyll Content of Sink leaves), CFSE (Chlorophyll Fluorescence of Source leaves), CFSK (Chlorophyll Fluorescence of Sink leaves), LA (Leaf Area), SN (Stolon Number), SW (Stolon Weight), TN (Tuber Number), TFW (Tuber Fresh Weight), TDW (Tuber Dry Weight), SFW (Shoot Fresh Weight), SDW (Shoot Dry Weight), RDW (Root Dry Weight), RWC (Relative Water Content)

Drought effects on the carbon partitioning at the molecular level

To gain insight in the response to drought of the carbon partitioning pathways, we investigated the effects of drought on expression of the genes involved in carbon partitioning at 28DOD in roots, stolons, source and sink leaves of cultivars grown under irrigated and drought conditions.

This included genes encoding key enzymes in the starch biosynthesis pathway (Fig.3). A more detailed illustration of the pathway is given in Supplementary Figure SF4, also showing the connection between source leaves and sink tissues in terms of sugar transport and starch storage.

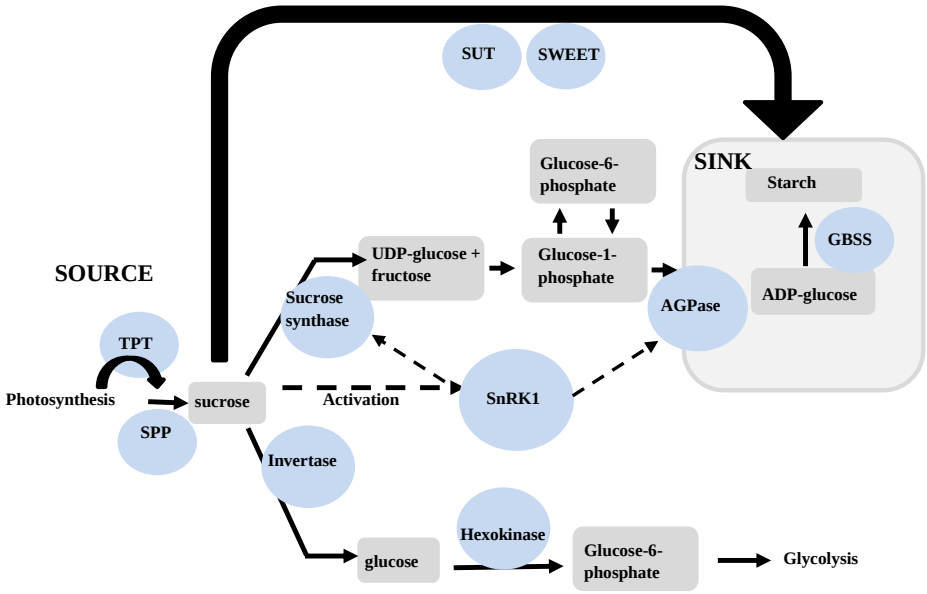
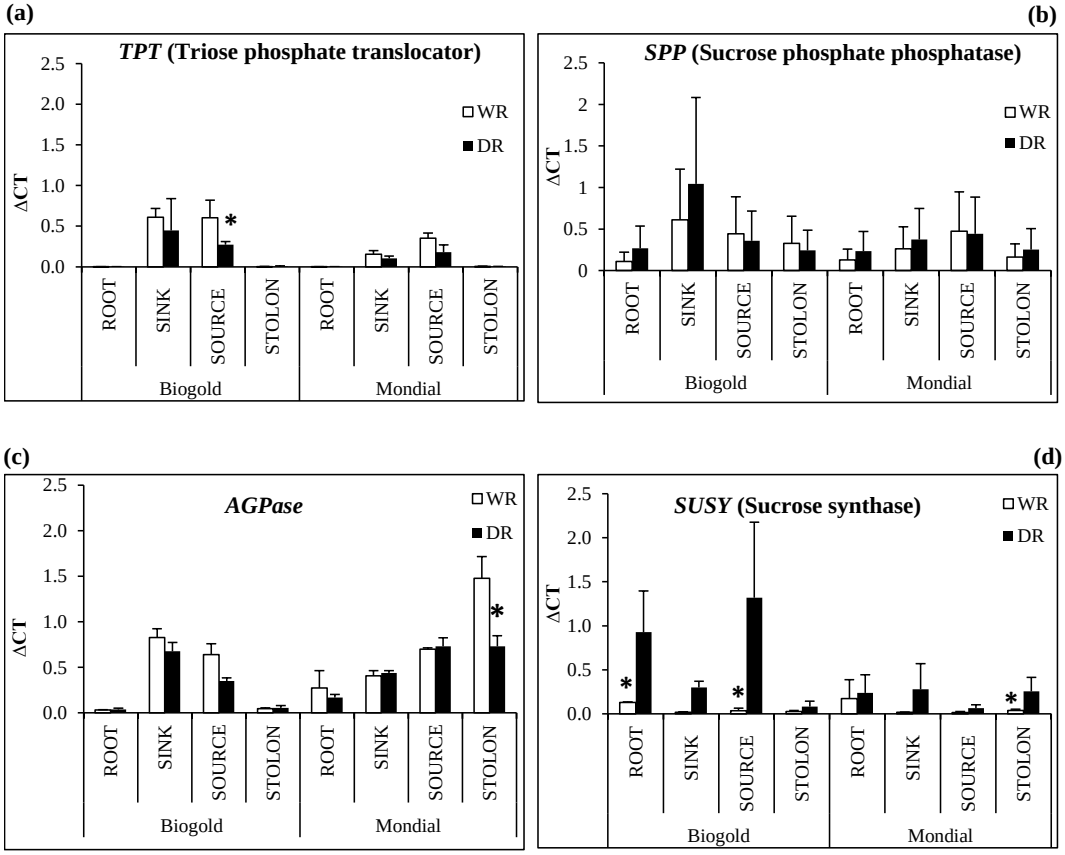


Figure 3: Simplified scheme of the starch biosynthesis pathway showing some of the genes and their complementary substrates. triose phosphate translocator (*TPT*), sucrose phosphate phosphatase (*SPP*), granule-bound starch synthase (*GBSS*), ADP-glucose pyrophosphorylase (*AGPase*), sucrose transporter (*SUT*), sucrose-will-eventually-be-exported-transporter (*SWEET*), SNF1-related protein kinase-1 (*SnRK1*). The circles represent genes while the rounded rectangles are the substrates or products of the gene (enzyme) activities.

The expression of the triose phosphate translocator (*TPT*) gene was measured to see whether drought affected the export of triose sugars from the plastids into the cytosol of source leaves. *TPT* was only expressed at appreciable levels in leaves, and tended to be decreased under drought conditions (Fig.4a). Sucrose phosphate phosphatase (*SPP*) catalyses the final step in sucrose biosynthesis (Huber & Huber, 1996). It was expressed in all tissues, under all conditions, and the effect of drought on its expression was minimal (Fig.4b). We also investigated the expression of important genes involved in sucrose metabolism in the cytosol: neutral invertases and sucrose synthases (*SUSY*). Both classes of genes were upregulated under drought. Interestingly, *SUSY* (Fig.4d) was ten-fold more upregulated under drought than the neutral invertases (Fig.4i). The gene expression of apoplastic invertase (*STIN8*), which regulates sucrose metabolism in the apoplast (Sturm, 1999), was not significantly changed under drought (Fig.4h). *SWEET10* is a plasma membrane sucrose transporter that transports

sucrose out of the cell, and is known to be highly expressed in all tissues of the plant (Manck-Götzenberger & Requena, 2016). We observed a significant reduction in the mRNA expression of *SWEET10* under drought in the source leaves of both cultivars (Fig.4f). The *SUT2* protein transports sucrose into the cell (Truernit, 2001). The *SUT2* transcript was upregulated in source and sink leaves in the tolerant cultivar Biogold (Fig.4g). The expression of the starch precursory gene *AGPase* (Fig.4c) and *GBSS* were remarkably reduced under drought stress in the underground tissues of the sensitive cultivar Mondial, but not in the tolerant cultivar Biogold. *GBSS* expression was even increased in Biogold stolons and roots (Fig.4e). Drought did not significantly affect the pyruvate decarboxylase gene (*PDC*) (Fig.4j), which is part of the plants' glycolytic/tricarboxylic acid cycles and may be indicative of the metabolic energy status (Perata et al., 2015).



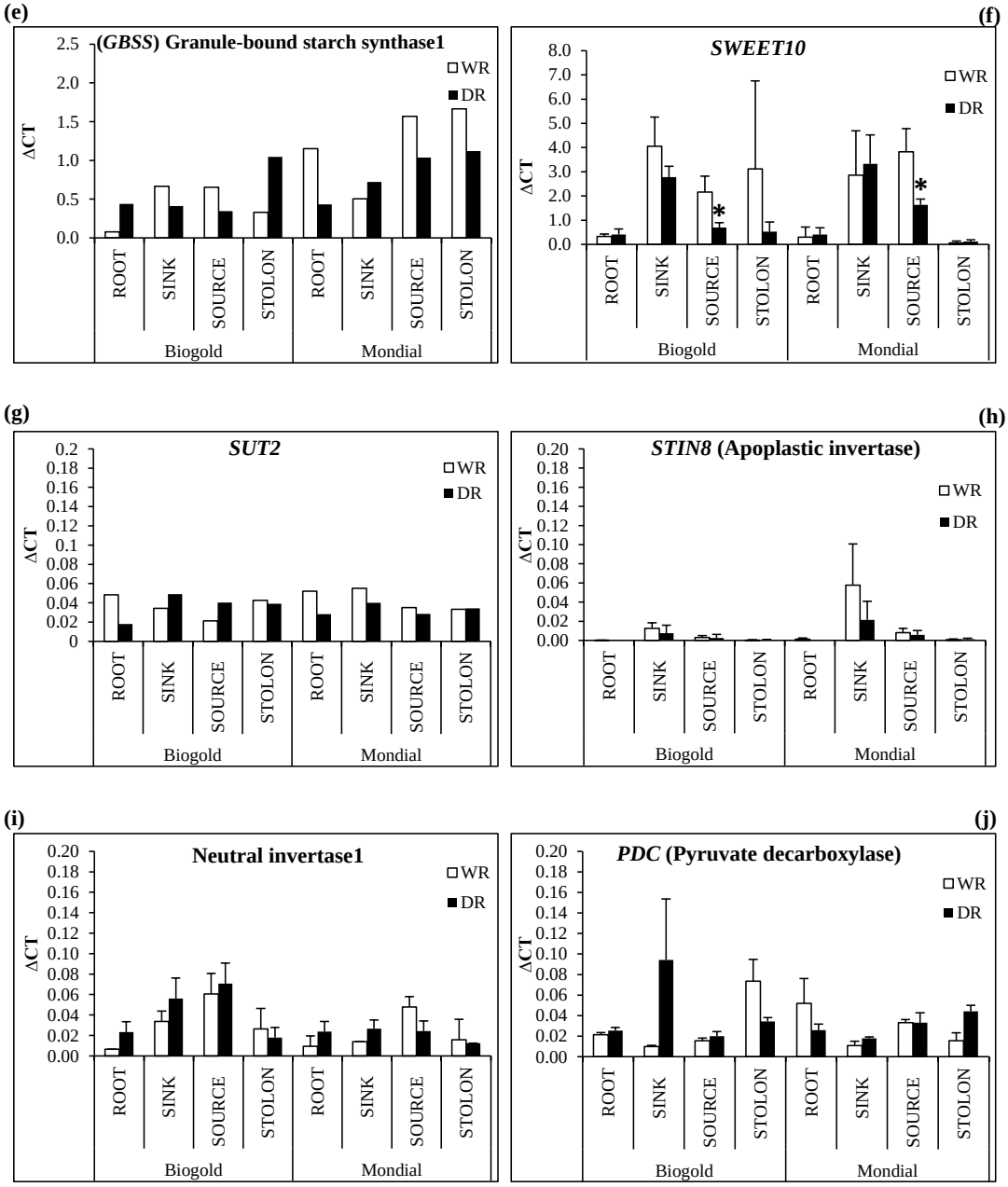


Figure 4: Gene expression levels (ΔC_t values) of genes of the starch biosynthesis pathway under control (WR) and drought (DR) conditions at 28DOD. (**a**) triose phosphate transferase (*TPT*), (**b**) sucrose phosphate phosphatase (*SPP*), (**c**) ADP-glucose pyrophosphorylase (*AGPase*), (**d**) sucrose synthase, (**e**) granule-bound starch synthase (*GBSS*), (**f**) sucrose-will-eventually-be-exported-transporter10 (*SWEET10*), (**g**) sucrose transporter2 (*SUT2*), (**h**) apoplastic invertase (*STIN8*), (**i**) neutral invertase1 and (**j**) pyruvate decarboxylase (PDC). Error bars are standard errors of the mean of 4 replicates. Pooled samples (Figs. "e", "g") have no error bars. Asterisks denote significant difference between WR and DR ($p \leq 0.05$).

Biochemical analyses

The gene expression results suggested that sucrose synthesis and metabolism of the source leaves were not significantly reduced under drought stress. We investigated this further with biochemical analysis of the sucrose content in leaf tissues of the same plants at 28DOD. Interestingly, we observed an increase in the sucrose content of source leaves in both cultivars (Fig.5a), but sucrose content did not show any increase in the sink leaves under drought. Furthermore, we investigated the content of starch in the leaves of these cultivars. Mondial synthesized and stored starch in its leaves, with higher amounts in the source leaf than in the sink leaf both under irrigated and drought conditions (Fig.5b). However, in Biogold leaves, starch was low and even not detected under drought stress.

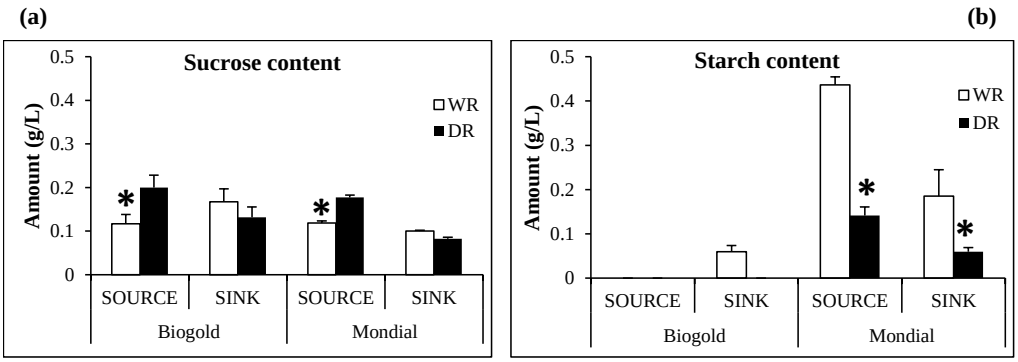


Figure 5: (**a**) Sucrose content in the source and sink leaves of Biogold and Mondial under irrigated (WR) and non-irrigated (DR) conditions, (**b**) Starch content in the source and sink leaves of Biogold and Mondial under irrigated (WR) and non-irrigated (DR) conditions, at 28DOD. Error bars represent standard errors of the mean. Asterisks denote significant difference between WR and DR.

Carbon partitioning and plant growth regulation

It has been demonstrated in previous studies that carbon (sugar) availability is highly associated with growth regulating signal molecules like trehalose-6-phosphate (T6P) and SNF1-related kinase1 (*SnRK1*) (Lastdrager et al., 2014; Tsai & Gazzarrini, 2014). T6P signals other growth regulatory molecules in the presence of sugars, triggering plant growth while repressing *SnRK1*. On the other hand, *SnRK1* is known to be activated in the presence of sucrose and to induce the expression of *SUSY* and *AGPase* in favour of starch synthesis (Fig.3). Therefore, we investigated the role of drought in this interaction between T6P and *SnRK1*, and how this would affect carbon partitioning and plant growth. Drought reduced the expression of T6P Synthase (*TPS*) in some tissues (Fig.6a), but this was not accompanied by upregulation of *SnRK1* in those tissues (Fig.6b).

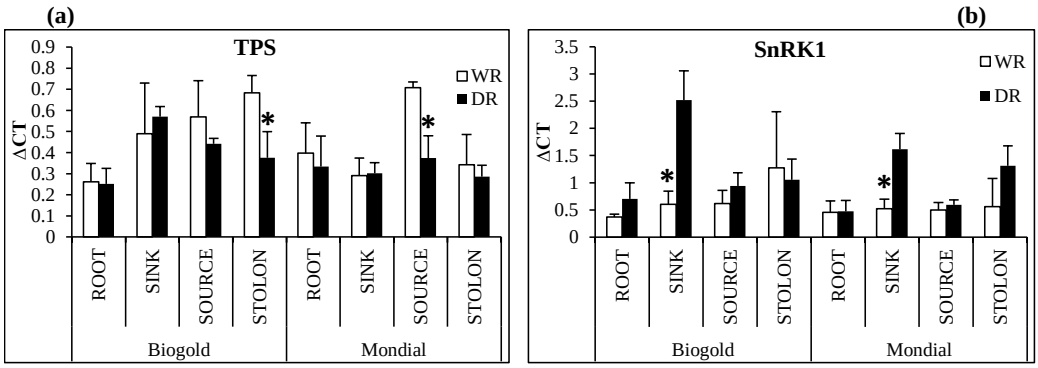


Figure 6: Gene expression of (**a**) trehalose-6-phosphate synthase (*TPS*) and (**b**) SNF1-related kinase1 (*SnRK1*) under irrigated (WR) and non-irrigated (DR) conditions at 28DOD. Error bars represent standard errors of the mean. Asterisks denote significant difference between WR and DR.

DISCUSSION AND CONCLUSIONS

Drought stress leads to huge losses in potato yield globally, especially in arid and semi-arid regions of the world. A significant proportion of the impact of drought on potato yield results from its impact on carbon partitioning (Bassam et al., 1990; Luitel et al., 2015). In view of the unfavourable predictions of climate change and more severe drought scenarios, an understanding of the mechanism of drought tolerance is important and equally urgent. The drought sensitivity of potato tuber production may be a direct effect on initial tuber formation, but drought is thought to highly impact tuber bulking (Lahlou et al., 2003). In this paper, we investigated the effect of drought on carbon partitioning from the leaves to the tubers in commercial cultivars. Our findings indicate that drought impacts different aspects of the carbon partitioning pathway in a genotype-dependent way.

Role of physiological drought responses on carbon partitioning

We studied the physiological changes in potato cultivars under drought by evaluating their leaf stomatal conductance, chlorophyll fluorescence and chlorophyll content. Drought conditions in our trials reduced the stomatal conductance of the potato leaves (Supplementary Fig.SF1). Stomatal closure is a mechanism that plants use to reduce water loss through transpiration (Haworth et al., 2016). The molecular basis of stomatal closure has been elaborated, and abscisic acid (ABA) as well as elevated CO_2 levels have been shown to play significant roles (Le et al., 2011). ABA signalling is induced by osmotic stress, and is also known to induce the expression of dehydrins (osmoprotectants), which function as chaperones in plant drought responses (Hanin et al., 2011). In our study, we observed that the expression of a dehydrin gene, *TAS14*, was upregulated under drought up to several hundred-fold (Supplementary Fig.SF3). This suggests that the potato cultivars in our dataset were severely stressed, and responded to the drought stress in an ABA-dependent manner, leading amongst others to closure of their

stomata. However, this water-conservatory mechanism affects other physiological aspects of plants and impacts on carbon partitioning as well, through reduced carbon assimilation (Haworth et al., 2016). The leaf-rolling phenotype of the plants in our study may be a response to reduced carbon assimilation. According to Pinto-Marijuan and Munne-Bosch (2014), a reduction in carbon assimilation requires an adapted reduction in intercepted photons of light energy in order to prevent oxidative stress arising from the accumulation of oxygen radicals (Pinto-Marijuan & Munne-Bosch, 2014). The damaging effect of excess light on the photosystems as inferred from chlorophyll fluorescence has been previously used as a measure of drought resilience in wheat plants exposed to rapid desiccation (Havaux & Lannoye, 1985). In our study, however, the chlorophyll fluorescence of drought-stressed plants did not significantly differ from those of the irrigated plants (Supplementary Fig.SF1). Jefferies (1994) demonstrated in field grown potato (*cv.* Maris Piper) that drought had no significant effect on PSII function because excess light energy was dissipated by photorespiration. The striking difference highlighted by Jefferies between his field drought and other controlled drought experiments may be linked to the rate of drought stress development, which is generally quite gradual in the field, and the severity of the stress (Jefferies, 1994). In our study, we attempted to mimic a field rainfall scenario by giving water to the stressed plants with a two-day interval between successive irrigations instead of a rapid dry out. Thus, the plants in our study showed no drought effect on PSII, similar to the field drought trials of Jefferies (1994).

In addition to leaf-rolling, the leaves showed a severe reduction in leaf expansion in response to the drought stress. This suggests that carbon partitioning towards leaf growth may have been affected by the drought. Due to this limitation in leaf expansion the leaves remained small, but also dark green in colour throughout the growing season. The dark green-coloured drought-stressed leaves also had higher chlorophyll density (Supplementary Fig.SF1). It is insightful to know whether this leaf area reduction was due to a preferential partitioning of carbon assimilates to other tissues of the plant or to some other reasons. This insight would require a combination of data from the phenotypic observations, but also the gene expression and metabolite analytical assays. In a review on understanding source-to-sink carbon partitioning in tomato (Osorio et al., 2014), the authors emphasized the need to combine molecular, physiological, but also the ecological information in order to understand this complex concept. In our study therefore, we explored the contributions of various aspects to gain additional understanding of potato carbon partitioning under drought stress.

Molecular keys to carbon partitioning during drought

In this study, we have used two potato cultivars with contrasting drought responses, Biogold (tolerance) and Mondial (sensitivity), to investigate carbon partitioning at the molecular level by monitoring the relative expression of the genes in the starch biosynthesis pathway. A major physiological difference between the two cultivars is that Biogold formed tubers under the drought stress condition while Mondial did not form tubers. Thus, the molecular changes we observed in these cultivars may be solely due to drought stress, or additionally due to presence versus absence of tuber sinks.

During photosynthesis, triose sugars are produced in the chloroplast and triose phosphate translocator (*TPT*), a transmembrane transport protein, exports the phosphorylated triose sugars

from the chloroplast into the cytosol (Heineke et al., 1994). In our study, the expression of *TPT* was down-regulated under drought (Fig.4a), suggesting that drought might reduce triose sugar export from the chloroplast into the cytosol. Alternatively, triose sugars from the chloroplast can also possibly be diverted from the cytosol and rather channelled towards starch biosynthesis in the plastid (Supplementary Fig.SF4). It is known that the chloroplast can store starch granules in its stroma (Laetsch, 1968). We detected starch in the leaves of Mondial under both irrigated and drought conditions, suggesting that the photosynthesized triose sugars in Mondial had to be shared between starch synthesis in the plastids and export into the cytosol for sucrose synthesis. In the case of Biogold, starch was not detected in the leaves suggesting that the *TPT* down-regulation may be linked to reduction in photosynthetic rate under drought. A previous study demonstrated that *TPT* antisense repression resulted in a 40-60% reduction in photosynthesis of transgenic potato plants (Riesmeier et al., 1993a). Despite the supposed reduction in triose sugar export into the cytosol, gene expression of *SPP* suggests that sucrose synthesis in the cytosol was not reduced under drought (Fig.4b). Rather, sucrose levels in the source leaves were higher under drought than under irrigated conditions (Fig.5a). Interestingly, the gene expression of neutral invertase and sucrose synthase (*SUSY*) suggest that sucrose breakdown into hexoses was increased in the cytosol of the source leaf under drought relative to under irrigated conditions in the tolerant cultivar, Biogold (Figs.4d and i). The abundance of sucrose in the source leaves (Fig.5) despite the relatively high metabolic rate of sucrose in this tissue (Fig.4), suggests that the excess non-metabolized sucrose was not being exported from the source leaves. Possibly, some of it was stored in the vacuoles to serve as osmolytes (Martinoia et al., 2012). In the sensitive cultivar, Mondial, sucrose breakdown in the cytosol was hardly affected by the drought treatment (Fig.4i). The reduced expression of *SWEET10* (Fig.4f) may indicate that sucrose export from the source leaf was reduced in both cultivars. Low sucrose export from the source leaves under drought was also evidenced by the absence of up-regulation of apoplastic invertase *STIN8* as compared to the cytosolic sucrose-metabolizing genes (Fig.4h). One of the points of distinction between the tolerant and sensitive cultivar was that the tolerant cultivar, Biogold, had an up-regulation in *SUT2* expression under drought (Fig.4g), indicating that Biogold may have favoured active export of sucrose from its source leaves during drought more than the sensitive cultivar, Mondial.

Interestingly, the sink leaves of both cultivars had lower sucrose content under drought (Fig.5a). There may be two reasons for the low sucrose content of sink leaves under drought. Firstly, the source leaves may have reduced sucrose export to the phloem (source-limitation), and secondly, another sink tissue may be prioritized instead of the sink leaves (sink strength drive). The expression patterns of *AGPase* and *GBSS* in the underground root and stolon tissues suggest that under drought, starch biosynthesis in these underground tissues was increased for Biogold but not for Mondial (Figs.4c and e). Biogold may therefore have partitioned its photo-assimilates preferentially to the underground tissues and only minimally to the sink leaves. However, Mondial did not show evidence of a preferential partitioning to underground tissues over the sink leaves. Moreover, Mondial had no tubers under drought (Fig.1). Rather, its formation of starch in leaves may indicate that a high proportion of its source leaf sugars were used for starch biosynthesis. That is, it probably did not export much sucrose from the source leaves. The possible role of the absence of tubers in its inability to export sugars from the source leaves can, however, not be inferred from our results.

Interference with the molecular regulation of carbon partitioning and plant growth

In a review, Smeekens (2017) showed that plant growth and drought tolerance were boosted in various plant systems by the exogenous application of T6P (Smeekens, 2017). T6P is a growth regulatory molecule that is highly responsive to carbon availability, and it signals other downstream growth regulators for the induction of plant growth (Lastdrager et al., 2014; Tsai & Gazzarrini, 2014). That review showed the importance of this non-membrane-permeable molecule in the drought response, but the mechanism of its activity is poorly understood (Smeekens, 2017). One of the known facts about T6P is that it represses the expression of *SnRK1* under normal conditions (Lastdrager et al., 2014). We used the gene expression of Trehalose-6-phosphate Synthase (*TPS*) and SNF1-related kinase1 (*SnRK1*) to investigate the role of T6P in the drought response of potato. The drought stress in our study attenuated the expression of *TPS*, reducing its expression in some tissues like the source leaves and stolon tissues (Fig.6a), which would presumably result in decreased levels of T6P under drought. The reduction in T6P production under drought may have contributed to the reduction in plant height observed in our study. However, tissue-specific downregulation of *TPS* (in source leaves and stolon tissue) was not typically accompanied by increased expression of *SnRk1*. Instead, *SnRK1* was significantly upregulated in sink leaves (Figs.6a and b). *SnRK1* is known to play a significant role as a key switch in plant sugar signalling, sugar metabolism and hormonal regulation (Xue-Fei et al., 2012). It is known as an inhibitor of plant growth in instances of nutrient stress because it represses ribosomal proteins thereby inhibiting translation (Lastdrager et al., 2014). There are, however, conflicting reports on the effects of sugars on *SnRK1* (in)activation. Some research findings report that sucrose and other sugars inactivate *SnRK1* (Baena-Gonzalez et al., 2007; Toroser et al., 2000), while the converse has also been reported (Jossier et al., 2009). In our study, we did not find indications that high levels of sucrose inactivate *SnRK1* because we measured both high sucrose content and *SnRK1* upregulation in various tissues of the potato plants under drought. Our findings do not suggest that *TPS* downregulates *SnRK1* during drought. The inability of T6P to downregulate *SnRK1* may not be due to lack of sucrose, because sucrose content was high under drought. Possibly, during drought a much higher production of T6P is required for drought tolerance, which may involve downregulating *SnRK1*. The exogenous application mentioned in the review of Smeekens (2017) may likely have provided the required T6P amounts for drought tolerance. Whether T6P downregulates *SnRK1* endogenously and whether this requires high levels of T6P still remains to be established.

SnRK1 activation is known to trigger the expression of sucrose synthase (*SUSY*) (McKibbin et al., 2006), which is vital in sucrose metabolism. Interestingly, *SUSY* expression in our study was strongly upregulated under drought, much more than the expression of neutral invertases (Figs.4d and i). Our findings do not suggest an association between *SnRK1* and *SUSY* expression in the respective tissues, but also do not provide sufficient data to argue against the activation of *SUSY* expression by *SnRK1*. Higher activity of *SUSY* can have implications for growth under stress conditions. For instance, it has been shown that *SUSY* overexpression in *Gossipium hirsutum* resulted in elevated concentrations of cellulose leading to cell wall thickening (Coleman et al., 2009). Similarly, a report of drought effects on cell wall properties,

with a reduced cell wall content and cellulose deconstruction into sugars (osmolytes) at the expense of turgor under drought in Miscanthus has been published (van der Weijde et al., 2017). The strong *SUSY* up-regulation in our study may therefore result in a strengthened cell wall to avoid a collapse of the cell structure due to loss of turgor.

Moreover, it has been reported that *SUSY*-mediated sucrose metabolism is more energy-efficient than invertase-mediated sucrose metabolism (Ferreira & Sonnewald, 2012). In instances of stress like the drought condition in our study, energy-saving mechanisms are highly advantageous to the plants. In fact, we investigated the effect of the drought stress on the tricarboxylic acid (TCA) cycle using the gene expression of pyruvate decarboxylase (*PDC*). Interestingly, the expression of *PDC* suggests that the plants were able to maintain their TCA cycle under drought (Fig.4j), which is in line with the proposed energy-efficiency of *SUSY*-mediated sucrose metabolism.

Recommended breeding targets for carbon partitioning during drought

In this study we have evaluated the effect of drought on carbon partitioning and plant growth in potato cultivars. Carbon partitioning strongly affects yield of potato, especially under stress conditions like drought (Fig.1). Therefore, optimizing carbon partitioning under drought through breeding can contribute considerably to the development of drought-tolerant potato cultivars. The results presented in this paper have given new insights in the adaptation of carbon partitioning to water-limiting conditions, and possibly point to targets for breeding for drought tolerance. Firstly, the rate of triose sugar export from the chloroplast into the cytosol of source leaves for sucrose synthesis can be optimized. As can be seen in Supplementary Figure SF4, a limited transport of triose sugars from the plastid into the cytosol leads to the synthesis and storage of starch granules in the plastids of the leaves, rather than targeting those sugars to the tuber or new leaf development. Secondly, the export of sucrose from source leaves can be targeted for breeding. This involves members of the SUT and SWEET gene families. Our findings show these transporters are critically affected under drought and this drought effect can impede sucrose transport from source leaves to sink tissues. It is essential that sucrose is transported from the source leaves to avoid feedback inhibition of photosynthesis (Paul & Foyer, 2001). Feedback inhibition in itself can be a protective mechanism plants use to avoid photo-respiratory damage (Tiwari et al., 2016). Therefore, an optimal breeding strategy for drought tolerance should consider maintaining a homeostatic balance between photosynthetic and transpiration rate, without compromising sugar transport to sink tissues, especially the tuber.

ACKNOWLEDGMENTS

We thank the following for the contribution of their experience and time in the greenhouse and laboratory experiments of this study: Andre Maassen, Bertus vander Laan, Peter Dinh Quy and Robert Okayo.

REFERENCES

Anithakumari, A. M. (2011). *Genetic dissection of drought tolerance in potato.* (Ph.D.), Wageningen University, Wageningen. Retrieved from http://edepot.wur.nl/165211

Araya, T., Noguchi, K., & Terashima, I. (2006). Effects of Carbohydrate Accumulation on Photosynthesis Differ between Sink and Source Leaves of Phaseolus vulgaris L. *Plant and Cell Physiology, 47*(5), 644-652. doi: 10.1093/pcp/pcj033

Atwell, B. J., Kriedemann, P. E., & Turnbull, C. G. N. (Eds.). (1999). *Plants in Action* (1 ed.). New Zealand: Macmillan Education Australia Pty Ltd, Melbourne, Australia.

Azcon-Bieto, J. (1983). Inhibition of photosynthesis by carbohydrates in wheat leaves. *Plant Physiol., 73*, 681-686. doi: 0032-0889/83/73/0681/06/$00.50/0

Bacon, M. (2009). *Water use efficiency in plant biology*: John Wiley & Sons.

Baena-Gonzalez, E., Rolland, F., Thevelein, J. M., & Sheen, J. (2007). A central integrator of transcription networks in plant stress and energy signalling. *Nature, 448*(7156), 938-942. doi: 10.1038/nature06069

Bassam, N., Dambroth, M., Loughman, B. C., Spitters, C. J. T., & Schapendonk, A. H. C. M. (1990). Evaluation of breeding strategies for drought tolerance in potato by means of crop growth simulation *Genetic Aspects of Plant Mineral Nutrition* (Vol. 42, pp. 151-161): Springer Netherlands.

Baur-hoch, B., Machler, F., & Nosberger, J. (1990). Effect of Carbohydrate Demand on the Remobilization of Starch in Stolons and Roots of White Clover (Trifolium repens L.) after Defoliation. *Journal of Experimental Botany, 41*(5), 573-578. doi: 10.1093/jxb/41.5.573

Blechschmidt-Schneider, S., Ferrar, P., & Osmond, C. B. (1989). Control of photosynthesis by the carbohydrate level in leaves of the C4 plant Amaranthus edulis L. *Planta, 177*(4), 515-525. doi: 10.1007/bf00392620

Braun, D. M., & Slewinski, T. L. (2009). Genetic Control of Carbon Partitioning in Grasses: Roles of Sucrose Transporters and Tie-dyed Loci in Phloem Loading. *Plant Physiology, 149*(1), 71-81. doi: 10.1104/pp.108.129049

Brunner, I., Herzog, C., Dawes, M. A., Arend, M., & Sperisen, C. (2015). How tree roots respond to drought. *Frontiers in Plant Science, 6*, 547. doi: 10.3389/fpls.2015.00547

Chen, L.-Q., Qu, X.-Q., Hou, B.-H., Sosso, D., Osorio, S., Fernie, A. R., & Frommer, W. B. (2012). Sucrose Efflux Mediated by SWEET Proteins as a Key Step for Phloem Transport. *Science, 335*(6065), 207-211. doi: 10.1126/science.1213351

CIP. (2013). Agricultural research for development: Potato facts and figures. Retrieved 2nd May, 2013, from http://cipotato.org/potato/facts

Coleman, H. D., Yan, J., & Mansfield, S. D. (2009). Sucrose synthase affects carbon partitioning to increase cellulose production and altered cell wall ultrastructure. *Proceedings of the National Academy of Sciences, 106*(31), 13118-13123. doi: 10.1073/pnas.0900188106

Comas, L. H., Becker, S. R., Cruz, V. M. V., Byrne, P. F., & Dierig, D. A. (2013). Root traits contributing to plant productivity under drought. *Frontiers in Plant Science, 4*, 442. doi: 10.3389/fpls.2013.00442

DaCosta, M., & Huang, B. (2006). Changes in Carbon Partitioning and Accumulation Patterns during Drought and Recovery for Colonial Bentgrass, Creeping Bentgrass, and Velvet Bentgrass. *J. Amer. Soc. Hort. Sci., 131*(4), 484 - 490.

De Schepper, V., De Swaef, T., Bauweraerts, I., & Steppe, K. (2013). Phloem transport: a review of mechanisms and controls. *Journal of Experimental Botany*. doi: 10.1093/jxb/ert302

Deblonde, P. M. K., & Ledent, J. F. (2001). Effects of moderate drought conditions on green leaf number, stem height, leaf length and tuber yield of potato cultivars. *European Journal of Agronomy, 14*(1), 31-41. doi: http://dx.doi.org/10.1016/S1161-0301(00)00081-2

Dickinson, C. D., Altabella, T., & Chrispeels, M. J. (1991). Slow-Growth Phenotype of Transgenic Tomato Expressing Apoplastic Invertase. *Plant Physiology, 95*(2), 420-425.

Farhad, M. S., Babak, A. M., Reza, Z. M., Hassan, R. S. M., & Afshin, T. (2011). Response of proline, soluble sugars, photosynthetic pigments and antioxidant enzymes in potato (Solanum tuberosum L.) to different irrigation regimes in greenhouse condition. *Australian Journal of Crop Science, 5*(1), 55-60.

Ferreira, S. J., & Sonnewald, U. (2012). The Mode of Sucrose Degradation in Potato Tubers Determines the Fate of Assimilate Utilization. *Frontiers in Plant Science, 3*, 23. doi: 10.3389/fpls.2012.00023

Gargallo-Garriga, A., Sardans, J., Pérez-Trujillo, M., Rivas-Ubach, A., Oravec, M., Vecerova, K., Urban, O., Jentsch, A., Kreyling, J., Beierkuhnlein, C., Parella, T., & Peñuelas, J. (2014). Opposite metabolic responses of shoots and roots to drought. *4*, 6829.

Geiger, D. R., & Servaites, J. C. (1994). Diurnal regulation of photosynthetic carbon metabolism in C3 plants. *Annual review of plant biology, 45*(1), 235-256.

Giaquinta, R. (1977). Phloem Loading of Sucrose: pH Dependence and Selectivity. *Plant Physiology, 59*(4), 750-755.

Gifford, R. M., & Evans, L. T. (1981). Photosynthesis, Carbon Partitioning, and Yield. *Annual Review of Plant Physiology, 32*(1), 485-509. doi: doi:10.1146/annurev.pp.32.060181.002413

Haase, N. (2003). Estimation of dry matter and starch concentration in potatoes by determination of under-water weight and near infrared spectroscopy. *Potato Research, 46*(3-4), 117-127. doi: 10.1007/bf02736081

Hanin, M., Brini, F., Ebel, C., Toda, Y., Takeda, S., & Masmoudi, K. (2011). Plant dehydrins and stress tolerance: Versatile proteins for complex mechanisms. *Plant Signaling & Behavior, 6*(10), 1503-1509. doi: 10.4161/psb.6.10.17088

Hannah, M. A., Zuther, E., Buchel, K., & Heyer, A. G. (2006). Transport and metabolism of raffinose family oligosaccharides in transgenic potato. *Journal of Experimental Botany, 57*(14), 3801-3811. doi: 10.1093/jxb/erl152

Havaux, M., & Lannoye, R. (1985). Drought resistance of hard wheat cultivars measured by a rapid chlorophyll fluorescence test. *The Journal of Agricultural Science, 104*(3), 501-504. doi: doi:10.1017/S0021859600044257

Haworth, M., Killi, D., Materassi, A., Raschi, A., & Centritto, M. (2016). Impaired Stomatal Control Is Associated with Reduced Photosynthetic Physiology in Crop Species Grown at Elevated [CO(2)]. *Frontiers in Plant Science, 7*, 1568. doi: 10.3389/fpls.2016.01568

Heineke, D., Kruse, A., Flügge, U.-I., Frommer, W. B., Riesmeier, J. W., Willmitzer, L., & Heldt, H. W. (1994). Effect of antisense repression of the chloroplast triose-phosphate translocator on photosynthetic metabolism in transgenic potato plants. *Planta, 193*(2), 174-180. doi: 10.1007/bf00192527

Huber, S. C., & Huber, J. L. (1996). Role and regulation of sucrose phosphate synthase in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol, 47*, 431-444.

Jefferies, R. A. (1994). Drought and chlorophyll fluorescence in field-grown potato (Solanum tuberosum). *Physiologia Plantarum, 90*(1), 93-97. doi: 10.1111/j.1399-3054.1994.tb02197.x

Jossier, M., Bouly, J. P., Meimoun, P., Arjmand, A., Lessard, P., Hawley, S., Grahame Hardie, D., & Thomas, M. (2009). SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signalling in Arabidopsis thaliana. *Plant J, 59*(2), 316-328. doi: 10.1111/j.1365-313X.2009.03871.x

Koch, K. (2004). Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Plant Biology, 7*, 235–246.

Laetsch, W. M. (1968). Chloroplast Specialization in Dicotyledons Posessing the C4-DIcarboxylic Acid Pathway of Photosynthetic CO2 Fixation. *American Journal of Botany, 55*(8), 875-883.

Lahlou, O., Ouattar, S., & Ledent, J. (2003). The effect of drought and cultivar on growth parameters, yield and yield components of potato. *Agronomie, 23* 257-268. doi: 10.1051/agro:2002089

Lalonde, S., Boles, E., Hellmann, H., Barker, L., Patrick, J. W., Frommer, W. B., & Ward, J. M. (1999). The Dual Function of Sugar Carriers: Transport and Sugar Sensing. *The Plant Cell, 11*(4), 707-726. doi: 10.1105/tpc.11.4.707

Lastdrager, J., Hanson, J., & Smeekens, S. (2014). Sugar signals and the control of plant growth and development. *J Exp Bot, 65*(3), 799-807. doi: 10.1093/jxb/ert474

Le, A., S, T., Je, O., Kk, T., Shanker, A., & Venkateswarlu, B. (2011). Stomatal Responses to Drought Stress and Air Humidity *Abiotic Stress in Plants - Mechanisms and Adaptations* (pp. Ch. 12). Rijeka: InTech.

Leisner, S. M., & Turgeon, R. (1993). Movement of virus and phototoassimlate in th ephloem: A comparative analysis. *BioEsays, 15*(11), 741-748. doi: DOI: 10.1002/bies.950151107

Lemoine, R. (2000). Sucrose transporters in plants: update on function and structure. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1465*(1–2), 246-262. doi: http://dx.doi.org/10.1016/S0005-2736(00)00142-5

Lemoine, R., Camera, S. L., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N., Bonnemain, J.-L., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrilla, J., & Durand, M. (2013). Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in Plant Science, 4*, 272. doi: 10.3389/fpls.2013.00272

Li, P., Dong, H., Liu, A., Liu, J., Sun, M., Wang, G., Zhang, S., Li, Y., & Mao, S. (2014). Diagnosis of Premature Senescence of Cotton Using SPAD Value. *Agricultural Sciences, 5*, 992-999. doi: doi: 10.4236/as.2014.511107.

Ling, Q., Huang, W., & Jarvis, P. (2011). Use of a SPAD-502 meter to measure leaf chlorophyll concentration in Arabidopsis thaliana. *Photosynth Res, 107*(2), 209-214. doi: 10.1007/s11120-010-9606-0

Liu, D. D., Chao, W. M., & Turgeon, R. (2012). Transport of sucrose, not hexose, in the phloem. *Journal of Experimental Botany*. doi: 10.1093/jxb/ers127

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods, 25*(4), 402-408. doi: 10.1006/meth.2001.1262

Luitel, B. P., Khatri, B. B., Choudhary, D., Paudel, B. P., Jung-Sook, S., Hur, O., Baek , H. J., Cheol, K. H., & Yul, R. K. (2015). Growth and yield characters of potato genotypes grown in drought and irrigated conditions of Nepal. *Int J Appl Sci Biotechnol,, Vol 3* ((3)), 513-519. doi: DOI: 10.3126/ijasbt.v3i3.13347

Maloney, V. J., Park, J.-Y., Unda, F., & Mansfield, S. D. (2015). Sucrose phosphate synthase and sucrose phosphate phosphatase interact in planta and promote plant growth and biomass accumulation. *Journal of Experimental Botany*. doi: 10.1093/jxb/erv101

Manck-Götzenberger, J., & Requena, N. (2016). Arbuscular mycorrhiza Symbiosis Induces a Major Transcriptional Reprogramming of the Potato SWEET Sugar Transporter Family. *Frontiers in Plant Science, 7*(487). doi: 10.3389/fpls.2016.00487

Marcelis, L. (1996). Sink strength as a determinant of dry matter partitioning in the whole plant. *Journal of Experimental Botany, 47*(suppl 1), 1281.

Martin, T., Frommer, W. B., Salanoubat, M. a., & Willmitzer, L. (1993). Expression of an Arabidopsis sucrose synthase gene indicates a role in metabolization of sucrose both during phloem loading and in sink organs. *The Plant Journal, 4*, 367-377. doi: 10.1046/j.1365-313X.1993.04020367.x

Martinoia, E., Meyer, S., Angeli, A. D., & Nagy, R. (2012). Vacuolar Transporters in Their Physiological Context. *Annual review of plant biology, 63*(1), 183-213. doi: 10.1146/annurev-arplant-042811-105608

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence-a practical guide. *Journal of Experimental Botany, 51*(345), 659-668.

McDowell, N. G. (2011). Mechanisms Linking Drought, Hydraulics, Carbon Metabolism, and Vegetation Mortality. *Plant Physiology, 155*(3), 1051-1059. doi: 10.1104/pp.110.170704

McKibbin, R. S., Muttucumaru, N., Paul, M. J., Powers, S. J., Burrell, M. M., Coates, S., Purcell, P. C., Tiessen, A., Geigenberger, P., & Halford, N. G. (2006). Production of high-starch, low-glucose potatoes through over-expression of the metabolic regulator SnRK1. *Plant Biotechnol J, 4*(4), 409-418. doi: 10.1111/j.1467-7652.2006.00190.x

Minchin, P. E., & Thorpe, M. R. (1996). What determines carbon partitioning between competing sinks? *J Exp Bot, 47 Spec No*, 1293-1296. doi: 10.1093/jxb/47.Special_Issue.1293

Moorby, J. (1994). Carbon Partitioning: Within and Between Organisms. (Environmental Plant Biology Series.) Edited by PollockC. J., FarrarJ. F. and GordonA. J.. Oxford: Bios Scientific Publishers (1992), pp. 258, £43.00, US$86.00, ISBN 1-872748-95-3. *Experimental Agriculture, 30*(1), 105. doi: 10.1017/s0014479700023905

Nazarian-Firouzabadi, F., & Visser, R. G. F. (2017). Potato starch synthases: Functions and relationships. *Biochemistry and Biophysics Reports, 10*, 7-16. doi: https://doi.org/10.1016/j.bbrep.2017.02.004

Nicolas, M. E., Lambers, H., Simpson, R. J., & Dalling, M. J. (1985). Effect of Drought on Metabolism and Partitioning of Carbon in Two Wheat Varieties Differing in Drought-tolerance. *Annals of Botany, 55*(5), 727-742.

Nicot, N., Hausman, J. F., Hoffmann, L., & Evers, D. (2005). Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. *J Exp Bot, 56*(421), 2907-2914. doi: 10.1093/jxb/eri285

Onillon, B., Durand, J. L., Gastal, F., & Tournebize, R. (1995). Drought effects on growth and carbon partitioning in a tall fescue sward grown at different rates of nitrogen fertilization. *European Journal of Agronomy, 4*(1), 91-99. doi: http://dx.doi.org/10.1016/S1161-0301(14)80020-8

Osorio, S., Ruan, Y.-L., & Fernie, A. R. (2014). An update on source-to-sink carbon partitioning in tomato. *Frontiers in Plant Science, 5*(516). doi: 10.3389/fpls.2014.00516

Paul, M. J., & Foyer, C. H. (2001). Sink regulation of photosynthesis. *Journal of Experimental Botany, 52*(360), 1383-1400.

Paul, Q., & Arthur, S. (Eds.). (1996). *Sucrose metablosim in sources and sinks* (Vol. 48): Taylor & Francis.

Perata, P., Voesenek, R., Sasidharan, R., & Pucciariello, C. (2015). *Plant responses to flooding*: Frontiers E-books.

Pinto-Marijuan, M., & Munne-Bosch, S. (2014). Photo-oxidative stress markers as a measure of abiotic stress-induced leaf senescence: advantages and limitations. *J Exp Bot, 65*(14), 3845-3857. doi: 10.1093/jxb/eru086

Rambal, S., Lempereur, M., Limousin, J. M., Martin-StPaul, N. K., Ourcival, J. M., & Rodríguez-Calcerrada, J. (2014). How drought severity constrains gross primary production(GPP) and its partitioning among carbon pools in a Quercus ilex coppice? *Biogeosciences, 11*(23), 6855-6869. doi: 10.5194/bg-11-6855-2014

Ricardo, C. P. P., & Aprees, T. (1970). Invertase activity during the development of carrot roots. *Phytochemistry, 9*, 239 - 247.

Riesmeier, J. W., Flügge, U. I., Schulz, B., Heineke, D., Heldt, H. W., Willmitzer, L., & Frommer, W. B. (1993a). Antisense repression of the chloroplast triose phosphate translocator affects carbon partitioning in transgenic potato plants. *Proc Natl Acad Sci U S A, 90*(13), 6160-6164.

Riesmeier, J. W., Hirner, B., & Frommer, W. B. (1993b). Potato sucrose transporter expression in minor veins indicates a role in phloem loading. *Plant Cell, 5*(11), 1591-1598. doi: 10.1105/tpc.5.11.1591

Roitsch, T. (1999). Source-sink regulation by sugar and stress. *Curr Opin Plant Biol, 2*(3), 198-206. doi: 10.1016/s1369-5266(99)80036-3

Roitsch, T., & González, M.-C. (2004). Function and regulation of plant invertases: sweet sensations. *Trends in plant science, 9*(12), 606-613. doi: http://dx.doi.org/10.1016/j.tplants.2004.10.009

Rolland, F., Moore, B., & Sheen, J. (2002). Sugar Sensing and Signaling in Plants. *The Plant Cell, 14*(Suppl), s185-s205. doi: 10.1105/tpc.010455

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A., Hilal, M., & Prado, F. E. (2009). Soluble sugars—Metabolism, sensing and abiotic stress: A complex network in the life of plants. *Plant Signaling & Behavior, 4*(5), 388-393.

Ross, H. A., & Davies, H. V. (1992). Sucrose Metabolism in Tubers of Potato (Solanum tuberosum L.): Effects of Sink Removal and Sucrose Flux on Sucrose-Degrading Enzymes. *Plant Physiology, 98*(1), 287-293.

Schulz, A., Kühn, C., Riesmeier, J. W., & Frommer, W. B. (1998). Ultrastructural effects in potato leaves due to antisense-inhibition of the sucrose transporter indicate an apoplasmic mode of phloem loading. *Planta, 206*(4), 533-543. doi: 10.1007/s004250050430

Sharkey, T. D. (2015). Understanding carbon partitioning and its role in determining plant growth. *Plant, Cell & Environment, 38*(10), 1963-1964. doi: 10.1111/pce.12543

Smart, R. E., & Bingham, G. E. (1974). Rapid Estimates of Relative Water Content. *Plant Physiology, 53*, 258 - 260.

Smeekens, S. (2017). Drought resistance: Spraying for yield. *Nat Plants, 3*, 17023. doi: 10.1038/nplants.2017.23

Stitt, M., & Sonnewald, U. (1995). Regulation of metabolism in transgenic plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol., 46*, 341-368.

Sturm, A. (1999). Invertases. Primary Structures, Functions, and Roles in Plant Development and Sucrose Partitioning. *Plant Physiology, 121*(1), 1-8. doi: 10.1104/pp.121.1.1

Sturm, A., & Tang, G. Q. (1999). The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Elsevier Science, 4*(10), 401-407.

Sun, J., Zhang, J., Larue, C. T., & Huber, S. C. (2011). Decrease in leaf sucrose synthesis leads to increased leaf starch turnover and decreased RuBP regeneration-limited photosynthesis but not Rubisco-limited photosynthesis in Arabidopsis null mutants of SPSA1. *Plant, Cell & Environment, 34*(4), 592-604. doi: 10.1111/j.1365-3040.2010.02265.x

Tanner, C. B. (1981). Transpiration Efficiency of Potato1. *Agronomy Journal, 73*(1), 59-64. doi: 10.2134/agronj1981.00021962007300010014x

Thorne, J. H., & Koller, H. R. (1974). Influence of Assimilate Demand on Photosynthesis, Diffusive Resistances, Translocation, and Carbohydrate Levels of Soybean Leaves. *Plant Physiology, 54*(2), 201-207. doi: 10.1104/pp.54.2.201

Tiwari, A., Mamedov, F., Grieco, M., Suorsa, M., Jajoo, A., Styring, S., Tikkanen, M., & Aro, E.-M. (2016). Photodamage of iron–sulphur clusters in photosystem I induces non-photochemical energy dissipation. *2*, 16035.

Tobias, D. J., Hirose, T., Ishimaru, K., Ishige, T., Ohkawa, Y., Kano-Murakami, Y., Matsuoka, M., & Ohsugi, R. (1999). Elevated Sucrose-phosphate Synthase Activity in Source Leaves of Potato Plants Transrformed with the Maize SPS Gene. *Plant Production Science, 2*(2), 92-99. doi: 10.1626/pps.2.92

Toroser, D., Plaut, Z., & Huber, S. C. (2000). Regulation of a plant SNF1-related protein kinase by glucose-6-phosphate. *Plant Physiol, 123*(1), 403-412.

Truernit, E. (2001). Plant physiology: The importance of sucrose transporters. *Current Biology, 11*(5), R169-R171. doi: http://dx.doi.org/10.1016/S0960-9822(01)00085-9

Tsai, A. Y. L., & Gazzarrini, S. (2014). Trehalose-6-phosphate and SnRK1 kinases in plant development and signaling: the emerging picture. *Frontiers in Plant Science, 5*, 119. doi: 10.3389/fpls.2014.00119

Turgeon, R., & Medville, R. (2004). Phloem Loading. A Reevaluation of the Relationship between Plasmodesmatal Frequencies and Loading Strategies. *Plant Physiology, 136*(3), 3795-3803. doi: 10.1104/pp.104.042036

van Bel, A. J. E., & Hess, P. H. (2008). Hexoses as phloem transport sugars: the end of a dogma? *Journal of Experimental Botany, 59*(2), 261-272. doi: 10.1093/jxb/erm294

van der Weijde, T., Huxley, L. M., Hawkins, S., Sembiring, E. H., Farrar, K., Dolstra, O., Visser, R. G. F., & Trindade, L. M. (2017). Impact of drought stress on growth and quality of miscanthus for biofuel production. *GCB Bioenergy, 9*(4), 770-782. doi: 10.1111/gcbb.12382

Velterop, J. S., & Vos, F. (2001). A Rapid and Inexpensive Microplate Assay for the Enzymatic Determination of Glucose, Fructose, Sucrose, L-Malate and Citrate in Tomato (Lycopersicon esculentum) Extracts and in Orange Juice. *Phytochemical Anal, 12*, 299-304. doi: DOI: 10.1002/pca.598

Wang Li, Cui Na, Zhang, K.-Y., Fan, H.-Y., & Li, T.-L. (2013). Research advance of sucrose phosphate synthase (SPS) in higher plant. *Int. J. Agric. Biol, 15*, 1221-1226.

Winter, H., & Huber, S. C. (2000). Regulation of Sucrose Metabolism in Higher Plants: Localization and Regulation of Activity of Key Enzymes. *Critical Reviews in Biochemistry and Molecular Biology, 35*(4), 253-289.

Xu, Z., Zhou, G., & Wang, Y. (2007). Combined effects of elevated CO2 and soil drought on carbon and nitrogen allocation of the desert shrub Caragana intermedia. *Plant and Soil, 301*(1), 87-97. doi: 10.1007/s11104-007-9424-0

Xue-Fei, D., Na, C., Li, W., Xiao-Cui, Z., Bo, Q., Tian-Lai, L., & Guo-Liang, Z. (2012). The SnRK protein family and the function of SnRK1 protein kinase. *Int. J. Agric. Biol, 14*, 575-579.

Zeeman, S. C., Smith, S. M., & Smith, A. M. (2004). The Breakdown of Starch in Leaves. *The New Phytologist, 163*(2), 247-261.

Zrenner, R., Salanoubat, M., Willmitzer, L., & Sonnewald, U. (1995). Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (Solanum tuberosum L.). *The Plant Journal, 7*(1), 97-107. doi: 10.1046/j.1365-313X.1995.07010097.x

EFFECT OF DROUGHT ON THE POTATO STEM

Ernest Aliche[1,2], Alena Prusova-Bourke[3], Mariam Ruiz[1], Marian Oortwijn[1], Henk van As[3], Richard G.F. Visser[1,2], C. Gerard van der Linden[1,2]

[1]Plant Breeding, Wageningen University & Research, Droevendaalsesteeg 1, 6708PB, Wageningen

[2]Graduate School Experimental Plant Sciences, Droevendaalsesteeg 1, 6708PB, Wageningen,

[3]Wageningen University & Research, Laboratory of Biophysics, Stippeneng 4, 6708 WE, Wageningen,

To be submitted...

ABSTRACT

The potato stem is an important communication channel between the assimilate-exporting source leaves and the terminal sink tissues of the plant. The stem is an equally essential pathway for the bidirectional transport of water and photo-assimilates between the roots and the shoots of the plant as well as a venue for a variety of metabolic processes. The stem accommodates the vascular tissue (xylem and phloem) through which the aforementioned transport processes are mediated. During environmental stress conditions like water scarcity, the performance (canopy growth and tuber yield) of potato is adversely affected. The role of the stem during such stresses is essential, however, still understudied. In this study, we investigated the role of the potato stem tissues of cultivated potato grown in the greenhouse under drought using a multi-disciplinary approach including physiological, biochemical, morphological, microscopic and magnetic resonance imaging techniques. We compared a number of characteristics of the lower and upper potato stem grown under drought and control conditions. The biggest difference was found in the lower stem regions of the plants grown under drought in comparison to the control plants. The light microscopy analysis of the potato stem sections revealed that plants exposed to the drought stress have higher total xylem conducting area than control plants. This increase in the total xylem conducting area was accompanied by an increase in the number of narrow-diameter xylem conduits and decrease in the number of large-diameter xylem conduits. This may present a potential breeding target for drought tolerance in potato.

INTRODUCTION

The potato crop and drought stress

Potato (*Solanum tuberosum* L.) is the world's 3rd most important food crop (Bradshaw, 2010). FAOSTAT (2014) estimated potato global production at 368 million tonnes, and a total global cultivation land area of about 20 million hectares (Haverkort et al., 2013). In the last decades, developing countries have recorded increased potato production which nominates potato as a potential crop for food security (Bradshaw, 2010). However, potato is also drought sensitive (Obidiegwu et al., 2015). Drought is gaining global concern in view of climate change scenarios and its huge negative impacts on agriculture (Eisenstein, 2013; Grayson, 2013; Heffernan, 2013). In the coming 30 – 90 years, PDSI (Palmer Drought Severity Index) predicts a global widespread drought resulting from reduced rainfall and increased evaporation (Dai, 2011). These drought warnings suggest strongly negative impacts for crop production, especially potato (Obidiegwu et al., 2015). Hijmans (2003) estimates that drought in potato will reduce yield by up to 32% globally between the years 2040 – 2069 (Hijmans, 2003). Therefore, research efforts toward improving potato yield under drought are increasing.

Stem complexity and roles

The role of the potato stem in drought tolerance has hardly been studied, even though the stem plays a vital role in the bidirectional transport of water, photo-assimilates and other products of metabolism, with the vascular tissue of the stem mediating these transports (Gartner, 1995).

The stem is a potential reserve water-pool to maintain leaf water potential in functional boundaries (Banik et al., 2016), The primary components of the stem vascular tissue are xylem and phloem. Xylem consists of tracheids (narrow tubes with tapered ends) and vessels (wider tubes but shorter than tracheids and joined end-to-end) (Myburg et al., 2001). Xylem transports water and nutrients from the soil to different plant parts. Xylem transport is driven by transpiration and therefore xylem operates under negative pressure (Giordano et al., 1978). Several theories were proposed that describe the complex mechanism of water transport through the xylem (Dixon & Joly, 1894). In general, water transport through the xylem involves the interaction of various stem components like parenchyma, cambium, phloem and other tissues (Canny, 1995; Holbrook & Zwieniecki, 2011; Tyree & Zimmermann, 2013).

The phloem transports photo-assimilates from source tissues to sink organs of the plant for growth, respiration and/or storage (Ryan & Asao, 2014). It has highly specialized living cells called sieve elements (SEs). The SEs have reduced cytoplasm, no nuclei and are interconnected by sieve pores that are wide enough to form a low-resistance tube-like pathway for photo-assimilates (Jensen et al., 2012; Schulz, 1998). The SEs maintain their viability by the association with companion cells (CC), thereby forming the SE-CC complexes (Oparka & Turgeon, 1999; Schulz & Thompson, 2001). The CC are nucleated cells which carry out metabolic functions and provide the energy required for phloem transport (Ruan, 2010). Phloem transport has been described as mass flow of assimilates driven by hydrostatic pressure (Knoblauch & Peters, 2010), based on velocity estimates of different molecules in phloem flow (Ruan, 2010). This mass flow is facilitated by an osmotic pressure gradient between source and sink tissues of the plant as proposed by Munch (Munch, 1930). Although there are debates about the exact mechanism driving phloem flow in plant systems (Spanner, 1970; Thaine, 1969), in herbaceous plants like potato, the mechanism of an osmotically generated pressure gradient is widely accepted (De Schepper et al., 2013; Knoblauch & Peters, 2013). The pressure gradient results from a reduction in the water potential of the phloem when assimilates are imported into the SE-CC complex. Water molecules then osmotically flow into the phloem from the neighbouring xylem vessels. The increased concentration of assimilates and subsequent influx of water molecules establishes a hydrostatic pressure in the phloem. This hydrostatic pressure drives the flow of assimilates along the sieve elements from the source toward the sink tissues. Based on this model, sufficient water potential in the xylem is required for transport through the phloem (Johnson et al., 1992). In fact, phloem sap concentrations of 34.5% (that is, the sugar concentration that can generate a sufficient osmotic gradient to create a driving hydrostatic pressure in solution would be about 34.5% wt/wt in the SEs) is optimal for transport efficiency (Jensen et al., 2013) .

