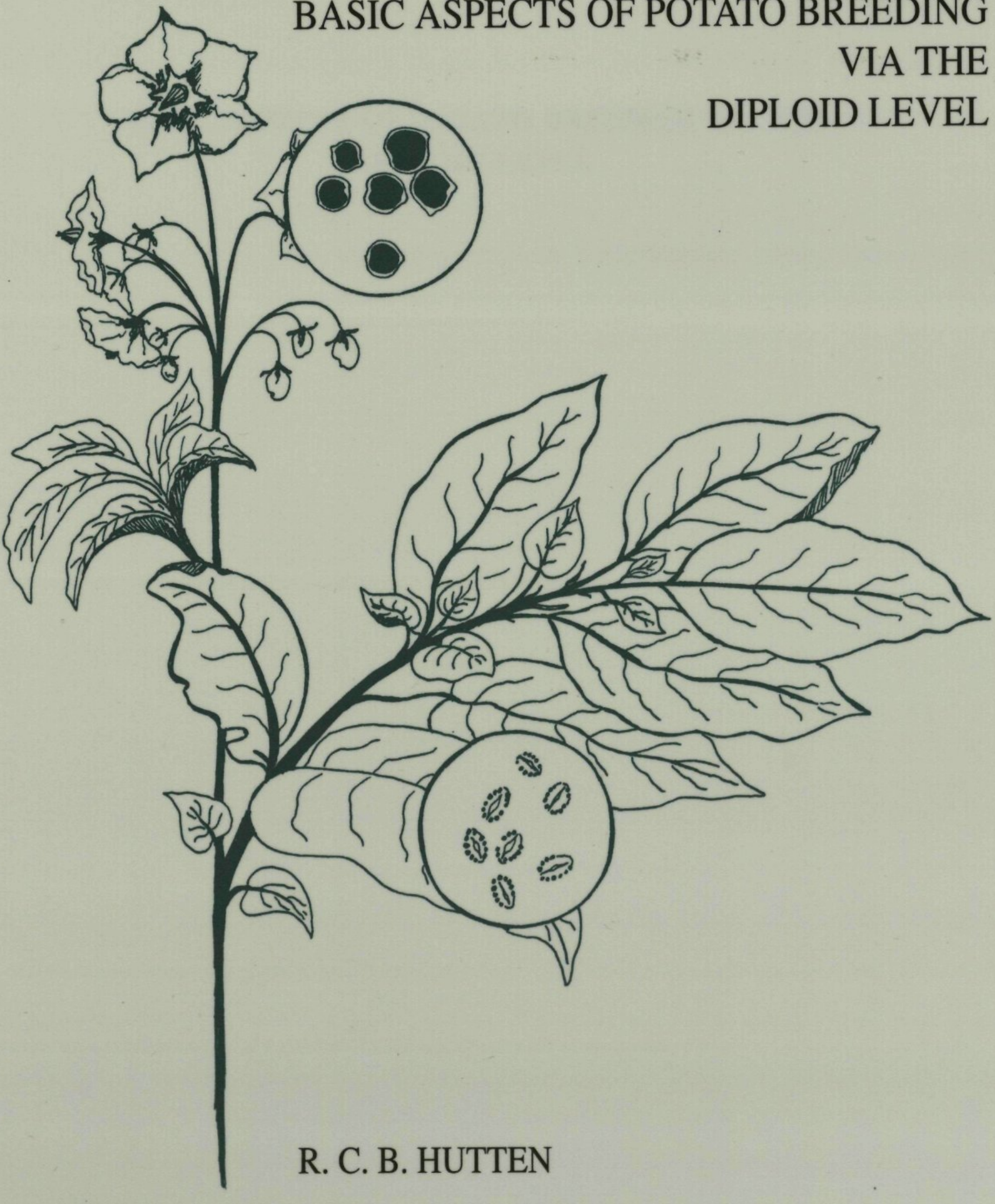
BASIC ASPECTS OF POTATO BREEDING
VIA THE
DIPLOID LEVEL



R. C. B. HUTTEN

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BASIC ASPECTS OF POTATO BREEDING VIA THE DIPLOID LEVEL

Proefschrift
ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
dr. C.M. Karssen,
in het openbaar te verdedigen
op woensdag 28 september 1994
des namiddags te vier uur in de Aula
van de Landbouwuniversiteit te Wageningen

This research was supported by C. Meijer BV, Hettema Zonen Kweekbedrijf BV, Stet en Slot Export BV, Handelmaatschappij