Xylem-phloem interaction under drought stress

Hydrodynamic interactions between the xylem and phloem have been demonstrated in different plant systems (Sevanto et al., 2011; Zwieniecki et al., 2004). These interactions become crucial under sub-optimal conditions like drought. During drought stress, the xylem is prone to cavitation (Pockman & Sperry, 2000; Tyree & Sperry, 1989). Cavitation is the formation of air bubbles in the xylem water-column in the regions of lower pressure (Vilagrosa et al., 2012). Cavitation breaks the water-columns along the transpiration stream and impairs the hydraulic

conductance of xylem vessels (Cochard & Tyree, 1990; Holtta et al., 2009; Lovisolo & schubert, 1998; Melcher et al., 2003; Torres-Ruiz et al., 2011; Twumasi et al., 2005). It also affects the characteristics of surrounding vessels. Xylem cavitation leads to a reduction in xylem flux and it is known to be associated with drought sensitivity of species like *F. excelsior* and *C. betulus* (Köcher et al., 2009; Schuldt, 2008). However, xylem cavitation can be reduced or eliminated by adapting the sugar concentration in the phloem sap (Lampinen & Noponen, 2003).

Water limitation stress affects phloem sieve tube elements as well as phloem sap transport (Sheikholeslam & Currier, 1977). Plants are not able to cope with the loss of turgor pressure in their SEs (van Bel, 2003b), and under water limitation stress, plants need to adopt methods to maintain turgor pressure. One of the methods speculated is an enhanced retrieval process of sucrose import from lateral sinks and surrounding source-cells into the sieve tube (De Schepper et al., 2013; Van Bel, 2003a). Those retrieved sucrose molecules generate negative water potential in the SEs, which attracts water from the neighbouring xylem to facilitate transport. Drought was shown to induce this interaction between phloem sap sugars and the water potential in the xylem vessels in *E. globulus* (Cernusak et al., 2003). However, drought can also lead to phloem transport failure through increased viscosity resulting from build-up of photo-assimilates in sieve tubes (Sevanto, 2014). This can occur in severe drought scenarios, when the overall amount of water available in the plant is drastically reduced. Furthermore, poor sieve tube wall permeability may also lead to viscosity build-up and impaired xylem-phloem transport interaction.

Optimal xylem-phloem transport interaction may be critical for potato yield because it is likely to affect the partitioning of assimilates to its food storage organ, the tuber. The underground sink tissues (roots, stolons and tubers) and above-ground sink leaves, meristem and flowers compete for photo-assimilates produced in the source leaves (Haverkort & MacKerron, 2012; Kooman & Rabbinge, 1996). The delivery of photo-assimilates to the prevailing sink tissue is mediated through the phloem. Potato phloem characteristically includes both external and internal phloem conduits (Banerjee et al., 2006). The external phloem borders the metaxylem and the internal phloem borders the protoxylem vessels. This suggests a high level of transport interaction between both phloem and xylem. A high phloem flux to xylem flux ratio in Solanaceae at night has been reported (Windt et al., 2006). This ratio is a measure of the fraction of xylem water that is used for phloem transport. Furthermore, diurnal rhythms occur in xylem-phloem transport interactions in potato (Baker & Moorby, 1969; Prusova, 2016). However, research efforts toward understanding the interactions of these vascular tissue components under drought conditions in potato are limited.

In the present study, we examined the drought responses of different regions of the potato stem in terms of their vascular tissue morphology and sap transport, and how drought affects the interaction between xylem transport and phloem transport. As these interactions are likely to be under diurnal control, we included both day time and night time measurements of xylem and phloem behaviour. The effect of drought on xylem morphology and flow, stomatal conductance and phloem transport was evaluated both under drought and control conditions. Our results

indicate that morphological changes in xylem diameter and density under drought may be associated with xylem flux and drought tolerance in potato.

MATERIALS AND METHODS

Plant material, growth and phenotyping

Four potato cultivars (Biogold, Festien, Hansa and Mondial) were grown from seed tubers in the greenhouses of Unifarm, Wageningen University & Research, The Netherlands. The cultivars were selected based on their drought responses in previous experiments (Chapters 2 - 4). Biogold showed the highest level of drought tolerance and Mondial the highest level of drought sensitivity in terms of tuberization and tuber yield in greenhouse pot trials. Seed tubers of the cultivars were provided by Dutch breeding companies. The tubers were pre-sprouted for a week prior to planting in 19cm diameter pots. The experiment was conducted from October 2015 through January 2016 under greenhouse conditions of 16/8hr day/night periods and 22.5/18.0^{o}C day/night temperatures. A staggered planting approach was adopted with the later maturing cultivars planted before the early maturing cultivars. This was done to synchronize stress application with plant phenology as much as possible. Thus, the planting sequence was as follows: firstly, Festien, then Mondial/Hansa, and lastly, Biogold, allowing 1 week in between planting slots. Drought was applied to three replicates of each cultivar after 1 week from the emergence of the last planted cultivar. The drought lasted for 7 weeks and a recovery treatment was given for 4 weeks. The drought-treated plants were given 75ml of water only when the soil water content dropped below 15% v/v (that is, volume of water per volume of soil). The soil water content percentage was determined based on information of drought stress range from the previous year (25%v/v - mild stress, ≤15%v/v – severe stress), measured with a Grodan Water Content Meter. The control plants received >200ml per day depending on the water amount used by the plant. Soil water reduction was monitored using Parrot Flower Power® sensors. After 21 days of drought treatment, stomatal conductance was measured using a Decagon SC-1 Leaf Porometer. Measurements were taken from source and sink leaves at 09:30-11:00hrs (daytime) and 19:00-21:00hrs (night time) The day length (16hr) period was maintained in the greenhouse compartment from 02:00-18:00hr, using artificial lighting when needed. Plant tissues were sampled after 28 days of drought, during the day and also at night, for biochemical analysis. Also, plant height was scored at three time points in the growing season: before drought application (H_0), seven weeks after drought application (H_1) and four weeks after recovery treatment (H_2). Increase in plant height during stress (ΔH-Str. = $H_1 - H_0$) and during recovery (ΔH-Rec. = $H_2 - H_1$) were determined. Plant height gives an indication of the distance of transport through the stem that water and sugars have to travel from source leaf regions to sink tissues.

Cross-section analysis

During tissue sampling, the lower and upper regions of the stem were sampled with sharp blades for cross-section analysis. Lower stem tissue was sampled at ~10cm above the soil surface, below the lowest leaf. The upper stem was sampled just below the sink leaves, that is, below

the 3rd open young leaf. The cut stem pieces, about 0.5cm in length, were immersed in 1ml of fixation buffer in Eppendorf tubes and stored overnight in the dark at 4°C. The fixation buffer was made of 5% gluteraldehyde and 0.1M phosphate buffer. Permeation of the fixation buffer into the tissues was ensured with a vacuum pump (Membran Vakuumpumpe Vacuubrand GMBH + CO). Samples were washed four times for 15 minutes in 0.1mM phosphate buffer, followed by four 15 minute washes in deionized water. Finally, the stem samples were dehydrated by subsequent washing in the following concentrations of ethanol: 10%, 30%, 50%, 70%, 96% and 100%. Each wash lasted 1hr except the 70% wash, which was only for 15 minutes. The washed samples were infiltrated with activated Kulzer Technovit 7100 (0.1M phosphate buffer, 0.1M KH_2PO_4 and 0.1M Na_2HPO_4, 100ml). Each sample was embedded in 15ml Technovit 7100 mixed with 1ml Hardener II. The setup was dried overnight to harden. The embedded stem was placed on the microtom, Reichert-Jung Leica Rijswijk (ZH) 2055 for sectioning. The cut transverse sections were placed on slides and stained with Toluidine-Blue. The slides were dried on Slide Warmer SW85. After slide preparation, the sections were visualized under the light microscope (Carl Zeiss D-7082 Oberkochen (Axiophot)). Section images were captured by a Nikon camera mounted on the microscope, and analysed with the ImageJ2 software package (Rueden et al., 2017). The xylem vessels were characterized into size classes and quantified. The size classes are: 0-20µm, 20-40µm, 40-60µm, 60-80µm and >80µm. Number of vessels per size class, surface area of vessels and xylem density per stem area were scored.

Sap extraction and biochemical analysis

Stem tissues of 2-3cm length were collected from upper and lower regions of the stem (see above). Sap was extracted using a centrifugation method (Hijaz & Killiny, 2014). Each tissue was quickly inserted in a spin column and placed in IEC Micromax Eppendorf Centrifuge 5417C. After 8 minutes centrifugation at 13,000rpm in the IEC Micromax Eppendorf Centrifuge 5417, sap was collected in Eppendorf tubes and stored at -80°C for biochemical analyses (Hijaz & Killiny, 2014). The stem tissues from which sap was collected were stored at -80°C. The sugar content of the sap and stem tissues was determined using a Boehringer Mannheim Sucrose/D-Glucose/D-Fructose kit (Kinkade, 1987). Each 1µl of sap was hydrolysed to completion with 1U β-fructosidase (Karley et al., 2002). The following sugars were quantified: sucrose, glucose and fructose.

Magnetic resonance imaging (MRI)

Xylem sap flow measurements were conducted in an MRI scanner, consisting of a vertically-orientated superconducting magnet with a 50 cm vertical free bore (Magnex, Oxford, UK). For induction and detection of the flow signal, a bird cage RF coil of 4 cm diameter was used inside a 1 T/m gradient set (Bruker, Karlsruhe, Germany) and controlled by the Avance console (Bruker, Karlsruhe, Germany) (Homan et al., 2007). Here we used a pulsed field gradient turbo spin echo (PFG-TSE) sequence for measurement of xylem sap displacement (Scheenen et al., 2000). For every pixel within an image we obtained a propagator (a displacement spectrum) (Scheenen et al., 2000). This propagator was analysed with an approach described elsewhere (Scheenen et al., 2000; Van As, 2006; Windt et al., 2006) and resulted in the following

parameters for each pixel: volume flow, amount of stationary water, flow conducting area (FCA) and average flow velocity. Flow measurements were carried out using the following imaging parameters: spectral width = 50 kHz, imaging matrix = 128x128 pixels, field of view = 17 x 17 mm, slice thickness = 3 mm, echo time = 5 ms. For the xylem measurements the specific parameters were: turbo factor: 8, number of averages: 2, repetition time: 2500 ms, displacement labelling time: 20 ms, gradient duration: 4 ms, 32 gradient steps, maximum gradient strengths: 400 $mT \cdot m^{-1}$ and acquisition time: 42 min. Data analysis was performed with IDL (ITT Visual Information Solutions, Boulder, Colorado, USA) using in-house processing, fitting and quantification routines.

For the MRI measurements, potato plants were grown in 19cm pots in the greenhouse under mild drought (<25%v/v, see above) until about 50m height. The inner walls of the 19cm pots were fitted with tubes that we used later on during the MRI measurements to cool the soil at night times. Prior to measurement, the stem was cleaned from old (lower) leaves (about 30cm) to be able to place the magnet close to the stem. The plants were mounted (one plant per measurement including two replicates per treatment per cultivar) in the MRI machine for scanning of the lower stem region a day prior to the measurement. During the measurements, the soil was cooled to about 18-19^{o}C at night by running cold water via the pot tubing. During the MRI measurements, stressed plants were watered with 100ml/24hr and control plants received 100ml/6hr. The following environmental features were monitored during the measurements: soil temperature, canopy temperature, light intensity, relative humidity and soil water potential.

RESULTS

Plant height adaptation and biomass ratio

The effect of plant height (size) on transport within the plant has been a subject of debate between scholars who support (Koch et al., 2004) or deny (West et al., 1999) any effects. Therefore, we investigated the increase in plant height of the four cultivars in this study during drought stress and after a recovery treatment. The seven-week period of stress coincided with the exponential growth phase of the plants, whereas during the recovery treatment the plants had passed the exponential growth phase. A comparison of the height increase between stressed and control plants during the seven weeks of stress, (ΔH-Str.), shows that growth rate was reduced under stress (Fig.1). This was significant in Biogold and Hansa. However, the height increase between stressed and control plants during recovery period, (ΔH-.Rec), showed no significant differences between both recovered and control plants (Fig.1). Additionally, tuber/shoot ratio at two time points, 28 and 77 days after drought (28DOD and 77DOD), varied among the cultivars suggesting differences in assimilate partitioning to tubers with time (Fig.1b). Remarkably, Biogold, showed no difference in tuber/shoot ratio between the two time points.

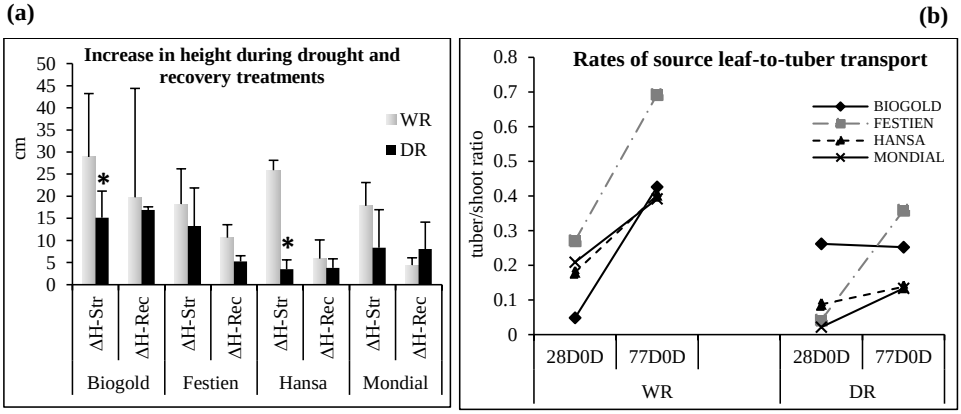


Figure 1: (**a**) Graph showing the increase in plant height after 7weeks of drought stress (ΔH-Str.) and after 4 weeks of recovery treatment (ΔH-Rec). The stress and recovery scores were compared with their respective control conditions. Error bars are standard deviations between three biological replicates. * = sig. ($p \leq 0.05$), (**b**) Tuber/shoot ratio of potato cultivars at two time points - 28 and 77 days of drought stress (DOD) under irrigated (WR) and non-irrigated (DR) conditions

Stomatal conductance

During water shortage one of the mechanisms plants use to manage water loss is stomatal closure (Osakabe et al., 2014). Therefore, stomatal conductance was measured in both source and sink leaves during the day (09:30-11:00hrs) and at night (19:00-21:00hrs).

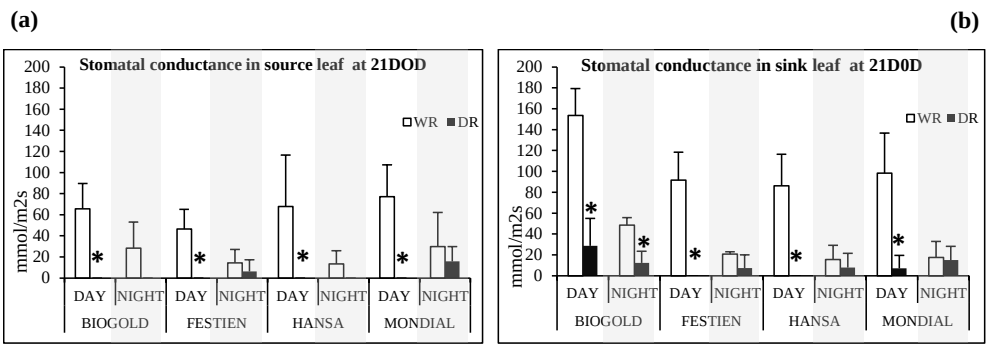


Figure 2: Stomatal conductance in (**a**) source and (**b**) sink leaves of four cultivars under (DR) drought and (WR) normal watering, during the day (09:30-11:00hrs) and at night. (19:00-21:00hrs). * sig. p-value ($\alpha=0.05$). Error bars = standard deviation between biological replicates, DOD = Days of drought. Light period: Artificial lighting (02:00) – Dawn (08:00) – Dusk (05:30)

All genotypes tested strongly reduced stomatal conductance under drought, with source leaves reducing it to a level that was even lower than the sink leaves (Fig.2). Under normal conditions the stomatal conductance was significantly reduced at night in both source and sink leaves.

Biogold and Mondial retained a moderately low level of conductance under drought in their sink leaves during both day and night, while Festien and Hansa only had measurable levels of stomatal conductance at night (Fig.2b). Biogold and Mondial from previous experiments had shown interesting contrasts in their drought response. Therefore, we further investigated xylem flow, vessels structure and sugar transport in the stem of Biogold and Mondial.

Xylem and phloem flow

The xylem and phloem flow in the lower stem region of 8-10 week-old potato plants was measured using a plant-dedicated MRI scanner (Windt et al., 2006). The drought stress inside the MRI scanner was four times milder in comparison to the greenhouse experiments of this paper due to technical limitations. The other environmental conditions like temperature, relative humidity and light intensity were set to mimic the greenhouse conditions. Each plant was placed into the MRI scanner several days prior to measurement to allow the plant to acclimate. Under both control and drought conditions the plants exhibited a typical diurnal xylem flow pattern, with highest values of all xylem flow characteristics (i.e., xylem volume flow, xylem average velocity and xylem flow conducting area) during the day and the lowest at night (Figs.3 and 5). The night values of xylem flow characteristics were much lower under drought than in control conditions (Figs.3 and 5). The peaks of xylem volume flow, xylem average velocity and xylem flow conducting area were much lower in the drought-stressed Mondial plant than in the water-limited Biogold plant (Fig.5).

Phloem flow characteristics did not show a significant difference between day and night, especially under drought stress (Figs.4 and 6). In control conditions, there was a tendency towards a day-night pattern, but the low signals made it difficult to discern a clear trend (Figs.4 and 6). Phloem volume flow was about 100- and 1000-fold less than xylem volume flow in Biogold and Mondial, respectively, under stress and control conditions. In both cultivars, phloem flow conducting area (FCA) was minimal and drought effects were not detected for the measured phloem flow characteristics (Figs.4 and 6).

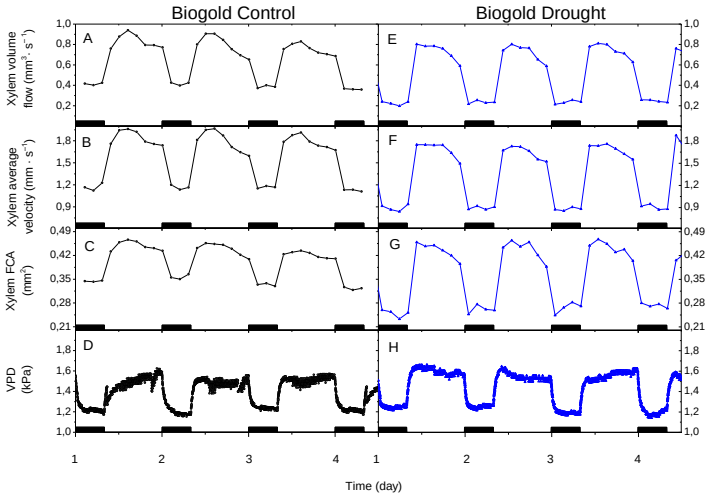


Figure 3: MRI measurements of xylem flow characteristics in the lower stem of Biogold under control and drought conditions. From the top of the plot: (**A**, **E**) volume flow of xylem sap, (**B**, **F**) average xylem flow velocity, (**C**, **G**) xylem flow conducting area and (**D**, **H**) vapour pressure deficit, all as a function of time. Black rectangles represent night-time.

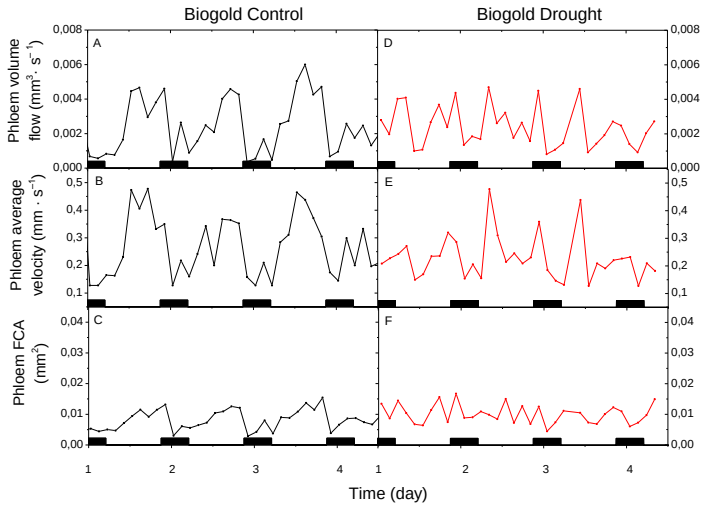


Figure 4: MRI measurements of phloem flow characteristics in the lower stem of Biogold under control and drought conditions. From the top of the plot: (**A**, **D**) volume flow of phloem sap, (**B**, **E**) average phloem flow velocity and (**C**, **F**) phloem flow conducting area, all as a function of time. Black rectangles represent night-time.

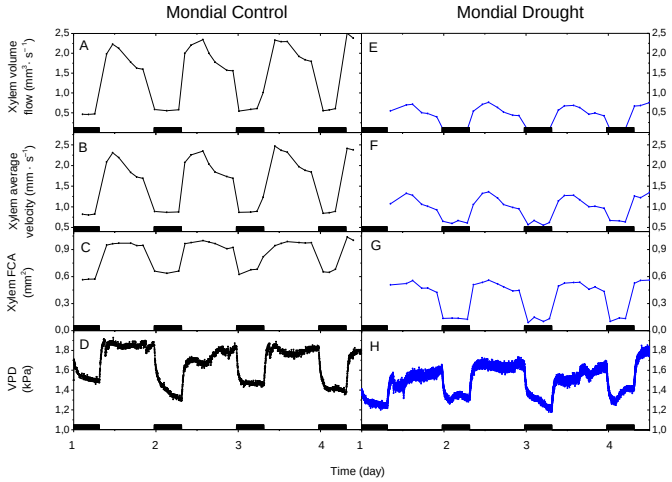


Figure 5: MRI measurements of xylem flow characteristics in the lower stem of Mondial under control and drought conditions. From the top of the plot: (**A**, **E**) volume flow of xylem sap, (**B**, **F**) average xylem flow velocity, (**C**, **G**) xylem flow conducting area and (**D**, **H**) vapour pressure deficit, all as a function of time. Black rectangles represent night-time.

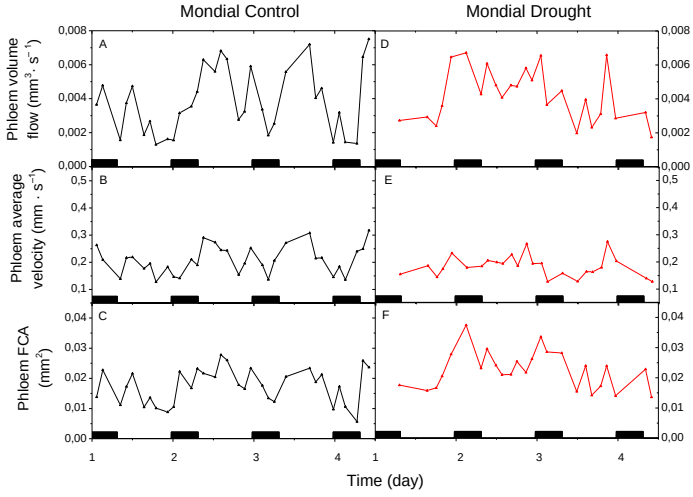


Figure 6: MRI measurements of phloem flow characteristics in the lower stem of Mondial under control and drought conditions. From the top of the plot: (**A**, **D**) volume flow of phloem sap, (**B**, **E**) average phloem flow velocity and (**C**, **F**) phloem flow conducting area, all as a function of time. Black rectangles represent night-time.

Xylem cross section

Three transverse sections of upper and lower stem regions of Biogold and Mondial were taken from three plants (replicates) per genotype to investigate the effect of drought on the morphology of transport vessels. The stem tissues sampled at 28 days after drought application were fixated, stained and sectioned. A microscopic view of the stem cross-section revealed that the tracheids in the lower stem of Biogold occupied a significantly larger surface area than in the upper stem (Fig.7). In Mondial, tracheids occupied a similar surface area in both upper and lower stem (Fig.7).

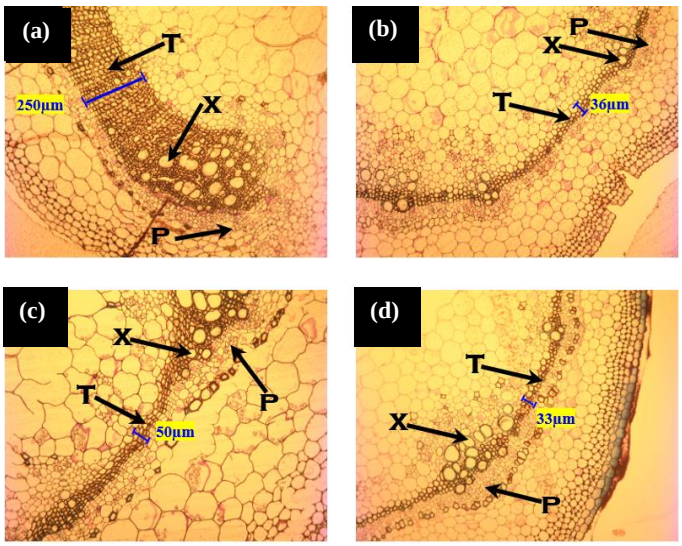


Figure 7: Stem cross sections at 28days after drought showing xylem tracheids (T) with blue lines indicating the thickness, xylem vessels (X), phloem vessels (P) and other cells. (**a**) Lower stem of Biogold, (**b**) Upper stem of Biogold, (**c**) Lower stem of Mondial and (**d**) Upper stem of Mondial.

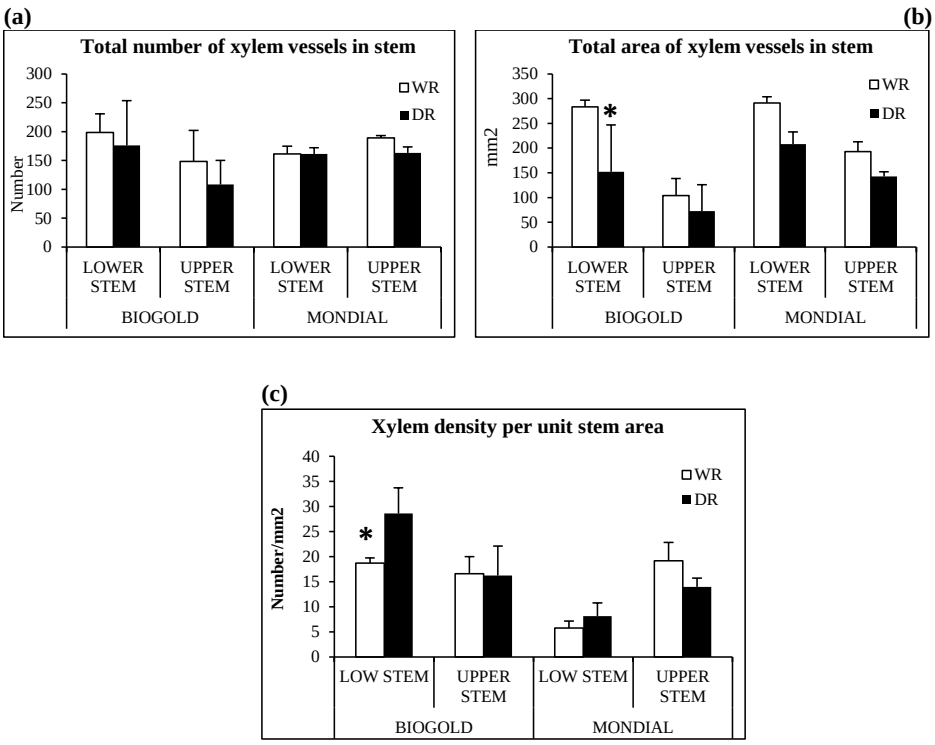


Figure 8: Graphs showing (**a**) mean total number of xylem vessels (**b**) mean total area of xylem vessels, (**c**) density of xylem vessels per unit area of stem cross section. Error bars are standard deviations between three biological replicates. *= sig. ($p \leq 0.05$).

There was no significant reduction in the total number of xylem vessels under drought in the upper and lower stem of both cultivars, but total area of xylem vessels was less under drought, and less in lower stem than upper stem (Fig.8b), although this xylem vessel area reduction was only significant in the lower stem of Biogold ($p \leq 0.05$). There was an increase in xylem density per unit area under drought in lower stem but not in the upper stem (Fig.8c). Also, the increase in xylem density in lower stem was significant in Biogold but not Mondial. The lower stem diameter of Mondial was significantly larger than the upper stem diameter, whereas in Biogold both regions of stem were about the same size (data not shown). By categorizing xylem vessels into size classes, the xylem vessel size composition and drought effects on xylem vessel size were further investigated.

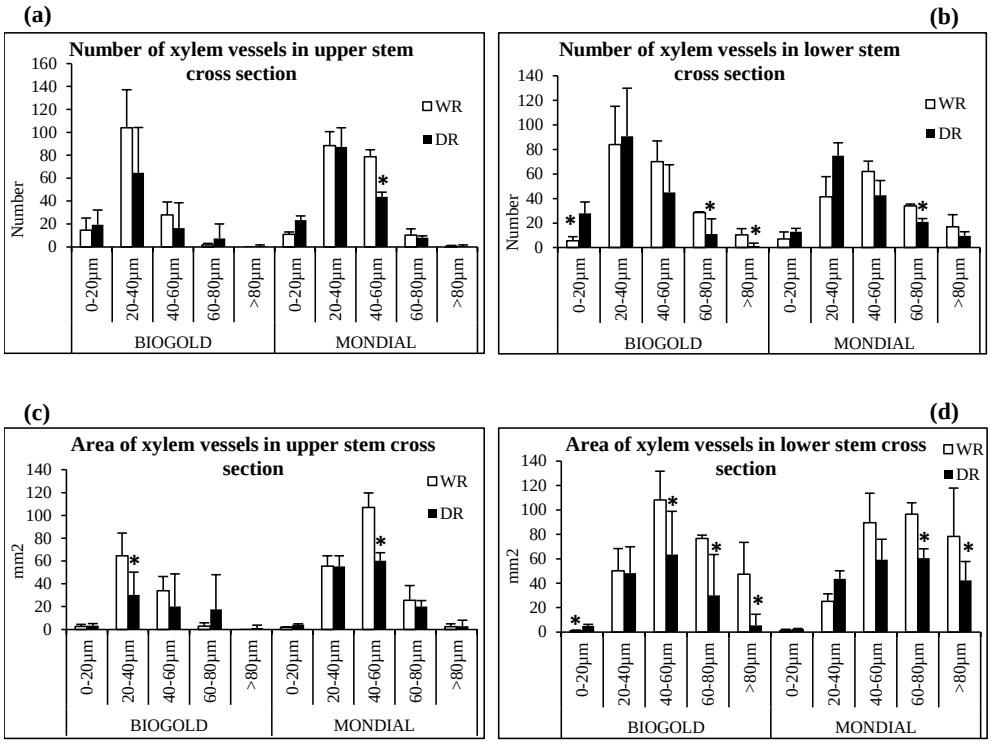


Figure 9: Graphs showing the number of xylem vessels per size class in (**a**) upper stem and (**b**) lower stem; and area of xylem vessels per size class in (**c**) upper and (**d**) lower stem regions. Error bars are standard deviations between three biological replicates. *= sig. ($p \leq 0.05$).

Intermediate-sized vessels (20-60µm) were the most abundant (Figs.9a and b). The lower stem generally had more vessels of the large size range (>80µm) than the upper stem. There was a tendency to more small-sized vessels (0-20µm) under drought in both cultivars (Fig.9a). In the lower stem, drought significantly reduced the number of vessels in size class >60µm in Biogold, In Mondial the reduction in number of vessels under drought was only significant in the 60-80µm size class (Fig.9b). The intermediate-sized vessels (20-60µm) generally contributed most to the conducting xylem area in the upper region of the stem (Fig.9c). Also, the effect of drought on xylem vessel area was observed only on the intermediate-sized vessels (Fig.9c). However, for the lower stem region the bigger-sized vessels (>60 µm) were significantly reduced under drought, and contributed more to the conducting xylem area than the smaller size classes (Fig.9d). Reduction in xylem vessel area of lower stem (Fig.8b) basically affected larger-sized vessels (Fig.9d).

Sugars transported in phloem sap

We quantified the content of the sugars, sucrose, glucose and fructose in sap extracted from vascular tissues, cambium and parenchyma of upper and lower regions of the stem of Biogold and Mondial, during the day and at night. The drought stress treatment did not significantly reduce the sucrose content in the sap (Fig.10). Remarkably, the lower stem of Mondial had more sucrose than the upper stem at night time (Figs.10a and c), but this was not observed for Biogold. In the upper stem, the time point (day or night) did not affect sucrose content of the sap. However, in the lower stem Mondial had a significantly higher amount of sucrose at night than during the day (Figs.10c and d).

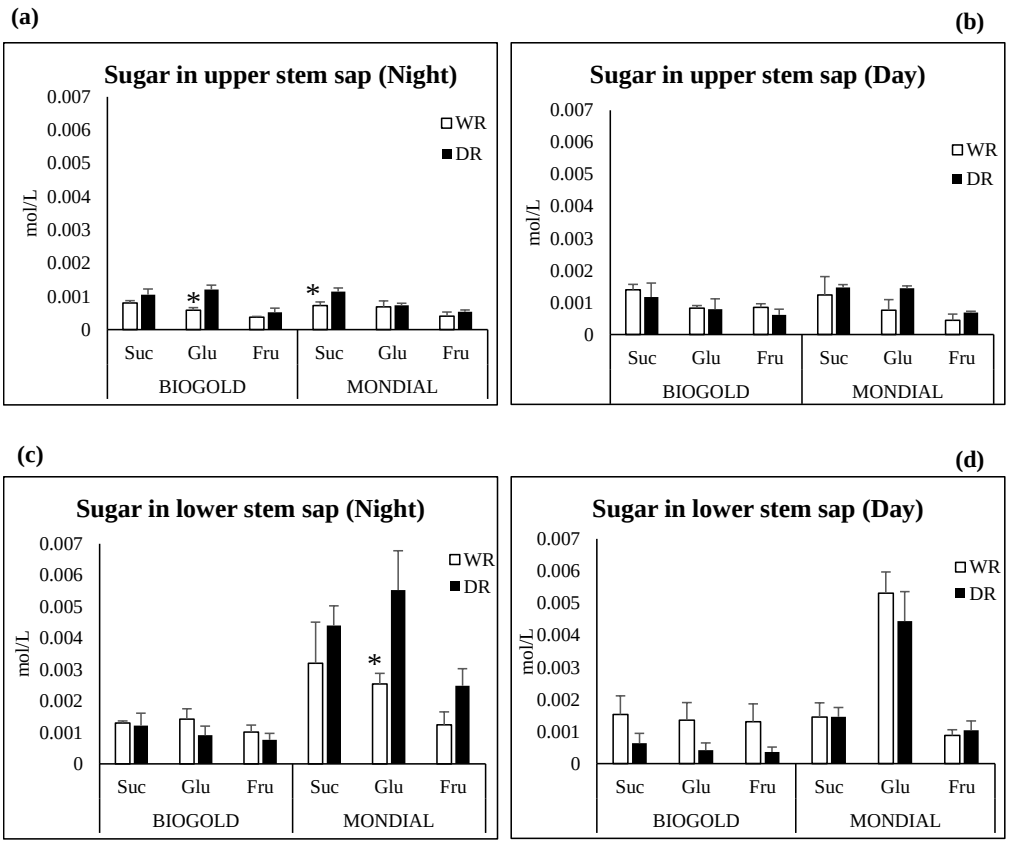


Figure 10: Sugar content of the stem sap of Biogold and Mondial in (**a**) upper stem at night, (**b**) upper stem in day time, (**c**) lower stem at night, (**d**) lower stem in day time. Error bars are standard deviations. Significance of drought and genotype effects are given differently for each sugar. For each sugar, asterisks show levels of significant differences.

The glucose content of the sap in the lower stem of Mondial was higher than that of Biogold (Figs.10c and d). Glucose content was significantly lower at night than during the day in the lower stem of Mondial under normal conditions (Figs.10c and d). Drought stress led to an increase in glucose content of sap only in Mondial, in the lower stem at night (Fig.10c), and in Biogold, the upper stem at night (Fig.10a). The fructose content of the sap in upper stem was not affected by drought treatment, day/night time points, or genotypic differences. However, in the lower stem, fructose content increased at night under drought in Mondial (Fig.10c); and was reduced in day time under drought in Biogold (Fig.10d). In summary, Biogold sugar content was less affected by drought and less variable from day to night than that of Mondial.

Sugars in stem structure

We also determined the sugar content of the stem tissues from which sap was extracted. Generally, the level of sugars in the stem tissue was 10-fold higher than in the sap (Figs.10 and 11). Only the sucrose content of upper stem of Mondial under drought stress differed between day and night (Figs.11a and b). Drought stress did not significantly affect the lower stem sucrose content of Biogold, but in the lower stem of Mondial, sucrose was totally absent under drought (Figs.11c and d). Sucrose content in the upper stem of Mondial was higher under drought during the day (Fig.11b). We observed a trend of reduced sucrose levels under drought relative to control in lower stem of both cultivars, whereas in the upper stem this was not the case.

Stem glucose content was not significantly affected by drought stress in Biogold, although there was a trend of glucose increase under drought in upper stem and decrease in the lower stem. Glucose content in the lower stem of Mondial was higher under drought than control (Fig.11c). Furthermore, there was a trend of reduced levels of fructose under drought in lower stem, which was different in the upper stem. This reduction was, however, only significant in the lower stem of Mondial during daytime (Fig.11d). Mondial showed a higher content of these sugars in the lower stem than Biogold.

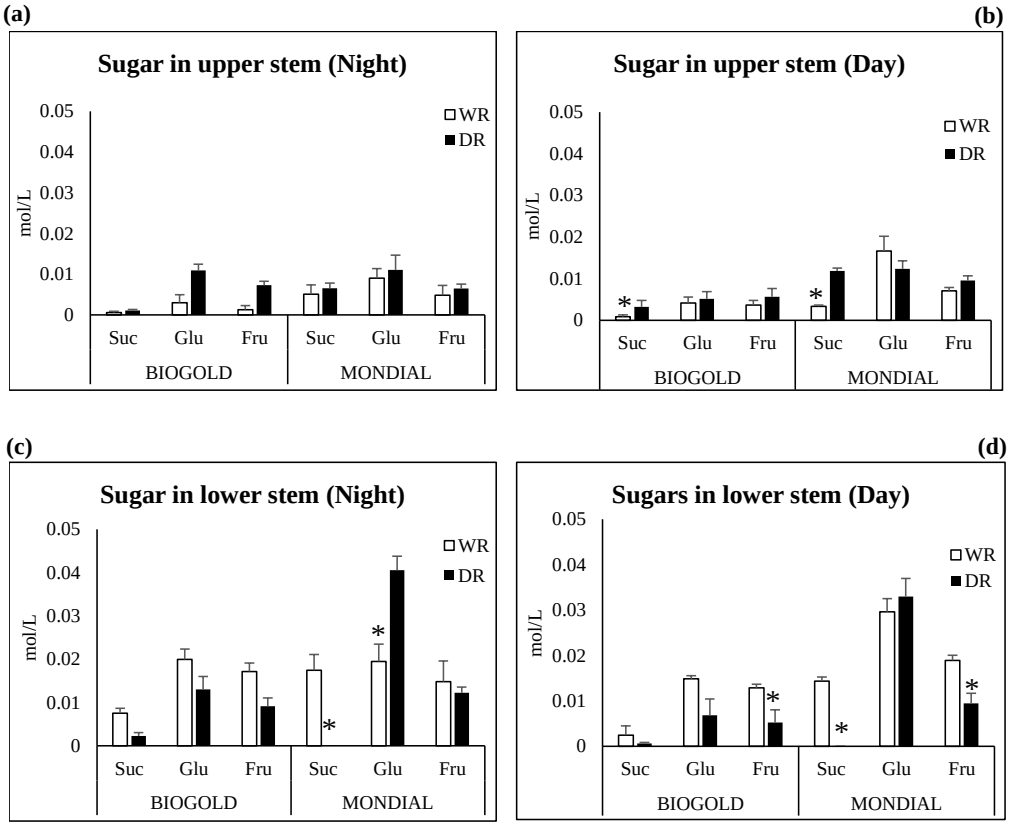


Figure 11: Sugar content of stem without sap in Biogold and Mondial. (**a**) upper stem at night, (**b**) upper stem in day time, (**c**) lower stem at night, (**d**) lower stem in day time. Error bars are standard deviations. Significances (asterisks) of drought and genotype effects are given differently for each sugar. For each sugar, asterisks show levels of significant differences.

DISCUSSION AND CONCLUSIONS

The stem provides support for plants and facilitates transport between different parts of the plant (Yan et al., 2016). Physiological and structural adaptations of the transport system to drought are likely to play a role in carbon partitioning, which may be of particular interest in potato considering the underground location of its major storage organ, the tuber. This understanding may contribute to further enhancement of the plant's ability to withstand drought conditions. Drought conditions interfere with stem structure and availability of transport materials (Banik et al., 2016; Zheng et al., 2009). We have gained additional insight in the adaptation of the transport system to drought conditions of potato using a multidisciplinary approach, combining physiological, biochemical, microscopic and MRI methodologies.

Potato growth adaptations

Reduction in plant height after a period of drought stress is often seen as a negative symptom of stress (Albiski et al., 2012; Luitel et al., 2015). Plant height reduction implies that drought reduces stem elongation (Farooq et al., 2009), and it may also affect the rate of proliferation of new leaves, which eventually reduces the photosynthetic capacity of the plant. However, our findings support the hypothesis that height reduction during drought may also serve an advantageous purpose for the plants – to reduce transport distance (Koch et al., 2004). A consideration of the Hagen-Poiseuille's equation shows that when the variables of the equation are kept constant, an increase in tube length causes a reduction in flow rate of liquid through the tube (Niklas, 2007). This suggests that under limited water availability like during drought, plant height reduction may be an important factor for efficient water and nutrient transport in plants. Plant height is regulated by auxin and it is known that sugars regulate auxin metabolism and transport (Ljung, 2013). The impact of drought stress on sugars impacts auxin activity, which stalls plant growth (Lastdrager et al., 2014). With reduced height, water and assimilates will not need to cover long distances to get to their needed destination. In our study, reduction of exponential growth of the plants (Fig.1a) may have aided the distribution of water, nutrients and assimilates. Therefore, height reduction under drought is both a stress symptom (Luitel et al., 2015) and an adaptive mechanism to cope with the stress in terms of water and assimilate transport (Koch et al., 2004).

Stomatal regulation of leaf transpiration and photosynthesis

The stomatal conductance measurements showed the source leaves just above the lower stem region closed their stomata earlier and much more than the sink leaves (Fig.2). Stomatal closure has implications for CO_2 exchange, transpiration pull and assimilate transport in the plant (Schapendonk et al., 1989; Wheeler et al., 1999). The stomatal closure of source leaves under drought may imply that sink leaves need to adapt carbon fixation rate, as was shown in another study where younger leaves maintained their stomatal conductance and photosynthesis despite the decline of these attributes in older leaves (Vos & Oyarzun, 1987). Adapted photosynthetic rate in sink leaves under drought may not necessarily suffice for continuous growth, but at least it may meet the metabolic energy requirements of the sink leaves. In another study on potato *cv.* Bintje in a growth chamber, photosynthesis was monitored under different CO_2 levels and the authors reported that photosynthesis in young leaves increased or decreased with respective increase or decrease in CO_2, more strongly than in older leaves (Katny et al., 2005). In such instances of limited or adapted photosynthesis leading to less sugar availability in the plant, and depending on the severity and duration of the drought, the source leaves in our study may have used up or transported the sugars they already photosynthesized prior to complete stomatal closure (Iwona et al., 2012). The impact of drought, which may be more severe on the source leaves than sink leaves, based on differences in stomatal closure (Fig.2), may lead to senescence and eventual leaf fall (Haverkort & Goudriaan, 1994).

Furthermore, the MRI study of the lower stem suggests that the day/night rhythm of xylem flow observed under normal conditions continued under a mild drought scenario, with strongly decreased flow at night (Figs.3 and 5). However, when the drought was severe (in the

greenhouse), some genotypes while closing their stomata completely during the day seemed to allow a low level of transpiration at night, and this was most obvious in the sink leaves (Fig.2b), suggesting that xylem flow was maintained to some extent in these plants. The adaptation of the plants' xylem flow to the severity of the drought conditions also affects the transport of photo-assimilates through the stem (Windt et al., 2006). The bulk flow theory has shown that xylem water influx into the phloem creates the hydrostatic pressure that drives assimilate transport through the phloem (Johnson et al., 1992). This implies in our study that under drought conditions with reduced xylem flow, photo-assimilate transport may also be limited. The MRI study of the two most contrasting genotypes showed a weak relationship trend between xylem flow and phloem flow patterns (Figs.3-6). The reason for the weak association between xylem and phloem flow patterns may be due to the size of the plants relative to the detection sensitivity of the MRI machine. In another MRI study on a three-month old tomato plant, it was shown that xylem volume flow decreased under drought, and phloem volume flow was equally reduced with a subsequent reduction in phloem flow velocity after two days (Prusova, 2016). The reduced xylem volume flow of *cv.* Mondial under drought in our study (Fig.5) might indicate that the drought sensitivity of Mondial may to some extent be related to reduced transport rates of assimilates.

The reason for maintenance of stomatal conductance in the night as observed in the sink leaves (Fig.2b) remains unclear, especially under drought. It is known in many plant species that stomata are not completely closed at night (Caird et al., 2007) (Fig.2). Advantages of stomatal opening at night when no photosynthesis occurs may include sustained nutrient transport (Snyder et al., 2003). It is also reported that when the stomata are open at night, the next day the stomatal conductance tends to increase at dawn (Snyder et al., 2003). Such increase in stomatal conductance at dawn may be beneficial for photosynthesis in plants growing in well-watered environments (Snyder et al., 2003). But under water-limiting conditions, keeping the stomata closed at night can avoid water loss, without the adverse effects of closed stomata when light is captured in photosynthesis. In fact, a genetic association has been found in grape vine between reduced transpiration rate at night and high biomass production per unit of water transpired, leading to high water use efficiency (Coupel-Ledru et al., 2016). Thus, plants may adapt the bulk flow theory at night by the adjustment of their phloem flow rates according to xylem flow rates (Windt et al., 2006), which also may result from the shutdown in photosynthesis at night, when there is no light to breakdown water and convert CO_2 molecules into carbon assimilates. Therefore, in our study, a high stomatal conductance at night under drought relative to irrigation (Fig.2) may result in low water use efficiency.

Adaptation of transport vessel size

Water and assimilate flow rates are partly influenced by the properties of the transport conduits (Kim et al., 2014; Thompson & Holbrook, 2003). In our study, the stem cross sections revealed some genotype-specific features that could potentially aid water transport management under drought conditions. The elaborate tracheid system in the lower stem of Biogold under both irrigated and non-irrigated condition is composed of narrow xylem tubes for water transport (Fig.7a). Xylem tracheids are the main water transport conduits in gymnosperms (Boutilier et al., 2014). Tracheids are xylem transport elements of narrow diameter, which are rarely used in

Angiosperms because the xylem vessels are well developed (Kim et al., 2014). However, during water scarcity, it has been demonstrated in tree species that tracheids are preferred water transport channels, since their narrow diameter provides water transport with adaptation against cavitation and embolism (Sperry et al., 1994). There are no reports on the role of tracheids in preventing cavitation and embolism in crop species under drought stress. But in our study, the abundance of these tracheids in Biogold may be an adaptation to facilitate improved transport under drought, and Biogold could still keep up water transport using the small-sized vessels and abundant tracheids. The small diameter of the tracheids also enhances adhesion forces between water molecules and walls of the tracheid, which aids in maintaining the water column as a means of reducing cavitation (Venugopal, 2016). This may suggest that breeding for potatoes with abundance of tracheids in the lower stem could improve the water management of the plant under drought stress conditions.

In other plants, lignification of the vessels has been observed under stress scenarios, making the walls of the vessels thicker (Kim et al., 2008; Sánchez-Aguayo et al., 2004). This may also result in reduced vessel diameter, but may at the same time increase the chances of embolisms (Gleason et al., 2016). Thickening of vessel walls was not observed in our study, but the reduction in the number of large sized vessels was remarkable in the lower stem (Fig.9b). This reduction in large-sized vessel area of the lower stem is a drought response mechanism that reduces the chances of drought-induced embolisms that may impair transport through the vessels (Cochard & Tyree, 1990). Reduction in vessel size is a drought response that is distinct from the numerous tracheids present under both irrigation and drought, though both tracheids and small-sized vessels serve the same function. Furthermore, increasing the total xylem conducting area by increasing the number of small-sized xylem elements may also be an effective adaptation to water scarcity. Moreover, larger surface area-to-volume ratio is essential in hydraulics (Zhang et al., 2016). Therefore, the increase in xylem density per unit stem area likely enhances water uptake from roots (Jacobsen et al., 2007). Additionally, our results seem to indicate that xylem vessels taper towards the shoot apex as seen from the fewer number of vessels of the large size class in the upper stem under both irrigation and drought (Figs.9a and b). It is known that water conduction is facilitated more readily in narrow ends of vessels (De Boer & Volkov, 2003). The tapering of the ends of the vessels means that adjoining vessel elements retain an intact water flow. Tapering of xylem elements is known in woody species to minimize the hydraulic energy cost of water transport (Anfodillo et al., 2006). Our finding may point to a similar xylem adaptation in potato, which facilitates water transport.

Sugar transport and structural sugars of the stem

It is important to note that assimilate transport may not be an exclusive function of the phloem vessel (Heizmann et al., 2001). Considerable amounts of sugars were also detected in xylem vessels of tomatoes (Die, 1962). Precise detection of assimilates in sap is still challenging despite great advances in metabolomics. A major aspect of the challenge lies in tissue sampling and preparation (Feist & Hummon, 2015). In the present study we have used a spin column in the collection of tissue sap to avoid tissue contamination as much as possible. We measured stem sap sucrose, glucose and fructose concentrations to estimate the amount of assimilates that are transported in all tissues of the stem to terminal sink tissues like tubers, root and young

leaves. Generally, the sucrose concentration of the stem sap was not reduced under drought (Fig.10). In Chapter 4 we observed that sucrose accumulated in mature (source) leaves under drought. The sucrose accumulation we observed under drought in the stem and in Chapter 4 in mature leaves may be suggesting that transport capacity and assimilate availability are matched, and transport of assimilates from source leaves into the stem transport vessels (see Chapter 4) may not be the only challenge. Another growth-limiting factor under drought may be the insufficiency of water in the transport vessels of the stem. Prusova et al. (2016) have shown similarity of trends between xylem and phloem flux under drought, indicating that xylem flow changes impact phloem transport. The low flow rate in the xylem under drought in our study (Figs.3 and 5), may therefore have severely impaired phloem transport of assimilates.

In the sap of the upper and lower stem, sucrose concentration was not higher than the amounts of its breakdown products, glucose and fructose, in both cultivars (Fig.10). Lui et.al (2012) reported that sucrose appeared to be the main sugar transported in plant species, although a negligible amount of hexoses was also detected in their study. On the other hand, there are indications that sucrose is not the only transportable form of sugar in plants, but hexoses (glucose and fructose) are also transported (van Bel & Hess, 2008). In our study, we observed that sucrose and the hexoses were equally present in the sap, suggesting that the sugar composition of assimilates that can be transported in potato may be quite dynamic. The amount of detectable sucrose and hexoses in the transported fraction may be affected by different factors including environmental conditions, time point in the day and in plant development, sensitivity of detection protocol (Duarte-Delgado et al., 2015).

The structural sugars measured in this study were 10-fold more abundant in the stem than in the sap (Figs.10 and 11). Unlike in the sap, the sucrose concentration of the stem structure was generally lower than the concentration of glucose and fructose (Fig.11). The sucrose in structural sugars of the stem may serve as a source for leakage retrieval during translocation (De Schepper et al., 2013). The leakage-retrieval hypothesis proposes that during transport, a part of the sugars are leaked from sap into lateral sinks like cambium for growth, while the rest continue to the intended terminal sink, and some of the leaked carbohydrates are retrieved from stem to sap for further transport in the sap (De Schepper et al., 2013). In this study, drought stress affected this sucrose reserve in the lower stem (lateral sink) more than in the upper stem (Fig.11). In fact, the sensitive genotype (Mondial) did not have any sucrose reserve under drought in the lower stem (Fig.11c and d). This may suggest an active mechanism of sucrose retrieval under drought into the transport stream, possibly for transport towards the sink tissues aboveground and underground. The terminal sink destination of such retrieved sucrose, or the presence/absence of a sink destination, may play a role in drought tolerance or sensitivity of the plant. Mondial may have prioritized upper shoot terminal sink, as shown from the high sucrose reserve in the upper stem under stress (Fig.11b) and tuber/shoot ratio at two time points, while Biogold invested more in tubers (Fig.1b). The direction of transport of the synthesized or retrieved sugars may depend on the driver of the assimilate transport (Lemoine et al., 2013). Divergent views have been reported about the transport of assimilates as either sink strength-driven (Marcelis, 1996; Wolswinkel, 1984) or source-driven (Farrar, 1993; Lemoine et al., 2013). Our investigation of two genotypes, Mondial and Biogold, with contrasting patterns of

drought response and transport, gives some insights related to this discussion. The tuber-to-shoot ratio at 28DOD indicated that in both plants, tubers were formed already but bulked differently through the growing season (Fig.1b). The relatively lower transport of assimilates to tubers in Mondial while maintaining shoot biomass seems to suggest a competition between tuber and aboveground tissues. According to Marcelis (1996), one of the signs of sink-driven partitioning is sink strength, which determines the competitiveness of one sink over others. Apparently, molecular determinants like invertases, sucrose synthases, sucrose transporters, which metabolize assimilates and feedback on the source tissue for more supply or an inhibition of photosynthesis, contribute to sink strength (Chapter 4) (Herbers & Sonnewald, 1998).

In summary, we have shown in this chapter that drought stress affects potato adversely and affects the water and assimilate transport system of the crop. We have investigated various transport mechanisms that may be targeted to alleviate the effects of drought stress. An adaptation of the characteristics of the stem vascular tissues, like the xylem diameter and density, could facilitate water and assimilate transport within the plant. Interestingly, plant height reduction during drought may not necessarily be a disadvantage, because it reduces the transport distance of resources in plants with adapted transport system. Based on our findings, another important feature to target for breeding is the preferential partitioning of assimilates to the tuber even under resource limitations like drought and possibly other stresses.

ACKNOWLEDGMENTS

We thank the following for the contribution of their experience and time in the greenhouse and laboratory experiments of this study: Edo Gerkema, Andre Maassen, Bertus vander Laan, Alex Super, Maarten Peters, Faline Plantenga, Abelenda Jose and Irma Hoendervangers.

REFERENCES

Albiski, F., Najla, S., Sanoubar, R., Alkabani, N., & Murshed, R. (2012). In vitro screening of potato lines for drought tolerance. *Physiol Mol Biol Plants*, *18*(4), 315-321. doi: 10.1007/s12298-012-0127-5

Anfodillo, T., Carraro, V., Carrer, M., Fior, C., & Rossi, S. (2006). Convergent tapering of xylem conduits in different woody species. *New Phytologist*, *169*(2), 279-290. doi: 10.1111/j.1469-8137.2005.01587.x

Baker, D. A., & Moorby, J. (1969). The Transport of Sugar, Water, and Ions into Developing Potato Tubers. *Annals of Botany*, *33*(4), 729-741.

Banerjee, A. K., Chatterjee, M., Yu, Y., Suh, S.-G., Miller, W. A., & Hannapel, D. J. (2006). Dynamics of a Mobile RNA of Potato Involved in a Long-Distance Signaling Pathway. *The Plant Cell*, *18*(12), 3443-3457. doi: 10.1105/tpc.106.042473

Banik, P., Zeng, W., Tai, H., Bizimungu, B., & Tanino, K. (2016). Effects of drought acclimation on drought stress resistance in potato (Solanum tuberosum L.) genotypes.

Environmental and Experimental Botany, 126, 76-89. doi: http://dx.doi.org/10.1016/j.envexpbot.2016.01.008

Boutilier, M. S. H., Lee, J., Chambers, V., Venkatesh, V., & Karnik, R. (2014). Water Filtration Using Plant Xylem. *PLoS ONE, 9*(2), e89934.

Bradshaw, J. E. (2010). *Root and Tuber Crops*: Springer New York.

Caird, M. A., Richards, J. H., & Donovan, L. A. (2007). Nighttime Stomatal Conductance and Transpiration in C(3) and C(4) Plants. *Plant Physiology, 143*(1), 4-10. doi: 10.1104/pp.106.092940

Canny, M. J. (1995). A New Theory for the Ascent of Sap—Cohesion Supported by Tissue Pressure. *Annals of Botany, 75*(4), 343-357. doi: 10.1006/anbo.1995.1032

Cernusak, L. A., Arthur, D. J., Pate, J. S., & Farquhar, G. D. (2003). Water relations link carbon and oxygen isotope discrimination to phloem sap sugar concentration in Eucalyptus globulus. *Plant Physiol, 131*(4), 1544-1554. doi: 10.1104/pp.102.016303

Cochard, H., & Tyree, M. T. (1990). Xylem dysfunction in Quercus: vessel sizes, tyloses, cavitation and seasonal changes in embolism. *Tree Physiol, 6*(4), 393-407.

Coupel-Ledru, A., Lebon, E., Christophe, A., Gallo, A., Gago, P., Pantin, F., Doligez, A., & Simonneau, T. (2016). Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. *Proc Natl Acad Sci U S A, 113*(32), 8963-8968. doi: 10.1073/pnas.1600826113

Dai, A. (2011). Drought under global warming: a review. *Wiley Interdisciplinary Reviews: Climate Change, 2*(1), 45-65. doi: 10.1002/wcc.81

De Boer, A. H., & Volkov, V. (2003). Logistics of water and salt transport through the plant: structure and functioning of the xylem. *Plant, Cell & Environment, 26*(1), 87-101. doi: 10.1046/j.1365-3040.2003.00930.x

De Schepper, V., De Swaef, T., Bauweraerts, I., & Steppe, K. (2013). Phloem transport: a review of mechanisms and controls. *Journal of Experimental Botany*. doi: 10.1093/jxb/ert302

Die, J. V. (1962). The distribution of carbohydrates in root and stem tissues of the tomato plant. *Acta Botanica Neerlandica, 2*, 418-424.

Dixon, H. H., & Joly, J. (1894). On the ascent of sap. *Annals of Botany 8*, 468–470.

Duarte-Delgado, D., Narváez-Cuenca, C.-E., Restrepo-Sánchez, L.-P., Kushalappa, A., & Mosquera-Vásquez, T. (2015). Development and validation of a liquid chromatographic method to quantify sucrose, glucose, and fructose in tubers of Solanum tuberosum Group Phureja. *Journal of Chromatography B, 975*, 18-23. doi: https://doi.org/10.1016/j.jchromb.2014.10.039

Eisenstein, M. (2013). Plant breeding: Discovery in a dry spell. *501*(7468), S7-S9.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development, 29*(1), 185-212. doi: 10.1051/agro:2008021

Farrar, J. F. (1993). Sink strength: What is it and how do we measure it? A summary. *Plant, Cell & Environment, 16*(9), 1045-1046. doi: 10.1111/j.1365-3040.1996.tb02061.x

Feist, P., & Hummon, A. B. (2015). Proteomic challenges: sample preparation techniques for microgram-quantity protein analysis from biological samples. *Int J Mol Sci, 16*(2), 3537-3563. doi: 10.3390/ijms16023537

Gartner, B. L. (1995). *Plant Stems: Physiology and Functional Morphology*: Elsevier Science.

Giordano, R., Salleo, A., Salleo, S., & Wanderlingh, F. (1978). Flow in xylem vessels and Poiseuille's law. *Canadian Journal of Botany, 56*(3), 333-338.

Gleason, S. M., Westoby, M., Jansen, S., Choat, B., Hacke, U. G., Pratt, R. B., Bhaskar, R., Brodribb, T. J., Bucci, S. J., Cao, K.-F., Cochard, H., Delzon, S., Domec, J.-C., Fan, Z.-X., Feild, T. S., Jacobsen, A. L., Johnson, D. M., Lens, F., Maherali, H., Martínez-Vilalta, J., Mayr, S., McCulloh, K. A., Mencuccini, M., Mitchell, P. J., Morris, H., Nardini, A., Pittermann, J., Plavcová, L., Schreiber, S. G., Sperry, J. S., Wright, I. J., & Zanne, A. E. (2016). Weak tradeoff between xylem safety and xylem-specific hydraulic efficiency across the world's woody plant species. *New Phytologist, 209*(1), 123-136. doi: 10.1111/nph.13646

Grayson, M. (2013). Agriculture and drought. *501*(7468), S1-S1.

Haverkort, A. J., de Ruijter, F. J., van Evert, F. K., Conijn, J. G., & Rutgers, B. (2013). Worldwide Sustainability Hotspots in Potato Cultivation. 1. Identification and Mapping. *Potato Research, 56*(4), 343-353. doi: 10.1007/s11540-013-9247-8

Haverkort, A. J., & Goudriaan, J. (1994). Perspectives of improved tolerance of drought in crops. *Aspects of Applied Biology, 38*.

Haverkort, A. J., & MacKerron, D. K. L. (2012). *Potato Ecology And modelling of crops under conditions limiting growth: Proceedings of the Second International Potato Modeling Conference, held in Wageningen 17–19 May, 1994*: Springer Netherlands.

Heffernan, O. (2013). The dry facts. *501*(7468), S2-S3.

Heizmann, U., Kreuzwieser, J., Schnitzler, J. P., Brüggemann, N., & Rennenberg, H. (2001). Assimilate Transport in the Xylem Sap of Pedunculate Oak (Quercus robur) Saplings. *Plant Biology, 3*(2), 132-138. doi: 10.1055/s-2001-12898

Herbers, K., & Sonnewald, U. (1998). Molecular determinants of sink strength. *Curr Opin Plant Biol, 1*(3), 207-216.

Hijaz, F., & Killiny, N. (2014). Collection and Chemical Composition of Phloem Sap from <italic>Citrus sinensis</italic> L. Osbeck (Sweet Orange). *PLoS ONE, 9*(7), e101830.

Hijmans, R. J. (2003). The effect of climate change on global potato production. . *Am J Potato Res 80*, 271–279.

Holbrook, N. M., & Zwieniecki, M. A. (2011). *Vascular Transport in Plants*: Elsevier Science.

Holtta, T., Cochard, H., Nikinmaa, E., & Mencuccini, M. (2009). Capacitive effect of cavitation in xylem conduits: results from a dynamic model. *Plant Cell Environ, 32*(1), 10-21. doi: 10.1111/j.1365-3040.2008.01894.x

Homan, N. M., Windt, C. W., Vergeldt, F. J., Gerkema, E., & Van As, H. (2007). 0.7 and 3 T MRI and Sap Flow in Intact Trees: Xylem and Phloem in Action. *Applied Magnetic Resonance, 32*(1), 157-170. doi: 10.1007/s00723-007-0014-3

Iwona, M., Sławomir, B., Magda, F., & Lech, R. (Eds.). (2012). *Plant Responses to Sugar Starvation, Carbohydrates*: InTech, .

Jacobsen, A. L., Agenbag, L., Esler, K. J., Pratt, R. B., Ewers, F. W., & Davis, S. D. (2007). Xylem density, biomechanics and anatomical traits correlate with water stress in 17 evergreen shrub species of the Mediterranean-type climate region of South Africa. *Journal of Ecology, 95*(1), 171-183. doi: 10.1111/j.1365-2745.2006.01186.x

Jensen, K., Mullendore, D., Holbrook, N. M., Bohr, T., Knoblauch, M., & Bruus, H. (2012). Modeling the hydrodynamics of phloem sieve plates. *Frontiers in Plant Science, 3*(151). doi: 10.3389/fpls.2012.00151

Jensen, K. H., Savage, J. A., & Holbrook, N. M. (2013). Optimal concentration for sugar transport in plants. *Journal of The Royal Society Interface, 10*(83). doi: 10.1098/rsif.2013.0055

Johnson, R. W., Dixon, M. A., & Lee, D. R. (1992). Water relations of the tomato during fruit growth. *Plant, Cell & Environment, 15*(8), 947-953. doi: 10.1111/j.1365-3040.1992.tb01027.x

Karley, A. J., Douglas, A. E., & Parker, W. E. (2002). Amino acid composition and nutritional quality of potato leaf phloem sap for aphids. *J Exp Biol, 205*(Pt 19), 3009-3018.

Katny, M. A., Hoffmann-Thoma, G., Schrier, A. A., Fangmeier, A., Jager, H. J., & van Bel, A. J. (2005). Increase of photosynthesis and starch in potato under elevated CO2 is dependent on leaf age. *J Plant Physiol, 162*(4), 429-438. doi: 10.1016/j.jplph.2004.07.005

Kim, H. K., Park, J., & Hwang, I. (2014). Investigating water transport through the xylem network in vascular plants. *Journal of Experimental Botany, 65*(7), 1895-1904.

Kim, Y.-H., Kim, C. Y., Song, W.-K., Park, D.-S., Kwon, S.-Y., Lee, H.-S., Bang, J.-W., & Kwak, S.-S. (2008). Overexpression of sweetpotato swpa4 peroxidase results in increased hydrogen peroxide production and enhances stress tolerance in tobacco. *Planta, 227*(4), 867-881. doi: 10.1007/s00425-007-0663-3

Kinkade, K. E. (1987). Boehringer Mannheim Organization. *Bio/Technology, 5*, 1339.

Knoblauch, M., & Peters, W. S. (2010). Munch, morphology, microfluidics - our structural problem with the phloem. *Plant Cell Environ, 33*(9), 1439-1452. doi: 10.1111/j.1365-3040.2010.02177.x

Knoblauch, M., & Peters, W. S. (2013). Long-distance translocation of photosynthates: a primer. *Photosynthesis Research, 117*(1), 189-196. doi: 10.1007/s11120-013-9867-5

Koch, G. W., Sillett, S. C., Jennings, G. M., & Davis, S. D. (2004). The limits to tree height. *Nature, 428*(6985), 851-854. doi: 10.1038/nature02417

Köcher, P., Gebauer, T., Horna, V., & Leuschner, C. (2009). Leaf water status and stem xylem flux in relation to soil drought in five temperate broad-leaved tree species with contrasting water use strategies. *Annals of Forest Science, 66*(1), 101-101. doi: 10.1051/forest/2008076

Kooman, P. L., & Rabbinge, R. (1996). An Analysis of the Relation between Dry Matter Allocation to the Tuber and Earliness of a Potato Crop. *Annals of Botany, 77*, 235 - 242.

Lampinen, M. J., & Noponen, T. (2003). Thermodynamic analysis of the interaction of the xylem water and phloem sugar solution and its significance for the cohesion theory. *Journal of Theoretical Biology, 224*(3), 285-298. doi: http://dx.doi.org/10.1016/S0022-5193(03)00165-6

Lastdrager, J., Hanson, J., & Smeekens, S. (2014). Sugar signals and the control of plant growth and development. *J Exp Bot, 65*(3), 799-807. doi: 10.1093/jxb/ert474

Lemoine, R., Camera, S. L., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N., Bonnemain, J.-L., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrilla, J., & Durand, M. (2013). Source-to-sink transport

of sugar and regulation by environmental factors. *Frontiers in Plant Science, 4*, 272. doi: 10.3389/fpls.2013.00272

Ljung, K. (2013). Auxin metabolism and homeostasis during plant development. *Development, 140*(5), 943-950. doi: 10.1242/dev.086363

Lovisolo, C., & schubert, A. (1998). Effects of water stress on vessel size and xylem hydraulic conductivity in Vitis vinifera L. . *Journal of Experimental Botany, 49*(321), 693-700.

Luitel, B. P., Khatri, B. B., Choudhary, D., Paudel, B. P., Jung-Sook, S., Hur, O., Baek , H. J., Cheol, K. H., & Yul, R. K. (2015). Growth and yield characters of potato genotypes grown in drought and irrigated conditions of Nepal. *Int J Appl Sci Biotechnol,, Vol 3* ((3)), 513-519. doi: DOI: 10.3126/ijasbt.v3i3.13347

Marcelis, L. (1996). Sink strength as a determinant of dry matter partitioning in the whole plant. *Journal of Experimental Botany, 47*(suppl 1), 1281.

Melcher, P. J., Zwieniecki, M. A., & Holbrook, N. M. (2003). Vulnerability of Xylem Vessels to Cavitation in Sugar Maple. Scaling from Individual Vessels to Whole Branches. *Plant Physiology, 131*(4), 1775-1780. doi: 10.1104/pp.102.012856

Munch, E. (1930). Die Stoffbewegungen in der Pflanze. *Fischer, Jena*.

Myburg, A. A., Lev-Yadun, S., & Sederoff, R. R. (2001). Xylem Structure and Function *eLS*: John Wiley & Sons, Ltd.

Niklas, K. J. (2007). Maximum plant height and the biophysical factors that limit it. *Tree Physiol, 27*(3), 433-440.

Obidiegwu, J. E., Bryan, G. J., Jones, H. G., & Prashar, A. (2015). Coping with drought: stress and adaptive responses in potato and perspectives for improvement. *Frontiers in Plant Science, 6*, 542. doi: 10.3389/fpls.2015.00542

Oparka, K. J., & Turgeon, R. (1999). Sieve elements and companion cells-traffic control centers of the phloem. *The Plant Cell, 11*(4), 739-750.

Osakabe, Y., Osakabe, K., Shinozaki, K., & Tran, L.-S. P. (2014). Response of plants to water stress. *Frontiers in Plant Science, 5*, 86. doi: 10.3389/fpls.2014.00086

Pockman, W. T., & Sperry, J. S. (2000). Vulnerability to xylem cavitation and the distribution of sonoran desert vegetation. *American Journal of Botany, 87*(9), 1287–1299.

Prusova, A. (2016). *Light on phloem (an MRI approach).* (Ph.D.), Wageningen UR, Wageningen.

Ruan, Y.-L. (Ed.). (2010). *Phloem transport* (2nd ed.). New Zealand: New Zealand Society of Plant Biologists.

Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics, 18*(1), 529. doi: 10.1186/s12859-017-1934-z

Ryan, M. G., & Asao, S. (2014). Phloem transport in trees. *Tree Physiol, 34*(1), 1-4. doi: 10.1093/treephys/tpt123

Sánchez-Aguayo, I., Rodríguez-Galán, J. M., García, R., Torreblanca, J., & Pardo, J. M. (2004). Salt stress enhances xylem development and expression of S-adenosyl-l-methionine synthase in lignifying tissues of tomato plants. *Planta, 220*(2), 278-285. doi: 10.1007/s00425-004-1350-2

Schapendonk, A. H. C. M., Spitters, C. J. T., & Groot, P. J. (1989). Effects of water stress on photosynthesis and chlorophyll fluorescence of five potato cultivars. *Potato Research, 32*(1), 17-32. doi: 10.1007/bf02365814

Scheenen, T. W., van Dusschoten, D., de Jager, P. A., & Van As, H. (2000). Microscopic displacement imaging with pulsed field gradient turbo spin-echo NMR. *J Magn Reson, 142*(2), 207-215. doi: 10.1006/jmre.1999.1916

Schuldt, B. (2008). *Seasonal response of tree xylem flux to climatic variation and experimental drought in Central Sulawesi.* Paper presented at the Tropical Rainforests and Agroforests Under Global Change: Proceedings ; International Symposium, October 5 - 9, 2008., Kuta, Bali, Indonesia.

Schulz, A. (1998). Phloem. Structure Related to Function. In H. D. Behnke, K. Esser, J. W. Kadereit, U. Lüttge, & M. Runge (Eds.), *Progress in Botany: Genetics Cell Biology and Physiology Ecology and Vegetation Science* (pp. 429-475). Berlin, Heidelberg: Springer Berlin Heidelberg.

Schulz, A., & Thompson, G. A. (2001). Phloem Structure and Function *eLS*: John Wiley & Sons, Ltd.

Sevanto, S. (2014). Phloem transport and drought. *J Exp Bot, 65*(7), 1751-1759. doi: 10.1093/jxb/ert467

Sevanto, S., HÖLttÄ, T., & Holbrook, N. M. (2011). Effects of the hydraulic coupling between xylem and phloem on diurnal phloem diameter variation. *Plant, Cell & Environment, 34*(4), 690-703. doi: 10.1111/j.1365-3040.2011.02275.x

Sheikholeslam, S. N., & Currier, H. B. (1977). Effect of Water Stress on Turgor Differences and 14C-Assimilate Movement in Phloem of Ecballium elaterium. *Plant Physiology, 59*(3), 381-383. doi: 10.2307/4264742

Snyder, K. A., Richards, J. H., & Donovan, L. A. (2003). Night-time conductance in C3 and C4 species: do plants lose water at night? *Journal of Experimental Botany, 54*(383), 861-865. doi: 10.1093/jxb/erg082

Spanner, D. C. (1970). The Electro-osmotic Theory of Phloem Transport in the Light of Recent Measurements on Heracleum Phloem. *Journal of Experimental Botany, 21*(2), 325-334. doi: 10.1093/jxb/21.2.325

Sperry, J. S., Nichols, K. L., June, E. M. S., & Eastlack, S. E. (1994). Xylem Embolism in Ring-Porous, Diffuse-Porous, and Coniferous Trees of Northern Utah and Interior Alaska. *Ecology, 75*(6), 1736-1752. doi: 10.2307/1939633

Thaine, R. (1969). Movement of Sugars through Plants by Cytoplasmic Pumping. *222*(5196), 873-875.

Thompson, M. V., & Holbrook, N. M. (2003). Scaling phloem transport: water potential equilibrium and osmoregulatory flow. *Plant, Cell & Environment, 26*(9), 1561-1577. doi: 10.1046/j.1365-3040.2003.01080.x

Torres-Ruiz, J. M., Diaz-Espejo, A., Chamorro, V., Fernández, J. E., Sebastiani, L., Minnocci, A., & Infante, J. M. (2011). *Influence of the water treatment on the xylem anatomy and functionality of current year shoots of olive trees.* Paper presented at the Acta Horticulturae.

Twumasi, P., Ieperen, W. v., Woltering, E. J., Emons, A. M. C., Schel, J. H. N., Meeteren, U. v., & Marwijk, D. v. (2005). Effects of water stress during growth of xylem anatomy,

xylem functioning and vase life in three Zinnia elegans cultivars. *Acta Horticulturae, 669*, 303-312.

Tyree, M. T., & Sperry, J. S. (1989). Vulnerability of Xylem to Cavitation and Embolism. *Annual Review of Plant Physiology and Plant Molecular Biology, 40*, 19-36. doi: 10.1146/annurev.pp.40.060189.000315

Tyree, M. T., & Zimmermann, M. H. (2013). *Xylem Structure and the Ascent of Sap*: Springer Berlin Heidelberg.

Van As, H. (2006). Intact plant MRI for the study of cell water relations, membrane permeability, cell-to-cell and long distance water transport. *Journal of Experimental Botany, 58*(4), 743-756.

Van Bel, A. J. E. (2003a). The phloem, a miracle of ingenuity. *Plant, Cell & Environment, 26*(1), 125-149. doi: 10.1046/j.1365-3040.2003.00963.x

van Bel, A. J. E. (2003b). Transport Phloem: Low Profile, High Impact. *Plant Physiology, 131*(4), 1509-1510.

van Bel, A. J. E., & Hess, P. H. (2008). Hexoses as phloem transport sugars: the end of a dogma? *Journal of Experimental Botany, 59*(2), 261-272. doi: 10.1093/jxb/erm294

Venugopal, S. (2016). *Biology-vol-II*.

Vilagrosa, A., Chirino, E., Peguero-Pina, J. J., Barigah, T. S., Cochard, H., & Gil-Pelegrín, E. (2012). Xylem Cavitation and Embolism in Plants Living in Water-Limited Ecosystems. In R. Aroca (Ed.), *Plant Responses to Drought Stress: From Morphological to Molecular Features* (pp. 63-109). Berlin, Heidelberg: Springer Berlin Heidelberg.

Vos, J., & Oyarzun, P. J. (1987). Photosynthesis and stomatal conductance of potato leaves-effects of leaf age, irradiance, and leaf water potential. *Photosynth Res, 11*(3), 253-264. doi: 10.1007/bf00055065

West, G. B., Brown, J. H., & Enquist, B. J. (1999). A general model for the structure and allometry of plant vascular systems. *Nature, 400*, 664. doi: 10.1038/23251

Wheeler, R. M., Mackowiak, C. L., Yorio, N. C., & Sager, J. C. (1999). Effects of CO2 on stomatal conductance: do stomata open at very high CO2 concentrations? *Ann Bot, 83*(3), 243-251. doi: 10.1006/anbo.1998.0813

Windt, C. W., Vergeldt, F. J., de Jager, P. A., & van As, H. (2006). MRI of long-distance water transport: a comparison of the phloem and xylem flow characteristics and dynamics in poplar, castor bean, tomato and tobacco. *Plant Cell Environ, 29*(9), 1715-1729. doi: 10.1111/j.1365-3040.2006.01544.x

Wolswinkel, P. (1984). Phloem unloading and 'sink strength': The parallel between the site of attachment of Cuscuta and developing legume seeds. *Plant Growth Regulation, 2*(4), 309-317. doi: 10.1007/bf00027290

Yan, Z., Li, P., Chen, Y., Han, W., & Fang, J. (2016). Nutrient allocation strategies of woody plants: an approach from the scaling of nitrogen and phosphorus between twig stems and leaves. *Scientific Reports, 6*, 20099. doi: 10.1038/srep20099

https://www.nature.com/articles/srep20099#supplementary-information

Zhang, Y.-J., Rockwell, F. E., Graham, A. C., Alexander, T., & Holbrook, N. M. (2016). Reversible Leaf Xylem Collapse: A Potential "Circuit Breaker" against Cavitation. *Plant Physiology, 172*(4), 2261-2274. doi: 10.1104/pp.16.01191

Zheng, X., Jitsuyama, Y., Terauchi, T., & Iwama, K. (2009). Effects of Drought and Shading on Non-structural Carbohydrate Stored in the Stem of Potato (Solanum tuberosum L.). *Plant Production Science, 12*(4), 449-452. doi: 10.1626/pps.12.449

Zwieniecki, M. A., Melcher, P. J., Feild, T. S., & Holbrook, N. M. (2004). A potential role for xylem–phloem interactions in the hydraulic architecture of trees: effects of phloem girdling on xylem hydraulic conductance. *Tree Physiol, 24*(8), 911-917. doi: 10.1093/treephys/24.8.911

EXPRESSION ANALYSES OF DROUGHT-STRESSED POTATO

Ernest B Aliche[1,2], Tim Gengler[1], Marian Oortwijn[1], Christian W. B. Bachem[1], Richard G. F. Visser[1], Theo Borm[1], C. Gerard van der Linden[1]

[1]Plant Breeding, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen.

[2] Graduate School Experimental Plant Sciences, Droevendaalsesteeg 1, 6708 PB, Wageningen

ABSTRACT

Drought stress tolerance is a complex trait of high importance in potato. The holistic genetic factors that contribute to drought response in the plant can hardly be captured by a simple study of candidate genes. However, whole-transcriptome analyses offer a more reliable approach of confirming known candidate genes and finding novel genes that are involved in drought responses of the plant. This approach was used in five cultivated potatoes to study the myriads of gene expression regulations that take place in leaf and tuber tissues at two time points during drought stress. Phenotypic measurements of shoot weight, tuber weight and stomatal conductance were scored during the growing season. The cultivars generally invested more in their tuber weight under stress in the earlier time point of drought than in their shoot weight. A paired-end RNA-seq dataset was analysed using the Tuxedo pipeline. Generally, the downregulated differentially expressed genes (DEGs) exceeded the upregulated DEGs. The plants under drought downregulated defence response genes to biotic agents and stress response genes to other abiotic stresses apart from drought-related stresses. The cultivars had their respective unique drought response DEGs, though Lady Rosetta and Jaerla shared more similarity in DEGs in the tuber and both cultivars also shared similar biomass measurements. We found association between the differential expression of a gene that regulates stomatal closure and maintenance of stomatal conductance under drought. A hormonal crosstalk between abscisic acid-, gibberellic acid- and cytokinin-mediated signalling pathways might be involved in signal transduction during the drought.

INTRODUCTION

Potato is an important food crop consumed by more than a billion people globally (CIP, 2013). According to FAOSTAT the global annual production of potato in 2013 was 368 million tonnes (FAOSTAT, 2014). The average global farm yield of potato was estimated at 18.4 tonnes per hectare (FAOSTAT, 2014; Haverkort et al., 2013). Potato contains most of the important vitamins and nutrients, and supports life better than most other crops (Davidson & Passmore, 1963; Reader, 2008). This makes it a relevant crop for balanced diets in the developed world and for food security in developing countries. Potato requires a maintenance of high soil moisture content at all stages of its growth in order to obtain high yields (Loon, 1981; Singh, 1969). Under such conditions of water availability, it is an efficient water user (Ati et al., 2012; Fakhari et al., 2013). However, under water limiting conditions (drought), potato yield is significantly reduced (Cantore et al., 2014).

Drought is a stress that results from an insufficiency of water. Drought is not a single or simple stress, but a complex stress that often predisposes the plant to other stresses (Whitmore & Whalley, 2009). The uncertainties of drought timing, duration and severity, which are caused by climate change, are part of the complexity in understanding drought stress (Hosseinizadeh et al., 2015; Jenkins & Warren, 2015). However, the major complexity of drought stress has been associated with the genomic composition of the plant (Blum, 2011). In cultivated potato, this includes the tetraploid genetics that makes the breeding for drought tolerance difficult due to the complex inheritance of traits after genetic crosses. Also, Anithakumari *et al.* (2011) demonstrated in diploid potato population that drought is controlled by many loci on the

genome that individually make small contributions to drought responses (Anithakumari et al., 2011). Therefore, mapping drought tolerance QTLs in the genome of cultivated potato may be quite complex and hardly exhaustive. In order to understand the basis of the drought response mechanisms in potato as well as in other crops, molecular research approaches have been adopted. These have resulted in the identification of genes that regulate various aspects of drought response (Dongjin et al., 2011; Gazendam et al., 2016; Obidiegwu et al., 2015; Szalonek et al., 2015; Vasquez-Robinet et al., 2008; Xu et al., 2014). However, the regulation of these drought-associated genes and their interactions at the molecular level are not yet clearly understood, at least partly because these genes are part of networks and act in concert (Ambrosone et al., 2017; Pieczynski et al., 2018), and most functional annotations depend on the over-expression or knock-down of single genes. These transgenic approaches, though informative, may not enhance our knowledge of the multiple interactions that affect the functionality of the single genes. Therefore, comprehensive molecular approaches that incorporate the entire genome of the plant are being implemented in the study of molecular mechanisms. These whole-genome-based molecular investigations have been made even more feasible by advancements in sequencing technology (Heather & Chain, 2016).

Through RNA sequencing, the transcripts from all active genes and their splice variants can be studied in depth (Anjum et al., 2016; Hoeijmakers et al., 2013; Li et al., 2015b; Morozova et al., 2009; Risso et al., 2014; Wang et al., 2009; Yang et al., 2016; Zyprych-Walczak et al., 2015). This transcriptomic or RNA-sequencing (RNA-seq) approaches reveal Differentially Expressed Genes (DEGs) that have been previously annotated, but also facilitates the discovery of novel transcripts (Liu et al., 2015b). In potato, RNA-seq has been applied in studies of the drought stress response of stolon tissues (Gong et al., 2015). The stolon tissues of the potato plant (*cv*. Ningshu 4) showed myriads of upregulated transcripts including heat shock proteins, dehydrins, aquaporin, protein phosphatases, sugar transporters and starch biosynthesis genes. Down-regulated transcripts included lipid transfer proteins, peroxidases and gibberellin-synthesis genes. In another transcriptomic study of the drought response in a diploid potato mapping population using microarrays, a transcriptional network of interactions among many genes was unveiled by a systems genetics approach (van Muijen et al., 2016). The integrative transcriptome, genetic and genomic analyses in that study led to the discovery of a master regulatory gene under drought, nuclear factor Y subunit C4 (*NFY-C4*), upstream of the potato drought response cascade. Also, a downstream gene, *TAS14* (an ABA-inducible dehydrin), was strongly induced by drought and correlated with drought recovery potential (van Muijen et al., 2016). Transcriptome analysis was also used to investigate variations in drought response mechanisms between two cultivars grown in the field (Evers et al., 2010). The field drought in Evers et al. (2010) repressed photosynthesis- and carbohydrate metabolism-related genes earlier in the sensitive genotype; and at longer duration of drought and these authors observed an induction of raffinose biosynthesis genes. Thus, transcriptomic analysis can be used to unveil networks of genes and pathways involved in drought response. In order to gain a more representative insight into the molecular drought response of potato, there is need to expand the study scope to include more genotypes, plant tissues and different plant growth stages.

In the present study, we used RNA-seq analysis to evaluate five different genotypes of cultivated potato for drought responses at two different time points and in two different tissues, mature leaf and young tuber. Our objective was to investigate the molecular basis for the contrasting drought responses we observed in the phenotypic study of these cultivars. We hypothesize that different molecular networks may play a role in potato during different time points and between the leaf and tuber tissues under drought stress. The outcomes of this study give insights on what pathways and molecular networks need to be prioritized for drought tolerance breeding in potato.

MATERIALS AND METHODS

Planting and drought application

A drought trial was conducted with seven potato cultivars selected based on their contrasting drought responses from previous experiments (Chapters 2 - 5) (Table 1). The cultivars are Bintje, Biogold, Hansa, Jaerla, Lady Rosetta, Mondial and Nicola. Potato seed tubers were obtained from the Dutch potato breeding companies HZPC Holland BV, C. Meijer, KWS POTATO and Averis seeds B.V. The seed tubers were pre-sprouted prior to sowing in ridges under a tunnel in the field at Unifarm, Wageningen University & Research. The ridges were set on clay soil and the field structure included two blocks: irrigated (WR) and non-irrigated (DR) treatments. In each block, the cultivars were randomized as plots within the blocks. Four plants of each genotype were planted in each plot. The spacing between plants in a row was 30cm and 75cm between rows. Border plants were planted in between plots in each row. Flower Power sensors (Parrot^{R}) were used to monitor environmental conditions: soil water content, temperature and light intensity. The irrigated and non-irrigated blocks were regularly given water by sprinkler irrigation from the planting date (22 June, 2016) till two weeks after emergence (22 July, 2016). Subsequently, irrigation was withheld from the non-irrigated block. The development of drought stress in the non-irrigated block was monitored using the sensors. Also, leaf tissues of the potato plants were sampled at several time-points for *TAS14* mRNA (known to be a good indicator for drought stress from a previous study, van Muijen et al 2016) expression analysis to monitor the development of drought stress in the plants.

Table 1: Drought response characteristics of the cultivars used in this study

Cultivars	Drought responses
Bintje	Relatively stable tuber number under drought in field trials (tolerant)
Biogold	Tuber formation under severe drought in the greenhouse (tolerant)
Hansa	High number of small-sized tubers under irrigation and drought conditions (sensitive)
Jaerla	Relatively high yield under early drought (Connantre 2014) (tolerant)
Lady Rosetta	Relatively high yield under early drought (Connantre 2014) (tolerant)
Mondial	High yielding under irrigation and yield losses during drought (sensitive)
Nicola	High number of tubers of different small sizes (sensitive)

Data collection and tissue sampling

Prior to drought application, canopy pictures were taken in order to evaluate the uniformity of plant growth. The pictures were taken with a digital camera mounted on a frame. After 28 days of drought application (28DOD), physiological traits (stomatal conductance and chlorophyll content) were scored. 28DOD was the first time point of data/tissue sampling, and a second sampling time point was at 56 days after drought application (56DOD). Fully expanded source leaves (5^{th} -6^{th} leaf from the apical meristem) and young tubers (about 2-3 cm diameter) were collected in aluminium foil at both time points. The tissues were immediately snap-frozen in liquid nitrogen and transferred to -80^{o}C until subsequent molecular analysis. The remaining tissues of the plant were harvested and the fresh weights of the shoot and tuber tissues were measured at the two time points. Tuber number was also counted and tuber sizes were graded. The shoot and tuber tissues were oven-dried at 105^{o}C for 72hrs and the dry weights were measured as well.

Sample preparation for RNA sequencing

Five of the cultivars were used for the RNA-seq study: Biogold, Hansa, Jaerla, Lady Rosetta and Nicola. The other two cultivars, Bintje and Mondial, were infected with late blight in the field. Therefore, we decided not to continue with them in the experiment. For RNA isolation, 50 mg of leaf or tuber tissues collected at 28DOD and 56DOD were homogenized in liquid nitrogen with a mortar. There was a total of 80 samples (5 cultivars x 2 treatments x 2 tissues x 2 time points x 2 plot replicates). RNA extraction was done by the addition of 500µl of Trizol for cell lysis and inhibition of the RNase activity. Spin columns were used to extract the RNA as described in the Qiagen RNeasy mini kit protocol (Qiagen, 2005). RNA was eluted from the columns with 30µl of RNase-free water. After one minute of incubation at room temperature the column was spun down at maximum speed to elute the RNA into a new 1.5ml Eppendorf tube. Qubit and Nanodrop measurements were used to determine the amount and quality of total RNA. Library preparation and paired-end Illumina sequencing were outsourced to Beijing Genomics Institute (BGI), Hong Kong.

Data Analyses

The Tuxedo pipeline was used for the RNA-seq analyses (Supplementary Fig.SF1) (Trapnell et al., 2012). The reads were aligned to the potato reference genome (DM-pseudomolecules v4.06) using Tophat version 2.1.1, which uses Bowtie-2.2 and Bowtie2Index files in the background for mapping reads within exons (Trapnell et al., 2012). For reads that span splice junctions, Tophat estimates the junction's splice sites and builds an index of splice sites in the transcriptome, thus mapping all reads appropriately. The mapped reads were assembled into transcripts using Cufflinks-2.2.1 (Trapnell et al., 2012). The resulting assemblies were merged together parsimoniously for each sample using the Cuffmerge package. Differential expression of transcripts in the merged assemblies was computed using the Cuffdiff package. The RNA-seq analysis was implemented with documentations in Linux OS (Debian). Graphical interpretations of the RNA-seq data were visualized using cummeRbund package in R studio version 3.0.1. We used the resulting Differentially Expressed Genes (DEGs) to do a hierarchical clustering of the cultivars for each tissue and time point by using the nearest neighbour joining

clustering method in StatistiXL version 2.0. Subsequently, we used Gene Ontology (GO-terms) generated from a comparison of six functional annotation pipelines (Trinotate HMM, Trinotate BLAST, OrthoMCL-UniProt, BLAST2GO, Phytozome and InterPro2GO) for a functional analysis of the DEGs in our RNA-seq dataset (Amar et al., 2014). The datasets generated from the phenotypic observations were analysed for genotypic and treatment variation using GENSTAT 17th edition.

RESULTS

Drought stress monitors

Drought was applied in our field trial by withholding irrigation from the drought-stressed block. We monitored soil water content percentage throughout the growth period. According to the data from the Parrot Flower Power environmental sensors, the applied drought coincided with the tuber initiation and tuber bulking stages of plant growth. The scale of soil moisture content range of the Parrot Flower Power was between 8% (very dry) to 45% (saturated) soil moisture content. For our study, we defined a field drought (water limitation) at 25% moisture level, and it progressed in severity until the final harvest of the plants (Fig.1). The control (irrigated) plants were maintained at 30% and above.

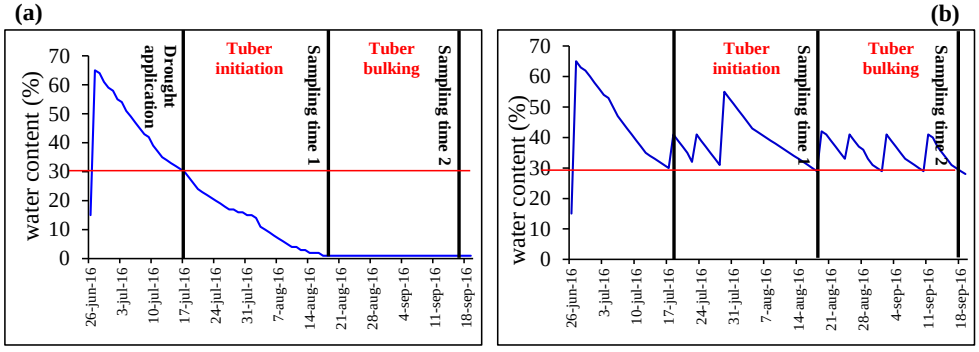


Figure 1: Soil water content percentage under (**a**) drought and (**b**) irrigated treatments. Data was collected from 6 sensors (Parrot Flower Power) from which the average was calculated.

Phenotypic response to drought (28DOD)

At 28DOD, plant canopy physiological and growth properties were impacted by the drought (Fig.2). The Shoot fresh weight in Hansa was most severely affected under drought (Fig.2a). Stomatal conductance was more reduced in Jaerla and Hansa than in the other cultivars, and least affected in Biogold (Fig.2b). The drought stress at 28DOD coincided with the tuberization stage of the potato phenology (Fig.1). Tuber weight of the cultivars was not reduced under drought at 28DOD (Fig.2d). However, tuber number per plant was affected especially in Nicola, Hansa and Biogold, while Lady Rosetta and Jaerla were less affected (Fig.2c).

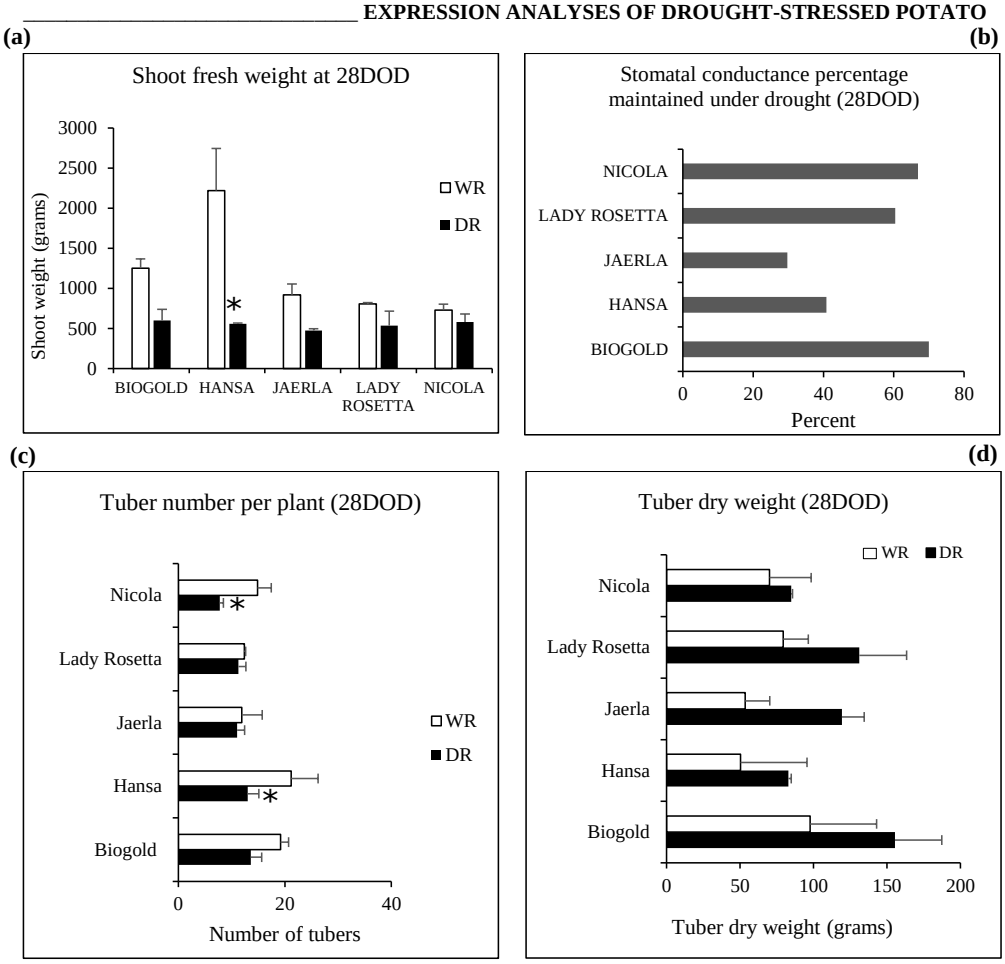


Figure 2: (**a**) Shoot fresh weight of cultivars under irrigation (WR) and drought (DR) at 28 DOD. Error bars represent standard error of the mean. Asterisk(s) represent significant difference between irrigated and drought-stressed plants ($p \leq 0.05$), (**b**) percentage of stomatal conductance under drought (28DOD) relative to irrigated condition, (**c**) number of tubers per plant at 28DOD, (**d**) tuber dry weight per plot at 28DOD. Error bars represent standard error of the means. Asterisks show significant difference between drought (DR) and irrigation (WR)

Overview of sequencing dataset

Among the 80 samples that were selected for sequencing, one sample failed in the library construction. RNA sequencing of the remaining 79 samples yielded reads of high quality phred scores that did not need trimming prior to the analyses. An average of 61million paired-end reads were produced for each sample. Each read length was 150bp resulting in a mean sequencing depth of 21.8x based on coverage computation (Lander & Waterman, 1988). After reads alignment, 60.6% of the reads on average mapped to the potato reference genome (Supplementary File 1). The number of differentially expressed genes (DEGs) between irrigated and non-irrigated conditions in the leaf and tuber tissues of all genotypes at the two time points are given in Table 2. Generally, downregulated genes outnumbered the upregulated genes

except at 56DOD in the leaf of Hansa and tuber of Lady Rosetta. We observed a remarkably high range of variation in the number of DEGs among the tissues and time points. Some samples did not yield DEGs while in others we observed thousands of DEGs. This variation in the number of DEGs may be attributed to two possibilities – some tissues or time points may have responded to the drought with less gene differential expression, or a low number of replicates (two) per sample in our study may have contributed to high deviations between replicates (Manga et al., 2016).

Consistent DEGs among Genotypes

We first investigated the DEGs for genes that were differentially expressed in all or most of the cultivars in the respective tissues and time points (Fig.3). These consistent DEGs likely represent genes that are generally essential in the drought response across the tested genotypic backgrounds. There were more consistent DEGs at 28DOD than at 56DOD, and the downregulated consistent DEGs outnumbered the upregulated consistent DEGs (Fig.3).

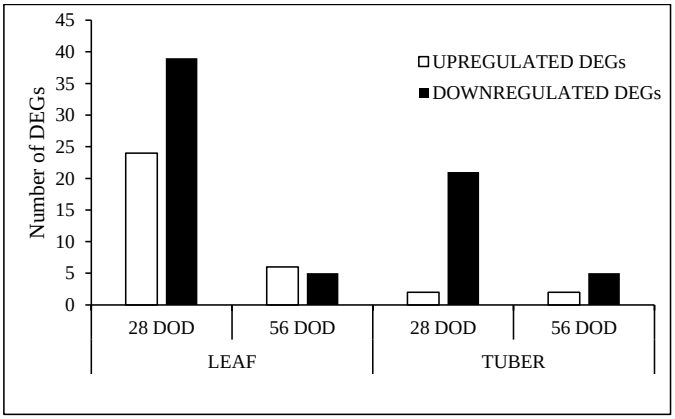


Figure 3: Number of DEGs consistent in all or most of the cultivars in leaf and tuber tissues at 28DOD and 56DOD.

Table 2: Number of DEGs, upregulated and downregulated genes, under drought in leaf and tuber tissues at two time points: (1) 28DOD and (2) 56DOD

Genotypes	Tissues	Time points	Total Genes	DEGs (#)	Upregulated Genes	Downregulated Genes
Biogold	Leaf	1	46,490	1,190 (1,387)	349	841
		2*	46,390	63 (94)	31	32
	Tuber	1	47,281	0	0	0
		2**	46,190	8,666 (10,856)	3,635	5,031
Hansa	Leaf	1	44,664	299 (372)	111	188
		2	45,368	73 (99)	46	27
	Tuber	1	45,548	151 (187)	13	138
		2	47,290	114 (128)	4	110
Jaerla	Leaf	1*	44,973	0	0	0
		2*	44,842	198(217)	8	190
	Tuber	1	47,064	8,912 (11,461)	3,389	5,523
		2**	48,896	341 (629)	87	254
Lady Rosetta	Leaf	1*	46,199	1,335 (1,868)	615	720
		2*	46,217	535 (634)	123	412
	Tuber	1	47,803	771 (1,087)	218	553
		2*	47,599	266 (296)	245	21
Nicola	Leaf	1	46,941	2,583 (3,604)	1,221	1,362
		2*	47,397	204(237)	59	145
	Tuber	1	46,821	156 (186)	39	117
		2	48,517	0	0	0

(#): the numbers in parenthesis represent the original output of DEGs that contained spurious genome coordinates including non-annotated regions and indistinguishably large number of genes. DOD means *days of drought*. * indicates time point or tissue in which at least a sample was of a low (Grade C) quality. ** indicates time point or tissue in which at least a sample was of the lowest (Grade D) quality

In the leaf tissues at 28DOD, the consistently upregulated genes included transcriptions factors, cytochrome P450, superoxide dismutase, abscisic acid and environmental stress-inducible protein (*TAS14*), delta 1-pyrroline-5-carboxylate synthetase, ninja-family protein, protein phosphatase 2C and an amino acid transporter. The consistently downregulated genes in leaves at 28DOD included defence response genes to biotic agents, extensin, non-specific lipid transfer proteins, an oligopeptide transporter, a UDP-galactose transporter, a major intrinsic protein, a MAP kinase kinase and peroxidases (Supplementary File 2). In the tuber tissues at 28DOD, the consistently upregulated genes were fructose-bisphosphate aldolase and glycine-rich protein; whereas the consistently downregulated genes included extensin, transcription factors like WRKYs, salt responsive proteins and ethylene-responsive element binding protein

(Supplementary File 2). At 56DOD, the consistently upregulated genes in leaf tissues were cytochrome P450, aquaporin, pectate lyase, polygalacturonase, a membrane protein and gibberellin-induced protein. The consistently downregulated genes were ethylene-responsive late embryogenesis-like protein, proline-rich protein, heat shock protein, pectin esterase and a DNA-binding protein (Supplementary File 2). In the tuber tissues at 56DOD the consistently upregulated genes were non-specific lipid transfer protein and abscisic acid and environmental stress-inducible protein dehydrin (*TAS14*), while the consistently downregulated genes were serine-pyruvate aminotransferase, apyrase, cysteine protease inhibitor, alkaline alpha-galactosidase seed imbibition protein and multicystatin (Supplementary File 2).

Highly expressed DEGs

We examined the DEGs of each cultivar for the most highly upregulated and most downregulated genes in the two tissues and time points (Supplementary File 3). For Biogold, we observed strong upregulation of genes related to ubiquitin-protein transferase activity, response to water deprivation and osmotic stress, and gibberellin degradation in the leaf at 28DOD. However, genes related to the defence response to biotic agents at this time point in the leaf were downregulated. Interestingly, at 56DOD genes encoding non-specific lipid transfer proteins in both leaf and tuber tissues were upregulated in Biogold. In Hansa, abscisic acid and environmental stress-inducible protein dehydrin (*TAS14*) and heat-shock proteins were highly upregulated to about 80- and 40-folds change, respectively, and genes involved in defence response to biotic agents were downregulated in leaf at 28DOD. A non-specific lipid-transfer protein was also highly upregulated by 30 folds in leaf at 56DOD. In the tuber of Hansa, fructose-bisphosphate aldolase was among the highest upregulated genes at 28DOD. Remarkably, a salt responsive protein in tuber was downregulated at 56DOD in Hansa. The highly upregulated DEGs of Jaerla included non-specific lipid transfer protein (11 folds) and abscisic acid and environmental stress-inducible protein dehydrin (*TAS14*) (75 folds). We observed a downregulation of an aquaporin TIP gene in leaf (28DOD) and tuber (56DOD) of Lady Rosetta. Furthermore, a methyl ketone synthase in leaf, and chlorophyll-associated genes and aldolase in tubers were upregulated at 28DOD in Lady Rosetta. Interestingly, a heat-shock protein was downregulated in the tuber of Nicola by 28 folds at 28DOD. The range of gene expression levels among the cultivars were high under irrigation (2770.03) and drought (37087.10), the lowest expression being zero in both conditions for some genes (Supplementary File 3).

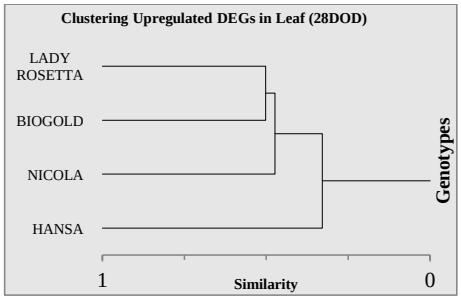
Comparison of phenotypic and molecular variation among cultivars

Due to the high level of differences in the number of DEGs among the samples, but also the genotypic difference in the highly expressed DEGs among cultivars in different tissues and at different time points, we decided to employ a forward genetics approach using the phenotypic traits collected during the experiment, to further investigate genotypic variation. This was done by combining phenotypes, hierarchical clustering of DEGs and GO-functional annotation of the DEGs.

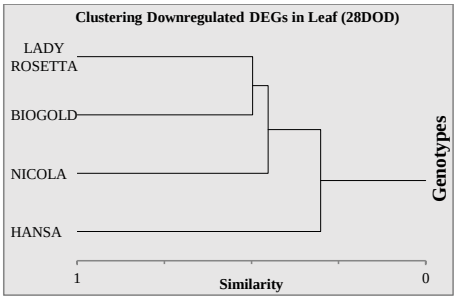
Canopy characteristics (28DOD)

Having observed phenotypic variations in shoot biomass and canopy stomatal conductance at 28DOD (Fig.2), we did a hierarchical clustering of the DEGs in leaf at this time point. The clustering showed Hansa as most distant from the rest of the cultivars at 28 DOD, indicating that the transcriptional changes induced by drought were different for this cultivar at this stage (Figs.4a and b). It should be noted that DEGs at this time point were surprisingly not detected in the leaf of Jaerla, which may be due to the significance cut-off settings we used across all samples in the analysis. However, we used all available DEGs for each cultivar at this time point, assuming that they were all equally stressed and may have responded according to their respective abilities to cope with drought. Thus, we considered the vast differences in the number of upregulated and downregulated DEGs among cultivars, as part of the variation in drought response.

(a)



(b)



(c) **Number of Upregulated DEGs (28DOD) - Leaf**

	B	H	J	L	N
B	349	32	0	189	197
H	32	111	0	37	75
J	0	0	0	0	0
L	189	37	0	615	349
N	197	75	0	349	1221

(d) **Number of Downregulated DEGs (28DOD) - Leaf**

	B	H	J	L	N
B	841	67	0	258	395
H	67	188	0	69	116
J	0	0	0	0	0
L	258	69	0	720	460
N	395	116	0	460	1362

Figure 4: (**a**) hierarchical cluster of upregulated DEGs in leaf at 28DOD, (**b**) hierarchical cluster of downregulated DEGs in leaf at 28DOD, (**c**) Number of upregulated DEGs overlapping between cultivars, (**d**) Number of downregulated DEGs overlapping between cultivars. The letters in (c) and (d) represent the cultivars: B (Biogold), H (Hansa), J (Jaerla), L (Lady Rosetta) and N (Nicola).

Based on the phenotypic differences (severe effects of drought on shoot weight and stomatal conductance in leaves at 28DOD (Fig.2)) and the hierarchical clustering, we chose to

investigate the molecular response of Hansa in more detail. We compared the drought response of Hansa with that of Biogold, which was the least affected in stomatal conductance under drought. Using the GO-based functional annotation, we found in the leaf tissue of Biogold that the (Extracellular Ca^{2+} sensing receptor gene (PGSC0003DMG400024508), which promotes stomatal opening and closure depending on its activation by extracellular calcium ($Ca^{2+}{}_o$) (Wang et al., 2012), was upregulated (2.24 fold change), while in Hansa this gene was not differentially expressed. The upregulation of this gene was also observed in Nicola (2.07-fold change), but it was not differentially expressed in Lady Rosetta.

Stomatal conductance is directly associated with photosynthetic activity (Aien et al., 2011). Therefore, we counted the number of times that genes with GO-terms relating to photosynthesis were found in both Biogold and Hansa (Fig.5). We thus found increased expression of genes involved in carbon fixation (photosynthesis-related) processes in drought-stressed Biogold leaves, while in Hansa they were mostly either not differentially expressed or downregulated.

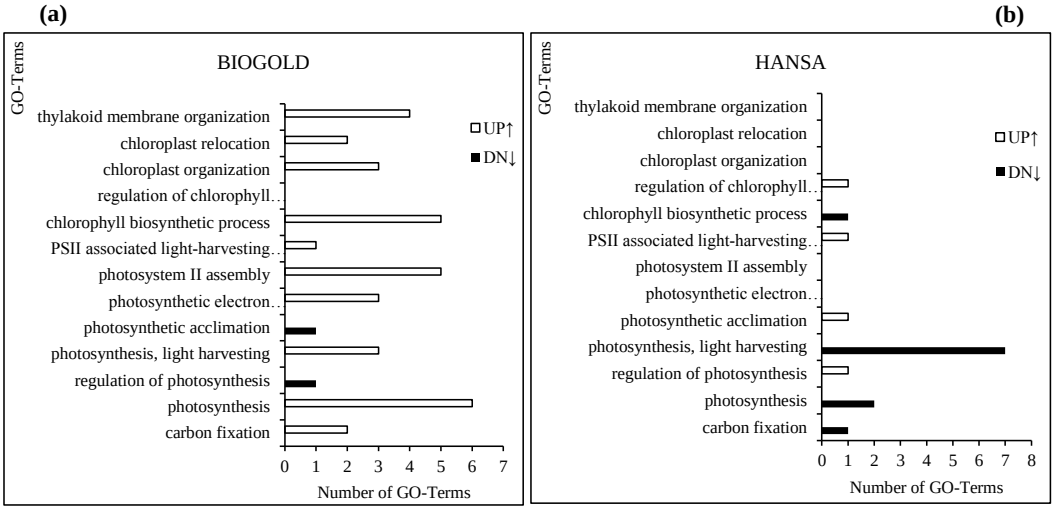


Figure 5: GO-based functional annotation showing the number of GO-terms for carbon fixation found in leaf at 28DOD in (**a**) Biogold, (**b**) Hansa. UP (Upregulation), DN (Downregulation), PS (Photosystem).

The upregulated photosynthesis-related genes in Biogold include phosphoenolpyruvate carboxylase, chlorophyll a/b binding protein, light-harvesting complex I protein, thylakoid soluble phosphoprotein, phytoene synthase and chloroplastic tetrapyrrole-binding protein. In Hansa, phosphoenolpyruvate carboxylase, chlorophyll a/b binding protein, photosystem Q(B) protein and NADPH protochlorophyllide oxidoreductase were downregulated. The only photosynthesis-related gene that was upregulated in Hansa is glucose-6-phosphate translocator, which was included in several GO-terms in Fig.5b.

Drought stress signalling and response (28DOD)

Because of the response in photosynthesis-related gene expression of Hansa (Fig.5b), together with the relatively high reduction in stomatal conductance (Fig.2a), we investigated the drought response of the transcriptome of Hansa in more detail. We found drought-induced upregulation of genes involved in hormonal signalling pathways like ABA signalling (protein phosphatase 2C, cytochrome P450, delta-1-pyrroline-5-carboxylase synthetase, among others), cytokinin signalling (two-component sensor protein histidine protein kinase, GATA transcription factor) and gibberellic acid signalling (gibberellin 2-oxidases) (Supplementary Table 1a). Several genes involved in hormone signalling pathways were downregulated, including ABA (ABA receptor *PYL4*), auxin (Glutathione-S-transferases) and ethylene (ethylene response factors and peroxidases) signalling pathways (Supplementary Table 1b). In comparison with Biogold, in which stomatal conductance was less reduced than in Hansa, we found upregulations in genes of the ABA signalling pathway including phospholipase D, calcium-dependent protein kinase and raffinose synthase (Supplementary Table 2a). Also, gibberellin 2-oxidases, gibberellin-induced protein, two-component sensor protein histidine protein kinase and auxin response factor were upregulated in Biogold (Supplementary Table 2a). However, an auxin biosynthesis gene was downregulated in Biogold (Supplementary Table 2b). A remarkable similarity between Hansa and Biogold is that Glutathione-S-transferase and the ethylene signalling pathway genes (ethylene response factors, peroxidases and MAP kinase kinase), were downregulated in leaves of both cultivars at 28DOD.

Overall, we found a lower number of upregulated and downregulated genes under drought in Hansa than in Biogold (Table 3). Interestingly, the abscisic acid and environmental stress-inducible protein (*TAS14*) was upregulated in both cultivars, though the fold change was higher in Hansa than Biogold (Table 3).

Tuberization (28DOD)

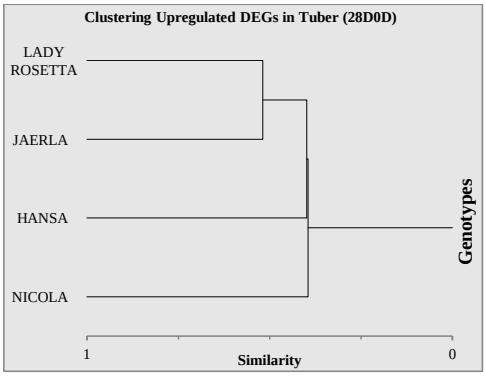
As a follow up on the phenotypic variation in tuber number at 28DOD (Fig.2c), we investigated the transcriptional variation in tuber tissues among the cultivars at this time point by hierarchical clustering. Biogold was not considered in this clustering as no DEGs were detected in Biogold tuber tissue. Lady Rosetta and Jaerla clustered together, as did Hansa and Nicola (Fig.6a and b). Based on the phenotypic variation in tuber number among the cultivars, and the distinct two by two clustering of the cultivars, we investigated the DEGs for genes involved in tuberization and carbon partitioning in Lady Rosetta and Jaerla, and compared them with those of Hansa and Nicola. Interestingly, we observed upregulations in the following genes in Lady Rosetta and Jaerla: *CONSTANS*, circadian clock coupling factor, stachyose synthase, galactinol synthase and early flowering protein; and additionally in Jaerla: sucrose phosphate synthase, UDP glucose epimerase, sugar transporters, hexokinases, trehalose-6-phosphate synthases, sucrose synthase, *BEL5* and *BEL29* proteins, vacuolar and neutral invertases, starch granule bound protein and apoplastic invertases. These upregulated genes of Lady Rosetta and Jaerla were not detected in Nicola and Hansa. On the other hand, the following tuberization/carbon partitioning-related genes were downregulated in Hansa: photoperiod responsive protein, tuber-specific and sucrose-responsive element binding protein and UDP-glucuronate-5-epimerase; and in Nicola, a hexokinase. The

downregulated genes in Lady Rosetta and Jaerla included induced stolon tip protein, sugar transporters, a neutral invertase and cellulose synthase.

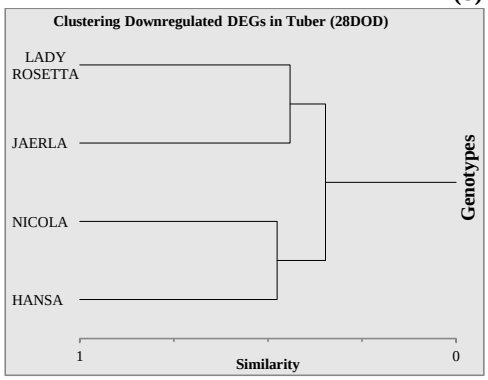
Table 3: Upregulated and downregulated DEGs involved in response to drought in the leaf of Hansa and Biogold at 28DOD

Genes	Annotations	Log2 fold change
Hansa		
PGSC0003DMG400003530	Abscisic acid and environmental stress-inducible protein TAS14	6.37
PGSC0003DMG400016742	Protein phosphatase 2C AHG3 homolog	2.04
PGSC0003DMG400012479	Nitrate transporter	1.90
PGSC0003DMG400015525	Histone H4	-3.34
PGSC0003DMG400023523	Histone H4	-3.30
PGSC0003DMG400009940	Endoplasmin homolog	-1.52
Biogold		
PGSC0003DMG400021683	E3 ubiquitin-protein ligase RMA1H1	4.61
PGSC0003DMG400007848	Phospholipase D	2.25
PGSC0003DMG400003530	Abscisic acid and environmental stress-inducible protein TAS14	2.02
PGSC0003DMG400018109	Raffinose synthase 2	1.68
PGSC0003DMG400016685	Receptor protein kinase CLAVATA1	1.63
PGSC0003DMG400010279	Digalactosyldiacylglycerol synthase 2, chloroplastic	1.08
PGSC0003DMG400009968	25 kDa protein dehydrin	-1.60
PGSC0003DMG401012256	Transcription factor	-1.43
PGSC0003DMG400029773	Ethylene-responsive transcriptional coactivator	-1.55
PGSC0003DMG400021331	PEN1	-4.88
PGSC0003DMG400020122	Circadian clock coupling factor ZGT	-1.33
PGSC0003DMG400017936	Late embryogenic abundant protein 5	-2.86
PGSC0003DMG400016285	Enoyl-CoA hydratase/isomerase family protein	-1.11
PGSC0003DMG400014417	Ethylene-responsive transcription factor 3	-2.83
PGSC0003DMG400010572	RNA-binding region-containing protein	-2.21
PGSC0003DMG400000731	Response to desiccation RD2	-1.48
PGSC0003DMG400000631	Lactoylglutathione lyase	-1.93

(a)



(b)



(c)

Number of Upregulated DEGs (28DOD) - Tuber

	B	H	J	L	N
B	0	0	0	0	0
H	0	13	4	4	1
J	0	4	3389	161	2
L	0	4	161	218	9
N	0	1	2	9	39

(d)

Number of Downregulated DEGs (28DOD) - Tuber

	B	H	J	L	N
B	0	0	0	0	0
H	0	138	88	4	28
J	0	88	5523	493	20
L	0	4	493	553	4
N	0	28	20	4	117

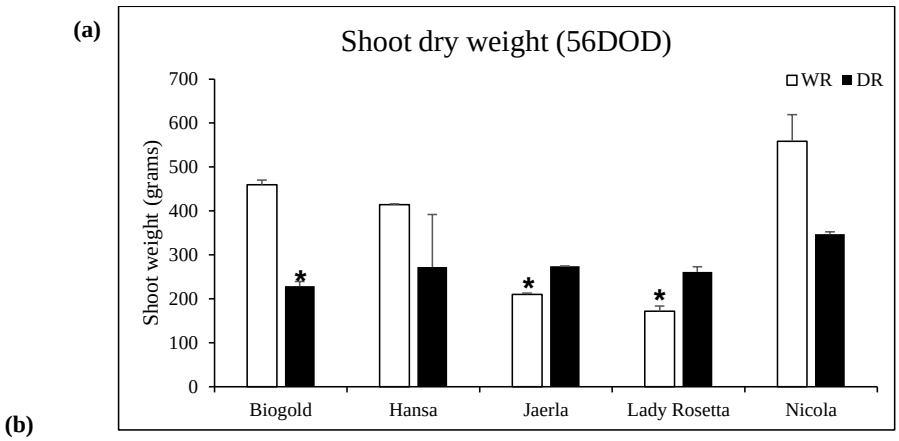
Figure 6: (**a**) hierarchical cluster of upregulated DEGs in tuber at 28DOD, (**b**) hierarchical cluster of downregulated DEGs in tuber at 28DOD, (**c**) Number of upregulated DEGs overlapping between cultivars in tuber, (**d**) Number of downregulated DEGs overlapping between cultivars in tuber. The letters in (c) and (d) represent the cultivars: B (Biogold), H (Hansa), J (Jaerla), L (Lady Rosetta) and N (Nicola).

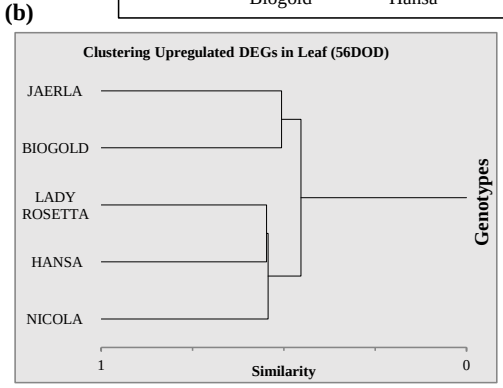
Shoot biomass (56DOD)

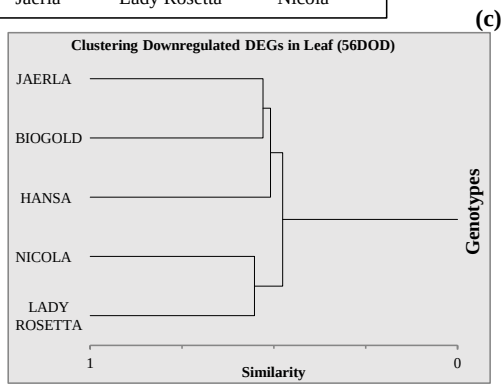
At harvest (56DOD) we scored shoot biomass to evaluate the impact of drought on various aspects of canopy development during the growing season. We found variations in shoot dry weight among the cultivars. The shoot weight increased under drought in Jaerla and Lady Rosetta, but significantly reduced in Biogold (Fig.7a). A hierarchical clustering of the cultivars using the DEGs enabled us to evaluate the transcriptional variation in drought response among them at this time point.

Interestingly, Jaerla and Lady Rosetta, which had increased shoot weight in phenotype, clustered in different clades. Therefore, we investigated the DEGs of all cultivars in order to infer their respective drought responses. We firstly considered the highly expressed DEGs (Supplementary File 3) and then the rest of the DEGs per genotype (fold changes are indicated here in parenthesis). In Biogold, the upregulated DEGs included non-specific lipid transfer proteins (10.6), lipid-binding proteins (7.3) and glycine-rich cell wall structural protein (7.1), but also abscisic acid and environmental stress-inducible protein (*TAS14*) (3.9); while citrate binding protein (-42.0), sucrose

synthase (-7.3) and lipoxygenase (-7.3), were downregulated. The downregulated genes in Jaerla were cellulose synthase (67.2), chlorophyll a/b binding protein (83.3), cytochrome P450 (10.4) and aquaporin (5.7). In Hansa we detected upregulation of non-specific lipid transfer protein (32.3), glycine-rich protein (23.0), sucrose synthase (4.0) and aquaporin (4.0), while the downregulated genes included ethylene-responsive LEA protein (10.3), pectin esterase (8.7), nitrate transporter (3.7). The upregulated genes in Nicola included gibberellin 2-oxidase (23), gibberellin-induced protein (3.59), pectate lyase (20.5), pectin esterase (7.2). The downregulated genes in Nicola included glycine-rich wall structural protein (31.5), protein phosphatase (18.3) and ABA hydroxylase (10.4). In Lady Rosetta some of the upregulated genes were protein phosphatase (12.5), cellulose synthase (11.8) and aquaporin (5.9), while the downregulated genes included senescence-specific cysteine protease (29.9) and protein phosphatase 2C (24.7). Remarkably, non-specific lipid transfer protein was commonly upregulated in leaves of three cultivars (Biogold, Jaerla and Hansa) at this time point. Lady Rosetta and Nicola shared 19 upregulated DEGs (Fig.7d), which include membrane proteins, aquaporin, cellulose synthase, pectate lyases, polygalacturonase, cytochrome P450. The 17 upregulated DEGs shared between Lady Rosetta and Hansa (Fig.7d) includes membrane proteins, glycine-rich protein, polygalacturonase, aquaporin and pectate lyase. The Lady Rosetta-Nicola-Hansa clade thus consisted of upregulations in membrane/cell wall-related gene expressions, while the Jaerla-Biogold clade had the non-specific lipid-binding protein in common. Hansa shared aspects of both groups.







(d)

Number of Upregulated DEGs (56DOD) - Leaf

	B	H	J	L	N
B	31	2	1	3	1
H	2	46	0	17	8
J	1	0	8	0	0
L	3	17	0	123	19
N	1	8	0	19	59

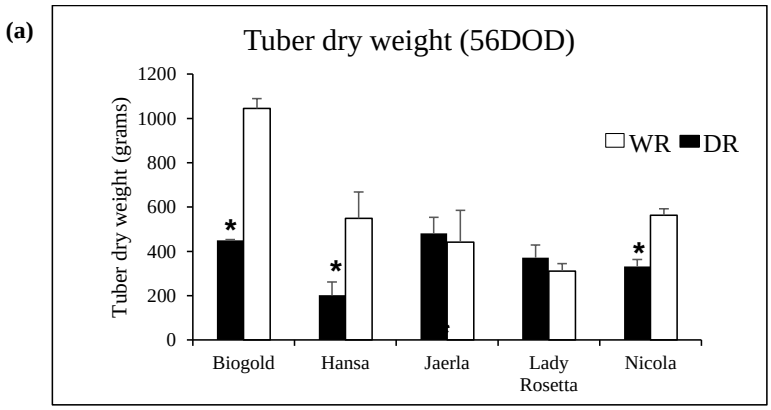
(e)

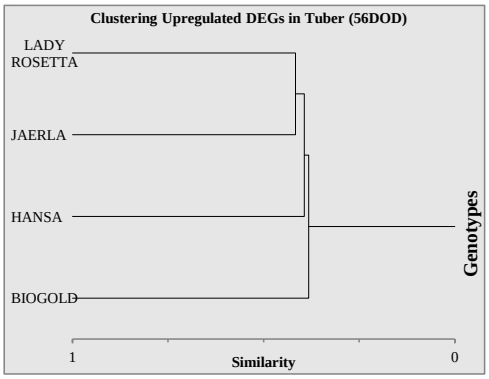
Number of Downregulated DEGs (56DOD) - Leaf

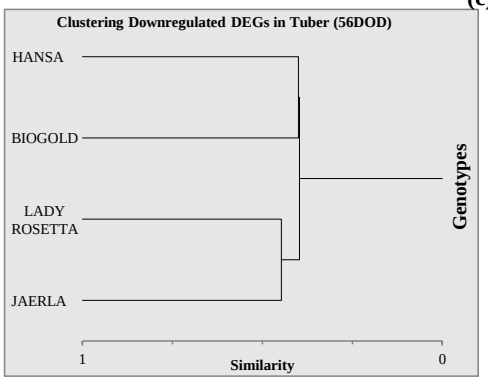
	B	H	J	L	N
B	32	3	6	1	0
H	3	27	4	5	3
J	6	4	190	1	0
L	1	5	1	412	47
N	0	3	0	47	145

Figure 7: **(a)** Shoot dry weight per plot at 56DOD. Error bars represent standard error of the mean. Asterisk(s) represent significant difference between irrigated and drought-stressed plants ($p \leq 0.05$), (**b**) hierarchical cluster of upregulated DEGs in leaf at 56DOD, (**c**) hierarchical cluster of downregulated DEGs in leaf at 56DOD, (**d**) Number of upregulated DEGs overlapping between cultivars in leaf at 56DOD, (**e**) Number of downregulated DEGs overlapping between cultivars in leaf at 56DOD. The letters in (c) and (d) represent the cultivars: B (Biogold), H (Hansa), J (Jaerla), L (Lady Rosetta) and N (Nicola).

Tuber yield (56DOD)







(d) **Number of Upregulated DEGs (56DOD) - Tuber**

	B	H	J	L	N
B	3635	2	29	63	0
H	2	4	0	0	0
J	29	0	87	6	0
L	63	0	6	245	0
N	0	0	0	0	0

(e) **Number of Downregulated DEGs (56DOD) - Tuber**

	B	H	J	L	N
B	5031	58	44	8	0
H	58	110	5	0	0
J	44	5	254	6	0
L	8	0	6	21	0
N	0	0	0	0	0

Figure 8: **(a)** tuber dry weight per plot at 56DOD. Error bars represent standard error of the mean. Asterisk(s) represent significant difference between irrigated and drought-stressed plants ($p \leq 0.05$), (**b**) hierarchical cluster of upregulated DEGs in tuber at 56DOD, (**c**) hierarchical cluster of downregulated DEGs in tuber at 56DOD, (**d**) number of upregulated DEGs overlapping between cultivars in tuber at 56DOD, (**e**) Number of downregulated DEGs overlapping between cultivars in tuber at 56DOD. The letters in (c) and (d) represent the cultivars: B (Biogold), H (Hansa), J (Jaerla), L (Lady Rosetta) and N (Nicola).

At harvest (56DOD), tuber weight was reduced in Hansa, Biogold and Nicola, while Jaerla and Lady Rosetta maintained tuber yield under drought (Fig.8a). We investigated the DEGs of the cultivars at this time point in order to evaluate variation in drought responses that may partly be associated with tuber yield. The two cultivars that maintained tuber yield, Jaerla and Lady Rosetta (Fig.8a) clustered on the same clade, distant from Hansa and Biogold (Fig.8b and c), and shared six genes for both the upregulated and downregulated DEGs. It is remarkable that we did not detect DEGs in tuber tissues of Nicola at this time point, which may be due to the significance threshold ($\alpha=0.05$) we used across all samples. The high number of DEGs detected for Biogold may be due to lack of a replicate sample (see, Materials & Methods section).

The six genes upregulated in both Jaerla and Lady Rosetta include: lipid-binding protein, non-specific lipid transfer protein, abscisic acid and environmental stress-inducible protein (*TAS14*), aquaporin, tonoplast intrinsic protein and L-asparaginase. The downregulated genes shared between Jaerla and Lady Rosetta were BURD domain-containing protein, serine-pyruvate aminotransferase, alkaline alpha-galactosidase seed imbibition protein, aquaporin TIP1 and two genes of unknown function. We further investigated the DEGs of the four cultivars at this time point for genes involved in carbon partitioning and tuber yield (based on the observed phenotypic variation in tuber yield) (Fig.8a), and other possible functional variation represented in DEGs.

We observed upregulation of sucrose synthase in Lady Rosetta (5.5 folds) and Biogold (2.6 folds), but it was not differentially expressed in Jaerla and Hansa. Sucrose phosphate synthase was upregulated in both Jaerla and Biogold by 2.2 folds, but not differentially expressed in Lady Rosetta and Hansa. We observed upregulation of neutral and apoplastic invertases only in Biogold (8 folds and 5 folds, respectively), but these invertases were not differentially expressed in Jaerla, Lady Rosetta and Hansa. Interestingly, an induced stolon tip protein was upregulated in Lady Rosetta (10 folds), down regulated in Hansa (18 folds), and not differentially expressed in both Jaerla and Biogold. Also, glucose-6-phosphate translocator was upregulated in Lady Rosetta (4 folds), downregulated in Biogold (3.4 folds), and not differentially expressed in Hansa and Jaerla.

1

DISCUSSION AND CONCLUSIONS

Drought stress is known to adversely affect potato tuber yield and productivity (Obidiegwu et al., 2015). The observed drought responses of potato are based on the molecular changes that occur after perception of drought. A good understanding of these molecular alterations and the implicated pathways will facilitate the identification of traits and genes that can be targeted in breeding for drought tolerant potato. In this study, we have investigated the molecular response and drought adaptation of potato in various genotypic backgrounds. We found a high level of variation in the number of differentially expressed genes among the cultivars in various tissues and time points. Also, genotypic differences in response to early stages of drought stress signalling may be linked to variations in downstream molecular response to drought.

Variation in differential gene expression

Differential gene expression is used to gain insight in the involvement of genes in phenotypic variation (Lovell et al., 2015). In this study, we observed high variation in the number of differentially expressed genes (DEGs) between tissues and time points among the cultivars, and in fact, we did not detect significant DEGs in three genotype-time point-tissue combinations (Table 2). A number of factors may be considered as possible reasons for differences in number of DEGs. Normalization has been shown to influence the detection of DEGs in RNA-seq analyses (Li et al., 2017; Zyprych-Walczak et al., 2015). Zyprych-Walczak et al. (2015) suggested that normalization approaches with minimal bias and variance between house-keeping genes across samples may enhance DEG detection. However, the authors mentioned that this would depend on the RNA-seq data structure. In our study we employed the Cufflinks Tuxedo pipeline, which uses the fragments per kilobase of transcripts per million mapped fragments (FPKM) normalization approach (Trapnell et al., 2012). FPKM normalizes read counts with transcript length to correct for differences between gene sizes; and it normalizes the counts for differences in volumes of sequencing reads across runs (Trapnell et al., 2012). Furthermore, another study has shown that filtering of low-expression genes can positively affect the detection of DEGs (Sha et al., 2015). A removal of 15-30% of genes with lowest average read count led to an identification of more DEGs than without filtering. However, the number of detected DEGs decreased beyond 30% filtering of low-expressed genes (Sha et al., 2015), suggesting that there is an optimal filtering range for low-expressed genes in RNA-seq analyses. Our approach incorporated this in the analyses. The quartile FPKM normalization we used scales sequence fragments using a ratio of 75 quartile fragment count to the mean 75 quartile value across libraries, thus boosting DEG detection including the low-expressed genes (Trapnell, 2014). Therefore, we have no basis to assume that the normalization or filtering thresholds may be the reasons for the vast variation in the dataset. However, another report has shown that an undetermined number of genes are undetected in RNA-seq studies because of their low expression in relation to the sequencing depth (García-Ortega & Martínez, 2015). In fact, Garcia-Ortega and Martinez (2015) suggested that given the current range of sequencing depths in RNA-seq experiments, about 10% of genes per library may remain undetected. In our study, we may not have accounted for the effect of our sequencing depth (21.8X) on the variations in DEG detected in tissues and time points. But we speculate that within the various tissues or time points, the cut off settings we used for differential expression may have

contributed to the variations in number of DEGs, depending on gene expression levels per sample. Furthermore, the use of two replicates per tissue and time point may have contributed to the vast variation in the dataset.

Transcriptional response to drought

Drought stress interferes with the homeostatic, physiological and metabolic balance of plants. Expression of a large number of genes changes upon exposure to drought stress to adapt to water limitation (Evers et al., 2010; Gong et al., 2015) and (Table 2). Generally a higher proportion of genes are downregulated than upregulated under drought (Gong et al., 2015) (Table 2), which may suggest a regulation of the plant's transcription machinery to prioritize the upregulation of genes needed for stress response.

The number of differentially expressed genes (DEGs) in our study were higher at 28DOD than at 56DOD, except in the tubers of Biogold and leaves of Jaerla, in which no (significantly) DEG were detected at 28DOD (Table 2). At the early stages of drought, shortly after the drought stress is perceived by the plants, many molecular pathways and systems of the plant would need to be adjusted to minimize adverse effects (Bechtold et al., 2016). But in the later stages of stress, plants may have adapted to the new conditions. In another study on diploid potato under drought, a higher number of DEGs were detected at the second sampling time point (9days after drought application) than at the first (4days after drought application) (Anithakumari, 2011). The contrast between our observation and that of Anithakumari (2011) may be because the two time points in her study were earlier than our first time point, and so both studies may have captured different stages of drought development in the plants. Moreover, the plants in Anithakumari (2011) may have perceived the stress differently in pots as compared to the plants in our study, which were grown in the field. Additionally, in our study, developmental changes in the plants between 28 and 56DOD may affect the transcriptome response to drought. However, it is not unlikely that the plants at 56DOD may have perceived a higher drought stress level (Fig.1).

The transcriptome responses of the cultivars were more similar to each other at 28DOD. Genes upregulated in leaf tissue in most cultivars at this early drought stage included abscisic acid and environmental stress-inducible protein (*TAS14*), superoxide dismutase and Delta 1-pyrroline-5-carboxylate synthetase (proline biosynthesis gene) (Supplementary File 2). *TAS14* is a dehydrin (Parra et al., 1996); a late embryogenesis abundant (LEA) protein that plays important protective roles during abiotic stresses (Gao et al., 2013; Hanin et al., 2011; Munoz-Mayor et al., 2012). In fact, *TAS14* has been reported as a bio-marker for drought stress perception of plants (van Muijen et al., 2016). The regulation of *TAS14* expression upon osmotic stress has been shown to involve an early accumulation of high amounts of ABA in leaves within short-term periods (Muñoz-Mayor et al., 2012). In our study, the upregulation of *TAS14* in the cultivars suggests that the plants had perceived the drought stress, and may be responding to adapt to the stress condition. The upregulation of superoxide dismutase in our study may point to plants' adaptive response to the supposedly perceived drought stress. Superoxide dismutase is involved in controlling oxidative stress by reducing the concentration of reactive oxygen species (ROS) (Alscher et al., 2002) during stress. Drought stress is known to trigger the build-

up of ROS (Cruz de Carvalho, 2008; You & Chan, 2015). Under drought, the role of superoxide dismutase has been reported in potato ROS tolerance response (V. Naraikina et al., 2014; Van der Mescht et al., 2007), suggesting that in our study, the upregulation of superoxide dismutase may indicate its involvement in stress adaptation of the plants. Likewise, proline biosynthesis is a known drought response feature in potato (Bündig et al., 2017). Proline accumulation during stress as an osmolyte is thought to facilitate balance in tissue turgor pressure in plants (Liang et al., 2013). However, the direct link of its role in drought stress to drought tolerance is yet to be demonstrated (Bündig et al., 2017). In fact, there are indications that proline accumulation may be post-transcriptionally regulated (Schafleitner et al., 2007). These reports on proline involvement in drought may suggest that in our study, the upregulation of proline biosynthesis gene may be indicative of drought stress perception and not necessarily a determinant of tolerance response.

Downregulated genes in both leaf and tuber tissues at 28DOD included genes implicated in salt tolerance, oxidative stress, heat and high light intensity response, but not genes for osmotic or drought response. The drought response genes upregulated in the tuber in our study at 28DOD included glycine-rich protein and fructose-bisphosphate aldolase (also known as aldolase). The protective role of glycine-rich protein in making compatible solute in drought response has been previously reported (Mangeon et al., 2010; Wani et al., 2013). On the other hand, aldolase is commonly known to be involved in both glycolysis, breaking down fructose bisphosphate into triose phosphates and gluconeogenesis, forming glucose from protein and lipid sources (Mininno et al., 2012). In a recent study in wheat (*Triticum aestivum*), cytosolic aldolase genes were reportedly upregulated under drought (osmotic) stress applied by 15% PEG 6000 treatment (Geng-Yin et al., 2017). Although the exact molecular mechanism of drought involvement of aldolase is yet unknown, its role in sucrose metabolism or sugar signalling during drought is probable. In another study, the alteration of hexose levels in potato tuber by the overexpression of bacterial xylose isomerase was shown to induce the catalytic activity of aldolase, which was linked to a higher tuber number and elevated sucrose synthesis and fluxes (Urbanczyk-Wochniak et al., 2003). Also, the catalytic properties of aldolase during gluconeogenesis and reported involvement in drought stress response (Lu et al., 2012), may suggest that in instances of limited sugar assimilate resources like drought, it could harness amino and fatty acid residues for sugar synthesis and eventual starch biosynthesis. However, further work will be required to investigate a possible role of aldolase in potato drought response in the tuber.

In the later stage of drought stress development (56DOD), leaves of most of the cultivars had differential expression of genes controlling cell wall modification, especially upregulation of genes involved in pectin degradation. Pectin reduces cell wall permeability and also supports its water holding capacity (Voragen et al., 2009). Pectin degradation may have contributed in part to our observation of softer leaves in the drought-stressed plots (data not shown). The membrane protein *UPF0497*, which controls the plasma membrane-cell wall junctions by forming a Casparian strip, was upregulated. The Casparian strip prevents extracellular diffusion thereby reducing water loss from plant cells under osmotic stress conditions (Chen et al., 2011). Casparian strips are characterized by the deposition of suberin and lignin in the cell wall, which

encrusts the cell wall and facilitates further deposition of phenolic compounds that ultimately block apoplastic transport (Chen et al., 2011). The involvement of Casparian strip in drought tolerance has not been reported, but there are reports of its involvement in cold tolerance (Yang et al., 2015). Also, aquaporin (plasma membrane intrinsic protein, a water transport channel) was upregulated under drought compared to control at this time point in the leaf of most cultivars. Increased expression of aquaporin PIP has been shown to enhance hydraulic conductivity in plants (Martre et al., 2002). However, transgenic study has also reported drought sensitivity in Arabidopsis plants overexpressing aquaporin gene from *Galega orientalis* (Li et al., 2015a). The upregulation of aquaporin PIP in our study may require further functional investigation to infer the effect on phenotype. Nonetheless, aquaporin upregulation seems to suggest the responsiveness of potato water channels to stress even when the stress is prolonged. Overall, the upregulations of pectin in leaves at 56DOD may suggest that the plant cell walls tended to become more vulnerable to drought as the stress elapsed. In another study, a longer duration of drought has been shown to impact on both tolerant and sensitive potato genotypes (Evers et al., 2010). However, the possible role of the Casparian strip in regulating water loss in potato would need further investigation. Remarkably, the downregulated genes in leaves at 56DOD relative to irrigated control plants included a heat shock protein, basic proline-rich protein and an ethylene-responsive LEA protein. These are genes known to play protective roles in drought stress response (Bündig et al., 2017; Hanin et al., 2011; Tang et al., 2016) and the downregulation of these genes is unexpected. In the tuber at 28DOD, genes for proline biosynthesis and LEA protein were upregulated (Supplementary File 2), and at 56DOD, the osmoprotective dehydrin (*TAS14*) and stress-responsive non-specific lipid transfer protein were upregulated in most cultivars. The protective roles of the known drought-associated genes among these upregulated DEGs have been described above. Likewise, the non-specific lipid transfer proteins may facilitate signal transduction through jasmonates during drought (Golldack et al., 2014)

Linking transcriptome to phenotypes

Drought is a complex trait partly because the drought response involves various phenotypic adjustments that are controlled by multiple genetic and molecular factors (Mir et al., 2012). One of the ways to disentangle this genetic complexity is by identifying specific phenotype(s) or phenotypic measurements that can be linked to DEGs (Sprenger et al., 2017). We investigated possible associations between phenotypic trait(s) and DEGs, and observed that the expression of extracellular Ca^{2+} sensing receptor known to regulate stomatal opening and closure (Wang et al., 2012), may be functionally linked to stomatal conductance under drought (Fig.2b). Stomatal closure during drought is generally assumed to be a drought tolerance mechanism (Le et al., 2011), mainly because it prevents transpirational loss of the limited water resource. Stomatal opening is associated with light intensity (Gray & Peirce, 1919). Light photons taken up by the leaf require sufficient CO_2 and water for photosynthesis. Stomatal closure that is not properly regulated in consideration of the light intensity can result in build-up of reactive oxygen species (ROS). It has been reported that under water limitation stress conditions with stomatal closure, the drop in CO_2 to O_2 ratio in the presence of light can lead to oxidative stress (Das & Roychoudhury, 2014). The extracellular Ca^{2+} sensing receptor might influence the

percentage maintenance of stomatal conductance under drought, and thus contribute to drought tolerance. For instance, the cultivar Biogold, which had upregulation of extracellular Ca^{2+} sensing receptor, may have harvested light photons and maintained its photosynthesis more readily than Hansa (Figs.5a and b).

Under conditions with limited resources like drought, photosynthetic activity needs to be balanced with water availability, and for a crop, the reduced assimilates need to be used to optimize yield (White et al., 2016). Partitioning of carbohydrates to tubers is of course preferred, but should be balanced with optimal growth of young leaves and roots, and maintenance of source leaves under these water-limited conditions. In our study we observed a drought-induced change in carbon partitioning of the cultivars based on shoot and tuber weights at 28DOD; the cultivars generally partitioned more assimilates to their tubers and less to their shoot under drought relative to irrigated conditions at this time point (Fig.2). However, the drought-stressed plants formed less tubers than irrigated plants (Fig.2c). The time point coincides with the time that new tubers are initiated and already formed tubers bulked (Fig.1). The tuberization stage has been reported as a critical stage during which drought stress impacts more severely on potato productivity (Muthoni & Kabira, 2016). Therefore, it is likely that the young tubers in our study formed a strong sink organ that facilitated the partitioning of assimilates to bulk them (Fernie & Willmitzer, 2001), even though the number of tubers was lower under drought (Fig.2a). Previous studies have reported reductions in tuber number under drought (Haverkort et al., 1990; Lahlou et al., 2003). Another study demonstrated that apoplastic invertase overexpression led to increased tuber sizes and lower tuber numbers, while a cytosolic invertase overexpression yielded the reverse result (Sonnewald et al., 1997). In our study, we observed genotypic variation in both the molecular aspects of tuberization and tuberization itself, inferred from tuber number (see section 'Tuberization (28DOD)'). The two genotypes with the lowest reduction in tuber number under drought (Fig.2c), Jaerla and Lady Rosetta, also showed upregulations in genes involved in tuberization - *BEL5* and *BEL29*, *CONSTANS*, but also invertases (apoplastic, cytosolic and vacuolar) and sucrose synthase. Previous studies have identified *BEL5* and *CONSTANS* to be photoperiod-dependent regulators of tuberization in potato (Gonzalez-Schain et al., 2012). *BEL5* is a mobile RNA that induces tuberization under short-day inductive photoperiods (Banerjee et al., 2006; Sharma et al., 2014), while *CONSTANS* represses mobile tuberization signals like *BEL5* and *SP6A*, thereby repressing tuberization in short-day photoperiod (Gonzalez-Schain et al., 2012). *BEL29*, on the other hand, has been shown to antagonize the tuber-inducing role of *BEL5* (Ghate et al., 2017). The upregulation of these short-day photoperiod-dependent genes in long day conditions (European summer) under drought in our study, may suggest a likely genotype-dependent interaction of drought with the tuberization pathway. Further dedicated studies will be required to understand this possible interaction in more details. The upregulated invertases and sucrose synthase in the tuber tissues in our study may have contributed to the regulation of assimilate bulking in the tuber (Fernie & Willmitzer, 2001).

At 56DOD more carbohydrate metabolism-related genes were upregulated in tuber tissue than in the leaves (data not shown). At this later time point the cultivars had a distinct response in carbon partitioning towards either tuber bulking or shoot maintenance (Figs.7a and 8a).

Remarkably, two cultivars, Jaerla and Lady Rosetta, maintained both their shoot and tuber weights at comparable level under drought stress and irrigated conditions (Figs.7a and 8a), and shared similarity in DEGs in their tuber but not in leaf (Figs.7b, 7c, 8b and 8c). We observed an upregulation of non-specific lipid transfer protein at this later time point in leaves of three of the cultivars (56DOD). Non-specific lipid transfer proteins have been reported to play various roles in plants including membrane stabilization and cell wall organization (Liu et al., 2015a). Induction of non-specific lipid proteins under drought conditions was reported in other crops although the mechanism of their drought involvement remains unclear (Giordani et al., 2011; Jang et al., 2002). Further studies may be required to understand the potential of the non-specific lipid transfer proteins for drought tolerance in potato.

Genes involved in sucrose metabolism and starch biosynthesis were upregulated in the tubers of the cultivars (data not shown). Sucrose metabolism and starch biosynthesis in tuber facilitate tuber growth (Geigenberger, 2003), which in turn increases sink strength and demand for more assimilates. This demand could facilitate the export of photosynthesized assimilates from leaves and prevent feedback inhibition of photosynthesis (Ayre, 2011). Although we observed differences in tuber yield in the genotypes at 56DOD (Fig.8), there was no clearly distinct gene expression pattern. Therefore, we have no evidence to infer that upregulation of sucrose metabolism and starch biosynthesis genes in tubers of Jaerla and Lady Rosetta also influenced shoot weight maintenance under stress. Our transcriptomic analyses also did not show any upregulation of photosynthesis-associated genes under drought relative to control plants in these two cultivars at 56DOD. In fact, chlorophyll a/b binding protein was downregulated in Jaerla, suggesting a possible reduction in light reception and excitation of the photosystems in photosynthesis (Pietrzykowska et al., 2014). Therefore, there is no evidence to suggest that Jaerla and Lady Rosetta were more actively photosynthesizing than for instance Biogold at this time point. We did observe that genes regulating leaf senescence were downregulated in Lady Rosetta at 56DOD, while genes that code for meristem initiation and growth (from leaf axils) were upregulated (data not shown), suggesting that shoot weight may have been maintained in Lady Rosetta due to second growths arising from the axils of older leaves (Zaag, 1992). However, we did not score this trait and so are not able to confirm any second growths in Lady Rosetta under drought.

Signalling pathways

Hormones play critical roles in the transduction of stress signals and induction of the required adaptation of the stress conditions (Müller & Munné-Bosch, 2015; Sah et al., 2016). In our study, genes involved in the various hormonal signalling pathways were upregulated at the first time point (Supplementary Table 1). The DEGs provided insights into the components of the signalling pathways that may have been involved in the potato drought stress response (Supplementary Tables 1 and 2). A comparison of the two most contrasting cultivars in terms of drought response at 28DOD, Hansa and Biogold, revealed that the ABA receptor *PYL4* was downregulated in Hansa, but not differentially expressed in Biogold. PYL ABA receptors are the first point of perception of the ABA hormone at the start of the ABA signalling cascades (Kline et al., 2010). Furthermore, protein phosphatase 2c (*PP2C*) was upregulated in Hansa but not in Biogold (Supplementary Tables 1 and 2). It has been reported that expression of *PYLs*

inhibits *PP2C* and thus, facilitates downstream ABA-signalling responses (Park et al., 2009). In our study, the upregulation of *PP2C* in Hansa may be due to the downregulation of *PYL4*, which may suggest that ABA signalling under drought was impaired in Hansa. Assuming that the differential expressions imply a dysfunctional ABA signalling in Hansa, we could link it to the undetected differential expression of the extracellular Ca^{2+} sensing receptor, which may have contributed to the unregulated stomatal conductance we observed (Fig.2). Genes that are further downstream of the ABA signalling pathway, e.g. phospholipase D (PLD) and calcium-dependent protein kinase (CDPK) were also upregulated in Biogold, suggesting that in this cultivar the ABA signalling pathway is activated (Grill & Himmelbach, 1998). Even at the later time point of stress, the ABA pathway appeared to be active in Biogold (data not shown). Furthermore, genes of the gibberellic acid- and cytokinin-mediated signalling pathways were upregulated at 28DOD in the leaf of Biogold and Hansa (Supplementary Tables 1 and 2). Gibberellic acid (GA) is a growth regulating hormone and GA signalling is now known to be modulated by the DELLA and SCARECROW-LIKE (SCL) transcription factors, which also integrate GA signalling responses into the ABA signalling pathway (Golldack et al., 2013). In fact, evidence of a convergent crosstalk between GA and ABA signalling with jasmonic acid (JA) has been reported under drought, and the DELLAs were shown to mediate the interface of these signalling pathways (Golldack et al., 2014). In our study, we did not detect differential expression of the DELLAs. Moreover, we did not detect differential expression for genes involved in GA biosynthesis. GA inactivating genes (Gibberellin 2-oxidases) (Lo et al., 2008), however, were upregulated in both Biogold and Hansa (Supplementary Tables 1 and 2). This may suggest that in our study, the ABA-pathway may have antagonized the GA pathway in Biogold. There are reports of ABA – GA antagonistic interaction in other plant systems (Rogers & Rogers, 1992; Ye & Zhang, 2012). However, in Hansa we cannot conclude on a possible interaction the ABA-GA pathways. We observed upregulation in a two-component sensor protein histidine protein kinase (HK) in Biogold and Hansa, which is involved in Cytokinin (CK) signalling (Nongpiur et al., 2012). HK functions as a receptor of CK and subsequently triggers downstream responses (Muller & Sheen, 2007). Cytokinin regulates cell differentiation and delays senescence (Wingler et al., 1998). Its involvement in drought tolerance has been reported in rice, where it coordinated the assimilation and regulation of carbon and nitrogen metabolism (Reguera et al., 2013), and in osmotic stress in both ABA-dependent and ABA-independent ways (Tran et al., 2010). Cytokinin was shown to interact with ABA, but reports on the nature of this interactions are contradictory. CK levels in plants were shown to be antagonistic to ABA levels, and under stress ABA would repress cytokinin signalling through AB1 (Huang et al., 2017). However, an indirect activation of cytokinin signalling under stress by alternative receptors like the histidine kinase family has been speculated as well (O'Brien & Benková, 2013). These may explain the upregulations of both the ABA and cytokinin signalling pathways in Biogold in our study, but our observation for Hansa questions this generalization. Crosstalk between CK and another hormone, auxin, has also been reported in root and shoot apical meristematic tissues of Arabidopsis during development (El-Showk et al., 2013). Cytokinin signalling was also reported to have an antagonistic role to auxin signalling during stress response (O'Brien & Benková, 2013). The above findings may partly explain our observation of more downregulated than upregulated genes in the auxin signalling pathway. However, the downregulation of auxin biosynthesis gene in Biogold and absence of its

differential expression in Hansa, may suggest different interactions between CK and auxin in the two cultivars. Antagonistic interactions were also reported between ABA accumulation and ethylene, with stress responses induced by ABA countered by ethylene and vice versa (Wilkinson et al., 2012). However, our findings do not give clear directions of interaction between these two hormones, even though many genes involved in ethylene signalling were downregulated. Based on our results, the hormone signalling pathway seems to be responsive to drought stress. But genotypic differences may play a significant role on the interaction among the various hormonal pathways.

In summary, the transcriptomic study provides additional insight into the molecular responses to drought, from drought perception to tolerance. Based on our results, it may be necessary to investigate more deeply into the interaction of drought with GA biosynthesis genes and the GA-mediated signalling pathway, considering the role of GA in tuberization. However, a focus on specific aspects of drought response would need dedicated experimental set up to directly link phenotype with the gene(s) involved. For further studies on GA signalling, we propose an experimental set up with intermittent sampling of leaf and young tuber tissues at seedling through tuberization stages in series of time points. Phenotypic sampling of stolon to tuber transition and leaf stomatal conductance may give insights on possible ABA-GA interaction that relates to tuberization. Transcriptomic analyses combined with metabolite assays could reveal the molecular actors that play a role, and possibly clarify any aspect of tuberization under drought that are transcriptional or post-transcriptional regulated.

REFERENCES

Aien, A., Khetarpal, S., & Pal, M. (2011). Photosynthetic Characteristics of Potato Cultivars Grown under High Temperature. *American-Eurasian J. Agric. & Environ. Sci*, *11*(5), 633-639.

Alscher, R. G., Erturk, N., & Heath, L. S. (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J Exp Bot*, *53*(372), 1331-1341.

Amar, D., Frades, I., Danek, A., Goldberg, T., Sharma, S. K., Hedley, P. E., Proux-Wera, E., Andreasson, E., Shamir, R., Tzfadia, O., & Alexandersson, E. (2014). Evaluation and integration of functional annotation pipelines for newly sequenced organisms: the potato genome as a test case. *BMC Plant Biology*, *14*(1), 329. doi: 10.1186/s12870-014-0329-9

Ambrosone, A., Batelli, G., Bostan, H., D'Agostino, N., Chiusano, M. L., Perrotta, G., Leone, A., Grillo, S., & Costa, A. (2017). Distinct gene networks drive differential response to abrupt or gradual water deficit in potato. *Gene*, *597*, 30-39. doi: 10.1016/j.gene.2016.10.024

Anithakumari, A. M. (2011). *Genetic dissection of drought tolerance in potato.* (Ph.D.), Wageningen University, Wageningen. Retrieved from http://edepot.wur.nl/165211

Anithakumari, A. M., Dolstra, O., Vosman, B., Visser, R. F., & Linden, C. G. (2011). In vitro screening and QTL analysis for drought tolerance in diploid potato. [Euphytica]. *181*(3), 357-369. doi: 10.1007/s10681-011-0446-6

Anjum, A., Jaggi, S., Varghese, E., Lall, S., Bhowmik, A., & Rai, A. (2016). Identification of Differentially Expressed Genes in RNA-seq Data of Arabidopsis thaliana: A Compound Distribution Approach. *Journal of Computational Biology*, *23*(4), 239-247. doi: 10.1089/cmb.2015.0205

Ati, A. S., Iyada, A. D., & Najim, S. M. (2012). Water use efficiency of potato (Solanum tuberosum L.) under different irrigation methods and potassium fertilizer rates. *Annals of Agricultural Sciences*, *57*(2), 99-103. doi: http://dx.doi.org/10.1016/j.aoas.2012.08.002

Ayre, B. G. (2011). Membrane-Transport Systems for Sucrose in Relation to Whole-Plant Carbon Partitioning. *Molecular Plant*, *4*(3), 377-394. doi: https://doi.org/10.1093/mp/ssr014

Banerjee, A. K., Chatterjee, M., Yu, Y., Suh, S.-G., Miller, W. A., & Hannapel, D. J. (2006). Dynamics of a Mobile RNA of Potato Involved in a Long-Distance Signaling Pathway. *The Plant Cell*, *18*(12), 3443-3457. doi: 10.1105/tpc.106.042473

Bechtold, U., Penfold, C. A., Jenkins, D. J., Legaie, R., Moore, J. D., Lawson, T., Matthews, J. S. A., Vialet-Chabrand, S. R. M., Baxter, L., Subramaniam, S., Hickman, R., Florance, H., Sambles, C., Salmon, D. L., Feil, R., Bowden, L., Hill, C., Baker, N. R., Lunn, J. E., Finkenstädt, B., Mead, A., Buchanan-Wollaston, V., Beynon, J., Rand, D. A., Wild, D. L., Denby, K. J., Ott, S., Smirnoff, N., & Mullineaux, P. M. (2016). Time-Series Transcriptomics Reveals That AGAMOUS-LIKE22 Affects Primary Metabolism and Developmental Processes in Drought-Stressed Arabidopsis. *The Plant Cell*, *28*(2), 345-366. doi: 10.1105/tpc.15.00910

Blum, A. (2011). Drought resistance – is it really a complex trait? *Functional Plant Biology*, *38*(10), 753-757. doi: http://dx.doi.org/10.1071/FP11101

Bündig, C., Vu, T. H., Meise, P., Seddig, S., Schum, A., & Winkelmann, T. (2017). Variability in Osmotic Stress Tolerance of Starch Potato Genotypes (Solanum tuberosum L.) as Revealed by an In Vitro Screening: Role of Proline, Osmotic Adjustment and Drought Response in Pot Trials. *Journal of Agronomy and Crop Science*, *203*(3), 206-218. doi: 10.1111/jac.12186

Cantore, V., Wassar, F., Yamaç, S. S., Sellami, M. H., Albrizio, R., Stellacci, A. M., & Todorovic, M. (2014). Yield and water use efficiency of early potato grown under different irrigation regimes. *International Journal of Plant Production*, *8*(3), 1735-8043.

Chen, T., Cai, X., Wu, X., Karahara, I., Schreiber, L., & Lin, J. (2011). Casparian strip development and its potential function in salt tolerance. *Plant Signaling & Behavior*, *6*(10), 1499-1502. doi: 10.4161/psb.6.10.17054

Cruz de Carvalho, M. H. (2008). Drought stress and reactive oxygen species. *Plant Signaling & Behavior*, *3*(3), 156-165. doi: 10.4161/psb.3.3.5536

Das, K., & Roychoudhury, A. (2014). Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers in Environmental Science*, *2*(53). doi: 10.3389/fenvs.2014.00053

Davidson, S., & Passmore, R. (1963). Human nutrition and dietetics. *Human nutrition and dietetics.*(2nd Edition).

Dongjin, S., Seok-Jun, M., Seyoun, H., Beom-Gi, K., Sang, R. P., Seong-Kon, L., Hye-Jin, Y., Hye, E. L., Hawk-Bin, K., Dongwon, B., Bu, Y. Y., & Myung-Ok, B. (2011). Expression of StMYB1R-1, a Novel Potato Single MYB-Like Domain transcription Factor, Increases Drought Tolerance. *Plant Physiology, 155*, 421–432.

El-Showk, S., Ruonala, R., & Helariutta, Y. (2013). Crossing paths: cytokinin signalling and crosstalk. *Development, 140*(7), 1373-1383. doi: 10.1242/dev.086371

Evers, D., Lefèvre, I., Legay, S., Lamoureux, D., Hausman, J.-F., Rosales, R. O. G., Marca, L. R. T., Hoffmann, L., Bonierbale, M., & Schafleitner, R. (2010). Identification of drought-responsive compounds in potato through a combined transcriptomic and targeted metabolite approach. *Journal of Experimental Botany, 61*(9), 2327-2343. doi: 10.1093/jxb/erq060

Fakhari, R., Tobeh, A., Hasanzadeh, N., Barghi, A., & Shiri, M. (2013). Studying effects of different irrigation levels and planting patterns on yield and water use efficiency in potato (Solanum tuberosum L.). *Intl. Res. J. Appl. Basic. Sci., 4*(7), 1941-1945.

FAOSTAT. (2014). Food and Agriculture Organization of United Nations *Potatoes*.

Fernie, A. R., & Willmitzer, L. (2001). Molecular and Biochemical Triggers of Potato Tuber Development. *Plant Physiology, 127*(4), 1459-1465. doi: 10.1104/pp.010764

Gao, W., Bai, S., Li, Q., Gao, C., Liu, G., Li, G., & Tan, F. (2013). Overexpression of TaLEA Gene from Tamarix androssowii Improves Salt and Drought Tolerance in Transgenic Poplar (Populus simonii × P. nigra). *PLoS ONE, 8*(6), e67462. doi: 10.1371/journal.pone.0067462

García-Ortega, L. F., & Martínez, O. (2015). How Many Genes Are Expressed in a Transcriptome? Estimation and Results for RNA-Seq. *PLoS ONE, 10*(6), e0130262. doi: 10.1371/journal.pone.0130262

Gazendam, I., Greyling, R., Laurie, R. N., Matsaunyane, L. B. T., Oelofse, D., & Rakuambo, J. (2016). *A transgenic approach to improve the drought tolerance of potato.* Paper presented at the Acta Horticulturae.

Geigenberger, P. (2003). Regulation of sucrose to starch conversion in growing potato tubers. *Journal of Experimental Botany, 54*(382), 457-465. doi: 10.1093/jxb/erg074

Geng-Yin, L., Guo, X.-G., Xie, L.-P., Xie, C.-G., Zhang, X.-H., Yang, Y., Xiao, L., Tang, Y.-Y., Pan, X.-L., Guo, A.-G., & Xu, H. (2017). Molecular Characterization, Gene Evolution, and Expression Analysis of the Fructose-1, 6-bisphosphate Aldolase (FBA) Gene Family in Wheat (Triticum aestivum L.). *Frontiers in Plant Science, 8*(1030). doi: 10.3389/fpls.2017.01030

Ghate, T. H., Sharma, P., Kondhare, K. R., Hannapel, D. J., & Banerjee, A. K. (2017). The mobile RNAs, StBEL11 and StBEL29, suppress growth of tubers in potato. *Plant Mol Biol, 93*(6), 563-578. doi: 10.1007/s11103-016-0582-4

Giordani, T., Buti, M., Natali, L., Pugliesi, C., Cattonaro, F., Morgante, M., & Cavallini, A. (2011). An analysis of sequence variability in eight genes putatively involved in drought response in sunflower (Helianthus annuus L.). *Theoretical and Applied Genetics, 122*(6), 1039-1049. doi: 10.1007/s00122-010-1509-0

Golldack, D., Li, C., Mohan, H., & Probst, N. (2013). Gibberellins and abscisic acid signal crosstalk: living and developing under unfavorable conditions. *Plant Cell Reports, 32*(7), 1007-1016. doi: 10.1007/s00299-013-1409-2

Golldack, D., Li, C., Mohan, H., & Probst, N. (2014). Tolerance to drought and salt stress in plants: Unraveling the signaling networks. *Frontiers in Plant Science, 5*(151). doi: 10.3389/fpls.2014.00151

Gong, L., Zhang, H., Gan, X., Zhang, L., Chen, Y., Nie, F., Shi, L., Li, M., Guo, Z., Zhang, G., & Song, Y. (2015). Transcriptome Profiling of the Potato (Solanum tuberosum L.) Plant under Drought Stress and Water-Stimulus Conditions. *PLoS ONE, 10*(5), e0128041. doi: 10.1371/journal.pone.0128041

Gonzalez-Schain, N. D., Diaz-Mendoza, M., Zurczak, M., & Suarez-Lopez, P. (2012). Potato CONSTANS is involved in photoperiodic tuberization in a graft-transmissible manner. *Plant J, 70*(4), 678-690. doi: 10.1111/j.1365-313X.2012.04909.x

Gray, J., & Peirce, G. J. C. F. p. d. A. (1919). The Influence of Light Upon the Action of Stomata and Its Relation to the Transpiration of Certain Grains. *American Journal of Botany, 6*(4), 131-155. doi: 10.2307/2435124

Grill, E., & Himmelbach, A. (1998). ABA signal transduction. *Curr Opin Plant Biol, 1*(5), 412-418. doi: https://doi.org/10.1016/S1369-5266(98)80265-3

Hanin, M., Brini, F., Ebel, C., Toda, Y., Takeda, S., & Masmoudi, K. (2011). Plant dehydrins and stress tolerance: Versatile proteins for complex mechanisms. *Plant Signaling & Behavior, 6*(10), 1503-1509. doi: 10.4161/psb.6.10.17088

Haverkort, A. J., de Ruijter, F. J., van Evert, F. K., Conijn, J. G., & Rutgers, B. (2013). Worldwide Sustainability Hotspots in Potato Cultivation. 1. Identification and Mapping. *Potato Research, 56*(4), 343-353. doi: 10.1007/s11540-013-9247-8

Haverkort, A. J., Van De Waart, M., & Bodlaender, K. B. A. (1990). The effect of early drought stress on numbers of tubers and stolons of potato in controlled and field conditions. *Potato Research, 33*(1), 89-96. doi: 10.1007/bf02358133

Heather, J. M., & Chain, B. (2016). The sequence of sequencers: The history of sequencing DNA. *Genomics, 107*(1), 1-8. doi: https://doi.org/10.1016/j.ygeno.2015.11.003

Hoeijmakers, W. A., Bartfai, R., & Stunnenberg, H. G. (2013). Transcriptome analysis using RNA-Seq. *Methods Mol Biol, 923*, 221-239. doi: 10.1007/978-1-62703-026-7_15

Hosseinizadeh, A., SeyedKaboli, H., Zareie, H., Akhondali, A., & Farjad, B. (2015). Impact of climate change on the severity, duration, and frequency of drought in a semi-arid agricultural basin. *Geoenvironmental Disasters, 2*(1), 23. doi: 10.1186/s40677-015-0031-8

Huang, X., Zhang, X., Gong, Z., Yang, S., & Shi, Y. (2017). ABI4 represses the expression of type-A ARRs to inhibit seed germination in Arabidopsis. *Plant J, 89*(2), 354-365. doi: 10.1111/tpj.13389

Jang, C., Kim, D., Bu, S., Kim, J., Lee, S., Kim, J., Johnson, J., & Seo, Y. (2002). Isolation and characterization of lipid transfer protein (LTP) genes from a wheat-rye translocation line. *Plant Cell Reports, 20*(10), 961-966. doi: 10.1007/s00299-001-0424-x

Jenkins, K., & Warren, R. (2015). Quantifying the impact of climate change on drought regimes using the Standardised Precipitation Index. *Theoretical and Applied Climatology, 120*(1), 41-54. doi: 10.1007/s00704-014-1143-x

Kline, K. G., Sussman, M. R., & Jones, A. M. (2010). Abscisic Acid Receptors. *Plant Physiology, 154*(2), 479-482. doi: 10.1104/pp.110.160846

Lahlou, O., Ouattar, S., & Ledent, J. (2003). The effect of drought and cultivar on growth parameters, yield and yield components of potato. *Agronomie, 23* 257-268. doi: 10.1051/agro:2002089

Lander, E. S., & Waterman, M. S. (1988). Genomic mapping by fingerprinting random clones: a mathematical analysis. *Genomics, 2*(3), 231-239.

Le, A., S, T., Je, O., Kk, T., Shanker, A., & Venkateswarlu, B. (2011). Stomatal Responses to Drought Stress and Air Humidity *Abiotic Stress in Plants - Mechanisms and Adaptations* (pp. Ch. 12). Rijeka: InTech.

Li, J., Ban, L., Wen, H., Wang, Z., Dzyubenko, N., Chapurin, V., Gao, H., & Wang, X. (2015a). An aquaporin protein is associated with drought stress tolerance. *Biochemical and Biophysical Research Communications, 459*(2), 208-213. doi: https://doi.org/10.1016/j.bbrc.2015.02.052

Li, P., Piao, Y., Shon, H. S., & Ryu, K. H. (2015b). Comparing the normalization methods for the differential analysis of Illumina high-throughput RNA-Seq data. *BMC Bioinformatics, 16*(1), 347. doi: 10.1186/s12859-015-0778-7

Li, X., Brock, G. N., Rouchka, E. C., Cooper, N. G. F., Wu, D., O'Toole, T. E., Gill, R. S., Eteleeb, A. M., O'Brien, L., & Rai, S. N. (2017). A comparison of per sample global scaling and per gene normalization methods for differential expression analysis of RNA-seq data. *PLoS ONE, 12*(5), e0176185. doi: 10.1371/journal.pone.0176185

Liang, X., Zhang, L., Natarajan, S. K., & Becker, D. F. (2013). Proline Mechanisms of Stress Survival. *Antioxidants & Redox Signaling, 19*(9), 998-1011. doi: 10.1089/ars.2012.5074

Liu, F., Zhang, X., Lu, C., Zeng, X., Li, Y., Fu, D., & Wu, G. (2015a). Non-specific lipid transfer proteins in plants: presenting new advances and an integrated functional analysis. *Journal of Experimental Botany, 66*(19), 5663-5681.

Liu, Y., Lin-Wang, K., Deng, C., Warran, B., Wang, L., Yu, B., Yang, H., Wang, J., Espley, R. V., Zhang, J., Wang, D., & Allan, A. C. (2015b). Comparative Transcriptome Analysis of White and Purple Potato to Identify Genes Involved in Anthocyanin Biosynthesis. *PLoS ONE, 10*(6), e0129148.

Lo, S.-F., Yang, S.-Y., Chen, K.-T., Hsing, Y.-I., Zeevaart, J. A. D., Chen, L.-J., & Yu, S.-M. (2008). A Novel Class of Gibberellin 2-Oxidases Control Semidwarfism, Tillering, and Root Development in Rice. *The Plant Cell, 20*(10), 2603-2618. doi: 10.1105/tpc.108.060913

Loon, C. D. (1981). The effect of water stress on potato growth, development, and yield. [American Potato Journal]. *58*(1), 51-69. doi: 10.1007/bf02855380

Lovell, J. T., Mullen, J. L., Lowry, D. B., Awole, K., Richards, J. H., Sen, S., Verslues, P. E., Juenger, T. E., & McKay, J. K. (2015). Exploiting Differential Gene Expression and Epistasis to Discover Candidate Genes for Drought-Associated QTLs in Arabidopsis thaliana. *The Plant Cell, 27*(4), 969-983. doi: 10.1105/tpc.15.00122

Lu, W., Tang, X., Huo, Y., Xu, R., Qi, S., Huang, J., Zheng, C., & Wu, C. A. (2012). Identification and characterization of fructose 1,6-bisphosphate aldolase genes in Arabidopsis reveal a gene family with diverse responses to abiotic stresses. *Gene, 503*(1), 65-74. doi: 10.1016/j.gene.2012.04.042

Manga, P., Klingeman, D. M., Lu, T.-Y. S., Mehlhorn, T. L., Pelletier, D. A., Hauser, L. J., Wilson, C. M., & Brown, S. D. (2016). Replicates, Read Numbers, and Other Important Experimental Design Considerations for Microbial RNA-seq Identified Using Bacillus thuringiensis Datasets. *Frontiers in Microbiology*, *7*(794). doi: 10.3389/fmicb.2016.00794

Mangeon, A., Junqueira, R. M., & Sachetto-Martins, G. (2010). Functional diversity of the plant glycine-rich proteins superfamily. *Plant Signaling & Behavior*, *5*(2), 99-104.

Martre, P., Morillon, R., Barrieu, F., North, G. B., Nobel, P. S., & Chrispeels, M. J. (2002). Plasma Membrane Aquaporins Play a Significant Role during Recovery from Water Deficit. *Plant Physiology*, *130*(4), 2101-2110. doi: 10.1104/pp.009019

Mininno, M., Brugière, S., Pautre, V., Gilgen, A., Ma, S., Ferro, M., Tardif, M., Alban, C., & Ravanel, S. (2012). Characterization of Chloroplastic Fructose 1,6-Bisphosphate Aldolases as Lysine-methylated Proteins in Plants. *Journal of Biological Chemistry*, *287*(25), 21034-21044. doi: 10.1074/jbc.M112.359976

Mir, R. R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R., & Varshney, R. K. (2012). Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theor Appl Genet*, *125*(4), 625-645. doi: 10.1007/s00122-012-1904-9

Morozova, O., Hirst, M., & Marra, M. A. (2009). Applications of new sequencing technologies for transcriptome analysis. *Annu Rev Genomics Hum Genet*, *10*, 135-151. doi: 10.1146/annurev-genom-082908-145957

Muller, B., & Sheen, J. (2007). Advances in Cytokinin Signaling. *Science*, *318*(5847), 68-69.

Müller, M., & Munné-Bosch, S. (2015). Ethylene Response Factors: A Key Regulatory Hub in Hormone and Stress Signaling. *Plant Physiology*, *169*(1), 32-41. doi: 10.1104/pp.15.00677

Munoz-Mayor, A., Pineda, B., Garcia-Abellan, J. O., Anton, T., Garcia-Sogo, B., Sanchez-Bel, P., Flores, F. B., Atares, A., Angosto, T., Pintor-Toro, J. A., Moreno, V., & Bolarin, M. C. (2012). Overexpression of dehydrin tas14 gene improves the osmotic stress imposed by drought and salinity in tomato. *J Plant Physiol*, *169*(5), 459-468. doi: 10.1016/j.jplph.2011.11.018

Muñoz-Mayor, A., Pineda, B., Garcia-Abellán, J. O., Antón, T., Garcia-Sogo, B., Sanchez-Bel, P., Flores, F. B., Atarés, A., Angosto, T., Pintor-Toro, J. A., Moreno, V., & Bolarin, M. C. (2012). Overexpression of dehydrin tas14 gene improves the osmotic stress imposed by drought and salinity in tomato. *J Plant Physiol*, *169*(5), 459-468. doi: http://dx.doi.org/10.1016/j.jplph.2011.11.018

Muthoni, J., & Kabira, J. N. (2016). *Potato Production under Drought Conditions: Identification of Adaptive Traits*.

Nongpiur, R., Soni, P., Karan, R., Singla-Pareek, S. L., & Pareek, A. (2012). Histidine kinases in plants: Cross talk between hormone and stress responses. *Plant Signaling & Behavior*, *7*(10), 1230-1237. doi: 10.4161/psb.21516

O'Brien, J. A., & Benková, E. (2013). Cytokinin cross-talking during biotic and abiotic stress responses. *Frontiers in Plant Science*, *4*, 451. doi: 10.3389/fpls.2013.00451

Obidiegwu, J. E., Bryan, G. J., Jones, H. G., & Prashar, A. (2015). Coping with drought: stress and adaptive responses in potato and perspectives for improvement. *Frontiers in Plant Science, 6*, 542. doi: 10.3389/fpls.2015.00542

Park, S.-Y., Fung, P., Nishimura, N., Jensen, D. R., Fujii, H., Zhao, Y., Lumba, S., Santiago, J., Rodrigues, A., Chow, T.-f. F., Alfred, S. E., Bonetta, D., Finkelstein, R., Provart, N. J., Desveaux, D., Rodriguez, P. L., McCourt, P., Zhu, J.-K., Schroeder, J. I., Volkman, B. F., & Cutler, S. R. (2009). Abscisic acid inhibits PP2Cs via the PYR/PYL family of ABA-binding START proteins. *Science, 324*(5930), 1068-1071. doi: 10.1126/science.1173041

Parra, M. M., Pozo, O., Luna, R., Godoy, J. A., & Pinto-Toro, J. A. (1996). Structure of the dehydrin tasl4 gene of tomato and its developmental and environmental regulation in transgenic tobacco. *Plant MolecularBiology, 32*, 453-460.

Pieczynski, M., Wyrzykowska, A., Milanowska, K., Boguszewska-Mankowska, D., Zagdanska, B., Karlowski, W., Jarmolowski, A., & Szweykowska-Kulinska, Z. (2018). Genomewide identification of genes involved in the potato response to drought indicates functional evolutionary conservation with Arabidopsis plants. *Plant Biotechnol J, 16*(2), 603-614. doi: 10.1111/pbi.12800

Pietrzykowska, M., Suorsa, M., Semchonok, D. A., Tikkanen, M., Boekema, E. J., Aro, E. M., & Jansson, S. (2014). The light-harvesting chlorophyll a/b binding proteins Lhcb1 and Lhcb2 play complementary roles during state transitions in Arabidopsis. *Plant Cell, 26*(9), 3646-3660. doi: 10.1105/tpc.114.127373

Qiagen. (2005). RNeasy® Plus Mini Handbook. In Qiagen (Ed.).

Reader, J. (2008). *Propitious esculent: The potato in world history*: Random House.

Reguera, M., Peleg, Z., Abdel-Tawab, Y. M., Tumimbang, E. B., Delatorre, C. A., & Blumwald, E. (2013). Stress-Induced Cytokinin Synthesis Increases Drought Tolerance through the Coordinated Regulation of Carbon and Nitrogen Assimilation in Rice. *Plant Physiology, 163*(4), 1609-1622. doi: 10.1104/pp.113.227702

Risso, D., Ngai, J., Speed, T. P., & Dudoit, S. (2014). Normalization of RNA-seq data using factor analysis of control genes or samples. *32*(9), 896-902.

Rogers, J. C., & Rogers, S. W. (1992). Definition and functional implications of gibberellin and abscisic acid cis-acting hormone response complexes. *Plant Cell, 4*(11), 1443-1451. doi: 10.1105/tpc.4.11.1443

Sah, S. K., Reddy, K. R., & Li, J. (2016). Abscisic Acid and Abiotic Stress Tolerance in Crop Plants. *Frontiers in Plant Science, 7*, 571. doi: 10.3389/fpls.2016.00571

Schafleitner, R., Gaudin, A., Gutierrez Rosales, R. O., Alvarado Aliaga, C. A., & Bonierbale, M. (2007). Proline accumulation and real time PCR expression analysis of genes encoding enzymes of proline metabolism in relation to drought tolerance in Andean potato. *Acta Physiologiae Plantarum, 29*(1), 19-26. doi: 10.1007/s11738-006-0003-4

Sha, Y., Phan, J. H., & Wang, M. D. (2015). Effect of low-expression gene filtering on detection of differentially expressed genes in RNA-seq data. *Conference proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual Conference, 2015*, 6461-6464. doi: 10.1109/EMBC.2015.7319872

Sharma, P., Lin, T., Grandellis, C., Yu, M., & Hannapel, D. J. (2014). The BEL1-like family of transcription factors in potato. *Journal of Experimental Botany, 65*(2), 709-723. doi: 10.1093/jxb/ert432

Singh, G. (1969). A review of the soil-moisture relationship in potatoes. *American Potato Journal, 46*(10), 398-403. doi: 10.1007/bf02869560

Sonnewald, U., Hajirezaei, M.-R., Kossmann, J., Heyer, A., Trethewey, R. N., & Willmitzer, L. (1997). Increased potato tuber size resulting from apoplastic expression of a yeast invertase. *Nature Biotechnology, 15*, 794.

Sprenger, H., Erban, A., Seddig, S., Rudack, K., Thalhammer, A., Le, M. Q., Walther, D., Zuther, E., Köhl, K. I., Kopka, J., & Hincha, D. K. (2017). Metabolite and transcript markers for the prediction of potato drought tolerance. *Plant Biotechnol J*, n/a-n/a. doi: 10.1111/pbi.12840

Szalonek, M., Sierpien, B., Rymaszewski, W., Gieczewska, K., Garstka, M., Lichocka, M., Sass, L., Paul, K., Vass, I., Vankova, R., Dobrev, P., Szczesny, P., Marczewski, W., Krusiewicz, D., Strzelczyk-Zyta, D., Hennig, J., & Konopka-Postupolska, D. (2015). Potato Annexin STANN1 Promotes Drought Tolerance and Mitigates Light Stress in Transgenic <italic>Solanum tuberosum</italic> L. Plants. *PLoS ONE, 10*(7), e0132683.

Tang, R., Zhu, W., Song, X., Lin, X., Cai, J., Wang, M., & Yang, Q. (2016). Genome-Wide Identification and Function Analyses of Heat Shock Transcription Factors in Potato. *Frontiers in Plant Science, 7*, 490. doi: 10.3389/fpls.2016.00490

Tran, L.-S. P., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2010). Role of cytokinin responsive two-component system in ABA and osmotic stress signalings. *Plant Signaling & Behavior, 5*(2), 148-150.

Trapnell, C. (2014). Cufflinks.cuffdiff (v6). Maryland: University of Maryland Center for Bioinformatics and Computational Biology.

Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., Pimentel, H., Salzberg, S. L., Rinn, J. L., & Pachter, L. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *7*(3), 562-578.

Urbanczyk-Wochniak, E., Leisse, A., Roessner-Tunali, U., Lytovchenko, A., Reismeier, J., Willmitzer, L., & Fernie, A. R. (2003). Expression of a bacterial xylose isomerase in potato tubers results in an altered hexose composition and a consequent induction of metabolism. *Plant Cell Physiol, 44*(12), 1359-1367.

V. Naraikina, N., S. Sin'kevich, M., N. Demin, I., A. Selivanov, A., Moshkov, I., & I. Trunova, T. (2014). *Changes in the activity of superoxide dismutase isoforms in the course of low-temperature adaptation in potato plants of wild type and transformed with Δ12-acyl-lipid desaturase gene* (Vol. 61).

Van der Mescht, A., De Ronde, J. A., Slabbert, M. M., & Oelofse, D. (2007). Enhanced drought tolerance in transgenic potato expressing the Arabidopsis thaliana Cu/Zn superoxide dismutase gene. *South African Journal of Science, 103*, 169-173.

van Muijen, D., Anithakumari, A. M., Maliepaard, C., Visser, R. G. F., & van der Linden, C. G. (2016). Systems genetics reveals key genetic elements of drought induced gene regulation in diploid potato. *Plant, Cell & Environment, 39*(9), 1895-1908. doi: 10.1111/pce.12744

Vasquez-Robinet, C., Mane, S. P., Ulanov, A. V., Watkinson, J. I., Stromberg, V. K., De Koeyer, D., Schafleitner, R., Willmot, D. B., Bonierbale, M., Bohnert, H. J., & Grene, R. (2008). Physiological and molecular adaptations to drought in Andean potato genotypes. *Journal of Experimental Botany, 59*(8), 2109-2123. doi: 10.1093/jxb/ern073

Voragen, A. G. J., Coenen, G.-J., Verhoef, R. P., & Schols, H. A. (2009). Pectin, a versatile polysaccharide present in plant cell walls. *Structural Chemistry, 20*(2), 263. doi: 10.1007/s11224-009-9442-z

Wang, W. H., Yi, X., Han, A., Liu, T., Chen, J., Wu, F., Dong, X., He, J., Pei, Z., & Zheng, H. (2012). Calcium-sensing receptor regulates stomatal closure through hydrogen peroxide and nitric oxide in response to extracellular calcium in Arabidopsis. *Journal of Experimental Botany, 63*(1), 177-190. doi: 10.1093/jxb/err259

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet, 10*(1), 57-63. doi: 10.1038/nrg2484

Wani, S. H., Singh, N. B., Haribhushan, A., & Mir, J. I. (2013). Compatible Solute Engineering in Plants for Abiotic Stress Tolerance - Role of Glycine Betaine. *Current Genomics, 14*(3), 157-165. doi: 10.2174/1389202911314030001

White, A. C., Rogers, A., Rees, M., & Osborne, C. P. (2016). How can we make plants grow faster? A source–sink perspective on growth rate. *Journal of Experimental Botany, 67*(1), 31-45.

Whitmore, A. P., & Whalley, W. R. (2009). Physical effects of soil drying on roots and crop growth. *Journal of Experimental Botany, 60*(10), 2845-2857. doi: 10.1093/jxb/erp200

Wilkinson, S., Kudoyarova, G. R., Veselov, D. S., Arkhipova, T. N., & Davies, W. J. (2012). Plant hormone interactions: innovative targets for crop breeding and management. *Journal of Experimental Botany, 63*(9), 3499-3509.

Wingler, A., von Schaewen, A., Leegood, R. C., Lea, P. J., & Paul Quick, W. (1998). Regulation of Leaf Senescence by Cytokinin, Sugars, and Light : Effects on NADH-Dependent Hydroxypyruvate Reductase. *Plant Physiology, 116*(1), 329-335.

Xu, Q., He, Q., Li, S., & Tian, Z. (2014). Molecular characterization of StNAC2 in potato and its overexpression confers drought and salt tolerance. *Acta Physiologiae Plantarum, 36*(7), 1841-1851. doi: 10.1007/s11738-014-1558-0

Yang, J., Ding, C., Xu, B., Chen, C., Narsai, R., Whelan, J., Hu, Z., & Zhang, M. (2015). A Casparian strip domain-like gene, CASPL, negatively alters growth and cold tolerance. *Scientific Reports, 5*, 14299.

Yang, S., Mercante, D. E., Zhang, K., & Fang, Z. (2016). An Integrated Approach for RNA-seq Data Normalization. *Cancer Informatics, 15*, 129-141. doi: 10.4137/cin.s39781

Ye, N., & Zhang, J. (2012). Antagonism between abscisic acid and gibberellins is partially mediated by ascorbic acid during seed germination in rice. *Plant Signal Behav, 7*(5), 563-565. doi: 10.4161/psb.19919

You, J., & Chan, Z. (2015). ROS Regulation During Abiotic Stress Responses in Crop Plants. *Frontiers in Plant Science, 6*, 1092. doi: 10.3389/fpls.2015.01092

Zaag, D. E. v. d. (1992). Potatoes and their cultivation in the Netherlands (pp. 47). The Hague: NIVAA (Netherlands Potato Consultative Institute).

Zyprych-Walczak, J., Szabelska, A., Handschuh, L., #xf3, rczak, K., Klamecka, K., Figlerowicz, M., & Siatkowski, I. (2015). The Impact of Normalization Methods on

RNA-Seq Data Analysis. *BioMed Research International, 2015*, 10. doi: 10.1155/2015/621690

CHAPTER 7

GENERAL DISCUSSION

The response of potato to drought stress varies depending on genotypic differences and variations in drought timing, duration and severity (Bassam et al., 1990). Among the various drought response strategies (escape, avoidance, etc.), tolerance has gained wide acceptance because stress tolerance characteristics are more often inherited independently than characteristics of other response strategies, and can thus be combined by convergent improvement (Kang & Priyadarshan, 2008). Also, stress tolerance protects plant cellular structures, which offers the plant a longer survival time in the field, and a possibility of stress recovery when environmental conditions improve (Kriz & Larkins, 2008; Xu et al., 2010). This protection of the plant cells and maintenance of its metabolic processes ultimately contribute to yield sustenance in such adverse conditions (Muthoni & Kabira, 2016). The potato drought tolerance response comprises several mechanisms (Boguszewska-Mańkowska et al., 2018). In-depth molecular analyses and physiological studies are required to understand the various mechanisms (Gong et al., 2015). In this thesis, "*Water-saving potatoes: Exploring and characterizing drought tolerance mechanisms*", I have investigated the drought response of potato varieties to gain insight into the response strategies and mechanisms that are best suited for maintenance of yield under water-limited conditions.

Potato is known as a drought-sensitive crop due to its shallow root system (van Loon, 1981; Yamaguchi & Tanaka, 1990), but also the vulnerability of its canopy to drought due to high transpiration rate and reduced leaf expansion (Manhas & Sukumaran, 1988; Weisz et al., 1994). However, we cannot fairly justify either the generalization of potato drought sensitivity (Obidiegwu et al., 2015), or its potential for drought tolerance improvement (Kappachery et al., 2013; Stevenson & Clark, 1937), until we understand the extent of variation in the crop. Unveiling the variation in the crop and its potential for drought tolerance improvement requires studies that involve many potato genotypes (Wishart et al., 2013). Crop improvement is a more realistic and sustainable option than environmental improvement that is limited by the cost and difficulty in managing some aspects of the environment, like the uncontrollable aspects (e.g., climate) (Cooper & Hammer, 1996). For instance, a potato cultivar with a growing season of 120 – 150 days would require no less than 500 to 700 mm of shallow irrigation water to produce an average yield of 40 tons/ha (FAOSTAT, 2008). In the Netherlands, potato yield is about 42 tons/ha with average rainfall of about 400 mm during the potato cultivation season and extra irrigation by farmers in summer (FAOSTAT, 2016). Extra irrigation efforts are often not affordable to tropical and sub-tropical farmers in the emerging world. Therefore, yields in stress- and resource-challenged regions are generally much lower (Low et al., 2015). Unfortunately, more severe and more frequent drought scenarios have been predicted in both the emerging and developed worlds, respectively (Dai, 2013). Considering the increasing consumption of potato in these regions (Van Der Zaag & Horton, 1983), drought tolerance improvement in the crop is highly needed.

The challenges for potato drought tolerance improvement include selection of the most appropriate parental lines and lack of reliable screening methods in the early generations of breeding (Caliskan, 2016). Also, the consideration of potato as having a low genetic diversity based on the overrepresentation of one or a few progenitors in the pedigree of many newer cultivars (Mori et al., 2015), could limit breeding efforts. In fact, studies that show the potential

of commercial cultivars for drought tolerance improvement are limited (Soltys-Kalina et al., 2016). The knowledge gap with respect to the drought tolerance potential of cultivars sets back drought tolerance breeding efforts, and the use of wild relatives for breeding could introduce unfavourable alleles (Mani & Hannachi, 2015a). Furthermore, as in other crops, the research and breeding focus still needs to shift from survival to acceptable yields under drought, and this requires an understanding of the underlying mechanisms involved, and a translation of those into selectable candidate genes (Krannich et al., 2015). Unfortunately, the lack of validation of markers so far generated for selecting drought tolerant genotypes is still limiting their use in breeding (Caliskan, 2016).

This thesis contributes towards solutions to some aspects of these challenges by unveiling the diversity in potato cultivars grown in Europe and presenting options of appropriate choice of drought tolerance traits and tools. Also, the findings presented in this thesis further our understanding of drought response-associated pathways and mechanisms. These various aspects are further discussed and include recommendations, in order to facilitate their implementation in potato drought tolerance breeding efforts.

FACTORS INFLUENCING POTATO DROUGHT TOLERANCE IMPROVEMENT

Complexity of drought stress tolerance

Drought stress tolerance is a complex trait mainly because of its quantitative inheritance, being controlled by numerous small effect loci (Mir et al., 2012). The vast genetic variation that underlies the diverse mechanisms of the drought response also complicates a deeper understanding of drought stress tolerance (Boguszewska-Mańkowska et al., 2018; Khorshidi-Benam & Hassanpanah, 2007). Furthermore, drought stress coincidence with different plant phenological stages triggers different responses (Martin et al., 1992; Zhang et al., 2018). Therefore, a multi-faceted approach is required in unravelling the complexity of drought stress in order to facilitate crop improvement. That is, dissecting drought tolerance into QTLs, traits, mechanisms, and possibly genes, in the respective growth stages. QTL studies have been used to dissect the genetic complexity of the potato drought response, which resulted in several loci associated with traits that contribute to tolerance (Anithakumari, 2011; Tessema, 2017). Also, association mapping approaches may be used to further dissect the genetic control of drought tolerance in cultivated potato under field conditions. Association mapping exploits the recombination events that have occurred in the evolutionary or breeding history of a species (Hall et al., 2010). The various generations of recombination of loci in linkage disequilibrium implies that linkage blocks become smaller leading to more fine-scale mapping than traditional QTL mapping (Nordborg & Tavare, 2002). Also, the allelic variation in the germplasm are further explored in association mapping (Zhu et al., 2008).

In this thesis I used association mapping to dissect the genetics of drought tolerance in a panel of 95 commercial cultivars representing the potato germplasm cultivated in Europe (Chapter 3). I found associations between different genomic regions and tuber yield traits under irrigation and drought conditions (Chapter 3 – Fig.8 & Supplementary Fig.SF1). The genetic control of tuber yield on chromosome 5, which we found in this thesis under normal growth conditions, has previously been reported (Anithakumari, 2011; Hurtado-Lopez et al., 2015; Schönhals et

al., 2016; Tessema, 2017). However, the association on chromosome 3 with marketable tuber size and parameters of tuber size distribution (spread and size class with maximum tuber number), is novel. The agronomic factors affecting tuber size distribution directly (number of tubers per stem, and dry matter production and tuber growth) and indirectly (planting density and number of stems per plant), have been previously described (Struik et al., 1990). Another older study speculated on a rapid regulation of the number of tuber sets that determine marketable tuber size, irrespective of the continuous formation and/or resorption of new tubers (Moorby & Milthorpe, 1975). These reports suggested that various aspects of tuber size distribution that eventually influence marketable tuber size are similarly regulated. The associated region on Chromosome 3 in our study contributes towards unravelling the genetic control of these traits. Further investigations may be required to understand the molecular mechanisms involved. Interestingly, I found a normal distribution in the allele dosage of the cultivars for this marker on Chromosome 3 (Fig.1), suggesting that there has been no obvious selection for this specific region, which opens up the possibility for further improvement through this locus. It also indicates a robust statistical relevance for this marker locus. This may be exploited for breeding marketable tuber size under drought conditions.

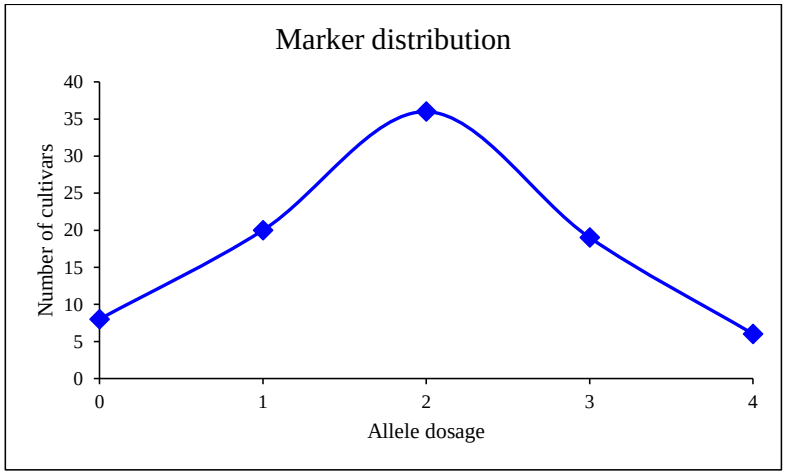


Figure 1: Normal distribution of allele dosages for the PotVar0030768 marker on Chromosome 3 of the potato genome showing the number of cultivars that contain each allele dosage (0: nulliplex, 1: simplex, 2: duplex, 3: triplex and 4: quadruplex).

Drought tolerance level in commercial cultivars

Wild potato is a rich resource for drought tolerance but is not the preferred option for breeding due to several factors like unwanted linkage drag, sexual incompatibilities and inapplicability of molecular markers (Halterman et al., 2016). On the other hand, genetic modification is currently not an option in Europe (Raybould & Poppy, 2012). A realistic option is to use advanced breeding material to improve drought and this requires an understanding of the drought tolerance potential of the cultivated germplasm. I have explored this using the European

cultivated potato in multi-location field trials. In the most drought-stressed field trial (Connantre 2015) we recorded an average of 54% and 51% total yield and marketable yield reductions, respectively (Chapters 2 and 3). The total rainfall in Connantre (2015) during the potato growing season was only 42 mm (Chapter 2 – Supplementary Fig.SF2). In another study conducted in Nepal using five CIP clones selected for drought tolerance, a German variety (NPI-106) and a Dutch variety (Desiree, also included in the cultivar set in this thesis), the drought-stressed plants were irrigated once for germination and they possibly received rain in the planting month prior to emergence (Luitel et al., 2015). I may assume that the amount of water the stressed block received was not more than the amount of rainfall in the planting month (February), that is, 45.1 mm and 26.6 mm in the two years of the trial, 2013 and 2014, respectively. The resulting reduction in marketable yield in that study was an average of 79%, which is a more severe reduction than the 51% reduction we recorded in the Connantre (2015) trial, despite the probable comparability of the stress levels in both studies. Among the genotypes used in Luitel et al. (2015), yield reductions in the supposedly tolerant CIP lines ranged from 67-81%, while yield in Desiree was reduced by 86%. This suggests that there is a level of drought tolerance in the cultivars we investigated that may be useful for crop improvement.

The variation in yield reduction in our study (Connantre 2015) ranged from 27% - 69% (Supplementary Table 1). Remarkably, the yield reduction of Desiree in our study was 57%, which is much lower than the 86% reduction reported in Luitel et al. (2015). The disparity between the two observations may be due to temperature differences between the two regions during the tuberization stages of the experiments. In our study the maximum mean temperature in June (tuberization period) was 24^{o}C, while in Luitel et al. (2015) it was 28^{o}C in April at tuber formation. A combined effect of drought stress and high temperatures has been shown to cause more severe reductions in tuber yield than single stress (Rykaczewska, 2013). Another factor may be the difference in altitude between both studies. The high altitudes in Nepal may have exposed the plants in Luitel et al. (2015) to higher vapour pressure deficits that could reduce stomatal conductance. High VPD is known to reduce stomatal opening thereby limiting carbon assimilation and photosynthesis (Romero et al., 2017). In another study in Belgium, 11% yield reduction was reported for Desiree under drought (Lahlou et al., 2003). However, in the study of Lahlou et al. (2003) the drought-stressed plants received at least 148mm of rainfall, which must have resulted in a much milder stress than in our study. Therefore, the response of Desiree varied in the different locations, indicating that the environment has a strong influence on response of potato plants.

The role of GxE interactions in drought tolerance improvement

Climatic differences between locations and environmental differences between years in the same location affect the drought response of different genotypes differently (Kooman et al., 1996). In our study, we observed significant GxE interaction (Chapter 2 – Table 1), suggesting a differential response of the cultivars to drought in the various locations. The GxE analyses using both Finlay Wilkinson's Regression (FWR) and GGE biplots revealed a trend of a decrease in tuber yield with lower water availability across environments in more than half of the cultivars (Chapter 2 – Fig.3b [Quadrants II and III]). However, tuber yield was not directly

linked to water availability in the few genotypes that clustered in Quadrant I (Chapter 2 – Fig.3b). These relatively stable and widely adapted cultivars may be important sources for improving potato drought tolerance (Supplementary Table 1). The success of a variety may be attributed to both its stability over years and wide adaptability to several environments, in addition to a high yield potential (Roy & Kharkwal, 2004). According to Ceccarelli et al. (1991), yield stability in plants is based on the mechanism of buffering whereby a heterozygous plant or a population of plants with slight genetic dissimilarity forms a homeostatic resistance to the effects of fluctuating environmental conditions. The eventual yield stability is a combined contribution of the interaction of multiple traits towards yield (Ceccarelli et al., 1991). In our study we assessed the yield stability of the cultivars in multi-year trials in the various locations. Although the 'Year' factor was significant based on the Analysis of variance (Chapter 2 – Table 1), there were no significant 'Genotype x Year' and 'Genotype x Location x Year' interactions. These indicate that the drought stress was different between years, for instance, the early and late droughts at Connantre in 2014 and 2015, respectively. However, the difference between years did not result in a differential response of the cultivars to drought. Therefore, GxY and GxExY interactions may not be as serious a concern as Genotype x Location (GxL) interaction for drought tolerance breeding in Western Europe. In fact, breeders generally prefer multi-location trials in a year to multi-year trials in the same location, as they usually assume that GxExY interaction is absent (Romagosa et al., 2013). Our finding suggests that this may be the case in potato as well. Each year presented a unique environment from different years in the same location (Chapter 2 – Fig.6), thus GxY interaction, just like GxL interaction, may be considered as an aspect of GxE interaction.

However, wide adaptability across different locations may be a challenge to drought tolerance in potato based on our findings (Chapter 2 – Table 1). The highest percentages of variation in our dataset were due to Location (37.92%) and Genotype x Location interaction (22.41%) (Chapter 2 – Table 1). This suggests that GxE interaction effects need to be carefully considered in drought tolerance improvement of potato. In a case study in the UK, potato yield increase from 34 t/ha in 1964 to 42.9 t/ha in 1976 was attributed to environmental improvement (4t/ha) and genotype replacements (5.5 t/ha), but with a negative GxE effect of -0.6 t/h, because the new cultivars were not better than the old ones in their responsiveness to environmental change (Simmonds, 1981). Such negative effects of GxE may be avoided by defining a range of target environments, as this determines the selection/assessment environment and thresholds of trait measurements required for selection (Bradshaw, 2016). In fact, in Japan, for instance, potato breeding has been partitioned according to the various climatic conditions in the country, which has facilitated an all-year-round cultivation of the crop (Mori et al., 2015). In addition, in a study on potato nitrogen use efficiency in Ethiopia, it was recommended to breed cultivars for two target mega environments under rain-fed and irrigation production systems at low NUE (Getahun, 2017). Breeding efforts with focus on targeted environments would equip breeders with knowledge of the drought timing, duration and likely severity in the region of interest. Also, based on the knowledge of the expected drought timing, breeders can choose the developmental stage to select for drought tolerance traits at the target environment. The GxE analyses in this thesis provide useful information for choice of cultivars and selection environments for drought tolerance improvement with the European potato germplasm

(Chapter 2 – Figs.3b & 4c). Cultivars in Quadrant I (Chapter 2 – Fig.3b) are resourceful for introducing high yield potential, stability and wide adaptation within target environments (Supplementary Table 1). Interestingly, about 50% of the late maturity cultivars in our dataset clustered in this Quadrant I, suggesting a possible involvement of maturity in drought tolerance.

Foliage maturity and drought tolerance

Potato foliage maturity describes the duration in days after emergence that a potato plant requires to have its canopy expand to maximum ground coverage, and the longevity of the maximum canopy cover until eventual senescence (Struik, 2010). Foliage maturity is scored in potato fields by breeders and agronomists using traits like onset of leaf senescence and duration of plant life cycle (Kloosterman et al., 2013). More recently, the greenness of canopy captured on camera can be used to infer foliage maturity (Rémi Ducreux, HZPC, France, personal communication). There is evidence of genetic control of foliage maturity on potato chromosome 5, which forms the basis for the observed variations in foliage growth (Visker et al., 2003). Interestingly, this genetic locus controlling foliage maturity is tightly linked to important traits in potato like late blight resistance, tuberization and yield (Kloosterman et al., 2013; Visker et al., 2003). Furthermore, Anithakumari et al. (2012) found a co-localization of some drought tolerance QTLs with this maturity locus on potato chromosome 5. The co-localizing drought tolerance QTLs were associated with shoot weight, tuber number and tuber weight under drought (Anithakumari et al., 2012). In this thesis, we observed that foliage maturity played an important role in the drought response of the cultivars in our dataset (Chapter 2 – Fig.8). The impact of foliage maturity was, however, influenced by the timing of drought in the growing season. The effect of foliage maturity differences on maximum canopy cover and exponential growth rate did not change under the late drought as compared to the irrigated conditions (Chapter 2 – Fig.8c & 8d). But during the early drought, which coincided with critical phases of canopy growth, the later maturity types were less affected than the early ones in terms of exponential canopy growth rate and maximum canopy cover. It is known from previous reports that early drought results in less canopy growth and affects early maturing cultivars more severely, while late drought hastens senescence and may be more severe on either early or late maturity types depending on how late the drought occurs (Bassam et al., 1990; van Loon, 1981). Early maturing genotypes may escape a very late drought (Bassam et al., 1990), but when the drought is not so late, like in our trial (Connantre 2015), early maturity types are vulnerable. In another study involving three cultivars of different maturity types in the field, early drought reportedly delayed full canopy development by reducing shoot growth (Chang et al., 2018). However, dependence of the shoot growth response on maturity was not observed by Chang et al. (2018), probably because of the small sample size used in that study. The early maturity types in our study may have been severely affected by the early drought due to their relatively shorter exponential growth phase than later maturity types. Therefore, late maturity may be more advantageous for canopy growth maintenance when drought occurs before the senescence of early-maturing genotypes.

The association of the maturity locus on potato Chromosome 5 with tuber yield (Kloosterman et al., 2013; Visker et al., 2003), may have contributed to the tuber weight differences under drought among the maturity classes in our study (Chapter 2 – Figs.8c and d). The findings from

Kloosterman *et al.* (2013) using diploid potato populations showed that an allele for early foliage maturity of the CYCLING DOF FACTOR (CDF) 1 gene on Chromosome 5 (*StCDF1.2*) induced early tuber initiation in a late-maturing genotype. More recently, another CDF allele on Chromosome 5 different from the *StCDF1.2* of Kloosterman et al. (2013), *StCDF1_snp1812*, was shown to improve tuber and starch yield in potato when present in triplex allele dosage (Schönhals et al., 2016). A role for CDFs in tuber yield under drought conditions in potato has not yet been reported. However, an overexpression of tomato CDF homologs has been shown to improve drought tolerance in *Arabidopsis* (Corrales et al., 2014). The above respective reports of CDF involvement with tuber yield and drought tolerance may point to their possible role as a component of the maturity locus in regulating tuber yield under drought. In our study, late-maturing cultivars had more tuber weight than the early maturity types under drought (Chapter 2 – Fig.8). Chang et al. (2018) reported that the late-maturing cultivar formed tubers earlier during an early drought than under control conditions, which promoted yield maintenance under drought. The molecular mechanism for the induction of early tuberization in the late cultivar in Chang et al. (2018) was not reported. Assuming that the early tuberization in Chang et al. (2018) is comparable to the early tuberization effect of *StCDF1.2* overexpression in late maturity background in Kloosterman et al. (2013), I may speculate a possible interaction of drought with CDF gene expression. Further research, however, will be needed to investigate drought-CDF interactions.

The advantage of late maturity types of potato in response to drought includes the possibility of a drought recovery and foliage second growths, which can lead to longer duration of light interception and dry matter production (Haverkort & Goudriaan, 1994). However, Soltys-Kalina et al. (2016) have argued that late maturity is not the main factor determining drought tolerance. In their study, a supposedly late maturing cultivar, Sequoia, was not as tolerant as the other late-maturing Katahdin half-sibs used in that study (Soltys-Kalina et al., 2016). The poor performance of *cv.* Sequoia under drought in that study may be due to the experimental approach – the plants were grown in cylindrical bags of limited dimensions. Nevertheless, a description of *cv.* Sequoia in potato varieties database shows that it is recommended for cultivation in non-irrigated regions (Wilson, 2010). Based on our results, I recommend a late maturity background to be considered for drought tolerance improvement in potato at least under temperate climate conditions with long days. Requirements may be different under conditions in which the growth season is relatively short. For such regions, drought tolerance in combination with intermediate or even early maturity is required. Therefore, further genetic analysis and fine-mapping is required to dissect the contribution of maturity from other traits contributing to drought tolerance in potato.

TARGET FEATURES TO IMPROVE FOR POTATO DROUGHT TOLERANCE

Growth balance between shoot and underground tissues

Coordination of aboveground foliage growth and belowground root and tuber growth in potato requires interaction and signalling between the different tissues of the plant (Ewing & Wareing, 1978; Jefferies, 1993; Swiezynski et al., 1978), in particular under stress conditions when

resources for growth are limiting. Studies of the molecular regulation of this interaction and signalling has provided new insights (Ghate et al., 2017; Jonik et al., 2012; Katoh et al., 2015). The activity of mobile RNAs was shown to regulate shoot growth and tuber yield in potato (Ghate et al., 2017). Overexpression of the long distance mobile RNA *StBEL5* in leaf tissue induced growth in underground tissues. Antagonistically, overexpression of mobile RNAs *StBEL11* and *StBEL29* in leaf inhibited tuber growth, while their *RNAi* lines enhanced overall tuber yield with no effect on shoot growth (Ghate et al., 2017). Also in another study, the overexpression of an *E. coli* pyrophosphatase and knockdown of ADP-glucose pyrophosphorylase (*AGPase*) in potato leaves were used to re-route photoassimilates to sink organs, simultaneously boosting sink capacity by overexpressing plastidic metabolite translocators in tubers (Jonik et al., 2012). These reports demonstrate that molecular interaction and signalling among tissues can be targets for manipulation and possibly also, breeding, to favour tuber yield.

In Chapter four of this thesis we investigated the molecular interaction between above- and belowground tissues in terms of carbon partitioning using genotypes with contrasting drought responses - tolerance (Biogold) and sensitivity (Mondial). The major contrast between Biogold and Mondial was the continued tuberization and bulking in Biogold under drought, while in Mondial tuberization and bulking was severely impaired, depending on the drought severity. The gene expression results showed that the assimilate transporters, triose phosphate translocator (*TPT*) and sucrose will eventually be exported transporter10 (*SWEET10*), were both downregulated in the two cultivars (Chapter 4 – Fig.4), suggesting that these were not the determinant factors for the genotypic variation. The activity of *TPT* in triose sugars export from the chloroplast may affect photosynthesis, but not tuber yield (Riesmeier et al., 1993; Schulz et al., 1993). Also, a link between *SWEET10* and potato tuberization was suggested (Abelenda, 2017; Timmermans, 2016), but this link has not yet been confirmed under drought. On the other hand, we found a contrasting response to drought between the two cultivars for the expression of starch biosynthesis genes, ADP-glucose pyrophosphorylase (*AGPase*) and Granule-bound starch synthase (*GBSS1*), in the belowground tissues (Chapter 4 – Fig.4). The upregulation of *AGPase* and *GBSS1* in the stolon tissues and absence of starch in the leaves of Biogold (Chapter 4 – Fig.5) may be linked to the presence of tubers on the plant. Likewise, the downregulations of these genes and starch accumulation in the leaves of Mondial (Chapter 4 – Fig.5), may be associated with lack of tubers. However, the causal relationships between the gene expressions, starch in the leaves and tuber yield phenotypes require further understanding.

We performed a slightly milder drought experiment in which we evaluated tuber/shoot ratio at two time points (Chapter 5 – Fig.1b), similar to the experiment discussed in chapter 4. The results suggest that genotypic differences and drought intensity may possibly play a role in the causal relationship between gene expression (of genes in carbon partitioning pathway and genes in starch biosynthesis), leaf starch storage and tuber yield. Biogold maintained a higher tuber-to-shoot ratio than Mondial between 28-77 days of stress (Chapter 5 – Fig.1b), suggesting that assimilate partitioning in Biogold favoured tuber yield under drought than in Mondial. Interestingly, tuberization was observed in all four genotypes in the mild drought experiment (Chapter 5 – Fig.1b), unlike under the more severe drought (Chapter 4 – Fig.1a). Therefore, I

speculate that the more severe drought stress may have induced a tuberization shutdown in Mondial, but not in Biogold. There are indications that drought inhibits tuberization as inferred from tuber number (Deblonde & Ledent, 2001; MacKerron & Jefferies, 1986; Struik & Van Voorst, 1986). Tuberization is a complex developmental process influenced by many factors including several regulatory proteins (*SP6A*, *BEL5*, etc.), hormones (GA, ABA, etc.) and metabolites (e.g., sucrose) (Mani & Hannachi, 2015b; Xu et al., 1998). Under the severe drought conditions of our study in Chapter 4, the levels of sucrose in the leaves of the two cultivars (Chapter 4 – Fig.5a) suggest that sucrose was not the limiting factor. Furthermore, starch accumulation in the leaf of Mondial both under irrigation and drought conditions (Chapter 4 – Fig.5b) suggests that the absence of tubers under drought may not be a justifiable reason for the starch accumulation. Genotype-specific characteristics with respect to the interaction of drought with the molecular factors regulating the tuberization process itself is therefore a possibility. We did a pilot study using transgenic diploid potato lines with overexpression or silencing constructs of CDF genes. The gene expression patterns we observed did not perfectly fit in the current tuberization model as described in (Kloosterman et al., 2013; Kloosterman et al., 2008). The CDF homologs *CDF1* and *CDF5*, seemed to respond to drought stress and possibly repress tuberization through *BEL5* and *SP6A*. The differences between the gene expression patterns in our pilot study and the current tuberization model suggests that the tuberization pathway may be more complex and might involve more genes than we currently know. Moreover, in the transcriptomic analyses (Chapter 6), we detected significant upregulation of genes that repress tuberization in cultivars that maintained tuber formation under drought, but not in the cultivars that were significantly affected in their tuber formation. These findings suggest a possible interaction of drought with tuberization. A further investigation of the tuberization-related genes under drought is required to gain more insights on the effects of drought on tuberization. This understanding will facilitate breeding for potato that can more readily balance shoot and below-ground growth, especially tuber yield.

Physiology and morphology of transport

Drought stress negatively impacts the physiological and morphological characteristics of potato (Li et al., 2017; Tourneux et al., 2003; Vasquez-Robinet et al., 2008). One of the early responses to drought stress is stomatal closure, which is the result of ABA-mediated signalling (Munemasa et al., 2015). Stomatal closure reduces water loss through transpiration as a drought adaptive measure (Waggoner & Simmonds, 1966). However, a lowered transpiration leads to reductions in soil water uptake by plant roots (Campbell et al., 1976; Saradadevi et al., 2017), which can negatively affect photosynthesis (Vos & Groenwold, 1989), but also xylem fluxes and therefore nutrient transport (Mahmud et al., 2014). In this thesis, we observed genotypic variation in stomatal closure under drought (Chapter 6 – Fig.2b). One of the genes that was upregulated in genotypes that maintained stomatal opening under drought is an extracellular Ca^{2+}-sensing receptor (*CAS*). Currently, there are no reports of *CAS* involvement in potato stomatal conductance, but the role of *CAS* signalling pathway in stomatal closure has been elaborated in *Arabidopsis* (Wang et al., 2014; Wang et al., 2012). *CAS* signalling involves an ABA cross-talk with a *CAS*-mediated induction of hydrogen peroxide (H_2O_2) and nitric oxide (NO) through high extracellular Ca^{2+} levels, which triggers cytosolic Ca^{2+} increase in guard

cells and eventual stomatal closure (Wang et al., 2012). *CAS* is localized in the thylakoid membrane of the chloroplast and is involved in regulating CO_2 availability and redox reactions of the chloroplast stroma (Hochmal et al., 2015). In Arabidopsis plants, *CAS* was also reported to be involved in the formation of photosynthetic electron transport system, leading to drought tolerance and water use efficiency (Wang et al., 2014). In our study, we observed *CAS* downregulation, stomatal closure and downregulation of photosynthesis-related genes in the same cultivar, Hansa, and a direct contrast of these characteristics in *cv*. Biogold (Chapter 6 – Fig.5). Based on the reported role of *CAS* in photosynthesis, our observation may point to a possible interaction of ABA-dependent and *CAS*-mediated stomatal closure in potato under drought, which may be associated with molecules regulating photosynthesis.

Furthermore, we observed morphological modifications of water-transport vessels in the stem associated with drought tolerance (Chapter 5). Cultivar Biogold had numerous tracheid elements in the lower stem region (Chapter 5 – Fig.7). Tracheids have not been reported in potato, but they are mentioned as a component of the tomato stem (van der Schoot & Bel, 1989), although their involvement in drought response is generally unknown in Solanaceae and other crops. In *Juniperus* species, however, decreasing size of tracheids was associated with decreasing vulnerability to cavitation under drought (Willson & Jackson, 2006). In crop species, breeding for narrow xylem vessels in roots of wheat enhanced yield under drought conditions by increasing the hydraulic resistance to water uptake, thereby moderating water use (Richards, 2006). Assuming a conservation of function across species, this may suggest that the tracheids and small vessel sizes in *cv*. Biogold in our study may have facilitated a maintenance of upward water flow under drought, in response to decreased (root) hydraulic pressure. Another study on potato xylem reported that potato plants grown at 15^{o}C had a lower number of xylem vessels than when grown at 20^{o}C, suggesting that indeed potato stem vessels may be responsive to environmental stresses (Harris, 2013). Based on our findings, it is recommended that structural components of the potato stem are considered as targets for improvement of water transport in order to cope with drought conditions. It has been shown that fluxes in the water transport vessels of the stem facilitate phloem transport (Prusova, 2016; Windt et al., 2006). Therefore, improved vessel sizes that maintain water transport may likely enhance assimilate transport as well.

Water use

On a global scale, agriculture is currently using about 70% of the world's total freshwater, and agricultural water requirements are speculated to increase by 15% in 2050 (Khokhar, 2017). However, the fresh water resource of the world is on the decline (Frankel, 2015). There is therefore a need for crops that can efficiently use water. Potato is known as an efficient water user under optimal conditions (Ati et al., 2012), and the dependence of potato yield on nutrient input is less under full irrigation (Badr et al., 2012). However, potato water use efficiency is challenged under drought conditions (Ankush et al., 2007; Dalla Costa et al., 1997). As a step towards managing potato water use efficiency under drought, CIP introduced the Partial Root-zone Drying (PRD) management practice (Adolfo et al., 2008). PRD technique involves alternating irrigation/dry-out on two halves of the potato root system during the growing season (CIP, 2013; Jovanovic & Stikic, 2018). The idea is to create an ABA-mediated drought signal

that trains the plant to acclimate its transpiration and subsequently use water more efficiently (Liu et al., 2008). Using PRD, tuber yield was maintained while using 50% of full irrigation water (Yactayo et al., 2013). However, PRD only works if initiated early (not later than six weeks after planting) (Yactayo et al., 2013), and even 50% of full irrigation capacity may not be affordable to resource-poor farmers in arid regions. Therefore, genetic improvement of potato WUE under drought is essential, and when combined with management practices may boost productivity. In our field trial at Connantre (2015), I computed total water use efficiency (WUE_T) as "Yield/water input" (Montgomery, 2016), under irrigation and drought stress, in order to evaluate the variation in the crop for water use under drought.

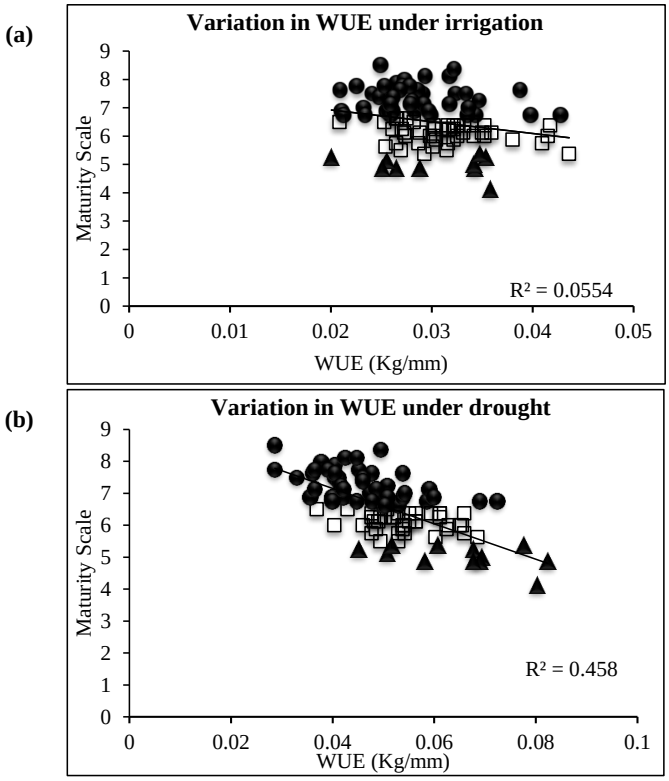


Figure 2: Variation in total water use efficiency (WUE_T) of 103 cultivars representing different maturity classes under (**a**) irrigation, (**b**) drought stress. Symbols show early maturity (circles), intermediate maturity (open squares) and late maturity types (triangles). Maturity scale ranges from 0 (late) to 9 (early).

I observed a variation range of 0.024 kg/mm and 0.053 kg/mm in WUE_T under irrigation and drought stress, respectively. That is, variation in WUE_T doubled under drought, and was more strongly associated with maturity than under irrigation (Fig.2). Higher WUE_T was recorded in the late-maturing compared to early-maturing plants. The advantage of a longer growth cycle on tuber yield (Kooman et al., 1996), may have provided the late-maturing cultivars in our study

more time for tuber bulking than the earlier maturity types. The variation in the potato for WUE under drought may be exploited to improve water use, but further studies are required to dissect the genetics of the contributory traits and to better understand the mechanisms involved (Ankush et al., 2007).

CONTRIBUTION TOWARDS DROUGHT TOLERANCE BREEDING IN POTATO

Traits and tools for potato drought tolerance selection

As a complex trait, drought tolerance improvement will depend on targeting the right traits that will contribute to tolerance (Khan, 2014; Khan et al., 2015). In this thesis, we investigated several potato shoot and tuber traits in the greenhouse and in the field, to discover traits that could contribute to potato drought tolerance improvement. The drought tolerance-associated traits based on our findings are highlighted below, including the feasibility and availability of tools for scoring them in the field.

Stomatal conductance has been extensively studied in potato and shown to decline under drought prior to reductions in photosynthesis (Vos & Groenwold, 1989; Vos & Oyarzún, 1987). Stomatal conductance is a more reliable measure of drought stress perception than, for instance, soil water content or leaf water potential, because it reports both non-hydraulic (hormonal) and hydraulic (water potential) stress signals (Jovović et al., 2016). In fact, stomatal closure can be detected at relatively high leaf water potential (-0.4 MPa and -0.6 MPa) (Dalla Costa et al., 1997). In this thesis we scored stomatal conductance on subsets of the cultivars grown in the greenhouse (6 cultivars – Chapter 4, Sup. Fig.SF1) and in a rain-out tunnel in the field (5 cultivars – Chapter 6 - Fig.2b), at four weeks after stress (28DOD). We observed a trend of similarity between both trials for genotypic drought responses in the cultivars grown in both trials, Biogold and Hansa. This might suggest that the greenhouse findings related to stomatal conductance in this study may be reliably translated to field conditions. In the greenhouse, we observed 85% and 75% reductions in stomatal conductance and tuber yield, respectively, for Biogold, while 94% and 100% reductions in stomatal conductance and tuber yield, respectively, were recorded for Hansa (Chapter 4 - Fig.1b & Supp. Fig.SF1). In the rain-out tunnel in the field, we observed 30% and 50% reductions in stomatal conductance for Biogold and Hansa, respectively, but tuber yield was not reduced in both cultivars (Chapter 6 - Fig.2b & d). Apparently, the drought stress in the greenhouse was more severe than in the rain-out tunnel. Possibly, a shutdown of stomatal conductance may negatively impact tuber yield. In another study using four genotypes with contrasting drought tolerance, the two drought-tolerant genotypes exhibited variant mechanisms of stomatal regulation – early stomatal closure, and delayed but enhanced stomatal closure, respectively (Boguszewska-Mańkowska et al., 2018). The findings of Boguszewska-Mankowska et al. (2018) and our study (Chapters 4 & 6) suggest a genotype-dependent regulation of stomatal conductance that is associated with drought tolerance. However, in a study of drought response of *Andigena* potato genotypes in the field, no association was found between stomatal conductance and tuber yield (Schafleitner et al., 2007). The contrasting result between our finding and Schafleitner et al. (2007) may be due to the differences in genotypic backgrounds between both studies. Also, in Schafleitner et al.

(2007) stomatal conductance was scored on leaf petioles, while in our study we scored the leaf abaxial surface. I recommend stomatal conductance measurements during drought stress, as a means of selecting for plants with regulated stomatal opening/closure as against plants with uncontrollably open or closed stomata. Keeping stomata open increases transpiration, which might need to be supported by deeper roots to give higher yields under drought. Deeper roots on plants that close their stomata means that the investment in roots may not pay off. Therefore, stomatal conductance is an important piece of the puzzle of drought tolerance, but it needs to be used in the context of water uptake and transport. Reliable scores of stomatal conductance in the field require methods that minimize the time lapse between individual observations. Therefore, using a hand-held porometer may not be feasible for screening large sets of plants. For such large screens, new developments in high throughput phenotyping, like the use of thermal infrared cameras for canopy temperature and stomatal behaviour, will be useful (Prashar & Jones, 2014).

Canopy growth traits are determinants of light interception by a plant, which affects photosynthesis and eventual tuber yield (Genet, 1985; Khurana & McLaren, 1982; Shah et al., 2004). Drought stress, however, reduces potato canopy growth leading to poor yield (Fleisher et al., 2008). Therefore, we investigated the canopy drought response by exploring various parameters of canopy growth. We found that the time-based parameters of canopy growth were critical for tuber yield (Chapter 2 – Fig.8c & d). That is, a delay in both attainment of exponential canopy growth rate and maximum canopy cover correlated with tuber yield under drought. This delay may suggest that canopy growth under drought is regulated in order to balance assimilate partitioning to various plant tissues, including tubers. In a study using three potato cultivars grown in the field, it was demonstrated that genotypic differences in final tuber yield were not due to the amount of radiation intercepted, but the efficiency in radiation use (Oliveira et al., 2016). Shorter stolons and larger tuber sink strength were reported for the highest yielding cultivar compared to the other cultivars. Another study of three potato cultivars in the field described the advantages of long duration of canopy stay-green phenotype for drought tolerance, suggesting that canopy stay-green implied a reduced degradation of chlorophyll (Rolando et al., 2015). These reports at least suggest that certain characteristics of canopy growth, in addition to light interception, can influence tuber yield. Accordingly, the parameters we have described in this thesis present leads towards exploiting canopy growth for drought tolerance. Therefore, I recommend the measurement of canopy cover during selection trials to obtain parameters through which foliage maturity, length of photosynthetic period and senescence, can be inferred. In terms of trait sampling, drones technology may become common and cheap in the near future (Adrienne, 2015), and could be used in capturing canopy growth images in order to extract parameters for selection in large fields (Ludovisi et al., 2017).

Marketable tuber yield of potato is the actual productivity of the crop and determines its relevance in the various market sectors (Liovi et al., 2008). Drought stress may drastically reduce marketable tuber yield (Hirut et al., 2017; Luitel et al., 2015; Sri Ranjan & Abbas, 2015). In our study, we evaluated marketable tuber yield alongside parameters of potato tuber size distribution (Chapter 3). We found that under drought, marketable tuber yield, size distribution spread of tuber number and the size class with the highest tuber number, were associated with

the same molecular marker on Chromosome 3 (Chapter 3 – Fig.8). This suggests that the tuber size distribution parameters may likely give indications of tuber yield marketability under drought. This is the first attempt at dissecting the genetics of marketable tuber yield in potato. An implementation of molecular markers associated with this region on chromosome 3 early in a breeding scheme could shorten the duration of the breeding program, and save costs of large phenotypic screening. Marker-assisted selection in potato is more commonly reported for disease resistance (Sliwka et al., 2010; Tiwari et al., 2013), than for abiotic stress tolerance, probably because of the quantitative nature of environmental stresses (Hospital, 2009). Also, the large genomic region of loci associated with stress tolerance traits make it difficult to implement them in breeding (Slater et al., 2013). The novel marker-trait association in this thesis may be further explored by genotyping and phenotyping more potato cultivars to investigate broad applicability of the marker for the associated traits, and to further narrow down the QTL interval. A successful validation of this marker and implementation in breeding selection schemes would mean that large numbers of potato lines can be screened and selections made for marketable tuber size, and wide spread of tuber size distribution, which are mapped to the same genomic region with size class with the highest number of tubers.

Xylem density and size in potato have not received much research attention even though there are several studies that have investigated the xylem with respect to ABA signalling under drought (Ahmadi et al., 2010; Liu et al., 2006). In this thesis, we investigated the possible roles of xylem morphology and distribution in the stem on drought response. We found that drought tolerance may be associated with a high xylem density in the lower stem (Chapter 5 – Fig.8). Also, in the drought tolerant cultivar with high xylem density, we observed numerous small-sized xylem elements (tracheids) (Chapter 5 – Fig.7). I speculate that the mechanism of drought resistance involving xylem density and size, may be the prevention of cavitation under drought. In another study on cavitation resistance using six Chaparral shrubs, it was shown that xylem density contributed to the prevention of cavitation (Jacobsen et al., 2005). Furthermore, there are indications that narrow xylem vessels are less vulnerable to freezing-induced cavitation (Lambers et al., 2013). Therefore, I recommend further studies in potato to investigate the potential, genetic variation and the expected contribution of xylem traits to drought tolerance. Until the genetic basis of this trait has been understood, I recommend that screening for xylem density and size be included in the latter phases of selection trials since it involves destructive assays. In this way, it could be used to screen for lines that can better manage their hydraulic conductance during drought.

Drought signalling pathways and response mechanisms

We investigated the transcriptome profile of potato cultivars with contrasting drought responses to gain insight on the molecular processes underlying genetic and phenotypic variation in the field. Firstly, the expression of genes of the ABA signalling pathway in the two most contrasting cultivars, Biogold and Hansa, provided insight in their molecular responses related to drought stress signalling. We found a downregulation of the ABA receptor *PYL4* in Hansa, and the same fold change in Hansa and Biogold for an upregulation of cytochrome P450 (*CYP707A4*) (Chapter 6 – Supp. Tables 1 and 2). The PYL(s) play a significant role as receptors of the stress-induced ABA, which is the first step in ABA-mediated stress signalling (Gonzalez-Guzman et

al., 2012; Ng et al., 2014). On the other hand, cytochrome P450 (*CYP707A4*) is known to encode ABA 8'-hydroxylases, which is involved in ABA degradation (Kushiro et al., 2004). The upregulation of cytochrome P450 (*CYP707A4*) in both cultivars may suggest that at the time of tissue sampling (28DOD – days of drought), ABA concentration may not be as high as at earlier moments of stress perception (there is no data to investigate ABA concentration at the beginning of the stress). But the downregulation of *PYL4* in Hansa may suggest an impairment of ABA sensing in Hansa. The PYL(s) form a tertiary complex after ABA recognition that inhibits protein phosphatase 2C (*PP2C*), thereby activating the expression of *PP2C* targets such as *SnRK2* that facilitate downstream stress responses (Gonzalez-Guzman et al., 2012; Hubbard et al., 2010; Park et al., 2009). In our study, *PP2C* was upregulated in Hansa, but downregulated in Biogold, possibly suggesting that downstream ABA signalling was more active in Biogold. In fact, we observed upregulations of phospholipase D (*PLD*), calcium-dependent protein kinase (*CDPK*) and ABC transporter gene in Biogold, but not in Hansa. *PLD* hydrolyses membrane lipids to produce phosphatidic acid (PA), which functions as second messenger in stress signalling (Wang, 2005). PA is a central signalling molecule that activates *CDPK* and also NADPH oxidase leading to ROS signalling that facilitates stomatal closure (Hemantaranjan, 2013; Munnik, 2009; Zhang et al., 2009). Moreover, the role of PLDs in the promotion of ROS production and mediation of plant response to ROS has been reported (Wang, 2005). Therefore, signalling in Biogold may have induced more downstream response genes than in Hansa. These variations in DEGs between Biogold and Hansa in our study suggest differences in ABA signalling mechanisms that may relate to variation in drought tolerance.

Genes of the Gibberellic Acid (GA) pathway (gibberellin 2-oxidase) and Cytokinin (CK) pathway (two-component sensor protein histidine protein kinase), were upregulated in both Hansa and Biogold. Gibberellin 2-oxidase is involved in GA degradation (Chen et al., 2016). Upregulation of gibberellin 2-oxidase at 28DOD may suggest that drought downregulated GA signalling in the plants. In maize, it was reported that exogenous application of GA under drought, but also CK, can alleviate stress effects during the vegetative growth phase (Akter et al., 2014). On the other hand, antagonistic interaction of ABA with GA has been reported (Rogers & Rogers, 1992). But the upregulation of the ABA degrading gene cytochrome P450 suggests that ABA repression of GA may not be concluded based on our data. In terms of the CK pathway, histidine kinases (HKs) are known as the primary cytokinin receptors in plants that facilitate further downstream cytokinin-mediated responses (Muller & Sheen, 2007). The upregulation of the two-component sensor protein histidine protein kinase in our study at 28DOD, may suggest that cytokinin signalling probably began at a relatively late stage of the drought. Indeed, a role for CK in delaying senescence has been reported (Wingler et al., 1998). This may suggest that the CK upregulation at 28DOD in our study may be necessary in delaying the aging of the plants, supporting a green canopy for a longer time.

Additionally, auxin hormonal pathway-responsive genes, glutathione-S-transferases (GSTs), were downregulated in both Biogold and Hansa, while small auxin-upregulated RNAs (*SAURs*) and indole-3-acetic acid (IAA) amido synthetase were downregulated only in Biogold. GSTs are involved in cell detoxification and responses to oxidative stress (Marrs, 1996). However, drought and salt stress tolerance have also been reported in *GST* knockout *Arabidopsis* lines,

and this was attributed to the combined effect of Glutathione and ABA (Chen et al., 2012). Furthermore, *SAURs* are auxin- and stress-related genes that when overexpressed resulted in less H_2O_2 and longer roots, thus conferring drought and salt tolerance in *Arabidopsis* (Guo et al., 2017). The downregulation of the auxin biosynthesis gene (IAA-amido synthetase (*GH3*.3) and *SAURs* suggests a repression of the auxin hormone pathway in our drought stressed potato plants. Likewise, genes of the ethylene hormonal pathway and ethylene response factors were downregulated under drought in both Biogold and Hansa. Complex interactions among the various hormonal pathways have been reported in literature (Abel et al., 1995; Cheng et al., 2009; Iqbal et al., 2017; Kovtun et al., 1998; Nordstrom et al., 2004; O'Brien & Benková, 2013; Rogers & Rogers, 1992; Ross et al., 2000; Weiss & Ori, 2007). Under drought stress, crosstalk between hormonal pathways may be more intense than under normal growth and development (Xiong et al., 2002). Our evaluation of the two contrasting genotypes, Biogold and Hansa, unveiled differences in drought signalling mechanisms, especially in the ABA pathway. The improvement of drought tolerance in potato may therefore involve targeting genes of the downstream signalling cascades in the ABA signalling pathway. Genes of the Cytokinin signalling pathway may essentially be targeted for canopy longevity in the field under drought. Also a balance between ABA degradation after stress perception and timely GA biosynthesis may be useful in improving leaf expansion, because GA facilitates growth (Pandey, 2017). Moreover, the role of GA in tuber formation has been described, which directly relates to tuber yield (Struik et al., 1989).

CONCLUSIONS AND THESIS IMPACT

Drought is an environmental challenge and will remain relevant for many decades due to the increasing impact of climate change on rainfall patterns in different regions of the globe. Potato is a widely consumed food crop of high importance in both the developed and emerging worlds. Therefore, this thesis on drought tolerance in potato is a timely investment that contributes towards enhancing potato production in the near future under water-limiting conditions. In line with the aims of this thesis, we have studied a representative set of potato cultivars grown in Europe for variation in their drought response that may be used to improve potato drought tolerance. Our conclusion is that there is untapped variation in the germplasm that can facilitate drought tolerance breeding, and so, we have provided the criteria to select progenitors of breeding schemes (Supplementary Table 1). We have provided measurable traits that may be used to select for drought-tolerant lines, and molecular marker association with marketable tuber yield that localizes on a locus (Chromosome 3) that is yet to be harnessed for breeding. Furthermore, we identified molecular pathways, physiological processes and morphological modifications that are associated with drought tolerance of potato. Our results thus form a platform that can be further advanced towards drought tolerance breeding in potato.

From a scientific perspective, the findings on the mechanisms of drought response may be implemented in other plant systems thereby preparing crops for the changing environmental conditions. It has led to a tentative model depicted in Fig. 3 as to how the potato plant may act under conditions when water becomes limited (drought). Breeders would benefit from the knowledge of these drought response mechanisms as well as of the Genotype-by-environment interactions and the breeder's toolbox generated from this study, which they may use in the

selection of the best performing lines in their breeding programs. Agronomists, growers and extension workers can utilize the knowledge on potato growth under different drought stress regimes to manage differences between growing seasons. Policy makers may use the mean yield per water resource input in different locations to set the thresholds on regulations for agricultural water use (irrigation) for expected level of yield under different drought severities in newly bred varieties. Therefore, this thesis may be of core interest to many stake holders including breeders, growers, agronomists, processing industry, researchers and policy makers.

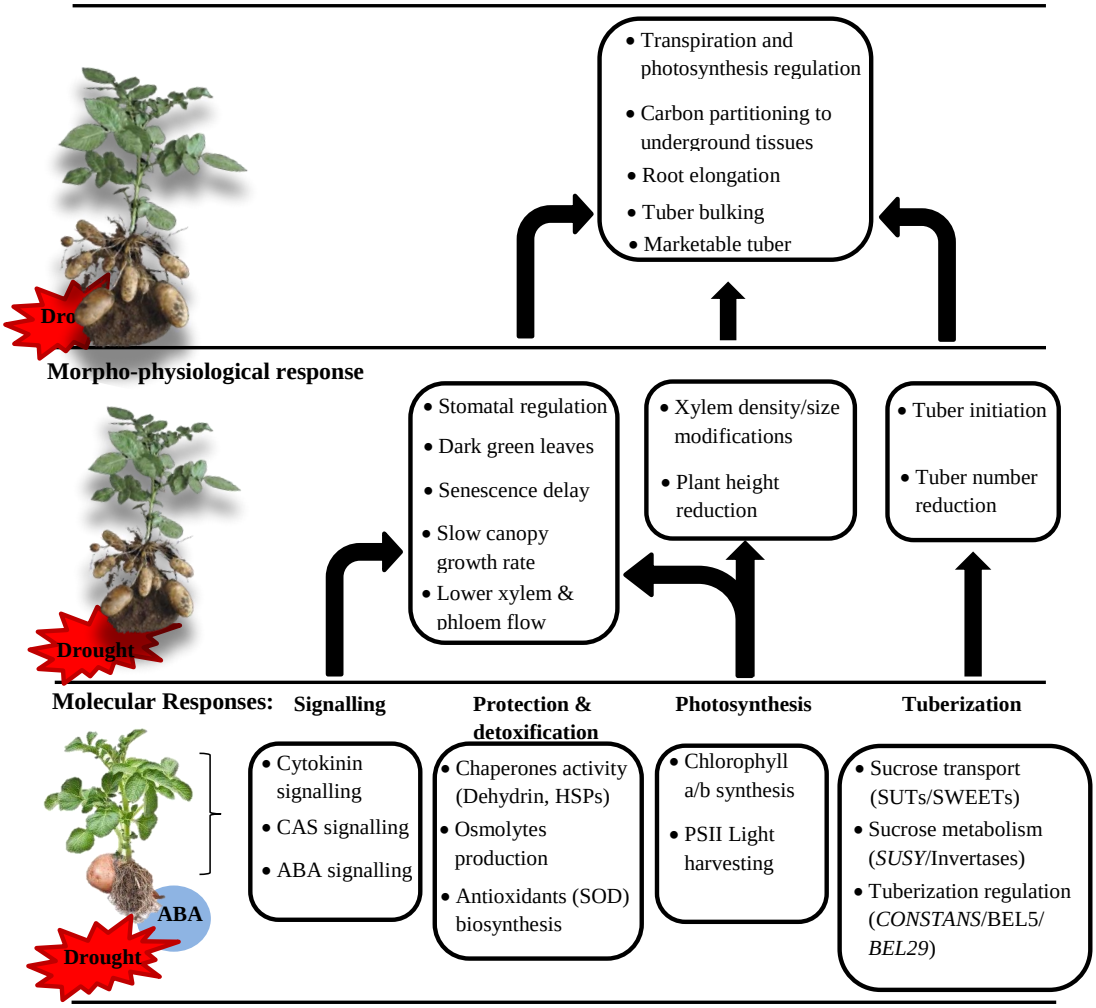


Figure 3: Model of potato drought response based on the findings from the multidisciplinary approach in this thesis. ABA (Abscisic acid), CAS (Calcium sensing), SOD (Superoxide dismutase), HSPs (heat shock proteins)

REFERENCES

Abel, S., Nguyen, M. D., Chow, W., & Theologis, A. (1995). ASC4, a Primary Indoleacetic Acid-responsive Gene Encoding 1-Aminocyclopropane-1-carboxylate Synthase in Arabidopsis thaliana: Structural characterization, expression in *Escherichia coli*, and expression characteristics in response to auxin. *Journal of Biological Chemistry, 270*(32), 19093-19099. doi: 10.1074/jbc.270.32.19093

Abelenda, J. A., Personal communication (2017). [SWEETs in potatoes and tuberization].

Adolfo, P., Rojas, G., Málaga, M., Mares, V., & Roberto, Q. (2008). *Partial root-zone drying: an alternative irrigation management to improve the water use efficiency of potato crops. Production Systems and the Environment Division*.

Adrienne, W. (2015). *A cost-benefit analysis of Amazon Prime Air*. (BSc.), University of Tennessee, Chattanooga. Retrieved from https://scholar.utc.edu/cgi/viewcontent.cgi?referer=http://www.google.nl/url?sa=t&rct=j&q=&esrc=s&source=web&cd=4&ved=0ahUKEwjL1dO66KXZAhVDUlAKHb50AgsQFghEMAM&url=http%3A%2F%2Fscholar.utc.edu%2Fcgi%2Fviewcontent.cgi%3Farticle%3D1051%26context%3Dhonors-theses&usg=AOvVaw2AOuqyN3z32D-4UAH8xVfT&httpsredir=1&article=1051&context=honors-theses

Ahmadi, S. H., Andersen, M. N., Plauborg, F., Poulsen, R. T., Jensen, C. R., Sepaskhah, A. R., & Hansen, S. (2010). Effects of irrigation strategies and soils on field-grown potatoes: Gas exchange and xylem [ABA]. *Agricultural Water Management, 97*(10), 1486-1494. doi: https://doi.org/10.1016/j.agwat.2010.05.002

Akter, N., Rafiqul Islam, M., Abdul Karim, M., & Hossain, T. (2014). Alleviation of drought stress in maize by exogenous application of gibberellic acid and cytokinin. *Journal of Crop Science and Biotechnology, 17*(1), 41-48. doi: 10.1007/s12892-013-0117-3

Anithakumari, A. M. (2011). *Genetic dissection of drought tolerance in potato*. (Ph.D.), Wageningen University, Wageningen. Retrieved from http://edepot.wur.nl/165211

Anithakumari, A. M., Nataraja, K. N., Visser, R. G. F., & van der Linden, C. G. (2012). Genetic dissection of drought tolerance and recovery potential by quantitative trait locus mapping of a diploid potato population. *Molecular Breeding, 30*(3), 1413-1429. doi: 10.1007/s11032-012-9728-5

Ankush, P., Alison, R., Dale, K., Hamlyn, J., Gavin, R., Paul, H., Timothy, G., Pete, H., Jim, M., Finlay, D., Philip, W., & Glenn, B. (2007). Water use efficiency in potato. Retrieved from Ankush.Prashar@scri.ac.uk website: http://www.hutton.ac.uk/webfm_send/676

Ati, A. S., Iyada, A. D., & Najim, S. M. (2012). Water use efficiency of potato (Solanum tuberosum L.) under different irrigation methods and potassium fertilizer rates. *Annals of Agricultural Sciences, 57*(2), 99-103. doi: http://dx.doi.org/10.1016/j.aoas.2012.08.002

Badr, M. A., El-Tohamy, W. A., & Zaghloul, A. M. (2012). Yield and water use efficiency of potato grown under different irrigation and nitrogen levels in an arid region. *Agricultural Water Management, 110*, 9-15. doi: https://doi.org/10.1016/j.agwat.2012.03.008

Bassam, N., Dambroth, M., Loughman, B. C., Spitters, C. J. T., & Schapendonk, A. H. C. M. (1990). Evaluation of breeding strategies for drought tolerance in potato by means of

crop growth simulation *Genetic Aspects of Plant Mineral Nutrition* (Vol. 42, pp. 151-161): Springer Netherlands.

Boguszewska-Mańkowska, D., Pieczyński, M., Wyrzykowska, A., Kalaji, H. M., Sieczko, L., Szweykowska-Kulińska, Z., & Zagdańska, B. (2018). Divergent strategies displayed by potato (Solanum tuberosum L.) cultivars to cope with soil drought. *Journal of Agronomy and Crop Science, 204*(1), 13-30. doi: 10.1111/jac.12245

Bradshaw, J. E. (2016). Genotype x Environment Interactions and Selection Environments *Plant Breeding: Past, Present and Future* (pp. 207-232). Cham: Springer International Publishing.

Caliskan, M. E. (2016). New challenges in potato breeding to cope with climate change: dual tolerance to heat and drought. *Agronomy Series of Scientific Research, 59*(2), 151-154.

Campbell, M. D., Campbell, G. S., Kunkel, R., & Papendick, R. I. (1976). A model describing soil-plant-water relations for potatoes. *American Potato Journal, 53*(12), 431-442. doi: 10.1007/bf02852657

Ceccarelli, S., Acevedo, E., & Grando, S. (1991). Breeding for yield stability in unpredictable environments: single traits, interaction between traits, and architecture of genotypes. *Euphytica, 56*(2), 169-185. doi: 10.1007/bf00042061

Chang, D. C., Jin, Y. I., Nam, J. H., Cheon, C. G., Cho, J. H., Kim, S. J., & Yu, H. (2018). Early drought effect on canopy development and tuber growth of potato cultivars with different maturities. *Field Crops Research, 215*, 156-162. doi: https://doi.org/10.1016/j.fcr.2017.10.008

Chen, J. H., Jiang, H. W., Hsieh, E. J., Chen, H. Y., Chien, C. T., Hsieh, H. L., & Lin, T. P. (2012). Drought and Salt Stress Tolerance of an Arabidopsis Glutathione S-Transferase U17 Knockout Mutant Are Attributed to the Combined Effect of Glutathione and Abscisic Acid. *Plant Physiology, 158*(1), 340-351. doi: 10.1104/pp.111.181875

Chen, S., Wang, X., Zhang, L., Lin, S., Liu, D., Wang, Q., Cai, S., El-Tanbouly, R., Gan, L., Wu, H., & Li, Y. (2016). Identification and characterization of tomato gibberellin 2-oxidases (GA2oxs) and effects of fruit-specific SlGA2ox1 overexpression on fruit and seed growth and development. *Horticulture Research, 3*, 16059.

Cheng, W. H., Chiang, M. H., Hwang, S. G., & Lin, P. C. (2009). Antagonism between abscisic acid and ethylene in Arabidopsis acts in parallel with the reciprocal regulation of their metabolism and signaling pathways. *Plant Mol Biol, 71*(1-2), 61-80. doi: 10.1007/s11103-009-9509-7

CIP. (2013). Increasing Water Efficiency for Potato Production. *International Potato Center*. https://cipotato.org/blog/increasing-water-efficiency/

Cooper, M., & Hammer, G. L. (1996). *Plant Adaptation and Crop Improvement*: CAB International.

Corrales, A.-R., Nebauer, S. G., Carrillo, L., Fernández-Nohales, P., Marqués, J., Renau-Morata, B., Granell, A., Pollmann, S., Vicente-Carbajosa, J., Molina, R.-V., & Medina, J. (2014). Characterization of tomato Cycling Dof Factors reveals conserved and new functions in the control of flowering time and abiotic stress responses. *Journal of Experimental Botany, 65*(4), 995-1012.

Dai, A. (2013). Increasing drought under global warming in observations and models. *3*(1), 52-58.

Dalla Costa, L., Delle Vedove, G., Gianquinto, G., Giovanardi, R., & Peressotti, A. (1997). Yield, water use efficiency and nitrogen uptake in potato: influence of drought stress. *Potato Research, 40*(1), 19-34. doi: 10.1007/bf02407559

Deblonde, P. M. K., & Ledent, J. F. (2001). Effects of moderate drought conditions on green leaf number, stem height, leaf length and tuber yield of potato cultivars. *European Journal of Agronomy*, *14*(1), 31-41. doi: http://dx.doi.org/10.1016/S1161-0301(00)00081-2

Ewing, E. E., & Wareing, P. F. (1978). Shoot, Stolon, and Tuber Formation on Potato (Solanum tuberosum L.) Cuttings in Response to Photoperiod. *Plant Physiology*, *61*(3), 348-353.

FAOSTAT. (2008). Potato and water resources. Retrieved from http://www.fao.org/potato-2008/en/potato/water.html

FAOSTAT. (2016). Food and Agriculture Organization of United Nations. Potatoes.

Fleisher, D. H., Timlin, D. J., & Reddy, V. R. (2008). Interactive Effects of Carbon Dioxide and Water Stress on Potato Canopy Growth and Development All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. *Agronomy Journal*, *100*, 711-719. doi: 10.2134/agronj2007.0188

Frankel, T. C. (2015). New NASA data show how the world is running out of water. Retrieved from https://www.washingtonpost.com/news/wonk/wp/2015/06/16/new-nasa-studies-show-how-the-world-is-running-out-of-water/?utm_term=.075b901d0de4

Genet, R. A. (1985). Potato Agronomy - An overview *Potato Agronomy* (Vol. 1). Lincoln: Crop Research Division.

Getahun, B. B. (2017). *Genetic diversity of potato for nitrogen use efficiency under low input conditions in Ethiopia.* (PhD), Wageningen University, Wageningen. Retrieved from http://edepot.wur.nl/420903C1 - NN08202,6748

Ghate, T. H., Sharma, P., Kondhare, K. R., Hannapel, D. J., & Banerjee, A. K. (2017). The mobile RNAs, StBEL11 and StBEL29, suppress growth of tubers in potato. *Plant Mol Biol*, *93*(6), 563-578. doi: 10.1007/s11103-016-0582-4

Gong, L., Zhang, H., Gan, X., Zhang, L., Chen, Y., Nie, F., Shi, L., Li, M., Guo, Z., Zhang, G., & Song, Y. (2015). Transcriptome Profiling of the Potato (Solanum tuberosum L.) Plant under Drought Stress and Water-Stimulus Conditions. *PLoS ONE, 10*(5), e0128041. doi: 10.1371/journal.pone.0128041

Gonzalez-Guzman, M., Pizzio, G. A., Antoni, R., Vera-Sirera, F., Merilo, E., Bassel, G. W., Fernández, M. A., Holdsworth, M. J., Perez-Amador, M. A., Kollist, H., & Rodriguez, P. L. (2012). Arabidopsis PYR/PYL/RCAR Receptors Play a Major Role in Quantitative Regulation of Stomatal Aperture and Transcriptional Response to Abscisic Acid. *The Plant Cell*, *24*(6), 2483-2496. doi: 10.1105/tpc.112.098574

Guo, Y., Jiang, Q., Hu, Z., Sun, X., Fan, S., & Zhang, H. (2017). Function of the auxin-responsive gene TaSAUR75 under salt and drought stress. *The Crop Journal*. doi: https://doi.org/10.1016/j.cj.2017.08.005

Hall, D., Tegström, C., & Ingvarsson, P. K. (2010). Using association mapping to dissect the genetic basis of complex traits in plants. *Briefings in Functional Genomics, 9*(2), 157-165. doi: 10.1093/bfgp/elp048

Halterman, D., Guenthner, J., Collinge, S., Butler, N., & Douches, D. (2016). Biotech Potatoes in the 21st Century: 20 Years Since the First Biotech Potato. *American Journal of Potato Research, 93*(1), 1-20. doi: 10.1007/s12230-015-9485-1

Harris, P. M. (2013). *The Potato Crop: The scientific basis for improvement*: Springer US.

Haverkort, A. J., & Goudriaan, J. (1994). Perspectives of improved tolerance of drought in crops. *Aspects of Applied Biology, 38*.

Hemantaranjan, A. (2013). *Advances In Plant Physiology Vol. 14*.

Hirut, B., Shimelis, H., Fentahun, M., Bonierbale, M., Gastelo, M., & Asfaw, A. (2017). Combining ability of highland tropic adapted potato for tuber yield and yield components under drought. *PLoS ONE, 12*(7), e0181541.

Hochmal, A. K., Schulze, S., Trompelt, K., & Hippler, M. (2015). Calcium-dependent regulation of photosynthesis. *Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1847*(9), 993-1003. doi: https://doi.org/10.1016/j.bbabio.2015.02.010

Hospital, F. (2009). Challenges for effective marker-assisted selection in plants. *Genetica, 136*(2), 303-310. doi: 10.1007/s10709-008-9307-1

Hubbard, K. E., Nishimura, N., Hitomi, K., Getzoff, E. D., & Schroeder, J. I. (2010). Early abscisic acid signal transduction mechanisms: newly discovered components and newly emerging questions. *Genes Dev, 24*(16), 1695-1708. doi: 10.1101/gad.1953910

Hurtado-Lopez, P. X., Tessema, B. B., Schnabel, S. K., Maliepaard, C., Van der Linden, C. G., Eilers, P. H. C., Jansen, J., van Eeuwijk, F. A., & Visser, R. G. F. (2015). Understanding the genetic basis of potato development using a multi-trait QTL analysis. *Euphytica, 204*(1), 229-241. doi: 10.1007/s10681-015-1431-2

Iqbal, N., Khan, N. A., Ferrante, A., Trivellini, A., Francini, A., & Khan, M. I. R. (2017). Ethylene Role in Plant Growth, Development and Senescence: Interaction with Other Phytohormones. *Frontiers in Plant Science, 8*, 475. doi: 10.3389/fpls.2017.00475

Jacobsen, A. L., Ewers, F. W., Pratt, R. B., Paddock, W. A., & Davis, S. D. (2005). Do Xylem Fibers Affect Vessel Cavitation Resistance? *Plant Physiology, 139*(1), 546-556. doi: 10.1104/pp.104.058404

Jefferies, R. A. (1993). Cultivar responses to water stress in potato: effects of shoot and roots. *New Phytologist, 123*(3), 491-498. doi: 10.1111/j.1469-8137.1993.tb03761.x

Jonik, C., Sonnewald, U., Hajirezaei, M. R., Flügge, U. I., & Ludewig, F. (2012). Simultaneous boosting of source and sink capacities doubles tuber starch yield of potato plants. *Plant Biotechnol J, 10*(9), 1088-1098. doi: 10.1111/j.1467-7652.2012.00736.x

Jovanovic, Z., & Stikic, R. (2018). Partial Root-Zone Drying Technique: from Water Saving to the Improvement of a Fruit Quality. *Frontiers in Sustainable Food Systems, 1*(3). doi: 10.3389/fsufs.2017.00003

Jovović, M., Jovanović, Z., & Stikić, R. (2016). *Physiological responses of potato exposed to drought stress: stomatal conductance, leaf water potential and growth parameters*. East Sarajevo.

Kang, M., & Priyadarshan, P. M. (2008). *Breeding Major Food Staples*: Wiley.

Kappachery, S., Yu, J. W., Baniekal-Hiremath, G., & Park, S. W. (2013). Rapid identification of potential drought tolerance genes from Solanum tuberosum by using a yeast functional screening method. *C R Biol, 336*(11-12), 530-545. doi: 10.1016/j.crvi.2013.09.006

Katoh, A., Ashida, H., Kasajima, I., Shigeoka, S., & Yokota, A. (2015). Potato yield enhancement through intensification of sink and source performances. *Breeding Science, 65*(1), 77-84. doi: 10.1270/jsbbs.65.77

Khan, A. (2014). *Dissecting the component traits of drought tolerance in potato to enhance productivity under stress conditions.* Paper presented at the Asociacion Latinoamericana de la Papa (ALAP). Memorias. 26, Bogota, Columbia.

Khan, M. A., Saravia, D., Munive, S., Lozano, F., Farfan, E., Eyzaguirre, R., & Bonierbale, M. (2015). Multiple QTLs Linked to Agro-Morphological and Physiological Traits Related to Drought Tolerance in Potato. *Plant Molecular Biology Reporter / Ispmb, 33*(5), 1286-1298. doi: 10.1007/s11105-014-0824-z

Khokhar, T. (2017). Globally, 70% of Freshwater is Used for Agriculture. Retrieved from https://blogs.worldbank.org/opendata/chart-globally-70-freshwater-used-agriculture

Khorshidi-Benam, M. B., & Hassanpanah, D. (2007). *Evaluation of different potato cultivars at different irrigation periods and diffferent drought stages*.

Khurana, S. C., & McLaren, J. S. (1982). The influence of leaf area, light interception and season on potato growth and yield. *Potato Research, 25*(4), 329-342. doi: 10.1007/bf02357290

Kloosterman, B., Abelenda, J. A., Gomez, M. d. M. C., Oortwijn, M., de Boer, J. M., Kowitwanich, K., Horvath, B. M., van Eck, H. J., Smaczniak, C., Prat, S., Visser, R. G. F., & Bachem, C. W. B. (2013). Naturally occurring allele diversity allows potato cultivation in northern latitudes. *Nature 495*(7440), 246-250.

Kloosterman, B., De Koeyer, D., Griffiths, R., Flinn, B., Steuernagel, B., Scholz, U., Sonnewald, S., Sonnewald, U., Bryan, G. J., Prat, S., Banfalvi, Z., Hammond, J. P., Geigenberger, P., Nielsen, K. L., Visser, R. G., & Bachem, C. W. (2008). Genes driving potato tuber initiation and growth: identification based on transcriptional changes using the POCI array. *Funct Integr Genomics, 8*(4), 329-340. doi: 10.1007/s10142-008-0083-x

Kooman, P. L., Fahem, M., Tegera, P., & Haverkort, A. J. (1996). Effects of climate on different potato genotypes 2. Dry matter allocation and duration of the growth cycle. *European Journal of Agronomy, 5*(3), 207-217. doi: https://doi.org/10.1016/S1161-0301(96)02032-1

Kovtun, Y., Chiu, W. L., Zeng, W., & Sheen, J. (1998). Suppression of auxin signal transduction by a MAPK cascade in higher plants. *Nature, 395*(6703), 716-720. doi: 10.1038/27240

Krannich, C. T., Maletzki, L., Kurowsky, C., & Horn, R. (2015). Network Candidate Genes in Breeding for Drought Tolerant Crops. *Int J Mol Sci, 16*(7), 16378-16400. doi: 10.3390/ijms160716378

Kriz, A. L., & Larkins, B. A. (2008). *Molecular Genetic Approaches to Maize Improvement*: Springer Berlin Heidelberg.

Kushiro, T., Okamoto, M., Nakabayashi, K., Yamagishi, K., Kitamura, S., Asami, T., Hirai, N., Koshiba, T., Kamiya, Y., & Nambara, E. (2004). The Arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. *Embo j, 23*(7), 1647-1656. doi: 10.1038/sj.emboj.7600121

Lahlou, O., Ouattar, S., & Ledent, J. (2003). The effect of drought and cultivar on growth parameters, yield and yield components of potato. *Agronomie, 23* 257-268. doi: 10.1051/agro:2002089

Lambers, H., Chapin, F. S., & Pons, T. L. (2013). *Plant Physiological Ecology*: Springer New York.

Li, J., Cang, Z., Jiao, F., Bai, X., Zhang, D., & Zhai, R. (2017). Influence of drought stress on photosynthetic characteristics and protective enzymes of potato at seedling stage. *Journal of the Saudi Society of Agricultural Sciences, 16*(1), 82-88. doi: http://dx.doi.org/10.1016/j.jssas.2015.03.001

Liovi, I., Josipovi, M., Imi, D., Krizmani, G., & Miji, A. (2008). Marketable tuber yield stability in potato. *Cereal Research Communications, 36*, 87-90.

Liu, F., Shahnazari, A., Andersen, M. N., Jacobsen, S. E., & Jensen, C. R. (2006). Physiological responses of potato (Solanum tuberosum L.) to partial root-zone drying: ABA signalling, leaf gas exchange, and water use efficiency. *J Exp Bot, 57*(14), 3727-3735. doi: 10.1093/jxb/erl131

Liu, F., Song, R., Zhang, X., Shahnazari, A., Andersen, M. N., Plauborg, F., Jacobsen, S.-E., & Jensen, C. R. (2008). Measurement and modelling of ABA signalling in potato (Solanum tuberosum L.) during partial root-zone drying. *Environmental and Experimental Botany, 63*(1), 385-391. doi: https://doi.org/10.1016/j.envexpbot.2007.11.015

Low, J., Nyongesa, M., Quinn, S., & Parker, M. (2015). *Potato and Sweetpotato in Africa: Transforming the Value Chains for Food and Nutrition Security*: CAB International.

Ludovisi, R., Tauro, F., Salvati, R., Khoury, S., Mugnozza Scarascia, G., & Harfouche, A. (2017). UAV-Based Thermal Imaging for High-Throughput Field Phenotyping of Black Poplar Response to Drought. *Frontiers in Plant Science, 8*(1681). doi: 10.3389/fpls.2017.01681

Luitel, B. P., Khatri, B. B., Choudhary, D., Paudel, B. P., Jung-Sook, S., Hur, O., Baek , H. J., Cheol, K. H., & Yul, R. K. (2015). Growth and yield characters of potato genotypes grown in drought and irrigated conditions of Nepal. *Int J Appl Sci Biotechnol,, Vol 3* ((3)), 513-519. doi: DOI: 10.3126/ijasbt.v3i3.13347

MacKerron, D. K. L., & Jefferies, R. A. (1986). The influence of early soil moisture stress on tuber numbers in potato. *Potato Research, 29*(3), 299-312. doi: 10.1007/bf02359959

Mahmud, A. A., Bazzaz, M., Khan, S. A., Hossain, A., & Kadian, M. S. (2014). Tuber yield, tuber quality and plant water status of potato under drought and well watered condition. *Global Journal of Science Frontier Research: D Agriculture and Veterinary. (USA). 14*(10), 101-107.

Manhas, J. S., & Sukumaran, N. P. (1988). Diurnal changes in net photosynthetic rate in potato in two environments. *Potato Research, 31*(3), 375-378. doi: 10.1007/bf02357871

Mani, F., & Hannachi, C. (2015a). Genomic Advances in Potato Drought Tolerance. *JCBPS, 5*(2), 1677-1699.

Mani, f., & Hannachi, C. (2015b). Recent Genomic and Proteomic Profile of tuberization in potato (Solanum tuberosum L.). *Journal of New Sciences, 15*(6), 15.

Marrs, K. A. (1996). The functions and regulation of glutathione s-transferases in plants. *Annu Rev Plant Physiol Plant Mol Biol, 47*, 127-158. doi: 10.1146/annurev.arplant.47.1.127

Martin, R. J., Jamieson, P. D., Wilson, D. R., & Francis, G. S. (1992). Effects of soil moisture deficits on yield and quality of 'Russet Burbank' potatoes. *New Zealand Journal of Crop and Horticultural Science, 20*(1), 1-9. doi: 10.1080/01140671.1992.10422319

Mir, R. R., Zaman-Allah, M., Sreenivasulu, N., Trethowan, R., & Varshney, R. K. (2012). Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theor Appl Genet, 125*(4), 625-645. doi: 10.1007/s00122-012-1904-9

Montgomery, J. (2016). Calculating water use indices to benchmark water use efficiency. In F. sheet (Ed.). Narrabri: Australian Cotton Industry.

Moorby, J., & Milthorpe, F. L. (Eds.). (1975). *Crop physiolog: some case histories*. Cambridge: Cambridge University Press.

Mori, K., Asano, K., Tamiya, S., Nakao, T., & Mori, M. (2015). Challenges of breeding potato cultivars to grow in various environments and to meet different demands. *Breeding Science, 65*(1), 3-16. doi: 10.1270/jsbbs.65.3

Munemasa, S., Hauser, F., Park, J., Waadt, R., Brandt, B., & Schroeder, J. I. (2015). Mechanisms of abscisic acid-mediated control of stomatal aperture. *Curr Opin Plant Biol, 28*, 154-162. doi: 10.1016/j.pbi.2015.10.010

Munnik, T. (2009). *Lipid Signaling in Plants*: Springer Berlin Heidelberg.

Muthoni, J., & Kabira, J. N. (2016). *Potato Production under Drought Conditions: Identification of Adaptive Traits*.

Ng, L. M., Melcher, K., Teh, B. T., & Xu, H. E. (2014). Abscisic acid perception and signaling: structural mechanisms and applications. *Acta Pharmacologica Sinica, 35*(5), 567-584. doi: 10.1038/aps.2014.5

Nordborg, M., & Tavare, S. (2002). Linkage disequilibrium: what history has to tell us. *Trends Genet, 18*(2), 83-90.

Nordstrom, A., Tarkowski, P., Tarkowska, D., Norbaek, R., Astot, C., Dolezal, K., & Sandberg, G. (2004). Auxin regulation of cytokinin biosynthesis in Arabidopsis thaliana: a factor of potential importance for auxin-cytokinin-regulated development. *Proc Natl Acad Sci U S A, 101*(21), 8039-8044. doi: 10.1073/pnas.0402504101

O'Brien, J. A., & Benková, E. (2013). Cytokinin cross-talking during biotic and abiotic stress responses. *Frontiers in Plant Science, 4*, 451. doi: 10.3389/fpls.2013.00451

Obidiegwu, J. E., Bryan, G. J., Jones, H. G., & Prashar, A. (2015). Coping with drought: stress and adaptive responses in potato and perspectives for improvement. *Frontiers in Plant Science, 6*, 542. doi: 10.3389/fpls.2015.00542

Oliveira, J., Brown, H., Gash, A., & Moot, D. (2016). *An Explanation of Yield Differences in Three Potato Cultivars* (Vol. 108).

Pandey, G. K. (2017). *Mechanism of Plant Hormone Signaling Under Stress, 2 Volume Set*: Wiley.

Park, S. Y., Fung, P., Nishimura, N., Jensen, D. R., Fujii, H., Zhao, Y., Lumba, S., Santiago, J., Rodrigues, A., Chow, T. F., Alfred, S. E., Bonetta, D., Finkelstein, R., Provart, N. J.,

Desveaux, D., Rodriguez, P. L., McCourt, P., Zhu, J. K., Schroeder, J. I., Volkman, B. F., & Cutler, S. R. (2009). Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science, 324*(5930), 1068-1071. doi: 10.1126/science.1173041

Prashar, A., & Jones, H. (2014). Infra-Red Thermography as a High-Throughput Tool for Field Phenotyping. *Agronomy, 4*(3), 397.

Prusova, A. (2016). *Light on phloem transport (an MRI approach).* Wageningen University, Wageningen. Retrieved from http://edepot.wur.nl/389397

Raybould, A., & Poppy, G. M. (2012). Commercializing genetically modified crops under EU regulations. *GM Crops & Food, 3*(1), 9-20. doi: 10.4161/gmcr.18961

Richards, R. A. (2006). Physiological traits used in the breeding of new cultivars for water-scarce environments. *Agricultural Water Management, 80*(1), 197-211. doi: https://doi.org/10.1016/j.agwat.2005.07.013

Riesmeier, J. W., Flügge, U. I., Schulz, B., Heineke, D., Heldt, H. W., Willmitzer, L., & Frommer, W. B. (1993). Antisense repression of the chloroplast triose phosphate translocator affects carbon partitioning in transgenic potato plants. *Proc Natl Acad Sci U S A, 90*(13), 6160-6164.

Rogers, J. C., & Rogers, S. W. (1992). Definition and functional implications of gibberellin and abscisic acid cis-acting hormone response complexes. *Plant Cell, 4*(11), 1443-1451. doi: 10.1105/tpc.4.11.1443

Rolando, J. L., Ramírez, D. A., Yactayo, W., Monneveux, P., & Quiroz, R. (2015). Leaf greenness as a drought tolerance related trait in potato (Solanum tuberosum L.). *Environmental and Experimental Botany, 110*, 27-35. doi: https://doi.org/10.1016/j.envexpbot.2014.09.006

Romagosa, I., Borràs-Gelonch, G., Slafer, G., & van Eeuwijk, F. (2013). Genotype by Environment environment/environmental Interaction genotype/genotyping by environment interaction and Adaptation environment/environmental adaptation. In P. Christou, R. Savin, B. A. Costa-Pierce, I. Misztal, & C. B. A. Whitelaw (Eds.), *Sustainable Food Production* (pp. 846-870). New York, NY: Springer New York.

Romero, A. P., Alarcón, A., Valbuena, R. I., & Galeano, C. H. (2017). Physiological Assessment of Water Stress in Potato Using Spectral Information. *Frontiers in Plant Science, 8*(1608). doi: 10.3389/fpls.2017.01608

Ross, J. J., O'Neill, D. P., Smith, J. J., Kerckhoffs, L. H., & Elliott, R. C. (2000). Evidence that auxin promotes gibberellin A1 biosynthesis in pea. *Plant J, 21*(6), 547-552.

Roy, D., & Kharkwal, M. C. (2004). Breeding for Wider Adaptability. In H. K. Jain & M. C. Kharkwal (Eds.), *Plant Breeding: Mendelian to Molecular Approaches* (pp. 573-584). Dordrecht: Springer Netherlands.

Rykaczewska, K. (2013). The Impact of High Temperature during Growing Season on Potato Cultivars with Different Response to Environmental Stresses. *American Journal of Plant Sciences, Vol.04No.12*, 8. doi: 10.4236/ajps.2013.412295

Saradadevi, R., Palta, J. A., & Siddique, K. H. M. (2017). ABA-Mediated Stomatal Response in Regulating Water Use during the Development of Terminal Drought in Wheat. *Frontiers in Plant Science, 8*, 1251. doi: 10.3389/fpls.2017.01251

Schafleitner, R., Gutierrez, R., Espino, R., Gaudin, A., Pérez, J., Martínez, M., Domínguez, A., Tincopa, L., Alvarado, C., Numberto, G., & Bonierbale, M. (2007). Field Screening for Variation of Drought Tolerance in Solanum tuberosum L. by Agronomical, Physiological and Genetic Analysis. [Potato Research]. *50*(1), 71-85. doi: 10.1007/s11540-007-9030-9

Schönhals, E. M., Ortega, F., Barandalla, L., Aragones, A., Ruiz de Galarreta, J. I., Liao, J. C., Sanetomo, R., Walkemeier, B., Tacke, E., Ritter, E., & Gebhardt, C. (2016). Identification and reproducibility of diagnostic DNA markers for tuber starch and yield optimization in a novel association mapping population of potato (Solanum tuberosum L.). *Theor Appl Genet, 129*, 767-785. doi: 10.1007/s00122-016-2665-7

Schulz, B., Frommer, W. B., Flugge, U. I., Hummel, S., Fischer, K., & Willmitzer, L. (1993). Expression of the triose phosphate translocator gene from potato is light dependent and restricted to green tissues. *Mol Gen Genet, 238*(3), 357-361.

Shah, S. F. A., McKenzie, B. A., Gaunt, R. E., Marshall, J. W., & Frampton, C. M. (2004). Effect of production environments on radiation interception and radiation use efficiency of potato (Solanum tuberosum) grown in Canterbury, New Zealand. *New Zealand Journal of Crop and Horticultural Science, 32*(1), 113-119. doi: 10.1080/01140671.2004.9514285

Simmonds, N. W. (1981). Genotype (G). Environment (E) and GE components of crop yields. *Experimental Agriculture, 17*, 355 - 362.

Slater, A. T., Cogan, N. O. I., & Forster, J. W. (2013). Cost analysis of the application of marker-assisted selection in potato breeding. *Molecular Breeding, 32*(2), 299-310. doi: 10.1007/s11032-013-9871-7

Sliwka, J., Jakuczun, H., Kaminski, P., & Zimnoch-Guzowska, E. (2010). Marker-assisted selection of diploid and tetraploid potatoes carrying Rpi-phu1, a major gene for resistance to Phytophthora infestans. *J Appl Genet, 51*(2), 133-140. doi: 10.1007/bf03195721

Soltys-Kalina, D., Plich, J., Strzelczyk-Żyta, D., Śliwka, J., & Marczewski, W. (2016). The effect of drought stress on the leaf relative water content and tuber yield of a half-sib family of 'Katahdin'-derived potato cultivars. *Breeding Science, 66*(2), 328-331. doi: 10.1270/jsbbs.66.328

Sri Ranjan, R., & Abbas, H. (2015). *Effect of soil moisture deficit on marketable yield and quality of potatoes* (Vol. 57).

Stevenson, F. J., & Clark, G. F. (1937). Breeding and genetics in potato improvement. *Yearbook*, 405-444.

Struik, P. C. (2010). Can Physiology Help Us to Combat Late Blight in Potato? *Potato Research, 53*(4), 277-287. doi: 10.1007/s11540-010-9164-z

Struik, P. C., Haverkort, A. J., Vreugdenhil, D., Bus, C. B., & Dankert, R. (1990). Manipulation of tuber-size distribution of a potato crop. *Potato Research, 33*(4), 417-432. doi: 10.1007/bf02358019

Struik, P. C., Kramer, G., & Smit, N. P. (1989). Effects of soil applications of gibberellic acid on the yield and quality of tubers ofSolanum tuberosum L. cv. Bintje. *Potato Research, 32*(2), 203-209. doi: 10.1007/bf02358233

Struik, P. C., & Van Voorst, G. (1986). Effects of droght on the initiation, yeild, and size distribution of tubers ofSolanum tuberosum L. cv. Bintje. *Potato Research, 29*(4), 487-500. doi: 10.1007/bf02357913

Swiezynski, K. M., Sykala, A., & Wroblewska, J. K. (1978). Differences in early growth of shoots and roots in potato clones. *Potato Research, 21*, 241-248.

Tessema, B. B. (2017). *Genetic studies towards elucidation of drought tolerance of potato.* Wageningen University, Wageningen. Retrieved from http://edepot.wur.nl/413763

Timmermans, M. M. A. (2016). *A novel crosstalk between flowering locus T signalling and sugar transport in Solanum tuberosum.* (MSc.), Wageningen Unversity & Research, Wageningen. Retrieved from http://edepot.wur.nl/383538

Tiwari, J. K., Siddappa, S., Singh, B. P., Kaushik, S. K., Chakrabarti, S. K., Bhardwaj, V., & Chandel, P. (2013). Molecular markers for late blight resistance breeding of potato: an update. *Plant Breeding, 132*(3), 237-245. doi: 10.1111/pbr.12053

Tourneux, C., Devaux, A., René Camacho, M., Mamani, P., & Ledent, J. F. (2003). *Effect of water shortage on six potato genotypes in the highlands of Bolivia (II): Water relations, physiological parameters*.

van der Schoot, C., & Bel, A. J. E. v. (1989). Morphogram: A Novel Diagram to Organize the Transitive Secondary Xylem Elements of Basal Tomato (Solanum lycopersicum) Internodes. *American Journal of Botany, 76*(4), 475-486. doi: 10.2307/2444343

Van Der Zaag, D. E., & Horton, D. (1983). Potato production and utilization in world perspective with special reference to the tropics and sub-tropics. *Potato Research, 26*(4), 323-362. doi: 10.1007/bf02356154

van Loon, C. D. (1981). The effect of water stress on potato growth, development, and yield. *American Potato Journal, 58*(1), 51-69. doi: 10.1007/bf02855380

Vasquez-Robinet, C., Mane, S. P., Ulanov, A. V., Watkinson, J. I., Stromberg, V. K., De Koeyer, D., Schafleitner, R., Willmot, D. B., Bonierbale, M., Bohnert, H. J., & Grene, R. (2008). Physiological and molecular adaptations to drought in Andean potato genotypes. *Journal of Experimental Botany, 59*(8), 2109-2123. doi: 10.1093/jxb/ern073

Visker, M., Keizer, L., Van Eck, H., Jacobsen, E., Colon, L., & Struik, P. (2003). Can the QTL for late blight resistance on potato chromosome 5 be attributed to foliage maturity type? *Theoretical and Applied Genetics, 106*(2), 317-325. doi: 10.1007/s00122-002-1021-2

Vos, J., & Groenwold, J. (1989). Characteristics of photosynthesis and conductance of potato canopies and the effects of cultivars and transient drought. *Field Crops Research, 20*(4), 237-250. doi: https://doi.org/10.1016/0378-4290(89)90068-3

Vos, J., & Oyarzún, P. J. (1987). Photosynthesis and stomatal conductance of potato leaves—effects of leaf age, irradiance, and leaf water potential. *Photosynthesis Research, 11*(3), 253-264. doi: 10.1007/bf00055065

Waggoner, P. E., & Simmonds, N. W. (1966). Stomata and Transpiration of Droopy Potatoes. *Plant Physiology, 41*(8), 1268-1271.

Wang, W. H., Chen, J., Liu, T. W., Chen, J., Han, A. D., Simon, M., Dong, X. J., He, J. X., & Zheng, H. L. (2014). Regulation of the calcium-sensing receptor in both stomatal movement and photosynthetic electron transport is crucial for water use efficiency and drought tolerance in Arabidopsis. *J Exp Bot, 65*(1), 223-234. doi: 10.1093/jxb/ert362

Wang, W. H., Yi, X., Han, A., Liu, T., Chen, J., Wu, F., Dong, X., He, J., Pei, Z., & Zheng, H. (2012). Calcium-sensing receptor regulates stomatal closure through hydrogen peroxide and nitric oxide in response to extracellular calcium in Arabidopsis. *Journal of Experimental Botany, 63*(1), 177-190. doi: 10.1093/jxb/err259

Wang, X. (2005). Regulatory Functions of Phospholipase D and Phosphatidic Acid in Plant Growth, Development, and Stress Responses. *Plant Physiology, 139*(2), 566-573. doi: 10.1104/pp.105.068809

Weiss, D., & Ori, N. (2007). Mechanisms of Cross Talk between Gibberellin and Other Hormones. *Plant Physiology, 144*(3), 1240-1246. doi: 10.1104/pp.107.100370

Weisz, R., Kaminski, J., & Smilowitz, Z. (1994). Water deficit effects on potato leaf growth and transpiration: Utilizing fraction extractable soil water for comparison with other crops. *American Potato Journal, 71*(12), 829-840. doi: 10.1007/bf02849378

Willson, C. J., & Jackson, R. B. (2006). Xylem cavitation caused by drought and freezing stress in four co-occurring Juniperus species. *Physiologia Plantarum, 127*(3), 374-382. doi: 10.1111/j.1399-3054.2006.00644.x

Wilson, G. (2010). Agriculture Victoria *Potato Varieties*. Melbourne, Victoria: Department of Environment and Primary Industries.

Windt, C. W., Vergeldt, F. J., de Jager, P. A., & van As, H. (2006). MRI of long-distance water transport: a comparison of the phloem and xylem flow characteristics and dynamics in poplar, castor bean, tomato and tobacco. *Plant Cell Environ, 29*(9), 1715-1729. doi: 10.1111/j.1365-3040.2006.01544.x

Wingler, A., von Schaewen, A., Leegood, R. C., Lea, P. J., & Paul Quick, W. (1998). Regulation of Leaf Senescence by Cytokinin, Sugars, and Light : Effects on NADH-Dependent Hydroxypyruvate Reductase. *Plant Physiology, 116*(1), 329-335.

Wishart, J., George, T. S., Brown, L. K., Ramsay, G., Bradshaw, J. E., White, P. J., & Gregory, P. J. (2013). Measuring variation in potato roots in both field and glasshouse: the search for useful yield predictors and a simple screen for root traits. *Plant and Soil, 368*(1), 231-249. doi: 10.1007/s11104-012-1483-1

Xiong, L., Schumaker, K. S., & Zhu, J.-K. (2002). Cell Signaling during Cold, Drought, and Salt Stress. *The Plant Cell, 14*(suppl 1), S165-S183. doi: 10.1105/tpc.000596

Xu, X., van Lammeren, A. A. M., Vermeer, E., & Vreugdenhil, D. (1998). The Role of Gibberellin, Abscisic Acid, and Sucrose in the Regulation of Potato Tuber Formation in Vitro. *Plant Physiology, 117*(2), 575-584.

Xu, Z., Zhou, G., & Shimizu, H. (2010). Plant responses to drought and rewatering. *Plant Signaling & Behavior, 5*(6), 649-654.

Yactayo, W., Ramirez, D., Gutierrez Rosales, R., Mares, V., Adolfo, P., & Roberto, Q. (2013). *Effect of partial root-zone drying irrigation timing on potato tuber yield and water use efficiency* (Vol. 123).

Yamaguchi, J., & Tanaka, A. (1990). Quantitative observation on the root system of various crops growing in the field. *Soil Science and Plant Nutrition, 36*(3), 483-493. doi: 10.1080/00380768.1990.10416917

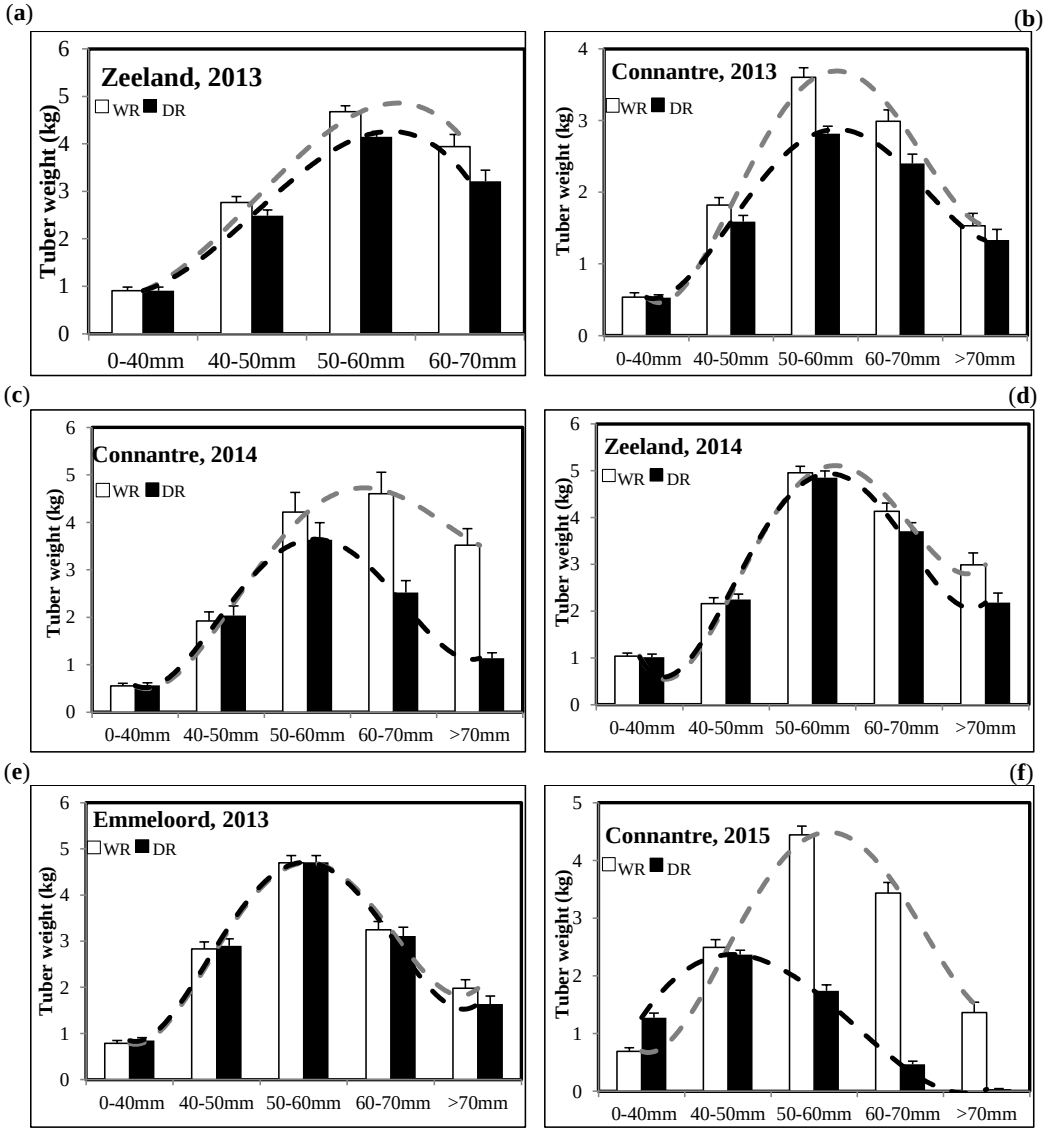
Zhang, S., Xu, X., Sun, Y., Zhang, J., & Li, C. (2018). Influence of drought hardening on the resistance physiology of potato seedlings under drought stress. *Journal of Integrative Agriculture, 17*(2), 336-347. doi: 10.1016/s2095-3119(17)61758-1

Zhang, Y., Zhu, H., Zhang, Q., Li, M., Yan, M., Wang, R., Wang, L., Welti, R., Zhang, W., & Wang, X. (2009). Phospholipase Dα1 and Phosphatidic Acid Regulate NADPH Oxidase Activity and Production of Reactive Oxygen Species in ABA-Mediated Stomatal Closure in Arabidopsis. *The Plant Cell, 21*(8), 2357-2377. doi: 10.1105/tpc.108.062992

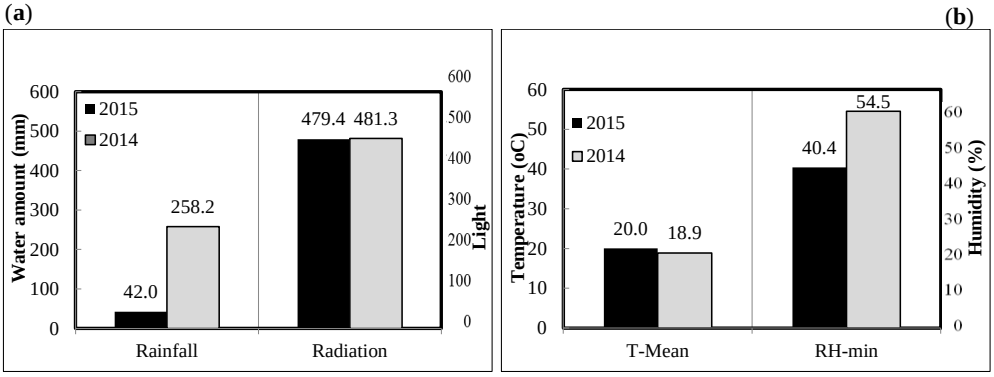
Zhu, C., Gore, M., Buckler, E. S., & Yu, J. (2008). Status and Prospects of Association Mapping in Plants. *The Plant Genome, 1*, 5-20. doi: 10.3835/plantgenome2008.02.0089

SUPPLEMENTARY MATERIAL

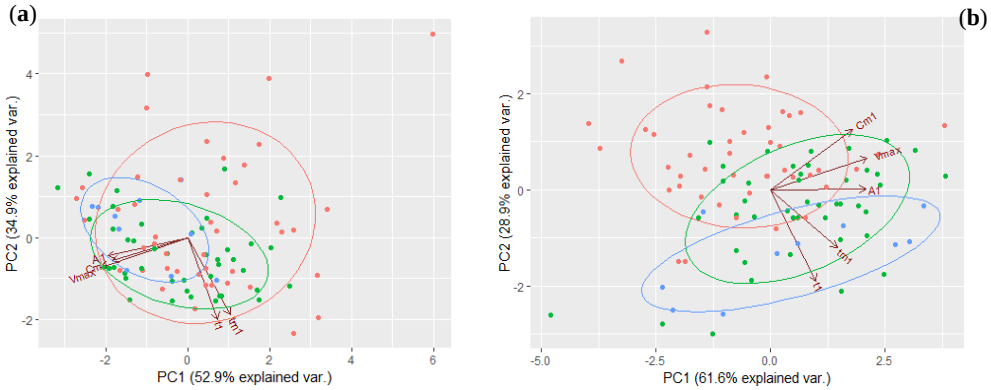
CHAPTER TWO



Supplementary Figure SF1 (a-f): Mean tuber weight per size class of 103 genotypes in different locations between 2013-2015 under irrigated (WR) and non-irrigated (DR) conditions. Error bars represent standard errors of the mean of each dataset.



Supplementary Figure SF2: Connantre environmental information -- (**a**) Comparison of total rainfall and radiation between 2014 vs. 2015. (**b**) Comparison of mean temperature and relative humidity between 2014 vs. 2015



Supplementary Figure SF3: Bi-plot of Connantre (2015) dataset showing growth parameters as vectors and cultivars as dots grouped according to their maturity classes (Early [red], Intermediate [green] and Late [blue]). (**a**) Irrigated treatment, (**b**) Non-irrigated treatment.

Supplementary Table 2: Comparison of growth parameters and yield of tolerant and sensitive cultivars of early and late maturity types under irrigated (WR) and non-irrigated (DR) conditions

CULTIVARS		**ADORA**	**HERMES**	**KARAKTER**	**FESTIEN**
MATURITY TYPES		Early	Early	Late	Late
DROUGHT RESPONSES		Sensitive	Tolerant	Sensitive	Tolerant
CANOPY GROWTH PARAMETERS					
Vmax (%)	WR	99.63	99.71	96.12	98.55
	DR	43.60	77.28	43.68	70.54
	%Δred	56.24	22.50	54.56	28.426
t_1 (td)	WR	66.85	68.23	71.09	68.21
	DR	59.49	65.39	68.40	68.86
	%Δred	11.02	4.16	3.79	-0.94
t_{m1} (td)	WR	38.04	38.09	41.29	37.55
	DR	27.88	34.17	35.28	37.01
	%Δred	26.71	10.29	14.55	1.43
C_{m1} (%/day)	WR	2.35	2.28	2.16	2.24
	DR	1.08	1.80	0.97	1.57
	%Δred	53.94	21.28	55.16	29.80
A_1 (m^2)	WR	3083.30	3191.40	3116.10	3182.70
	DR	1336.00	2468.40	1469.50	2333.90
	%Δred	56.67	22.66	52.84	26.67
TBW (kg)	WR	10.70	14.40	10.95	12.35
	DR	3.30	6.75	5.85	7.95
	%Δred	69.16	53.13	46.56	35.63

%Δred: percentage reduction in trait under drought = [(WR- DR)/WR] * 100

Supplementary Table 1: commercial cultivars (genotypes) and their respective pedigree and maturity/structure groups

Cultivar or breeder's clone	Reg. Year	Country of origin	Parentage	Market niche	Structure groups	Maturity
Adora	1990	HOL	Primura x Alcmaria	fresh consumption	Fresh consumption	Early
Agata	1990	HOL	BM 52-72 x Sirco	fresh consumption	Fresh consumption	Early
Agria	1985	GER	Quarta x Semlo	processing industry	Processing	Intermediate
Almera	1999	HOL	BM 77-2102 x AR 80-31-20	fresh consumption	Fresh consumption	Intermediate
Altus	2008	HOL	KA 87-2306 x KARTEL	advanced breeder's clone	Starch	Late
Ambition	2007	HOL	ADORA x QUINTA	advanced breeder's clone	Processing	Intermediate
Arizona	2009	HOL	UK 150-19D22 x MASCOTTE		**Undetermined**	Early
Asterix	1991	HOL	Cardinal x VE 70-9	processing industry	Undetermined	Intermediate
Atlantic	1976	USA	Wauseon x Lenape	processing industry	Undetermined	Intermediate
Avano	2005	HOL	KA 89-3516 x STABILO	advanced breeder's clone	Starch	Late
Avarna	2005	HOL	KA 89-3516 x STABILO	advanced breeder's clone	Starch	Late
Aveka	2001	HOL	KARDENT x KARTEL	advanced breeder's clone	Starch	Intermediate
Aventra	2005	HOL	unknown	advanced breeder's clone	Starch	Intermediate
Axion	2008	HOL	KA 87-2306 x STABILO	advanced breeder's clone	Starch	Late
Bartina	1988	HOL	Saturna x ZPC 62-75	fresh consumption	Undetermined	Intermediate
Bellini	2001	HOL	Mondial x Felsina	fresh consumption	Processing	Intermediate
Bintje	1910	HOL	Munstersen x Jaune d'or (= Fransen)	fresh consumption	Undetermined	Early
Biogold	2004	HOL	Novita x HZ 87 P 200	processing industry	Fresh consumption	Early
Bionica	2008	HOL	PENTLAND IVORY x CMK 88-169-005	advanced breeder's clone	Fresh consumption	Early
Caesar	1990	HOL	Monalisa x Ropta B 1178	processing industry	Undetermined	Intermediate
Canberra	2007	HOL	LATONA x RED SCARLETT	advanced breeder's clone	Fresh consumption	Early
Challenger	2008	HOL	AZIZA x VICTORIA	advanced breeder's clone	Processing	Intermediate
Charlotte	1981	FRA	Hansa x Danae	fresh consumption	Rest	Early
Colomba						Early
Cupido	1995	HOL	W 72-22-496 x ESTIMA	advanced breeder's clone	Rest	Early

Desiree	1962	HOL	Urgenta x Depesche	fresh consumption	Undetermined	Intermediate
EL Mundo	2010	HOL	HEO 950251-84 x VALOR		**Fresh consumption**	Intermediate
Elkana	1978	HOL	MARA x PROMINENT	advanced breeder's clone	Starch	Intermediate
Eurostar	2003	HOL	AGRIA x VK 69-491		**Processing**	Intermediate
Everest	2006	HOL	SPUNTA x MARADONNA	advanced breeder's clone	Processing	Early
Fabula	1997	HOL	Monalisa x Hudson	fresh consumption	Undetermined	Intermediate
Festien	2000	HOL	KARTEL x KA 80-1920	starch industry	Starch	Late
Fontane	1999	HOL	Agria x AR 76-34-3	processing industry	Undetermined	Intermediate
Hansa	1957	GER	OBERARNBACHER FRUHE x FLAVA	fresh consumption	Undetermined	Intermediate
Hermes	1973	AUT	DDR 5158 x SW 163/55	processing industry	Fresh consumption	Early
Innovator	1999	HOL	Shepody x RZ 84-2580	processing industry	Fresh consumption	Early
Inova	1999	HOL	NICOLA x IMPALA	fresh consumption	Fresh consumption	Early
Jaerla	1969	HOL	Sirtema x MPI 19268	fresh consumption	Fresh consumption	Early
Jazzy						Early
Karakter	1996	HOL	KA 77-0133 x AM 78-3736	advanced breeder's clone	Starch	Late
Karnico	1987	HOL	Astarte x AM 66-42	starch industry	Starch	Late
Kastelli	2011	HOL	MONDIAL x FELSINA		**Processing**	Intermediate
Kennebec	1948	USA	USDA B 127 x USDA 96-56	ancient cultivar	Processing	Intermediate
Kondor	1984	HOL	KONST 61-333 x WILJA	fresh consumption	Fresh consumption	Intermediate
Kuras	1996	HOL	BRDA (=PG 285) x VK 69-491	starch industry	Undetermined	Late
Kuroda	1998	HOL	AR 76-199-3 x KONST 80-1407	fresh consumption	Fresh consumption	Early
Labadia	2001	HOL	MONDIAL x VAN GOGH	advanced breeder's clone	Rest	Early
Lady Amarilla	2007	HOL	AGRIA x HERMES	advanced breeder's clone	Processing	Early
Lady Anna	2010	HOL	CMK 93-042-005 x FONTANE		Undetermined	Intermediate
Lady Blanca	2007	HOL	LADY OLYMPIA x CMK 91-088-016	advanced breeder's clone	Processing	Early
Lady Britta	2008	HOL	SATURNA x PK 87-204-13		Undetermined	Early
Lady Christel	1996	HOL	WS 73-3-391 x Mansour	fresh consumption	Rest	Early
Lady Claire	1996	HOL	Agria x KW 78-34-470	processing industry	Processing	Early
Lady Felicia	1997	HOL	AGRIA x W 72-22-496	advanced breeder's clone	Undetermined	Early
Lady Jo	2001	HOL	LADY AMELIA x VE 74-45	advanced breeder's clone	Processing	
Lady Lenora	2010	HOL	BONANZA x LADY JO		**Undetermined**	Early

Lady Olympia	1996	HOL	AGRIA x KW 78-34-470	processing industry	Processing	Intermediate
Lady Rosetta	1988	HOL	CARDINAL x VTN 62-33-3	processing industry	Undetermined	Early
Leonardo	2010	HOL	TRA 89-462 x BOLESTA		**Processing**	Intermediate
Liseta	1988	HOL	Spunta x VE 66-295	fresh consumption	Processing	Early
Marabel	1993	HOL	NENA x MA 75-364	fresh consumption	Fresh consumption	Early
Marfona	1977	HOL	PRIMURA x KONST 51-123	fresh consumption	Fresh consumption	Early
Maris Piper	1963	GB	Y 22/6 x (ARRAN CAIRN x HERALD)	processing industry	Fresh consumption	Intermediate
Markies	1997	HOL	FIANNA x AGRIA	fresh consumption	Processing	Late
Melody	2001	HOL	VE 74-45 x W 72-22-496	advanced breeder's clone	Rest	Intermediate
Merano	2006	HOL	BR 90-0024 x HZ 89 JK 8	fresh consumption	Starch	Intermediate
Monalisa	1982	HOL	Bierma A 1-287 x Colmo	fresh consumption	Undetermined	Early
Mondial	1987	HOL	SPUNTA x VE 66-295	fresh consumption	Undetermined	Intermediate
Mozart	2003	HOL	REDSTAR x CAESAR		Undetermined	Early
Musica	2007	HOL	CMK 93-042-005 x LADY CHRISTL	advanced breeder's clone	Fresh consumption	Early
Nicola	1973	GER	CLIVIA x 6430/101	fresh consumption	Fresh consumption	Intermediate
Nomade	1995	HOL	Elles x AM 78-3704	starch industry	Starch	Intermediate
Orchestra	2007	HOL	MARADONNA x CUPIDO	advanced breeder's clone	Fresh consumption	Early
Pentland Dell	1961	GB	Roslin Chania x Roslin Sasamua	processing industry	Rest	Intermediate
Picasso	1994	HOL	Cara x Ausonia	fresh consumption	Fresh consumption	Intermediate
Piccolostar	2007	HOL	AUSONIA x VE 74-120		**Fresh consumption**	Early
Premiere	1979	HOL	Civa x Provita	processing industry	Undetermined	Early
Ramos	2000	HOL	AGRIA x VK 69-491	fresh consumption	Processing	Intermediate
Red Scarlett	1999	HOL	ZPC 80-239 x IMPALA	fresh consumption	Fresh consumption	Early
Rodeo	1999	HOL	MONDIAL x BIMONDA		Undetermined	Intermediate
Russet Burbank	1908	USA	mutant van Burbank	processing industry	Undetermined	Intermediate
Sagitta	1961	DDR	SCHWALBE x LU. 55.459/5 N	advanced breeder's clone	Fresh consumption	Intermediate
Santana	1994	HOL	Spunta x VK 69-491	processing industry	Fresh consumption	Early
Sante	1983	HOL	Y 66-13-636 x AM 66-42	fresh consumption	Rest	Early
Saturna	1964	HOL	Maritta x (Record x CPC 1673-1adg)	processing industry	Undetermined	Early
Seresta	1994	HOL	AM 78-3704 x Sonata	starch industry	Undetermined	Intermediate
Shepody	1980	CAN	Bake King x F58050	processing industry	Processing	Early

Sifra	2008	HOL	MONDIAL x ROBINTA	advanced breeder's clone	Processing	Intermediate
Soprano	2005	HOL	SPUNTA x CMK 90-002-002	advanced breeder's clone	Processing	Early
Spunta	1968	HOL	Bea x USDA 96-56	fresh consumption	Processing	Intermediate
Starga	2000	HOL	SL 75-905 x AM 78-3704	advanced breeder's clone	Starch	Intermediate
Sylvana	2008	HOL	FABULA x XANTIA	advanced breeder's clone	Processing	Intermediate
Terra Gold	2004	HOL	CMK 87-206-001 x FRESCO	advanced breeder's clone	Undetermined	Intermediate
Timate	1984	HOL	Elvira x AM 66-42	fresh consumption	Fresh consumption	Early
Valiant	1987	GB	G 1/46 x D 42/8	advanced breeder's clone	Starch	Late
VR 808	2009	HOL	LADY CLAIRE x ATLANTIC		Processing	Intermediate
Vtn 62-33-3	/	HOL	((V 24/20 x ULSTER KNIGHT)1 x PROFIJT)15 x (VRN I-3 x PROFIJT)5	progenitor clone	Starch	Early
Winston	1992	GB	KISMET x DXMP 70	fresh consumption	Rest	Early

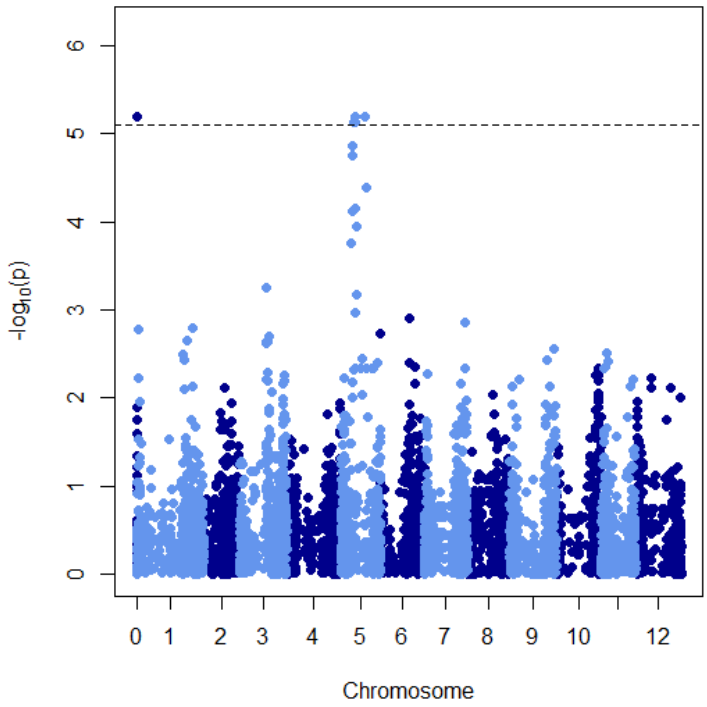
Supplementary Table 2: tuber size distribution parameters profile and drought response grade (DRG) of cultivars at the Connantre 2015 trial

		Irrigated (WR)						Non-Irrigated (DR)						Drought Tolerance (DT)						
CULTIVARS	MATURITY	TBN ms	TBN spread	TBN mf	TBW mcs	TBW spread	TBW mf	TBN ms	TBN spread	TBN mf	TBW mcs	TBW spread	TBW mf	TBN ms	TBN spread	TBN mf	TBW mcs	TBW spread	TBW mf	DRG*
AVANO	L	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	6
EUROSTAR	I	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	6
LABADIA	E	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	6
AVARNA	L		√	√			√	√	√	√	√	√	√	√	√	√	√	√	√	6
AVEKA	I			√			√	√	√	√	√		√	√	√	√	√	√	√	6
AXION	L					√		√	√	√	√	√	√	√	√	√	√	√	√	6
CAESAR	I	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	6
FESTIEN	L	√	√	√	√		√	√	√	√	√		√	√	√	√	√	√	√	6
FONTANE	I	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	6
KARAKTER	L	√		√			√	√	√	√	√		√	√	√	√	√	√	√	6
KURODA	E	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	6
LADY OLYMPIA	I	√	√	√			√	√	√	√	√	√	√	√	√	√	√	√	√	6
LEONARDO	I	√		√			√	√	√	√	√	√	√	√	√	√	√	√	√	6
MARFONA	E	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√	√	6
MERANO	I	√		√			√	√	√	√	√	√	√	√	√	√	√	√	√	6
PENTLAND DELL	I											√		√	√	√	√	√	√	6
SERESTA	I							√	√	√	√		√	√	√	√	√	√	√	6
STARGA	I													√	√	√	√	√	√	6
TIMATE	E					√			√		√	√	√	√	√	√	√	√	√	6
VALIANT	L					√		√	√	√	√	√	√	√	√	√	√	√	√	6
AGRIA	I	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√	5
ALTUS	L		√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		√	5
AMBITION	I	√	√	√	√		√	√	√	√	√	√	√		√	√	√	√	√	5
ATLANTIC	I	√	√	√	√	√	√	√	√	√	√	√	√		√	√	√	√	√	5
BELLINI	I													√	√	√	√		√	5
EL MUNDO	I	√	√	√	√	√	√	√	√	√	√		√	√	√	√	√		√	5
ELKANA	I	√	√	√	√		√	√	√	√	√		√	√	√	√	√		√	5
KURAS	L	√	√	√	√		√	√	√	√	√	√	√		√	√	√	√	√	5
MARKIES	L	√		√			√	√	√	√	√		√	√	√	√	√		√	5
MONDIAL	I					√		√	√	√	√		√	√	√	√	√		√	5
MOZART	E	√	√	√	√		√	√	√	√	√	√	√		√	√	√	√	√	5
NOMADE	I	√	√	√	√	√	√	√	√	√	√		√	√	√	√	√		√	5
PICASSO	I	√	√	√	√		√	√	√	√	√		√		√	√		√	√	5
RODEO	I													√	√		√	√	√	5
SAGITTA	I	√		√			√	√	√	√	√	√	√		√	√	√	√	√	5
WINSTON	E	√	√	√	√		√	√	√	√	√	√	√	√		√	√	√	√	5
EVEREST	E	√	√	√	√		√	√	√	√	√	√	√		√	√		√	√	4
KARNICO	L					√			√	√		√		√	√	√		√		4
MARIS PIPER	I		√		√	√	√	√	√	√	√		√	√		√	√		√	4
SANTE	E	√	√	√	√	√	√	√	√	√	√	√	√		√	√		√	√	4

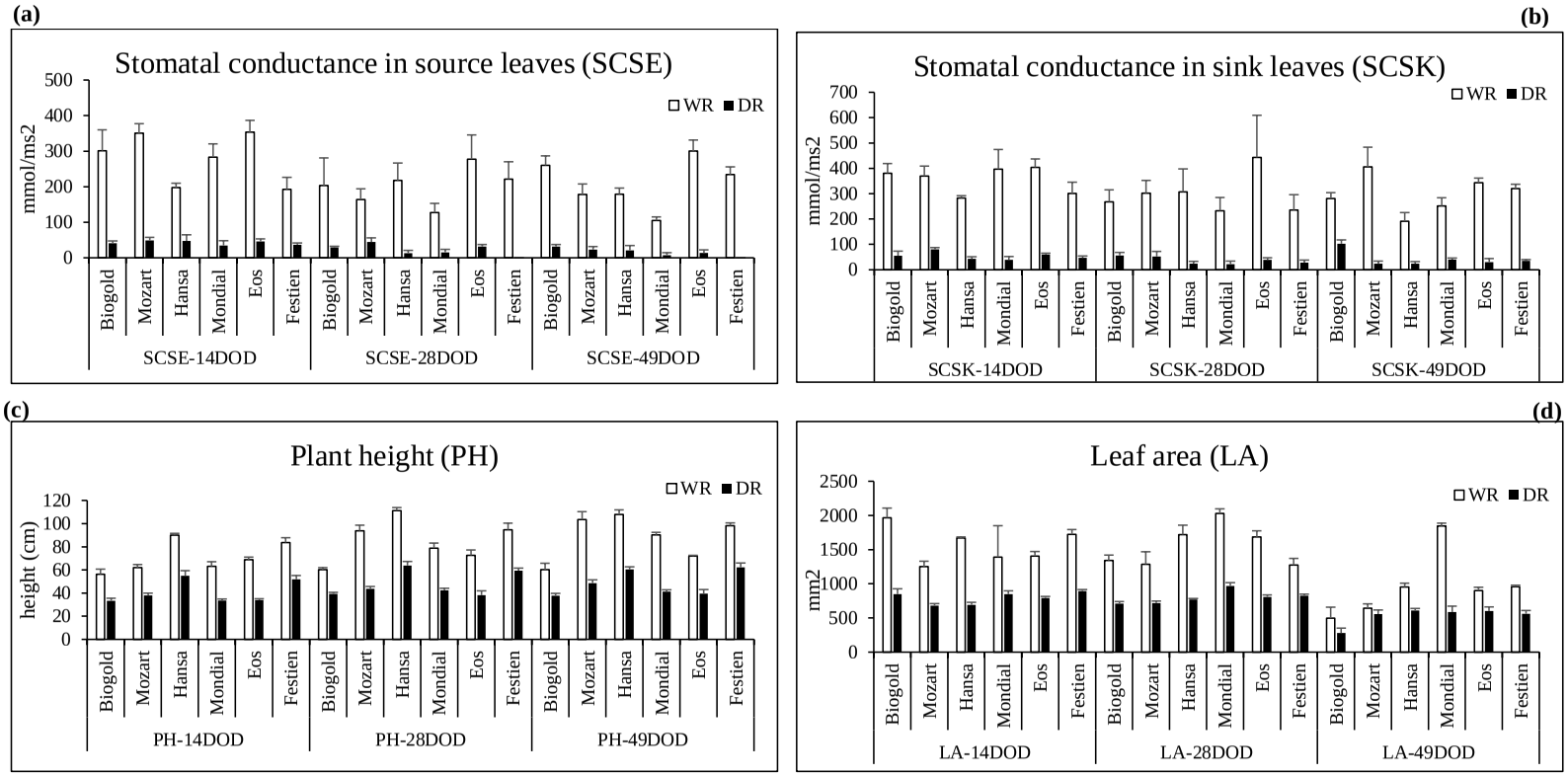
SHEPODY	E	√	√	√	√	√	√	√	√	√	√	√	√	√		√	√		√		√	4
TERRAGOLD	I	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√					√	4
ADORA	E													√			√	√				3
HERMES	E	√	√	√	√		√	√	√	√	√	√	√		√			√	√			3
INNOVATOR	E							√						√	√		√					3
KASTELLI	I	√	√	√	√		√	√	√	√	√		√			√	√				√	3
KENNEBEC	I	√	√	√	√	√	√	√	√	√	√	√	√		√	√					√	3
LADY CLAIRE	E													√	√		√					3
ORCHESTRA	E	√	√	√	√	√	√	√	√	√	√	√	√			√		√	√			3
PICCOLO STAR	E													√			√	√				3
RAMOS	I													√	√		√					3
SYLVANA	I	√	√	√	√	√	√	√	√	√	√	√	√		√	√					√	3
VR 808	I													√	√		√					3
BARTINA	I	√	√	√	√	√	√	√	√	√	√	√	√			√					√	2
DESIREE	I	√	√	√	√	√	√	√	√			√			√	√						2
FABULA	I	√	√	√	√	√	√	√	√	√	√	√	√			√					√	2
JAERLA	E	√	√	√	√	√	√	√	√	√	√	√	√			√					√	2
JAZZY	E													√			√					2
KONDOR	I	√	√	√	√	√	√	√	√	√	√	√	√			√					√	2
LADY AMARILLA	E														√			√				2
LADY ROSETTA	E	√	√	√	√	√	√	√	√		√	√	√					√	√			2
MELODY	I	√	√	√	√	√	√	√	√	√	√	√	√			√					√	2
RED SCARLETT	E											√		√				√				2
ALMERA	I	√	√	√	√		√	√	√						√							1
ASTERIX	I	√	√	√	√		√											√				1
AVENTRA	I						√											√				1
BINTJE	E																	√				1
BIOGOLD	E											√						√				1
BIONICA	E																	√				1
CHARLOTTE	E																√					1
LADY BRITTA	E		√				√					√						√				1
LADY CHRISTL	E																	√				1
LADY FELICIA	E			√			√											√				1
MARABEL	E																	√				1
SANTANA	E																	√				1
SATURNA	E					√						√		√								1
SIFRA	I	√	√	√	√	√	√					√						√				1
SOPRANO	E																	√				1
SPUNTA	I	√	√	√	√		√											√				1
AGATA	E																					0
ARIZONA	E	√	√	√	√	√	√															0
CANBERRA	E					√																0
CHALLENGER	I																					0
COLOMBA	E	√	√	√	√	√	√					√										0
CUPIDO	E		√				√															0
HANSA	I		√			√																0
INOVA	E																					0
LADY ANNA	I																					0

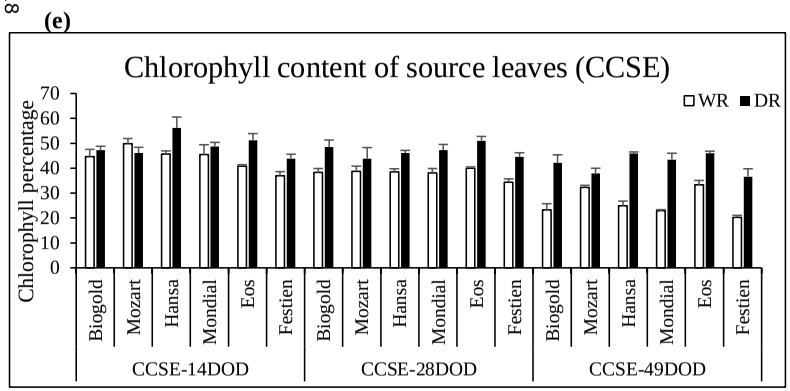
LADY LENORA	E		√	√		0
LISETA	E		√			0
MONALISA	E	√	√	√		0
MUSICA	E					0
NICOLA	I					0
PREMIERE	E		√			0
RUSSET BURBANK	I					0
VTN2 62- 33- 3	E		√			0

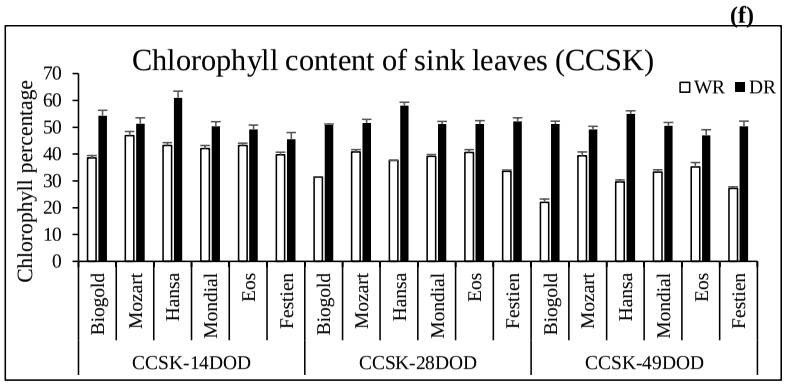
√ (above average value), *DRG (number of times a cultivar has above-average tuber size parameter with respect to drought tolerance column)

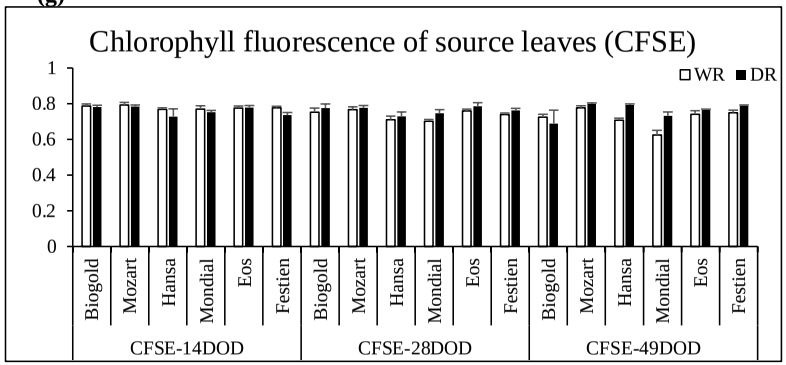


Supplementary Figure SF1: Manhattan plot showing significant association of tuber fresh weight under irrigated conditions with SNP markers in close proximity to the *StCDF*/Maturity locus on Chromosome-5.

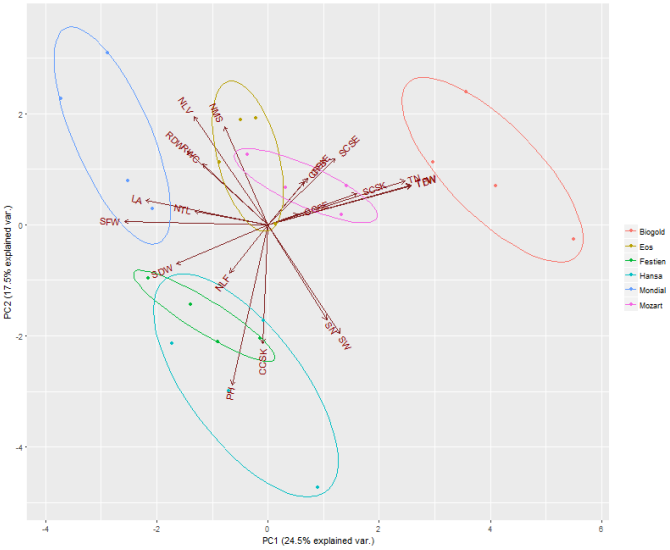

(a)
Stomatal conductance in source leaves (SCSE)
mmol/ms2
WR DR
Biogold Mozart Hansa Mondial Eos Festien
SCSE-14DOD
SCSE-28DOD
SCSE-49DOD
(b)
Stomatal conductance in sink leaves (SCSK)
mmol/ms2
SCSK-14DOD
SCSK-28DOD
SCSK-49DOD
(c)
Plant height (PH)
height (cm)
PH-14DOD
PH-28DOD
PH-49DOD
(d)
Leaf area (LA)
mm2
LA-14DOD
LA-28DOD
LA-49DOD



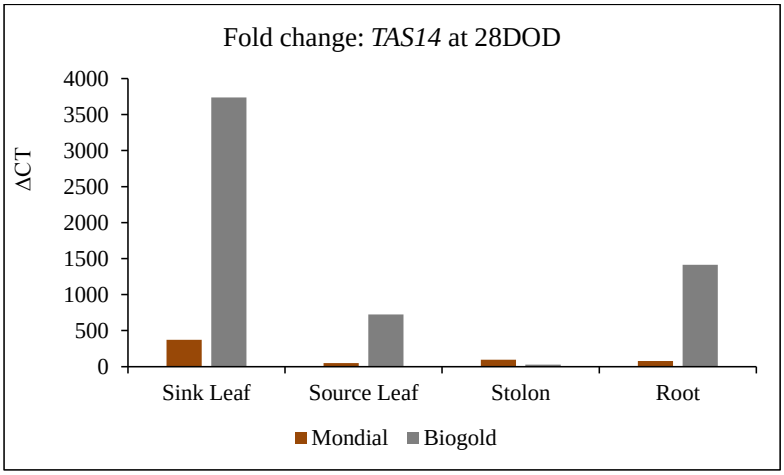




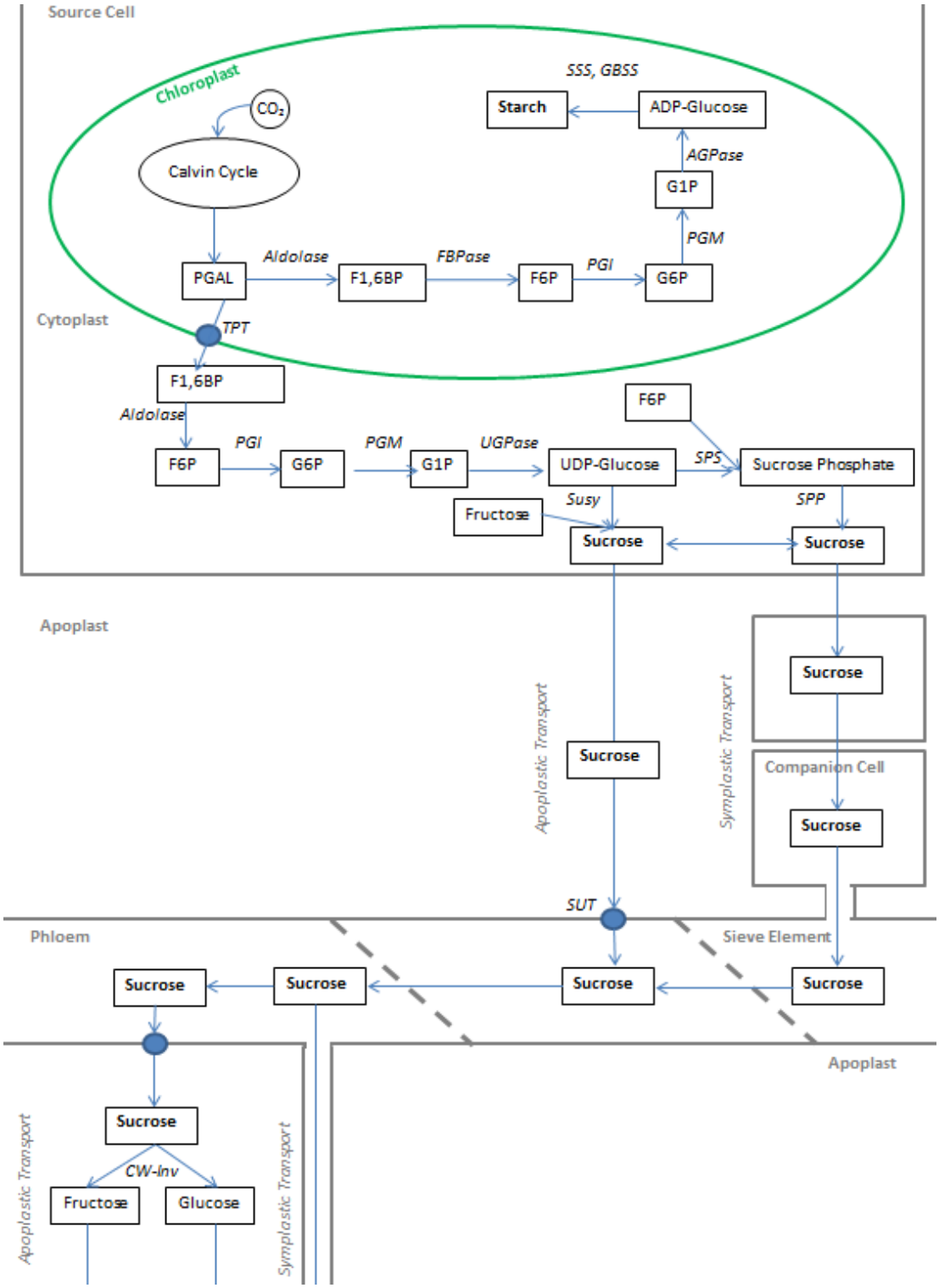
Supplementary Figure SF1: Graphs of (**a**) Stomatal conductance in source leaves (SCSE), (**b**) Stomatal conductance in source leaves (SCSK), (**c**) Plant Height (PH), (**d**) Leaf area (LA), (**e**) Chlorophyll content of source leaves (CCSE), (**f**) Chlorophyll content of sink leaves (CCSK) and (**g**) Chlorophyll fluorescence in source leaves (CFSE), under irrigated (WR) and drought (DR) conditions in the greenhouse at 14-, 28- and 49- days of drought (DOD). Error bars show standard errors of the mean of the biological replicates.

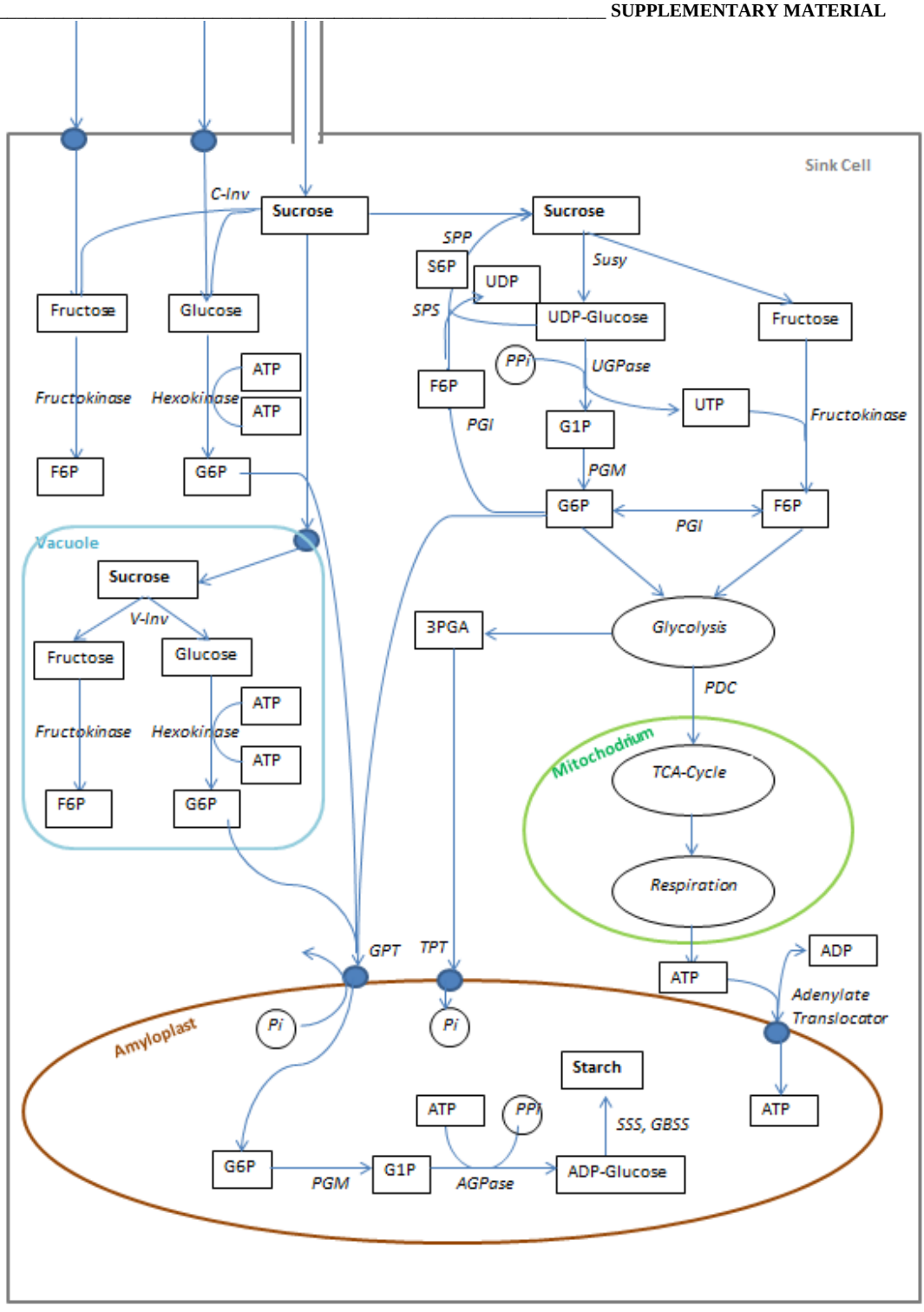


Supplementary Figure SF 2: PCA biplot of potato cultivars in drought treatment at 28DOD showing the clustering of the various cultivars. The traits are PH (Plant Height), NMS (Number of Stems), NLV (Number of leaves), NLF (Number of leaflets), NTL (Total number of leaves, that is, leaves plus leaflets), SCSE (Stomatal conductance of Source leaves), SCSK (Stomatal Conductance of Sink leaves), CCSE (Chlorophyll Content of Source leaves), CCSK (Chlorophyll Content of Sink leaves), CFSE (Chlorophyll Fluorescence of Source leaves), CFSK (Chlorophyll Fluorescence of Sink leaves), LA (Leaf Area), SN (Stolon Number), SW (Stolon Weight), TN (Tuber Number), TFW (Tuber Fresh Weight), TDW (Tuber Dry Weight), SFW (Shoot Fresh Weight), SDW (Shoot Dry Weight), RDW (Root Dry Weight), RWC (Relative Water Content)



Supplementary Figure SF3: Fold change of *TAS14* gene expression in various tissues of Mondial and Biogold under irrigated (control) vs. drought stress conditions at 28DOD.

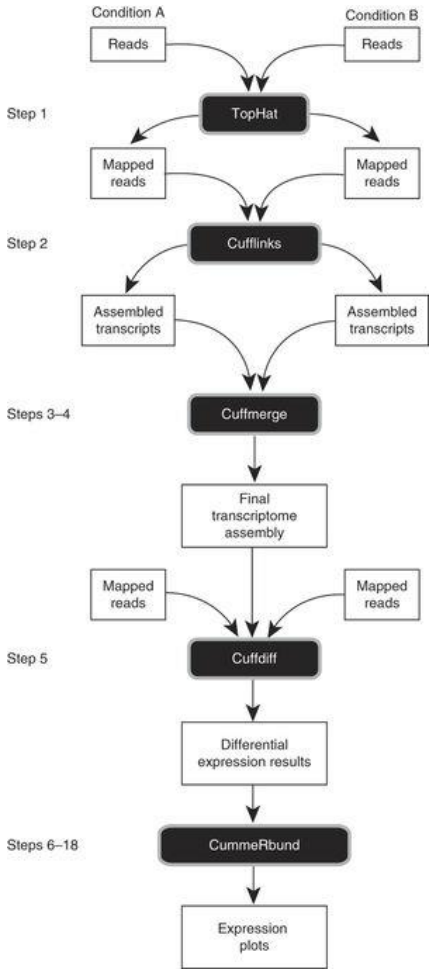

Source Cell
Chloroplast
CO_2
Calvin Cycle
SSS, GBSS
Starch
ADP-Glucose
AGPase
G1P
PGM
PGAL
Aldolase
F1,6BP
FBPase
F6P
PGI
G6P
Cytoplast
TPT
F1,6BP
Aldolase
F6P
PGI
G6P
PGM
G1P
UGPase
UDP-Glucose
F6P
SPS
Sucrose Phosphate
Fructose
Susy
SPP
Sucrose
Sucrose
Apoplast
Sucrose
Companion Cell
Sucrose
Apoplastic Transport
Symplastic Transport
Sucrose
SUT
Phloem
Sieve Element
Sucrose
Sucrose
Sucrose
Sucrose
Apoplast
Sucrose
CW-Inv
Fructose
Glucose
Apoplastic Transport
Symplastic Transport



Supplementary Figure SF4: Detailed starch biosynthesis pathway

Supplementary Table 1. Primers used for the gene expression study (including primers of result not shown in this report)

Genes	Product length		Sequence 5' toward 3'
APRT	121	F	GAACCGGAGCAGGTGAAGAA
		R	GAAGCAATCCCAGCGATACG
TAS14	118	F	CAACAGCAGCTTCGTCGATC
		R	CATGTCCTCCTCCTGGCATC
SUSY		F	AAAGCTGCTGAGCTCATGGT
		R	AGTCATCAGCCTCTCCGAGT
Sus3	200	F	GAACTTGTTAACGGAACTTCC
		R	TAGTGGTGAACTCGAAGAAAC
Sus4-T	112	F	AACTCAGTGCACCATTGCCC
		R	ATCAGCGGTAAACTGGGACG
N Inv1	133	F	TCCCATCGGCTCTTGCTTTT
		R	GCGGGCATCAATCCTTTTCC
N Inv2	106	F	GCCCTGTGGATGTGTCTCAA
		R	TGTCTTGGCCTTTCCAGCAA
N Inv3	122	F	TGCCAATCCAAGTGCAGCTA
		R	TCTGGGGACTTTTTGGACCG
Pain1.1-T	148	F	TTCAGTACCGGTGTTGGACG
		R	GAAGAGTCGTGCTGCTCCAT
STIN8	272	F	TTGGATGCCTCATACAACAAG
		R	CCTTTCTTGACATCGTCATTG
AGPase	163	F	AGAGAAACCGCAAGGAGAGC
		R	CCCAGGGAACTTGTCACGAA
PGM1	248	F	CCCACATCTGCTGCTCTTGA
		R	TTGAGTGGCCAACCTTCCTC
TPT	151	F	ATGCGGCATCAACAGGGAC
		R	GCACGGCTTAATGGATTCCC
UGPase-1	137	F	TACGGAAGACTTTGCCCCAC
		R	TGGGAGTAACCTTTGCTAGTAGTG
Aden.Tr-2	127	F	GCTGTATCAAAGACCGCTGC
		R	TCCTCCCAAAACATTCGCCA
PDC	169	F	TCTGAGTTTGAAGGAAGGCCA
		R	ACAGAGGTCTGTCCTGTTGA
Mal.Dehy.	151	F	GGGACTGAATGGGGTTCCAG
		R	CACCCTCATTGTGAGAGGGA
SUT1	127	F	TTCCATAGCTGCTGGTGTTC
		R	TACCAGAAATCGGTCCACAA
SUT2	135	F	GGCATTCCTCTTGCTGTAACC
		R	GCGAGACAACCATCTGAGGTAC
SUT4	125	F	GCTCTTGGGCTTGGACAAGGC
		R	GGCTGGTGAATTGCCTCCACC
SWEET10	134	F	ACTCCAGGTGTAATTGTGAAGGA
		R	CACGTGGCAACCTGTGTTTT
FRK1	158	F	CCCAAACCCACCTTTGGTCT
		R	GAGGTCCACCAGGAGCTCTA
SPS	175	F	TCTTGGTCGTCAGACAAGGC
		R	CAATGGAACCCTCTGCCCTT
GPT2	188	F	ATGCCCTTGGTGCTGCTATT
		R	AGGCACCTTCATTCACCGAG
PGI1	195	F	CTTCTTTGCGCAGCCAGATG
		R	TGGACTGCAACTCTGTGCTC
Aldo	160	F	ACTTGTGGAAAGCGTTTGGC
		R	TGCGTCCATCAACGGTAGAC
SPP2	184	F	ATGCATCAGAGAGGTGTGCC
		R	TGCTCAATTTCTCCACGCCT
RbcL	181	F	TCTGCGAATCCCTGTTGCTT
		R	AAGTCCACCGCGAAGACATT
GBSS1	195	F	CTTGTTGTGTCAAGCAGCCC
		R	TGGATGCAGAAAGCGACCTT
GBSS2	291	F	GTGCAAGCATATCTTTTACTTGTGA
		R	CACACGGTTCCCTCCGTAAA
TSP	190	F	TTGGCGATGCTTTGTCCAGA
		R	CAGTCGGAGTCCACAGGTTC
SnRK1	156	F	GTAGCCAATGAGACAGGCGT
		R	TGGCTCCTTTGCGAAATCCT



Supplementary Figure SF1: Tuxedo pipeline describing the workflow used in the RNA-seq data analysis.

Supplementary Table 1a: Upregulated DEGs in the leaf of Hansa at 28DOD involved in drought stress signalling

Genes	Annotations	Log2 fold change
ABA signalling		
PGSC0003DMG400016742	Protein phosphatase 2C AHG3 homolog	2.04
PGSC0003DMG400028315	Ninja-family protein AFP2	1.90
PGSC0003DMG400025795	Cytochrome P450	1.68
PGSC0003DMG402026767	Delta 1-pyrroline-5-carboxylate synthetase	2.15
PGSC0003DMG400001598	Snakin-2	1.51
Cytokinin signalling		
PGSC0003DMG402022640	Two-component sensor protein histidine protein kinase	2.23
PGSC0003DMG400000280	GATA transcription factor 21	1.32
Gibberellic acid signalling		
PGSC0003DMG400021292	Gibberellin 2-oxidase 3	3.22
PGSC0003DMG400002068	Gibberellin 2-oxidase 3	1.91
Auxin signalling		
PGSC0003DMG400012479	Nitrate transporter	1.90
Ethylene signalling		
PGSC0003DMG400012305	DNA binding protein	2.65

Supplementary Table 1b: Downregulated DEGs involved in hormonal signalling in the leaf of Hansa at 28DOD

Genes	Annotation	Log2 fold change
ABA signalling		
PGSC0003DMG400023949	Abscisic acid receptor PYL4	-2.56012
PGSC0003DMG400014232	Calnexin	-1.36766
PGSC0003DMG400031119	Stress-induced protein	-1.66195
PGSC0003DMG401020908	Plasma intrinsic protein 2,1	-1.47755
cytokinin signalling		
gibberellic signalling		
Auxin signalling		
PGSC0003DMG400014232	Calnexin	-1.36766
PGSC0003DMG400002169	Glutathione-S-transferase	-1.55268
PGSC0003DMG400002167	Glutathione S-transferase	-1.88683
ethylene signalling		
PGSC0003DMG400013401	Ethylene-responsive transcription	undefined
PGSC0003DMG400016006	Pti4	-1.63856
PGSC0003DMG400016004	Ethylene response factor 4	-2.46122
PGSC0003DMG400033696	MAPKK	-1.59723
PGSC0003DMG400032199	Peroxidase	-1.83573
PGSC0003DMG400032147	Peroxidase	-2.08473
PGSC0003DMG400019435	Wound-induced protein WIN1	-3.01746

Supplementary Table 2a: Upregulated DEGs in the leaves of Biogold involved in drought stress signalling at 28DOD

Genes	Annotations	Log2 fold change
ABA signalling		
PGSC0003DMG400026077	Calcium-dependent protein kinase	1.6
PGSC0003DMG400028315	Ninja-family protein AFP2	1.26
PGSC0003DMG400025795	Cytochrome P450	1.52
PGSC0003DMG400010279	Digalactosyldiacylglycerol synthase 2, chloroplastic	1.08
PGSC0003DMG402026767	Delta 1-pyrroline-5-carboxylate synthetase	1.84
PGSC0003DMG400001598	Snakin-2	1.49
PGSC0003DMG400007848	Phospholipase D	2.24
PGSC0003DMG400018109	Raffinose synthase 2	1.68
PGSC0003DMG400025226	Endonuclease/exonuclease/phosphatase family	1.78
Cytokinin signalling		
PGSC0003DMG402022640	Two-component sensor protein histidine protein	1.42
PGSC0003DMG400002722	Cellulose synthase-like A1	2.31
PGSC0003DMG400002190	Minichromosome maintenance factor	1.45
PGSC0003DMG400011837	Minichromosome maintenance factor	1.23
Gibberellic acid signalling		
PGSC0003DMG401019533	Gip1	2.75
PGSC0003DMG400002068	Gibberellin 2-oxidase 1	3.37
PGSC0003DMG400008833	Expansin	1.69
Auxin signalling		
PGSC0003DMG400012261	Auxin response factor 9	1.67
PGSC0003DMG400011603	Germin	1.16
PGSC0003DMG400013010	24K germin	3.11
PGSC0003DMG400024755	Xyloglucan endotransglucosylase/hydrolase 1	1.69
PGSC0003DMG400024643	Flavonoid 3'-monooxygenase	1.35
PGSC0003DMG400018110	Alliin lyase	1.49
Ethylene signalling		
PGSC0003DMG400012305	DNA binding protein	2.21
PGSC0003DMG400021683	E3 ubiquitin-protein ligase RMA1H1	4.61

Supplementary Table 2b: Downregulated DEGs involved in hormonal signalling in the leaf of Biogold at 28DOD

Genes	Annotation	Log2 fold change
ABA signalling		
PGSC0003DMG400027174	Protein phosphatase 2c	-3.47
PGSC0003DMG400035643	Drought-induced protein SDi	-2.61
PGSC0003DMG400030058	MAP kinase	-1.95
PGSC0003DMG400006788	Conserved gene of unknown function	undefined
PGSC0003DMG400023949	Abscisic acid receptor PYL4	-2.60
PGSC0003DMG400023814	Kinase family protein	-1.65
PGSC0003DMG400021331	PEN1	-4.88
PGSC0003DMG400020122	Circadian clock coupling factor ZGT	-1.33
PGSC0003DMG400019873	Phytoalexin-deficient 4-2 protein	-1.06
PGSC0003DMG400016285	Enoyl-CoA hydratase/isomerase family protein	-1.11
PGSC0003DMG400015927	UDP-arabinose 4-epimerase 1	-1.33
PGSC0003DMG400012138	N-rich protein	-1.75
PGSC0003DMG400008149	Calcium-dependent protein kinase 4	-1.06
PGSC0003DMG400007634	Serine-threonine protein kinase, plant-type	-2.04
PGSC0003DMG400007613	Alternative oxidase	-4.52
PGSC0003DMG400007258	Tyrosine specific protein phosphatase and dual	-1.52
PGSC0003DMG400005969	Gene of unknown function	-2.78
PGSC0003DMG400025931	GTP-binding protein alpha subunit, gna	-2.29
PGSC0003DMG400026646	Kinase	-1.50
PGSC0003DMG400032273	N-acetyltransferase	-4.39
PGSC0003DMG400031119	Stress-induced protein	-1.63
PGSC0003DMG400024693	Lipoxygenase	-2.60
PGSC0003DMG400010572	RNA-binding region-containing protein	-2.21
cytokinin signalling		
gibberellic acid signalling		
auxin signalling		
PGSC0003DMG400002179	Glutathione S-transferase	undefined
PGSC0003DMG400020139	Auxin-induced protein 22B	-1.24
PGSC0003DMG400013765	ATP binding protein	-1.54
PGSC0003DMG400011012	Glutathione-S-transferase	-3.54
PGSC0003DMG400002174	Glutathione S-transferase	-1.69
PGSC0003DMG400002172	Glutathione S-transferase T2	-1.30
PGSC0003DMG400002167	Glutathione S-transferase	-3.44
PGSC0003DMG400003228	SAUR family protein	-1.80
PGSC0003DMG400003227	SAUR family protein	-2.09
PGSC0003DMG400025022	Avr9/Cf-9 rapidly elicited protein 20	-3.12
PGSC0003DMG400043394	Conserved gene of unknown function	-2.71
PGSC0003DMG400026159	Ccd1	-2.77
PGSC0003DMG400024978	Indole-3-acetic acid-amido synthetase GH3.3	-3.05
PGSC0003DMG400021331	PEN1	-4.88
PGSC0003DMG400013879	Quinone reductase family protein	-1.28
ethylene signalling		
PGSC0003DMG400010753	Ethylene response factor 5	-2.83

PGSC0003DMG400029773	Ethylene-responsive transcriptional coactivator	-1.55
PGSC0003DMG400026821	Ethylene-responsive transcription factor 4	-1.87
PGSC0003DMG400026232	Avr9/Cf-9 rapidly elicited protein 1	-3.54
PGSC0003DMG400025282	AP2/ERF domain-containing transcription factor	-4.20
PGSC0003DMG400014417	Ethylene-responsive transcription factor 3	-2.83
PGSC0003DMG400010750	ERF transcription factor	-1.46
PGSC0003DMG400005899	CCR4-associated factor	-4.37
PGSC0003DMG400007947	WRKY transcription factor 2	-1.43
PGSC0003DMG400005909	Regulator of gene silencing	-4.46
PGSC0003DMG400001923	Matrix metalloprotease 1	-2.30
PGSC0003DMG400011169	Senescence-associated protein	-1.74
PGSC0003DMG400033696	MAPKK	-1.39
PGSC0003DMG400024160	Phospholipase A1	-3.69
PGSC0003DMG400023814	Kinase family protein	-1.65
PGSC0003DMG400022929	Aspartate aminotransferase	-2.50
PGSC0003DMG400023414	LEM3 (Ligand-effect modulator 3) family protein	-1.21
PGSC0003DMG400021331	PEN1	-4.88
PGSC0003DMG400020365	Conserved gene of unknown function	-1.24
PGSC0003DMG400019435	Wound-induced protein WIN1	-3.18
PGSC0003DMG400016285	Enoyl-CoA hydratase/isomerase family protein	-1.11
PGSC0003DMG400015927	UDP-arabinose 4-epimerase 1	-1.33
PGSC0003DMG400007634	Serine-threonine protein kinase, plant-type	-2.04
PGSC0003DMG400007613	Alternative oxidase	-4.52
PGSC0003DMG400007258	Tyrosine specific protein phosphatase and dual	-1.52
PGSC0003DMG400032199	Peroxidase	-1.71
PGSC0003DMG400032147	Peroxidase	-2.06
PGSC0003DMG400030462	Avr9/Cf-9 rapidly elicited protein 216	-2.64

Supplementary File 1: Overview of all sequenced samples showing number of sequenced and mapped reads

Samples	Genotypes	Tissues	Trt	Time point	Left reads	Mapped left reads	Right reads	Mapped right reads	Mapping rate(%)	Read length(bp)	Genome size(bp)	Seq. Depth	Exptal. Depth
BLD11	Biogold	Leaf	DR	1	63535123	42626031	63535123	37780744	63.3	150	840000000	22.7	14.4
BLD12	Biogold	Leaf	DR	1	62504972	42014989	62504972	37271798	63.4	150	840000000	22.3	14.2
BLD21	Biogold	Leaf	DR	2	65700068	43148483	65700068	38070723	61.8	150	840000000	23.5	14.5
BLD22	Biogold	Leaf	DR	2	72094758	47936465	72094758	43012785	63.1	150	840000000	25.7	16.2
BLW11	Biogold	Leaf	WR	1	71525660	48231813	71525660	42916301	63.7	150	840000000	25.5	16.3
BLW12	Biogold	Leaf	WR	1	65460881	42872066	65460881	38515104	62.2	150	840000000	23.4	14.5
BLW21	Biogold	Leaf	WR	2	60428456	40449606	60428456	33467364	61.2	150	840000000	21.6	13.2
BLW22	Biogold	Leaf	WR	2	62105022	41168615	62105022	34615581	61.0	150	840000000	22.2	13.5
BTD11	Biogold	Tuber	DR	1	57566071	38487937	57566071	33133003	62.2	150	840000000	20.6	12.8
BTD12	Biogold	Tuber	DR	1	67027502	44763675	67027502	37928002	61.7	150	840000000	23.9	14.8
BTD21	Biogold	Tuber	DR	2									
BTD22	Biogold	Tuber	DR	2	64469750	39233845	64469750	31054754	54.5	150	840000000	23.0	12.6
BTW11	Biogold	Tuber	WR	1	65227334	43807046	65227334	38151941	62.8	150	840000000	23.3	14.6
BTW12	Biogold	Tuber	WR	1	67724977	44855702	67724977	38623957	61.6	150	840000000	24.2	14.9
BTW21	Biogold	Tuber	WR	2	46016049	28711181	46016049	21766805	54.8	150	840000000	16.4	9.0
BTW22	Biogold	Tuber	WR	2	53178515	34364138	53178515	26433935	57.2	150	840000000	19.0	10.9
HLD11	Hansa	Leaf	DR	1	54936815	35610670	54936815	27765275	57.7	150	840000000	19.6	11.3
HLD12	Hansa	Leaf	DR	1	53856034	34805915	53856034	27695523	58.0	150	840000000	19.2	11.2
HLD21	Hansa	Leaf	DR	2	52520233	34916893	52520233	30137366	61.9	150	840000000	18.8	11.6
HLD22	Hansa	Leaf	DR	2	62952808	41923699	62952808	35626860	61.6	150	840000000	22.5	13.8
HLW11	Hansa	Leaf	WR	1	59620520	38648931	59620520	29116222	56.8	150	840000000	21.3	12.1
HLW12	Hansa	Leaf	WR	1	47076451	30562423	47076451	23687258	57.6	150	840000000	16.8	9.7
HLW21	Hansa	Leaf	WR	2	66255272	44457915	66255272	37791999	62.1	150	840000000	23.7	14.7
HLW22	Hansa	Leaf	WR	2	50373220	33871516	50373220	27766744	61.2	150	840000000	18.0	11.0
HTD11	Hansa	Tuber	DR	1	59036303	38177152	59036303	32007277	59.4	150	840000000	21.1	12.5
HTD12	Hansa	Tuber	DR	1	62126164	39148481	62126164	32693759	57.8	150	840000000	22.2	12.8
HTD21	Hansa	Tuber	DR	2	51453715	33888229	51453715	28764873	60.9	150	840000000	18.4	11.2
HTD22	Hansa	Tuber	DR	2	62222804	41485673	62222804	35941404	62.2	150	840000000	22.2	13.8
HTW11	Hansa	Tuber	WR	1	65397696	43392848	65397696	36883313	61.4	150	840000000	23.4	14.3
HTW12	Hansa	Tuber	WR	1	55505494	36269923	55505494	31008695	60.6	150	840000000	19.8	12.0
HTW21	Hansa	Tuber	WR	2	54702687	35947776	54702687	30159705	60.4	150	840000000	19.5	11.8
HTW22	Hansa	Tuber	WR	2	61688810	38882188	61688810	33428575	58.6	150	840000000	22.0	12.9

JLD11	Jaerla	Leaf	DR	1	60356380	39823106	60356380	33503976	60.7	150	840000000	21.6	13.1
JLD12	Jaerla	Leaf	DR	1	59051313	38703813	59051313	32439869	60.2	150	840000000	21.1	12.7
JLD21	Jaerla	Leaf	DR	2	47544410	31166096	47544410	26460471	60.6	150	840000000	17.0	10.3
JLD22	Jaerla	Leaf	DR	2	50055840	33874601	50055840	28567992	62.4	150	840000000	17.9	11.2
JLW11	Jaerla	Leaf	WR	1	65368633	40864970	65368633	35380529	58.3	150	840000000	23.3	13.6
JLW12	Jaerla	Leaf	WR	1	60331258	39001988	60331258	33342596	60.0	150	840000000	21.5	12.9
JLW21	Jaerla	Leaf	WR	2	51754325	34952515	51754325	28036670	60.9	150	840000000	18.5	11.2
JLW22	Jaerla	Leaf	WR	2	61660353	41282972	61660353	34348456	61.3	150	840000000	22.0	13.5
JTD11	Jaerla	Tuber	DR	1	53552629	35221899	53552629	29642068	60.6	150	840000000	19.1	11.6
JTD12	Jaerla	Tuber	DR	1	57944026	38334462	57944026	32093568	60.8	150	840000000	20.7	12.6
JTD21	Jaerla	Tuber	DR	2	49778566	32562204	49778566	27639963	60.5	150	840000000	17.8	10.8
JTD22	Jaerla	Tuber	DR	2	64405275	42461704	64405275	35583085	60.6	150	840000000	23.0	13.9
JTW11	Jaerla	Tuber	WR	1	57495376	39381573	57495376	33380886	63.3	150	840000000	20.5	13.0
JTW12	Jaerla	Tuber	WR	1	58492842	39588140	58492842	33149048	62.2	150	840000000	20.9	13.0
JTW21	Jaerla	Tuber	WR	2	73798273	47902992	73798273	39287733	59.1	150	840000000	26.4	15.6
JTW22	Jaerla	Tuber	WR	2	58317336	36551802	58317336	30217234	57.2	150	840000000	20.8	11.9
LLD11	Lady Rosetta	Leaf	DR	1	63732321	42306396	63732321	36974692	62.2	150	840000000	22.8	14.2
LLD12	Lady Rosetta	Leaf	DR	1	65527756	43253944	65527756	37274500	61.4	150	840000000	23.4	14.4
LLD21	Lady Rosetta	Leaf	DR	2	59072154	38847685	59072154	34788505	62.3	150	840000000	21.1	13.1
LLD22	Lady Rosetta	Leaf	DR	2	74573137	49749296	74573137	43715777	62.7	150	840000000	26.6	16.7
LLW11	Lady Rosetta	Leaf	WR	1	54294444	35489361	54294444	31662722	61.8	150	840000000	19.4	12.0
LLW12	Lady Rosetta	Leaf	WR	1	61530837	40347550	61530837	35670696	61.8	150	840000000	22.0	13.6
LLW21	Lady Rosetta	Leaf	WR	2	59902650	39892813	59902650	34334048	62.0	150	840000000	21.4	13.3
LLW22	Lady Rosetta	Leaf	WR	2	56528807	37859521	56528807	32808758	62.5	150	840000000	20.2	12.6
LTD11	Lady Rosetta	Tuber	DR	1	51988310	32909321	51988310	28419020	59.0	150	840000000	18.6	11.0
LTD12	Lady Rosetta	Tuber	DR	1	69006704	43288343	69006704	38319791	59.1	150	840000000	24.6	14.6
LTD21	Lady Rosetta	Tuber	DR	2	71449799	45561242	71449799	41292651	60.8	150	840000000	25.5	15.5
LTD22	Lady Rosetta	Tuber	DR	2	65235130	40806279	65235130	36970360	59.6	150	840000000	23.3	13.9
LTW11	Lady Rosetta	Tuber	WR	1	53190153	33761760	53190153	28004896	58.1	150	840000000	19.0	11.0
LTW12	Lady Rosetta	Tuber	WR	1	48900964	30952393	48900964	26978137	59.2	150	840000000	17.5	10.3
LTW21	Lady Rosetta	Tuber	WR	2	65911647	41825844	65911647	37895708	60.5	150	840000000	23.5	14.2
LTW22	Lady Rosetta	Tuber	WR	2	52828336	34110489	52828336	30124300	60.8	150	840000000	18.9	11.5
NLD11	Nicola	Leaf	DR	1	63306015	41857620	63306015	35078336	60.8	150	840000000	22.6	13.7
NLD12	Nicola	Leaf	DR	1	66784982	44080632	66784982	37686711	61.2	150	840000000	23.9	14.6
NLD21	Nicola	Leaf	DR	2	65101616	43038494	65101616	36201863	60.9	150	840000000	23.3	14.2
NLD22	Nicola	Leaf	DR	2	67902860	45745402	67902860	38152645	61.8	150	840000000	24.3	15.0
NLW11	Nicola	Leaf	WR	1	70815658	47174835	70815658	38325625	60.4	150	840000000	25.3	15.3

NLW12	Nicola	Leaf	WR	1	68197828	45952281	68197828	38020621	61.6	150	840000000	24.4	15.0
NLW21	Nicola	Leaf	WR	2	63149344	41495871	63149344	34680215	60.3	150	840000000	22.6	13.6
NLW22	Nicola	Leaf	WR	2	60032121	38898419	60032121	31468608	58.6	150	840000000	21.4	12.6
NTD11	Nicola	Tuber	DR	1	59690620	39075072	59690620	32615347	60.1	150	840000000	21.3	12.8
NTD12	Nicola	Tuber	DR	1	88671160	60185739	88671160	49395911	61.8	150	840000000	31.7	19.6
NTD21	Nicola	Tuber	DR	2	59572821	39569318	59572821	34588483	62.2	150	840000000	21.3	13.2
NTD22	Nicola	Tuber	DR	2	66215299	42189739	66215299	37746796	60.4	150	840000000	23.6	14.3
NTW11	Nicola	Tuber	WR	1	81163944	52862345	81163944	46683640	61.3	150	840000000	29.0	17.8
NTW12	Nicola	Tuber	WR	1	69822258	45196448	69822258	39813222	60.9	150	840000000	24.9	15.2
NTW21	Nicola	Tuber	WR	2	62199328	42058122	62199328	35381586	62.3	150	840000000	22.2	13.8
NTW22	Nicola	Tuber	WR	2	49169986	31738871	49169986	27940055	60.7	150	840000000	17.6	10.7

Treatment (Trt): (DR – Non-irrigated; WR – Irrigated)

Time points: (1 – 4weeks (that is, 28days) of drought; 2 – 8weeks (that is, 56days) of drought)

Supplementary File 2: Consistent Differentially Expressed Genes

Time-point 1 of leaf tissue (28dod): upregulated genes in most cultivars (B-Biogold, H-Hansa, L-lady Rosetta, J-Jaerla, N-Nicola)

GENES	ANNOTATIONS	MOLECULAR FUNCTION	BIOLOGICAL PROCESS	CELLULAR COMPARTMENT	CULTIVARS
PGSC0003DMG400000350	Zinc finger CCCH domain-containing protein 20	BINDING ACTIVITY ==> Metal (--> Zinc) ion binding, DNA (--> Sequence-specific) binding -> Transcription factor activity	Leaf senescence	Nucleus	BHLN
PGSC0003DMG400000984	3-oxo-5-alpha-steroid 4-dehydrogenase family protein	OXIDOREDUCTASE ACTIVITY==>3-oxo-5-alpha-steroid 4-dehydrogenase activity, acting on the CH-CH group of donors	lipid metabolic process	Cytoplasm, chloroplast envelope, integral component of membrane	BHLN
PGSC0003DMG400001598	Snakin-2	STRESS/DEFENSE RESPONSE==> response to abscisic acid, response to gibberellin, response to brassinosteroid, anthocyanin accumulation in tissues in response to UV light	polysaccharide biosynthetic process, tryptophan catabolic process, indole acetic acid biosynthetic process, multidimensional cell growth, cell tip growth, root hair elongation, cell wall organization	Extracellular region==>plant-type cell wall	BHLN
PGSC0003DMG400003084	Two-component response regulator ARR8	SIGNALING==>phosphorelay response regulator activity--> phosphorelay signal transduction			BHLN
PGSC0003DMG400005848	MYB transcription factor MYB92	BINDING ACTIVITY==>DNA (-->chromatin) binding			BHLN
PGSC0003DMG400009112	Protein phosphatase 2C 8	CATALYTIC ACTIVITY==>phosphoprotein phosphatase activity-->protein serine/threonine phosphatase activity-->protein dephosphorylation			BHLN
PGSC0003DMG400011538	Amino acid transporter	TRANSPORT ACTIVITY==>symporter activity --> acidic/neutral amino acid transmembrane transporter activity --> aspartate transport, tryptophan transport		Plasma membrane==>integral component of membrane	BHLN
PGSC0003DMG400012305	DNA binding protein	BINDING ACTIVITY==>DNA (-->sequence-specific/templated) binding -->transcription factor/regulation activity, transcription factor import to nucleus, SIGNALING==>cytokinin/ethylene-activated signalling pathways	Root development, Cotyledon development	Nucleus, cytoplasm	BHLN
PGSC0003DMG400017730	FtsH protease	BINDING ACTIVITY==>ATP binding (ATP-dependent peptidase activity, ATPase activity --> microtubule-severing ATPase	PSII associated light-harvesting complex II	Chloroplast==>integral component of membrane	BHLN

		activity), Protein binding (DNA helicase activity, metalloendopeptidase activity-->protein folding -->proteolysis), Zinc ion binding. STRESS RESPONSE==> response to heat, high light intensity, H2O2	catabolic process, regulation of apoptotic process, cell division		
PGSC0003DMG400017779	Proline-rich receptor kinase PERK7	PROTEIN PHOSPHORYLATION==>protein kinase activity-->protein serine/threonine kinase activity. BINDING ACTIVITY==>ATP binding			BHLN
PGSC0003DMG400020850	Beta-tubulin 16	BINDING ACTIVITY==> protein binding (protein polymerization), GTP binding (GTPase activity). STRUCTURAL FUNCTION==>structural constituent of cytoskeleton-->cytoskeleton organization. STRESS RESPONSE==>response to salt stress and cadmium ion	Gluconeogenesis, Microtubule-based movement/process, Proteasomal protein catabolic process	Cell wall, nucleolus, vacuole, Golgi apparatus, microtubule, plasma membrane, tubulin complex	BHLN
PGSC0003DMG400024935	Conserved gene of unknown function	BINDING ACTIVITY==>ATP binding, mismatched DNA binding (-->mismatch repair complex)	meiosis 1==>reciprocal meiotic recombination, chromosome segregation and organization, regulation of biological quality	Nucleus	BHLN
PGSC0003DMG400025795	Cytochrome P450	BINDING ACTIVITY==>Oxygen binding, heme binding, iron ion binding -->electron carrier activity. OXIDOREDUCTASE ACTIVITY==>acting on paired donors, with incorporation or reduction of molecular oxygen. STRESS RESPONSE==>response to red light, ABA metabolic process-->ABA 8'-hydrolase activity	Release of seed from dormancy, oxidation-reduction process		BHLN
PGSC0003DMG400026363	Conserved gene of unknown function	Response to sucrose, response to UV-B, response to karrikin	Flavonoid biosynthetic process	Nucleus	BHLN
PGSC0003DMG400027453	Ribonuclease t2	BINDING ACTIVITY==>RNA binding-->ribonuclease T2 activity			BHLN
PGSC0003DMG400027577	Superoxide dismutase	BINDING ACTIVITY==>Metal ion binding. OXIDOREDUCTASE ACTIVITY==>Superoxide metabolic process-->Superoxide dismutase activity			BHLN
PGSC0003DMG400027916	Inositol-3-phosphate synthase	Inositol-3-phosphate synthase activity-->inositol & phospholipid biosynthesis	Signalling		BHLN
PGSC0003DMG400028315	Ninja-family protein AFP2	STRESS RESPONSE==>abscisic acid-activated signalling pathway	Signal transduction	Nucleus	BHLN
PGSC0003DMG400030381	Nodulin	Involved in transport of nutrients, solutes, amino acids			BHLN
PGSC0003DMG401014665	Mitochondrial phosphate carrier protein	STRESS RESPONSE==>Response to salt stress	Transport	mitochondrial inner membrane, integral component of membrane	BHLN

PGSC0003DMG402022640	Two-component sensor protein histidine protein kinase	BINDING ACTIVITY==>DNA-templated protein binding-->protein targeting to membrane, regulation of transcription, positive regulation of flavonoid biosynthetic process. PHOSPHORYLATION==> protein serine/threonine kinase activity. SIGNALLING==>phosphorelay signal transduction system, cytokinin-activated signalling pathway, red light signalling pathway-->regulation of circadian rhythm, intracellular signal transduction	Embryo development ending in seed dormancy	Nucleus, cytoplasm	BHLN
PGSC0003DMG402026767	Delta 1-pyrroline-5-carboxylate synthetase	OXIDOREDUCTASE ACTIVITY==>glutamate-5-semialdehyde dehydrogenase activity. STRESS RESPONSE==>response to ABA, hyperosmotic salinity. PHOSPHORYLATION==>glutamate 5-kinase activity. BINDING ACTIVITY==>ATP binding	L-proline biosynthetic process, cellular amino acid biosynthetic, metabolic process, pollen development, embryo development ending in seed dormancy	cytoplasm, mitochondrion, cytosol, plasmodesma, chloroplast	BHLN
xPGSC0003DMG400000248	HB1	Response to hypoxia functioning in oxygen binding in the cell wall and plasma membrane.			BHLN
xPGSC0003DMG400003530	Abscisic acid and environmental stress-inducible protein TAS14	TAS14, Dehydrin involved in response to water stress			BHLN

Time-point 1 of leaf tissue (28dod): downregulated genes in most cultivars

GENES	ANNOTATIONS	MOLECULAR FUNCTION	BIOLOGICAL PROCESS	CELLULAR COMPARTMENT	CULTIVARS
PGSC0003DMG400000519	Glucan endo-1,3-beta-glucosidase, acidic isoform GI9	DEFENSE==>response to biotic stimulus, plant-type hypersensitive response. HYDROLYSIS==>hydrolase activity, hydrolyzing O-glycosyl compounds, glucan endo-1,3-beta-D-glucosidase activity	carbohydrate metabolic process	extracellular region, proteinaceous extracellular matrix, extracellular space	BHLN
PGSC0003DMG400000621	NtEIG-A1 protein	BINDING==>copper ion binding, electron carrier activity			BHLN
PGSC0003DMG400000783	Extensin	STRUCTURAL==>structural constituent of cell wall, plant-type cell wall organization		Cell wall	BHLN
PGSC0003DMG400001528	Class II chitinase	DEFENSE==>defence response, response to biotic stimulus, chitinase activity	cell wall macromolecule catabolic process, polysaccharide catabolic process, chitin catabolic process		BHLN
PGSC0003DMG400001948	Copalyl diphosphate synthase	BINDING==>magnesium ion binding. LYASE ACTIVITY==>hydro-lyase activity. Terpene synthase activity	metabolic process, copal-8-ol diphosphate(3-) biosynthetic process, geranylgeranyl diphosphate catabolic process	Intracellular membrane-bounded organelle, cytoplasmic part	BHLN
PGSC0003DMG400002167	Glutathione S-transferase	BINDING==>protein binding. TRANSFERASE==>glutathione transferase activity. DEFENSE==>defence response, response to biotic stimulus. SIGNALING==>auxin-activated signalling pathway			BHLN
PGSC0003DMG400005410	NAD(P)H:quinone oxidoreductase	OXIDOREDUCTASE==>NAD(P)H dehydrogenase (quinone) activity. DEFENSE==>response to salt stress, defence response to bacterium		plasma membrane, chloroplast stroma	BHLN
PGSC0003DMG400006276	NtPRp27	DEFENSE RESPONSE			BHLN
PGSC0003DMG400008673	Endochitinase (Chitinase)	BINDING==>chitin binding, chitinase activity. DEFENSE==>defence response	polysaccharide catabolic process, cell wall macromolecule catabolic process, chitin catabolic process	extracellular space	BHLN
PGSC0003DMG400010131	Nb cell death marker	Endopeptidase inhibitor activity			BHLN
PGSC0003DMG400010283	Class I chitinase				BHLN

PGSC0003DMG400010638	S-adenosylmethionine-dependent methyltransferase	METHYLATION==>methyltransferase activity	metabolic process		BHLN
PGSC0003DMG400011745	VQ motif-containing protein	Regulate plant developmental processes			BHLN
PGSC0003DMG400011949	Non-specific lipid-transfer protein	BINDING==>lipid binding-->lipid transport. STRESS RESPONSE==> response to stress			BHLN
PGSC0003DMG400012875	Protein disulfide isomerase L-2	OXIDOREDUCTASE==>protein disulfide oxidoreductase activity, protein disulfide isomerase activity, dolichyl-diphosphooligosaccharide-protein glycotransferase activity, protein folding, N-terminal protein myristoylation, cell redox homeostasis, electron carrier activity, Golgi vesicle transport. STRESS RESPONSE==>response to oxidative stress, antioxidant activity, response to endoplasmic reticulum stress,	Glycerol ether metabolic process, cellulose biosynthetic process	Mitochondrion, vacuolar membrane, endoplasmic reticulum, endoplasmic reticulum lumen, plasma membrane, chloroplast	BHLN
PGSC0003DMG400013227	Osmotin	DEFENSE==>defence response to fungus, killing of cells of other organism			BHLN
PGSC0003DMG400013399	Conserved gene of unknown function	DEFENSE==>protein folding, systemic acquired resistance. STRESS RESPONSE==>response to heat, response to high light intensity, response to endoplasmic reticulum stress, response to hydrogen peroxide		endoplasmic reticulum, Golgi apparatus	BHLN
PGSC0003DMG400014368	PAR-1c protein	Uncharacterized protein			BHLN
PGSC0003DMG400014558	Conserved gene of unknown function				BHLN
PGSC0003DMG400015228	Peptide methionine sulfoxide reductase	OXIDOREDUCTASE==>peptide-methionine (S)-S-oxide reductase activity, acting on a sulphur group of donors, disulfide as acceptor, protein repair. STRESS RESPONSE==>response to oxidative stress			BHLN
PGSC0003DMG400019435	Wound-induced protein WIN1	BINDING==> ribonuclease activity, chitin binding. DEFENSE==>response to virus, systemic acquired resistance, response to ethylene, defence response to fungus, defence response to bacterium, incompatible interaction, response to herbivore. STRESS RESPONSE==> response to salt stress		extracellular region	BHLN
PSC0003DMG400019526	Zinc finger protein	BINDING==>nucleic acid binding, zinc ion binding, protein binding (ubiquitin-protein transferase activity, protein ubiquitination)			BHLN

PGSC0003DMG400020017	Lichenase	HYDROLYSIS==>hydrolase activity, hydrolyzing O-glycosyl compounds, glucan endo-1,3-beta-D-glucosidase activity. DEFENSE==>defence response	Carbohydrate metabolic process	vacuole	BHLN
PGSC0003DMG400020271	Ubiquitin-protein ligase	BINDING==>zinc ion binding, ligase activity		integral component of membrane	BHLN
PGSC0003DMG400023949	Abscisic acid receptor PYL4	DEFENSE==>defence response, response to biotic stimulus, receptor activity, protein homodimerization activity. SIGNALING==>abscisic acid binding, abscisic acid-activated signalling pathway		nucleus, cytoplasm	BHLN
PGSC0003DMG400024081	Oligopeptide transporter	TRANSPORT==>transmembrane transport-->oligopeptide transporter activity, oligopeptide transport-->amino acid transport		plasma membrane	BHLN
PGSC0003DMG400024197	Major intrinsic protein 2	TRANSPORT==>transporter activity		membrane, integral component of membrane	BHLN
PGSC0003DMG400025063	Class IV chitinase	BINDING==>chitin binding, chitinase activity	carbohydrate metabolic process, chitin catabolic process, cell wall macromolecule catabolic process		BHLN
PGSC0003DMG400025616	UDP-galactose transporter	TRANSPORT==>transmembrane transport--> (pyrimidine nucleotide-sugar transmembrane transport, UDP-glucose transmembrane transporter activity, UDP-glucose transport). DEFENSE==> protein folding, endoplasmic reticulum unfolded protein response, systemic acquired resistance, STRESS RESPONSE==>response to heat, response to high light intensity, response to hydrogen peroxide	Embryo sac development, pollen development	integral component of Golgi membrane, integral component of endoplasmic reticulum membrane	BHLN
PGSC0003DMG400029830	Glucan endo-1,3-beta-D-glucosidase	HYDROLYSIS==>hydrolase activity, hydrolyzing O-glycosyl compounds, glucan endo-1,3-beta-D-glucosidase activity. DEFENSE==>defence response. BINDING==>cation binding	carbohydrate metabolic process	apoplast	BHLN
PGSC0003DMG400030006	PAR-1b protein	Uncharacterized protein			BHLN
PGSC0003DMG400031119	Stress-induced protein	STRESS RESPONSE==>response to salt stress, abscisic acid			BHLN
PGSC0003DMG400032199	Peroxidase	OXIDOREDUCTASE==> peroxidase activity, hydrogen peroxide catabolic process. DEFENSE==> response to virus, response to ethylene. STRESS RESPONSE==>response to oxidative stress. BINDING==>heme binding, metal ion binding		extracellular region	BHLN
PGSC0003DMG400032250	Non-specific lipid-transfer protein	BINDING==>lipid binding. TRANSPORT==>lipid transport			BHLN

PGSC0003DMG400033634	Fatty acid desaturase	OXIDOREDUCTASE==>oxidoreductase activity acting on paired donors with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water, unsaturated fatty acid biosynthetic process	lipid metabolic process	integral component of membrane, endoplasmic reticulum membrane,	BHLN
PGSC0003DMG400033696	MAPKK	DEFENSE==> defence response to oomycetes, response to wounding, systemic acquired resistance, defence response to bacterium. PHOSPHORYLATION==> protein kinase activity, protein phosphorylation, protein kinase activator activity, protein autophosphorylation, protein serine/threonine kinase activity, positive regulation of protein kinase activity. STRESS RESPONSE==> response to salt stress, SIGNALING==>salicylic acid mediated signalling pathway, ethylene-activated signalling pathway, auxin polar transport. BINDING==>ATP binding	ethylene biosynthetic process, camalexin biosynthetic process		BHLN
PGSC0003DMG400033882	Acidic endochitinase	HYDROLYSIS==>hydrolase activity, hydrolyzing O-glycosyl compounds, chitinase activity, lysozyme activity	carbohydrate metabolic process, polysaccharide catabolic process, chitin catabolic process		BHLN
PGSC0003DMG400039214	Arachidonic acid-induced DEA1	DEFENSE==>systemic acquired resistance, defence response to fungus		cytoplasmic vesicle	BHLN
PGSC0003DMG402000506	Alpha-DOX2	OXIDOREDUCTASE==>peroxidase activity, oxidoreductase activity acting on single donors with incorporation of molecular oxygen incorporation of two atoms of oxygen, STRESS RESPONSE==>response to oxidative stress, BINDING==>heme binding.			BHLN
xPGSC0003DMG400025877	Twi1 protein	Glucosyltransferase, transferase activity			BHLN

Time-point 1 of tuber tissue (28dod): upregulated genes in most cultivars

GENES	ANNOTATIONS	MOLECULAR FUNCTION	BIOLOGICAL PROCESS	CELLULAR COMPARTMENT	CULTIVARS
PGSC0003DMG400003548	Fructose-bisphosphate aldolase	fructose-bisphosphate aldolase activity	glycolytic process		HJL
PGSC0003DMG400031535	Glycine-rich protein A3	response to stimulus, abiotic (osmotic) stress responses			HJLN

Time-point 1 of tuber tissue (28dod): downregulated genes in most cultivars

GENES	ANNOTATIONS	MOLECULAR FUNCTION	BIOLOGICAL PROCESS	CELLULAR COMPARTMENT	CULTIVARS
PGSC0003DMG400000783	Extensin	STRUCTURE==>structural constituent of cell wall, plant-type cell wall organization		Cell wall	HJL
PGSC0003DMG400000811	RAV transcription factor	BINDING==>DNA-templated binding, sequence-specific DNA binding, transcription factor activity, regulation of transcription			HJN
PGSC0003DMG400002899	AP2/ERF domain-containing transcription factor	BINDING==>DNA-templated binding, sequence-specific DNA binding, transcription factor activity, regulation of transcription			HJN
PGSC0003DMG400003948	Stress-associated protein 6	BINDING==>DNA binding, zinc ion binding		plasmodesma	HJN
PGSC0003DMG400005835	WRKY transcription factor-30	BINDING==>DNA-templated binding, sequence-specific DNA binding, transcription factor activity, regulation of transcription			HJN
PGSC0003DMG400005899	CCR4-associated factor	BINDING==> nucleic acid binding, poly(A)-specific ribonuclease activity, nuclear-transcribed mRNA poly(A) tail shortening, RNA modification. DEFENSE==>response to wounding, response to mechanical stimulus, defence response to insect, response to chitin, defence response to bacterium. SIGNALING==>ethylene-activated signalling pathway	ethylene biosynthetic process, vegetative to reproductive phase transition of meristem	Nucleus	HJN
PGSC0003DMG400010136	Stigma expressed protein	Endopeptidase inhibitor activity			JLN
PGSC0003DMG400010462	Bcl-2-associated athanogene	BINDING==>protein binding, chaperone binding			JLN
PGSC0003DMG400010713	Salt responsive protein 2	Involved in response to salt stress			HJN
PGSC0003DMG400013984	TMV induced protein 1-2	BINDING==>protein binding			HJL
PGSC0003DMG400015534	ZPT2-13	BINDING==>metal ion binding			HJN
PGSC0003DMG400016003	Ethylene responsive element binding protein C2	BINDING==>DNA-templated binding, sequence-specific DNA binding, transcription factor activity, regulation of transcription			HJN
PGSC0003DMG400016828	Phosphate-responsive 1 family protein			extracellular region	HJL

PGSC0003DMG400018182	Ring finger protein	TRANSFERASE==> ubiquitin-protein transferase activity. DEFENSE==> defence response to bacterium, response to chitin. BINDING==>protein binding, zinc ion binding, incompatible interaction		plasma membrane, integral component of membrane	HJN
PGSC0003DMG400021508	C2H2-type zinc finger protein	STRESS RESPONSE==>response to stress, response to abiotic stimulus. BINDING==>metal ion binding			HJN
PGSC0003DMG400024100	Conserved gene of unknown function				HJN
PGSC0003DMG400026232	Avr9/Cf-9 rapidly elicited protein 1	BINDING==>DNA-templated binding, sequence-specific DNA binding, positive regulation of transcription, transcription factor activity, STRESS RESPONSE==>response to cold, DEFENSE==> response to chitin. SIGNALING==>ethylene-activated signalling pathway		Nucleus	HJN
PGSC0003DMG400030808	Phi-1 protein	STRUCTURE==>plant-type cell wall. response to brassinosteroid			HJL
PGSC0003DMG400041029	Bcl-2-associated athanogene	BINDING==>protein binding, chaperone binding			JLN
xPGSC0003DMG400000332	Salt responsive protein 2	Involved in response to salt stress			HJN
xPGSC0003DMG400028520	WRKY transcription factor 1	Transcription regulation, sequence-specific DNA binding			HJN

Time-point 2 of leaf tissue (56dod): upregulated genes in most cultivars

GENES	ANNOTATIONS	MOLECULAR FUNCTION	BIOLOGICAL PROCESS	CELLULAR COMPARTMENT	CULTIVARS
PGSC0003DMG400001374	Cytochrome P450 85A1	OXIDOREDUCTASE==>monooxygenase activity, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen. BINDING==>iron ion binding, heme binding, electron carrier activity	brassinosteroid biosynthetic process	integral component of membrane	BLN
PGSC0003DMG400006183	Aquaporin	TRANSPORT==> transporter activity, transport, water channel activity. STRESS RESPONSE==>response to abscisic acid. BINDING==>protein binding		nucleus, vacuole, plasmodesma, membrane, integral component of membrane, anchored component of plasma membrane	HLN

PGSC0003DMG400010771	Pectate lyase	Involved in plant cell wall degradation			HLN
PGSC0003DMG400013516	Polygalacturonase	CATALYTIC ACTIVITY==>polygalacturonase activity, galacturan 1,4-alpha-galacturonidase activity	carbohydrate metabolic process	extracellular region	HLN
PGSC0003DMG400027047	UPF0497 membrane protein	Regulates membrane-cell wall junctions and localized cell wall deposition		plasma membrane, integral component of membrane	HLN
xPGSC0003DMG401019533	Gip1	Antioxidant activity			HLN

Time-point 2 of leaf tissue (56dod): downregulated genes in most cultivars

GENES	ANNOTATIONS	MOLECULAR FUNCTION	BIOLOGICAL PROCESS	CELLULAR COMPARTMENT	CULTIVARS
PGSC0003DMG400000066	Ethylene-responsive late embryogenesis	DEFENSE==>response to wounding, defence response to fungus. STRESS RESPONSE==>response to desiccation, response to high light intensity		plasma membrane	HLN
PGSC0003DMG400026885	GI11736	Basic proline-rich protein-like			BHJ
PGSC0003DMG400027750	Hsc70	BINDING==>ATP binding. Response to stress	cell morphogenesis		HLN
PGSC0003DMG401019255	Pectin esterase 3	CATALYTIC ACTIVITY==>cell wall modification, pectin esterase activity, aspartyl esterase activity, enzyme inhibitor activity, negative regulation of catalytic activity.	fruit ripening, pectin catabolic process	extracellular region, cell wall	BHJ
PGSC0003DMG402019343	DNA binding protein	BINDING==>DNA-templated binding, sequence-specific DNA binding, transcription factor activity, regulation of transcription. STRESS RESPONSE==>response to stress, response to heat		nucleus, cytoplasm	HJL

Time-point 2 of tuber tissue (56dod): upregulated genes in most cultivars

GENES	ANNOTATIONS	MOLECULAR FUNCTION	BIOLOGICAL PROCESS	CELLULAR COMPARTMENT	CULTIVARS
PGSC0003DMG400011954	Non-specific lipid-transfer protein 1	BINDING==>lipid binding. STRESS RESPONSE==>response to stress. TRANSPORT==>lipid transport			BJL
xPGSC0003DMG400003530	Abscisic acid and environmental stress-inducible protein TAS14	Dehydrin involved in response to water stress			BJL

Time-point 2 of tuber tissue (56dod): downregulated genes in most cultivars

GENES	ANNOTATIONS	MOLECULAR FUNCTION	BIOLOGICAL PROCESS	CELLULAR COMPARTMENT	CULTI VARS
PGSC0003DMG400004652	Serine-pyruvate aminotransferase	HOMEOSTASIS==>serine-pyruvate transaminase activity, alanine-glyoxylate transaminase activity, response to fructose, cellular cation homeostasis, serine-glyoxylate transaminase activity. TRANSPORT==>water transport, divalent metal ion transport. STRESS RESPONSE==>response to salt stress. BINDING==>pyridoxal phosphate binding	metabolic process, photorespiration	peroxisome, plasma membrane, chloroplast stroma, apoplast	BJL
PGSC0003DMG400007335	Apyrase 3	BINDING==>nucleotide binding. HYDROLYIS==>hydrolase activity		membrane	BHJ
PGSC0003DMG400010137	Cysteine protease inhibitor 1	Endopeptidase inhibitor activity			BHJ
PGSC0003DMG400022258	Alkaline alpha-galactosidase seed imbibition protein	BINDING==>RNA binding, RNA-directed DNA polymerase activity, RNA-dependent DNA biosynthetic process. HYDROLYSIS==>hydrolase activity, hydrolyzing O-glycosyl compounds. Galactinol-raffinose galactosyltransferase activity			BJL
PGSC0003DMG400026899	Multicystatin	Cysteine-type endopeptidase inhibitor activity, negative regulation of peptidase activity			BHJ

Supplementary File 3: Highly differentially expressed genes in the cultivars in leaf (L) and tuber (T) tissues at 28DOD (1) and 56DOD(2). B-Biogold, H-Hansa, L-Lady Rosetta, J-Jaerla, N-Nicola)

GENES	IR	NI	log2 fold	Sample	annotation
PGSC0003DMG400021683	0.69	16.93	4.607	BL1	E3 ubiquitin-protein ligase RMA1H1
PGSC0003DMG400008000	2.25	36.42	4.016	BL1	L-asparaginase
PGSC0003DMG400002068	0.72	7.39	3.368	BL1	Gibberellin 2-oxidase 1
PGSC0003DMG400013754	0.00	3.37		BL1	Conserved gene of unknown function
PGSC0003DMG400021437	0.33	3.32	3.347	BL1	Gene of unknown function
PGSC0003DMG400020017	36.34	0.29	-6.984	BL1	Lichenase
PGSC0003DMG400010859	9.03	0.11	-6.343	BL1	Lipoxygenase
PGSC0003DMG400010131	44.29	0.79	-5.815	BL1	Nb cell death marker
PGSC0003DMG400003044	313.31	5.74	-5.770	BL1	Osmotin
PGSC0003DMG400013984	8.95	0.00		BL1	TMV induced protein 1-2
PGSC0003DMG400011953	2.55	28.36	3.475	BL2	Non-specific lipid-transfer protein 2
PGSC0003DMG400011954	5.90	63.93	3.438	BL2	Non-specific lipid-transfer protein 1
PGSC0003DMG402020132	2.47	23.06	3.223	BL2	Non-specific lipid-transfer protein
PGSC0003DMG400022131	5.51	39.68	2.847	BL2	Lipid binding protein
PGSC0003DMG400031535	14.28	99.03	2.794	BL2	Glycine-rich protein A3
PGSC0003DMG400003993	50.73	1.19	-5.418	BL2	Citrate binding protein
PGSC0003DMG400008099	41.84	1.21	-5.116	BL2	KiTH-2
PGSC0003DMG400040317	8.19	0.79	-3.374	BL2	Endochitinase
PGSC0003DMG400000871	3.43	0.38	-3.179	BL2	Quinonprotein alcohol dehydrogenase
PGSC0003DMG400020999	2.19	0.30	-2.880	BL2	Lipoxygenase
PGSC0003DMG400020774	2.10	15993.20	12.893	BT2	(Protein MLO)
PGSC0003DMG400000513	0.04	8.58	7.711	BT2	Stachyose synthase
PGSC0003DMG400000066	1.16	220.41	7.574	BT2	Ethylene-responsive late embryogenesis
PGSC0003DMG400012978	0.28	51.18	7.511	BT2	Gene of unknown function
PGSC0003DMG400014320	0.36	59.35	7.367	BT2	Nonspecific lipid-transfer protein
PGSC0003DMG400008980	235.65	0.24	-9.941	BT2	Basic 7S globulin 2 small subunit

PGSC0003DMG400002046	123.45	0.24	-8.997	BT2	Aspartic proteinase nepenthesin-1
PGSC0003DMG400037894	193.80	0.44	-8.782	BT2	Aspartic proteinase nepenthesin-1
PGSC0003DMG400016283	35.39	0.12	-8.257	BT2	Knolle
PGSC0003DMG400015402	523.13	0.00	inf	BT2	Cytochrome P450
PGSC0003DMG400003530	3.90	322.65	6.372	HL1	Abscisic acid and environmental stress-inducible protein TAS14
PGSC0003DMG400009255	1.06	40.49	5.254	HL1	Small heat-shock protein homolog protein
PGSC0003DMG400017730	1.35	34.07	4.655	HL1	FtsH protease
PGSC0003DMG400028624	1.42	26.07	4.195	HL1	Small heat-shock protein
PGSC0003DMG400030340	1.93	32.92	4.092	HL1	17.6 kD class I small heat shock protein
PGSC0003DMG400043403.1	169.61	0.00	inf	HL1	S-locus-specific glycoprotein S13
PGSC0003DMG400029201	25.75	0.74	-5.118	HL1	Sesquiterpene synthase 2
PGSC0003DMG400010369	31.42	1.26	-4.643	HL1	Iron-regulated transporter 1
PGSC0003DMG400010131	38.31	1.63	-4.557	HL1	Nb cell death marker
PGSC0003DMG400040260	17.31	0.77	-4.487	HL1	Glucan endo-1,3-beta-glucosidase, basic isoform 1
PGSC0003DMG400014320	1.07	34.57	5.014	HL2	Nonspecific lipid-transfer protein
PGSC0003DMG400029727	1.04	28.70	4.791	HL2	Conserved gene of unknown function
PGSC0003DMG400031529	0.58	13.35	4.528	HL2	Glycine-rich protein
PGSC0003DMG400029866	0.71	13.63	4.263	HL2	Amino acid transporter
PGSC0003DMG400004170	13.67	172.33	3.656	HL2	Asparagine synthetase [glutamine-hydrolyzing]
PGSC0003DMG402019343	11.18	0.95	-3.562	HL2	DNA binding protein
PGSC0003DMG400000066	30.46	2.95	-3.367	HL2	Ethylene-responsive late embryogenesis
PGSC0003DMG400028809	10.19	1.15	-3.148	HL2	2-oxoglutarate-dependent dioxygenase
PGSC0003DMG401019255	19.71	2.26	-3.122	HL2	Pectinesterase 3
PGSC0003DMG400026779	125.83	15.96	-2.979	HL2	PAS/LOV protein A
PGSC0003DMG400018833	1.68	16.03	3.255	HT1	Conserved gene of unknown function
PGSC0003DMG400030255	8.37	49.85	2.574	HT1	Sn-1 protein
PGSC0003DMG400031535	14.22	74.23	2.384	HT1	Glycine-rich protein A3
PGSC0003DMG400003548	8.56	44.08	2.364	HT1	Fructose-bisphosphate aldolase
PGSC0003DMG400019269	2.17	9.25	2.092	HT1	Organic cation transporter
PGSC0003DMG400008000	40.44	15.89	-1.348	HT1	L-asparaginase
PGSC0003DMG401003156	24.51	9.22	-1.410	HT1	Glycosyltransferase, CAZy family GT2

PGSC0003DMG400013282	57.97	21.41	-1.437	HT1	Tonoplast dicarboxylate transporter
PGSC0003DMG400005130	25.41	9.27	-1.455	HT1	Conserved gene of unknown function
PGSC0003DMG400013022	28.53	10.18	-1.486	HT1	Calcium-transporting ATPase 2, plasma membrane-type
PGSC0003DMG400021877	29.56	528.03	4.159	HT2	Xyloglucan endo-transglycosylase
PGSC0003DMG400024219	3.82	17.31	2.180	HT2	Inositol-1,4,5-triphosphate-5-phosphatase
PGSC0003DMG400023011	20.89	85.01	2.025	HT2	Conserved gene of unknown function
PGSC0003DMG400024205	199.13	703.78	1.821	HT2	Pit1 protein
PGSC0003DMG400043403.1	2770.03	6.59	-8.716	HT2	S-locus-specific glycoprotein S13
PGSC0003DMG400002161	49.96	0.94	-5.740	HT2	Conserved gene of unknown function
PGSC0003DMG400026899	47.63	1.10	-5.436	HT2	Multicystatin
PGSC0003DMG400010713	73.86	2.21	-5.063	HT2	Salt responsive protein 2
PGSC0003DMG400022836	63.28	1.92	-5.041	HT2	Histone H3.2
PGSC0003DMG400009871	24.96	109.52	2.133	JL2	Allergen Pru du 2.04
PGSC0003DMG400001671	22.99	95.22	2.050	JL2	Peptide transporter
PGSC0003DMG400020823	0.00	2.13	Inf	JL2	Non-specific lipid-transfer protein
PGSC0003DMG400003360	0.00	0.42	Inf	JL2	Copper ion binding protein
PGSC0003DMG400023100	0.00	1.74	Inf	JL2	CDPK adapter protein 1
PGSC0003DMG400019517	435.41	1.03	-8.717	JL2	Chitin-binding lectin 1
PGSC0003DMG400013010	636.72	1.70	-8.545	JL2	24K germin
PGSC0003DMG400006880	48.12	0.35	-7.094	JL2	DRT100
PGSC0003DMG400011751	299.82	3.41	-6.457	JL2	2-oxoglutarate-dependent dioxygenase
PGSC0003DMG400033084	804.03	9.65	-6.381	JL2	Chlorophyll a/b-binding protein (cab-12)
PGSC0003DMG400014293	0.05	12.58	7.918	JT1	Low-temperature-induced 65 kDa protein
PGSC0003DMG400024707	0.09	11.60	6.941	JT1	Luminal binding protein
PGSC0003DMG400014234	0.08	6.49	6.419	JT1	Conserved gene of unknown function
PGSC0003DMG400003530	12.79	962.49	6.234	JT1	Abscisic acid and environmental stress-inducible protein TAS14
PGSC0003DMG400015356	0.11	7.64	6.161	JT1	NADPH:protochlorophyllide oxidoreductase
PGSC0003DMG400008980	174.89	0.45	-8.616	JT1	Basic 7S globulin 2 small subunit
PGSC0003DMG400011323	239.87	0.88	-8.089	JT1	Lipid binding protein
PGSC0003DMG400026934	58.12	0.28	-7.712	JT1	Conserved gene of unknown function
PGSC0003DMG400012444	17.36	0.11	-7.367	JT1	ATP binding protein

PGSC0003DMG401016475	15.13	0.10	-7.204	JT1	Multicopper oxidase
PGSC0003DMG400009467	1.34	27.90	4.384	JT2	Metal ion binding protein
PGSC0003DMG400015196	0.38	5.33	3.813	JT2	Conserved gene of unknown function
PGSC0003DMG400008056	13.71	161.28	3.556	JT2	Gene of unknown function
PGSC0003DMG400011954	4.90	54.02	3.461	JT2	Non-specific lipid-transfer protein 1
PGSC0003DMG400003530	1.02	11.13	3.444	JT2	Abscisic acid and environmental stress-inducible protein TAS14
PGSC0003DMG400025158	28.44	0.54	-5.716	JT2	Divinyl ether synthase
PGSC0003DMG400003228	46.79	1.52	-4.943	JT2	SAUR family protein
PGSC0003DMG400012589	57.86	2.15	-4.748	JT2	Cationic peroxidase
PGSC0003DMG400018688	54.16	2.09	-4.693	JT2	(O-methyltransferase)
PGSC0003DMG400005982	20.50	1.07	-4.255	JT2	Conserved gene of unknown function
PGSC0003DMG400012305	0.60	25.42	5.393	LL1	DNA binding protein
PGSC0003DMG401025908	0.63	24.45	5.282	LL1	Methylketone synthase Ib
PGSC0003DMG400002443.1	47.60	1354.09	4.830	LL1	Gene of unknown function
PGSC0003DMG402025908	0.42	10.19	4.589	LL1	Methylketone synthase Ib
PGSC0003DMG400029839	0.82	16.79	4.361	LL1	Type-a response regulator
PGSC0003DMG400005985	131.17	2.37	-5.793	LL1	Sn-2 protein
PGSC0003DMG400016847	222.93	4.04	-5.786	LL1	Conserved gene of unknown function
PGSC0003DMG400001948	18.05	0.35	-5.695	LL1	Copalyl diphosphate synthase
PGSC0003DMG400010131	39.62	0.80	-5.629	LL1	Nb cell death marker
PGSC0003DMG400028182	125.00	0.00	inf	LL1	Aquaporin TIP1
PGSC0003DMG400025665	0.88	135.76	7.275	LL2	(Conserved gene of unknown function)
PGSC0003DMG400013015	0.74	19.11	4.683	LL2	N-acetyltransferase
PGSC0003DMG400004875	2.22	54.71	4.624	LL2	DNA binding protein
PGSC0003DMG400007458	0.51	10.69	4.397	LL2	RAV
PGSC0003DMG400019517	7.18	118.98	4.050	LL2	Chitin-binding lectin 1
PGSC0003DMG400045603	106.52	0.90	-6.895	LL2	Regulator of gene silencing
PGSC0003DMG400020261	46.98	0.53	-6.467	LL2	Polcalcin Jun o
PGSC0003DMG401001341	16.49	0.28	-5.906	LL2	Conserved gene of unknown function
PGSC0003DMG400011073	56.12	1.02	-5.776	LL2	Avr9/Cf-9 rapidly elicited protein 137
PGSC0003DMG400002899	107.04	2.15	-5.637	LL2	AP2/ERF domain-containing transcription factor

PGSC0003DMG400003548	1.00	51.10	5.682	LT1	Fructose-bisphosphate aldolase
PGSC0003DMG400026500	0.62	26.78	5.432	LT1	Type I (26 kD) CP29 polypeptide
PGSC0003DMG400019508	0.89	35.23	5.309	LT1	Chlorophyll a/b binding protein
PGSC0003DMG400008488	0.90	26.56	4.891	LT1	Chloroplast pigment-binding protein CP29
PGSC0003DMG400021287	1.06	25.93	4.607	LT1	Chlorophyll a-b binding protein 8, chloroplastic
PGSC0003DMG400019344	17.30	0.09	-7.525	LT1	DNA binding protein
PGSC0003DMG400011783	8.06	0.20	-5.346	LT1	Cembratrienol synthase 2a
PGSC0003DMG400037894	52.82	1.53	-5.110	LT1	Aspartic proteinase nepenthesin-1
PGSC0003DMG400006713	10.99	0.41	-4.758	LT1	Cembratrienol synthase 2a
PGSC0003DMG400011323	96.56	3.93	-4.619	LT1	Lipid binding protein
PGSC0003DMG400019517	1.56	348.84	7.806	LT2	Chitin-binding lectin 1
PGSC0003DMG400011752	0.70	113.26	7.331	LT2	Cellulose synthase
PGSC0003DMG400011740	0.50	52.13	6.700	LT2	SGA
PGSC0003DMG400021142	0.71	58.82	6.380	LT2	DWARF1/DIMINUTO
PGSC0003DMG400011751	3.13	251.23	6.328	LT2	2-oxoglutarate-dependent dioxygenase
PGSC0003DMG400026222	18.43	0.93	-4.306	LT2	Major pollen allergen Ory s 1
PGSC0003DMG400019386	35.54	3.23	-3.462	LT2	Phenylalanine ammonia-lyase
PGSC0003DMG400007880	11.90	1.11	-3.423	LT2	Senescence-associated protein
PGSC0003DMG400011185	65.06	7.14	-3.188	LT2	Low-molecular-weight cysteine-rich protein LCR78
PGSC0003DMG400028182	24.18	3.17	-2.930	LT2	Aquaporin TIP1
PGSC0003DMG400027274	0.85	20733.50	14.571	NL1	EMB1674
PGSC0003DMG400003530	0.54	125.60	7.868	NL1	Abscisic acid and environmental stress-inducible protein TAS14
PGSC0003DMG400000248	1.76	80.34	5.516	NL1	HB1
PGSC0003DMG403024610	0.15	5.14	5.120	NL1	Conserved gene of unknown function
PGSC0003DMG400012479	0.14	4.62	5.054	NL1	Nitrate transporter
PGSC0003DMG402024140	16.13	0.47	-5.104	NL1	PAE
PGSC0003DMG400020017	15.97	0.49	-5.030	NL1	Lichenase
PGSC0003DMG400016996	6.61	0.31	-4.402	NL1	Nitrate transporter
PGSC0003DMG400006255	5.79	0.31	-4.228	NL1	(Verticillium wilt disease resistance protein)
PGSC0003DMG400001550.0	16.61	0.93	-4.156	NL1	TSI-1 protein
PGSC0003DMG400039627.3	5.32	37087.10	12.766	NL2	(Cf-2.2)

PGSC0003DMG400002068	0.55	12.65	4.513	NL2	Gibberellin 2-oxidase 1
PGSC0003DMG400027047	5.90	69.03	3.548	NL2	UPF0497 membrane protein
PGSC0003DMG400007621	36.65	407.23	3.474	NL2	GAST1 protein
PGSC0003DMG400006880	1.19	11.54	3.272	NL2	DRT100
PGSC0003DMG400014311	151.64	1.41	-6.752	NL2	CRT binding factor 3
PGSC0003DMG400030810	41.26	1.28	-5.013	NL2	Conserved gene of unknown function
PGSC0003DMG400031523	21.40	0.68	-4.977	NL2	Glycine-rich cell wall structural protein 1.8
PGSC0003DMG400008248	36.24	1.31	-4.794	NL2	Wound-responsive AP2 like factor 2
PGSC0003DMG400000910	13.63	0.54	-4.666	NL2	AP2/ERF domain-containing transcription factor
PGSC0003DMG400019149	1.78	33.14	4.218	NT1	Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic
PGSC0003DMG400019584	2.63	40.00	3.926	NT1	Ribulose bisphosphate carboxylase small chain 1, chloroplastic
PGSC0003DMG400022573	0.39	4.83	3.646	NT1	ATP binding protein
PGSC0003DMG400013516	7.02	59.84	3.092	NT1	Polygalacturonase
PGSC0003DMG400012019	9.45	61.81	2.709	NT1	DC1.2 homologue
PGSC0003DMG400030339	93.58	0.76	-6.952	NT1	17.6 kD class I small heat shock protein
PGSC0003DMG400009255	70.32	1.02	-6.112	NT1	Small heat-shock protein homolog protein
PGSC0003DMG400028624	14.61	0.27	-5.756	NT1	Small heat-shock protein
PGSC0003DMG400014847	39.94	1.37	-4.861	NT1	Protein AIG2
PGSC0003DMG400020718	64.69	2.27	-4.833	NT1	Heat-shock protein

CHAPTER SEVEN

Supplementary Table 1: Total yield (TBW) and marketable yield (MTBW) of cultivars under irrigated (WR) and non-irrigated (DR) conditions at Connantre (2015)

CULTIVARS	MATURITY	MTBW-WR	MTBW-DR	TBW-WR	TBW-DR	TBW Red(%)	QUADRANT
ADORA	Early	7.40	1.02	10.70	3.30	69.16	III
MARABEL	Early	9.99	1.23	13.85	5.70	58.84	III
LADY FELICIA	Early	10.09	1.21	13.65	4.90	64.10	IV
PREMIERE	Early	7.58	0.62	12.60	5.15	59.13	III
BIOGOLD	Early	8.36	0.97	11.75	4.35	62.98	III
AGATA	Early	6.56	0.16	11.40	4.65	59.21	III
CANBERRA	Early	8.38	1.02	11.65	4.50	61.37	IV
JAERLA	Early	8.86	1.86	9.70	3.30	65.98	IV
LADY CHRISTL	Early	4.71	0.25	11.95	5.20	56.49	III
MONALISA	Early	7.25	0.64	10.90	4.20	61.47	III
COLOMBA	Early	14.42	2.07	16.65	6.20	62.76	II
JAZZY	Early	0.82	0.00	9.00	4.15	53.89	III
LADY CLAIRE	Early	6.65	0.81	11.55	4.65	59.74	III
CHARLOTTE	Early	3.45	0.00	10.35	3.80	63.29	III
CUPIDO	Early	10.93	0.44	13.90	4.75	65.83	III
INOVA	Early	5.92	0.14	12.50	4.65	62.80	III
LISETA	Early	9.07	0.55	14.35	5.30	63.07	I
LADY BRITTA	Early	7.90	1.11	10.65	4.75	55.40	III
SANTE	Early	8.94	2.09	11.25	5.30	52.89	IV
ARIZONA	Early	12.52	1.50	14.90	5.85	60.74	II
RED SCARLETT	Early	7.21	1.05	12.05	4.80	60.17	III
LABADIA	Early	11.02	3.64	13.65	6.80	50.18	I
MUSICA	Early	8.30	0.56	12.55	5.60	55.38	II
SOPRANO	Early	7.09	0.34	11.15	4.20	62.33	IV
WINSTON	Early	9.51	2.16	12.00	4.85	59.58	III
LADY AMARILLA	Early	6.50	0.39	10.00	5.45	45.50	III
ORCHESTRA	Early	12.62	3.08	14.45	6.25	56.75	I
BIONICA	Early	7.21	0.44	10.95	4.60	57.99	III
LADY LENORA	Early	6.97	0.71	12.15	4.85	60.08	III
LADY ROSETTA	Early	8.97	2.56	11.05	6.20	43.89	III
MARFONA	Early	8.24	3.12	9.05	4.10	54.70	IV
PICCOLO STAR	Early	6.52	0.99	11.25	6.90	38.67	III
SATURNA	Early	7.56	1.26	12.75	5.85	54.12	III
BINTJE	Early	13.08	1.90	18.35	8.35	54.50	II
EVEREST	Early	14.02	3.32	14.75	5.80	60.68	II
HERMES	Early	12.81	3.13	14.40	6.75	53.13	I
INNOVATOR	Early	7.20	1.56	11.15	5.55	50.22	III
MOZART	Early	11.42	2.80	12.85	5.15	59.92	II
SANTANA	Early	4.32	0.44	9.15	4.60	49.73	IV
SHEPODY	Early	8.96	2.77	10.05	5.50	45.27	III
TIMATE	Early	12.13	3.08	17.10	7.95	53.51	II

KURODA	Early	10.87	4.29	11.80	6.10	48.31	II
SERESTA	Int.	8.09	2.56	11.40	5.75	49.56	III
VTN2 62- 33- 3	Early	6.68	0.27	11.35	5.80	48.90	III
ATLANTIC	Int.	8.04	2.58	8.95	4.25	52.51	IV
LADY ANNA	Int.	8.48	0.87	12.10	4.95	59.09	III
VR 808	Int.	5.81	1.12	10.85	5.85	46.08	III
AGRIA	Int.	13.33	4.22	15.10	7.05	53.31	II
ALMERA	Int.	12.45	1.80	13.95	5.50	60.57	II
CAESAR	Int.	11.73	3.75	13.55	6.35	53.14	II
FONTANE	Int.	11.42	4.47	13.80	7.60	44.93	I
HANSA	Int.	8.70	0.79	12.95	7.05	45.56	II
KASTELLI	Int.	12.84	2.89	14.55	6.50	55.33	II
RAMOS	Int.	9.33	1.99	14.25	6.85	51.93	III
SIFRA	Int.	15.38	1.49	17.90	6.10	65.92	II
AMBITION	Int.	11.97	3.84	13.00	5.95	54.23	II
BELLINI	Int.	6.09	1.61	11.20	5.70	49.11	II
FABULA	Int.	12.26	3.52	13.30	5.50	58.65	II
LADY OLYMPIA	Int.	9.25	4.13	11.60	7.05	39.22	I
NICOLA	Int.	6.80	0.15	13.80	5.80	57.97	IV
SPUNTA	Int.	12.03	1.80	13.60	6.00	55.88	II
EL MUNDO	Int.	10.89	3.88	14.20	5.55	60.92	II
LEONARDO	Int.	8.94	2.74	11.70	6.35	45.73	III
MELODY	Int.	10.93	2.51	12.35	5.65	54.25	II
SAGITTA	Int.	11.93	2.47	15.40	6.50	57.79	II
SYLVANA	Int.	13.41	3.10	15.00	6.50	56.67	II
AVEKA	Int.	8.95	2.52	11.75	6.20	47.23	III
AVENTRA	Int.	9.43	1.23	12.80	6.25	51.17	II
EUROSTAR	Int.	13.20	4.55	15.10	7.25	51.99	II
KENNEBEC	Int.	12.59	2.79	13.95	5.30	62.01	II
MARIS PIPER	Int.	10.97	3.26	14.65	7.55	48.46	II
PICASSO	Int.	15.87	3.95	17.80	7.50	57.87	II
TERRAGOLD	Int.	11.77	3.57	12.95	4.65	64.09	II
CHALLENGER	Int.	9.51	0.73	16.30	7.20	55.83	II
DESIREE	Int.	10.05	1.96	13.05	5.60	57.09	II
KONDOR	Int.	12.74	3.91	13.80	6.20	55.07	I
ASTERIX	Int.	10.95	1.93	13.55	6.25	53.87	II
BARTINA	Int.	15.95	5.34	17.55	7.60	56.70	II
ELKANA	Int.	9.35	4.23	11.35	6.10	46.26	III
RUSSET BURBANK	Int.	6.74	0.15	12.30	5.50	55.28	III
NOMADE	Int.	10.57	4.23	12.90	6.95	46.12	III
PENTLAND DELL	Int.	6.88	2.73	10.90	7.90	27.52	III
MERANO	Int.	9.22	2.72	11.55	6.10	47.19	III
STARGA	Int.	7.06	1.73	13.50	5.70	57.78	III
MARKIES	Late	11.76	2.82	14.90	5.95	60.07	II
MONDIAL	Int.	10.27	4.13	18.70	8.95	52.14	II
RODEO	Int.	8.90	2.38	12.55	7.00	44.22	II

AVANO	Late	13.19	5.20	15.15	7.80	48.51	I
KARNICO	Late	5.43	1.45	8.60	5.20	39.53	I
KARAKTER	Late	8.61	2.47	10.95	5.85	46.58	II
AXION	Late	9.53	4.46	14.65	8.00	45.39	II
ALTUS	Late	12.49	5.58	14.70	9.50	35.37	I
AVARNA	Late	8.66	4.06	11.35	6.70	40.97	III
FESTIEN	Late	10.62	4.91	12.35	7.95	35.63	III
VALIANT	Late	6.23	3.93	10.75	7.80	27.44	I
KURAS	Late	13.48	5.11	15.35	9.25	39.74	I

TBW and MTBW are given in Kg/plot. A plot comprises 8 plants in 1.05m^2. TBW Red: percentage reduction in tuber weight. Quadrant: Cluster location based on Chapter 2 – Fig.3b

Drought stress is a global challenge that impacts on crop growth and reduces harvestable yield. The effects of drought on potato growth indicate that cultivated potato is a drought-sensitive crop. Due to the global importance of the potato as a food security crop, research efforts have been dedicated to understanding the interaction of drought stress with potato. Myriads of research reports have confirmed the drought sensitivity of potato, and several reasons for the drought sensitivity of the crop have been disclosed. However, the potential of the cultivated potato for drought tolerance improvement remained elusive. This knowledge gap has been addressed in this thesis, "Water-saving potatoes: Exploring and characterizing drought tolerance mechanisms", and some solutions and recommendations have been presented.

We collected 103 potato cultivars, representing the cultivated potato germplasm of Europe, for drought studies. In **Chapter two** we aimed at understanding the impact of drought on canopy development in this cultivar set, and how this drought-canopy growth interaction relates to tuber yield. We set up drought experiments at three different locations in the Netherlands and at Connantre (France) for three years, because we wanted to investigate the level of yield stability and adaptability of the cultivars across different climatic zones, and any Genotype-by-environment (GxE) interactions. We monitored canopy development during the growing season by taking pictures of the canopy ground cover weekly. We analysed the picture images and extracted parameters of canopy growth, which enabled us to interpret the effects of drought on canopy growth characteristics. We correlated canopy growth parameters with tuber yield traits, and found that a slower canopy growth rate was beneficial for tuber yield maintenance under drought. We found the late-maturing cultivars more drought-tolerant than the early maturity types. We also observed a significant GxE interaction, which we further investigated using both Finlay Wilkinson's regression and GGE biplots. We identified cultivars with wide adaptability across locations and others with stable yield in different years at the same location. Interestingly, the last two years at Connantre, 2014 and 2015, provided us a platform to study the effects of early and late drought on canopy growth and tuber yield. The late drought coincided with the tuber bulking stage of potato development and reduced tuber yield more severely than the early drought. However, we found a high genotypic variation in drought response among the cultivars.

Furthermore, we studied the tuber size distribution data generated by grading the harvested tubers of the 103 cultivars grown at the different locations, in **Chapter three**. We used the tuber grading data to model tuber size distribution under irrigation and non-irrigation. We extracted

model parameters of the distribution and used them to interpret the tuber bulking capacity of the cultivars under drought. We also calculated marketable tuber size using the tuber size grading data, and we found that the drought reduced total tuber yield and marketable tuber yield by the same percentage. We subsequently used a 14K SNP marker array for an association mapping of the tuber yield and size distribution traits with molecular markers. We found a significant association between a region on potato Chromosome 3 and the following traits: marketable fraction of tuber fresh weight under non-irrigated condition, marketable fraction of tuber number under non-irrigated condition, size distribution spread of tuber number and the size class where maximum tuber number was observed.

Based on the variations in the performances of the cultivars under drought relative to irrigation in the field, we selected a subset of cultivars with contrasting drought responses for in-depth studies in the greenhouse. In **Chapter four**, we studied carbon partitioning in the plants as a follow up to the observed drought effects on canopy growth and tuber yield in the field. We grew the plants in 19cm pots and during drought application, we mimicked the field drought scenario as much as possible. We irrigated the drought stress block once every two days, while monitoring the soil water content which was kept at 20±4 v/v of soil. The irrigation approach exposed the plants to early drought which persisted till late in the season. Therefore, we could capture molecular responses both at the early and later stages of drought development. We observed variations in drought responses among the genotypes based on phenotypic observation. Tuber formation was shut down under drought in most of the cultivars. We sampled tissues from the plants at 28DOD (days of drought) for a molecular investigation of genes of the starch biosynthetic pathway. We found that genes involved in sugar transport were repressed under drought. Also, starch biosynthesis genes, *AGPase* and GBSS, were downregulated in the root and stolon tissues of the sensitive cultivar, Mondial, but upregulated in these underground tissues in the tolerant cultivar, Biogold. However, starch accumulated in the leaves of Mondial both under irrigation and non-irrigation conditions, but not in the leaves of Biogold. The results of the gene expression studies suggested that assimilate transport between source and sink tissues may be a challenge to carbon partitioning in potato.

We further investigated assimilate and water transport in **Chapter five**, using a deep phenotyping imaging technique, Magnetic Resonance Imaging (MRI), to study phloem flow and xylem flow. The plants were firstly grown in the greenhouse in 19cm pots and drought was applied prior to measurements in the MRI. We sampled stem sap and stem tissues from a subset of the plants that were not studied in the MRI, and analysed the composition of sucrose and hexoses in the stem sap and stem. We also used a microscopy technique to study the stem cross section and the properties of the vascular tissues for transport. Our results indicate that a high density of small-diameter xylem tissues (vessels and tracheids) facilitates water transport during drought. Also, we found that the sink destination of assimilates under drought would depend on water availability in the xylem tissues for phloem transport, but also on the sink strength. The absence of a strong sink may induce starch accumulation in the source leaf tissue.

The observations we made in the field and greenhouse studies gave insights as to the drought response mechanisms of the various genotypes. But we needed to understand the molecular basis for the observed contrasting responses. Therefore, we embarked on a transcriptomic study

of leaf and tuber samples from five contrasting genotypes grown in a tunnel in the field at two times points, 28DOD and 56DOD, in **Chapter six**. We gathered phenotypic data of the plants while they were growing in the tunnel, which we used to further our understanding of the gene expressions from transcriptomic analyses. We used the Tuxedo pipeline (Cufflinks) to analyse the RNA-seq data, and implemented a gene ontology (GO-)based functional annotation in order to understand the implicated pathways and mechanisms of drought response. There were vast differences in the number of differentially expressed genes among the cultivars and between time points and tissues. We found variations in the hormonal signalling cascades among the cultivars. Our results suggest a possible crosstalk between the ABA signalling cascade and Calcium-sensing, which may be involved in the regulation of stomatal opening and closure during drought. Cultivar-specific cascades of drought response mechanisms could be inferred from the transcriptomic study, which has boosted our understanding of the phenotypic variations among the genotypes.

ACKNOWLEDGEMENTS

The findings reported in this thesis are a product of the contribution of many resource persons who invested their time, expertise and criticisms, which facilitated my completion of this thesis. I want to specially appreciate my supervisor, Gerard van der Linden, for his relentless dedication to my PhD. Thank you, Gerard, for sharing ideas from the planning of experiments till the final thesis. I really enjoyed our discussion sessions, and I commend your friendliness, versatility in knowledge and precision. Also, I am very grateful to my promotor, Richard Visser, for his overview of my project, setting apart enough time amidst his busy schedule to join in meetings with partners and make inputs toward the completion of this thesis. Many thanks to Marian Oortwijn for her dedicated assistance in the laboratory, greenhouse and field experiments. Marian, I appreciate your sacrifice during the long hours of phenotyping and sampling of tissues. I am also thankful to Christian Bachem for his involvement and contribution to the project in all meetings with our partners, and for providing mutants for experiments when needed. I immensely want to appreciate the partnering companies in this project for their in-kind contribution, and for organizing the field trials, sharing ideas and resources: C. Meijer (Guus Heselmans), HZPC Holland BV (Maurice Schehr, Jan de Haas, Remi Ducreux), KWS POTATO (Jeroen van Soesbergen, Remko Koeman, Abco de Buck, Emmet Dalton) and Averis Seeds BV (Nick de Vetten, Johan Hopman, Nico Rookmaker and Hellen Lensing). I appreciate Theo Borm for sharing his expertise in the analyses of the RNA-seq experiment of this thesis. I thank Herman van Eck for providing the SNP dataset that was used in this thesis. Thanks to our collaborators from the Biophysics Department, Alena Prusova, Edo Gerkema and Henk van As, for assisting with Magnetic Resonance Imaging (MRI) experiments. Thanks a lot, Alena, for your inputs in the writing of the MRI chapter. Many thanks to the MSc students who contributed their efforts and ideas in the course of their Masters' degree theses to this project: Jonathan Kalisvaart, Nasrin Sultana, Robert Okayo, Tom Theeuwen, Mariam Ruiz, Irma Hoendervangers and Tim Gengler.

I heartily want to thank Abelenda Jose, Christos Kissoudis, Peter Dinh, Michela Appiano, Rafael Chan, Cesar Ospina, Peter Vos, Johan Willemsen, Daniela Bustos, Biructawit Tessema, Jeroen Berg and Faline Plantenga, for sharing ideas, literature resources, scripts, equipment and suggestions that were helpful at different points in my thesis. I am grateful to the Unifarm personnel for looking after my plants in the greenhouse and for the lively atmosphere of their friendliness that motivated my enjoyment of the greenhouse trials: Andre Maassen, Bertus Laan, Maarten Peters, late Alexander Super, Wim vander Slikke, Herman Meurs, Gerard Derks and late Gerda van Engelenhoven. I appreciate the technicians at Plant Breeding for being available to assist with equipment, orders and protocols: Isolde Bertram, Annemarie Dechesne, Annelies Loonen, Linda Kodde and Johan Bucher.

I dearly appreciate the members of the Abiotic Stress Group for assisting with data collection in my greenhouse experiments and for their inputs during presentations that contributed in

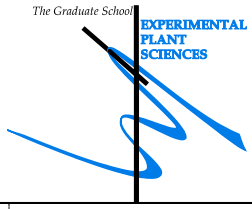
shaping my thesis: Hanneke van der Schoot, Elly Janssen, Dolstra Oene, Clemens van de Wiel, Charlie Chen, Viviana Jaramillo, Engin Yucel and Sri Sunarti. I am grateful to my friends from the Amazing Grace Parish who assisted me with tissue collection even during night hours: Sebastian Yanore, James Ledo, Pascal Agro, Richmond Addo, Edward Oppong, Ernest Dandi, Joshua Amar, Gerard Okonkwo, Gilbert Cito, Adefemi Orodeji and Lotte van Rangelrooij. I also appreciate the friends of my MSc students for assisting with sampling: Avit and Gideon. I am heartily grateful to my pastors and brethren of Amazing Grace Parish for sharing words of encouragement and their own PhD experiences, which motivated me during challenging times of my PhD: Pastors Farai & Busi, Elton &Edna Zvinavashe, Ikenna & Ijeoma Ngene, Sunday & Maureen Makama and Hans Kok. I also want to thank family friends who supported me with looking after my family when I travelled for conferences and meetings: Raymond and Homtapwa, Doris Verschuur, Gerbert and Janet, Pastor Adesuwa and Lotte Heiligers.

I am unequivocally grateful to my wife, Florence Taaka, for standing with me all through my PhD, assisting during data collection of large experiments, reading through the texts of this thesis, and taking care of our son, Great, to enable me finalize my thesis. I owe immeasurable gratitude to my parents, Mr and Mrs Michael Aliche, for giving me the opportunity of education despite other equally important family needs they had to meet, and for encouraging me to do a PhD. I thank my siblings for every encouragement and moral support they gave me in the course of my PhD: Charles Aliche, Chinyere and Pastor Victory Ikpeama, Martin Aliche, Anthony Aliche, Loretta and Pastor Clem Uzor. I am grateful to Rev. K. C. Eze, Rev. Felix Akara, Dr Ngozi Abu, Prof. Nkechinyere Nweze and Elijah Odii for their consistent moral support during the various stages of my PhD. I profoundly also want to thank my best friend whom I have taken as a family member, Parakletos, for sharing his ideas at different points in the thesis, assisting with tissue sampling and data collection, and challenging me to keep improving on every aspect of this thesis in a friendly way.

Ernest Beckee Aliche was born on 12 September 1984 at Oloko in Abia State, Nigeria. He obtained his BSc in Botany with 1st Class Honours at the University of Nigeria, Nsukka (UNN) in 2009. After his graduation, he participated in the National Youth Service at Government Girls Secondary School, Gusau, Zamfara State, Nigeria, during which he worked as a Physics teacher. He subsequently got a temporary position at the Department of Botany, UNN as a Graduate Assistant (2010 – 2011). In August 2011, he enrolled in Wageningen University and Research for his MSc study in Plant Sciences, with specialization in Plant Breeding and Genetic resources. His MSc thesis was on characterizing potato lines overexpressing phospholipase C genes for response to biotic and abiotic stresses, *in vitro* and in the greenhouse. After the completion of his MSc in September 2013, Ernest began his PhD research in Plant Breeding, Wageningen University and Research. His PhD work on exploring and characterizing drought tolerance mechanisms in cultivated potato is presented in this thesis. From March 1, 2018 Ernest is working as a postdoctoral researcher at the Plant Hormone Biology Group, Swammerdam Institute for Life Sciences, University of Amsterdam. He is investigating the role of Strigolactones on yield improvement in maize under drought stress conditions.

Education Statement of the Graduate School

Experimental Plant Sciences



Issued to: **Ernest Beckee Aliche**
Date: **13 June 2018**
Group: **Laboratory of Plant Breeding**
University: **Wageningen University & Research**

1) Start-up phase	*date*
► **First presentation of your project**	
Title: Water-saving potatoes, world-saving potatoes	03 Dec 2013
► **Writing or rewriting a project proposal**	
Title: Water-saving potatoes	01 Sep-30 Oct 2013
► **Writing a review or book chapter**	
► **MSc courses**	
► **Laboratory use of isotopes**	
Subtotal Start-up Phase	*7.5 credits**

2) Scientific Exposure	*date*
► **EPS PhD student days**	
EPS PhD student day, Leiden, NL	29 Nov 2013
EPS PhD student day 'Get2Gether', Soest, NL	29-30 Jan 2015
EPS PhD student day 'Get2Gether', Soest, NL	28-29 Jan 2016
► **EPS theme symposia**	
EPS theme 4 symposium 'Genome Biology', Wageningen, NL	13 Dec 2013
EPS theme 3 symposium 'Metabolism & Adaptation', Wageningen, NL	11 Mar 2014
EPS Theme 1 symposium 'Developmental Biology of Plants', Wageningen, NL	21 Jan 2016
► **NWO Lunteren days and other National Platforms**	
Annual meeting 'Experimental Plant Sciences', Lunteren, NL	14-15 Apr 2014
Annual meeting 'Experimental Plant Sciences', Lunteren, NL	13-14 Apr 2015
Annual meeting 'Experimental Plant Sciences', Lunteren, NL	11-12 Apr 2016
Annual meeting 'Experimental Plant Sciences', Lunteren, NL	10-11 Apr 2017
► **Seminars (series), workshops and symposia**	
Seminars:	
Seminar "How to Write a World-class Paper"	17 Oct 2013
Flying seminar Ortrun Mittelsten Scheid	19 Nov 2014
Bioinformatics seminar	24 Nov 2014
Invited seminar 'The application of plant biotechnology for preventing diseases'	04 Dec 2014
Flying seminar by Prof.dr. George Coupland, 'Seasonal flowering in annual and perennial plants'	19 Jan 2015
Workshops:	
ExPectationS 2014: Communication and Ethics in Science	28 'Mar 2014
Exploiting & understanding Solanaceous genomes, Wageningen, NL	13-14 'Oct 2014
Plant Breeding Research Day 2014	30 'Sep 2014
Plant Breeding Research Day 2015	29 'Sep 2015
Symposia:	
PhD symposium: Healthy Food and Living Environment, Wageningen, NL	10 'Dec 2013
EPS symposium: Omics Advances for Academia & Industry, Wageningen, NL	11 'Dec 2014
► **Seminar plus**	
► **International symposia and congresses**	
European Plant Science Retreat, Amsterdam, NL	01-04 'Jul 2014
European Association for Potato Research (EAPR), 19th Triennial Conference, Brussels, Belgium	06-11 Jul 2014
12th solanaceae genome workshop SOL 2015, Bordeaux, France	25-29 Oct 2015
Interdrought-V Conference, Hyderabad, India	21-25 Feb 2017
► **Presentations**	
Poster: EPS meeting, Lunteren, NL	14-15 Apr 2014
Poster: EAPR 2014, Brussels, Belgium	06-11 Jul 2014
Poster: EPS meeting, Lunteren, NL	13-14 Apr 2015
Poster: EPS meeting, Lunteren, NL	11-12 Apr 2016
Talk: 12th solanaceae genome workshop SOL 2015, Bordeaux, France	29 Oct 2015
Poster: Interdrought-V Conference, Hyderabad, India	21-25 Feb 2017
Talk: EPS meeting, Lunteren, NL	10-11 Apr 2017
Talk: Guest presentation at Solynta Potato Breeding, Wageningen, NL	16 Aug 2017
► **IAB interview**	
► **Excursions**	
KWS, Emmeloord, NL	27 Nov 2013
HZPC, Connantre, France	11-13 Aug 2014
Company visit to Genetwister & In2Care, Wageningen, NL	19 Sep 2014
Company visit to Tomatoworld, Westland, NL	14 Oct 2016
Subtotal Scientific Exposure	*21.9 credits**

3) In-Depth Studies	*date*
► **EPS courses or other PhD courses**	
Advanced course 'The power of RNA-seq', Wageningen, NL	16-18 Dec 2013
Course 'Entrepreneurship in and outside science', Wageningen, NL	08, 15 Sep-06, 13 Oct 2014
Advanced course 'The power of RNA-seq', Wageningen, NL	10-12 Feb 2016
► **Journal club**	
► **Individual research training**	
Subtotal In-Depth Studies	*3.0 credits**

4) Personal development	*date*
► **Skill training courses**	
WGS Course 'Career Orientation', Wageningen, NL	04, 11, 18, 25, 31 Oct 2016
WGS course 'Scientific Publishing', Wageningen, NL	13 Apr 2017
WGS course 'Project & Time Management, Wageningen, NL	16, 30 May-27 Jun 2017
► **Organisation of PhD students day, course or conference**	
► **Membership of Board, Committee or PhD council**	
Subtotal Personal Development	*3.3 credits**

TOTAL NUMBER OF CREDIT POINTS*	**35.7**

Herewith the Graduate School declares that the PhD candidate has complied with the educational requirements set by the Educational Committee of EPS which comprises of a minimum total of 30 ECTS credits

** A credit represents a normative study load of 28 hours of study.*

The research described in this thesis was financially supported by the Topsector (TKI Tuinbouw en Uitgangsmaterialen, number 262), Averis Seeds, C. Meijer, HZPC Holland B.V. and KWS POTATO.

Financial support from Plant Breeding, Wageningen University and Research, for printing this thesis is gratefully acknowledged.

Cover design by MEMO360°+

Printed by Digiforce || ProefschriftMaken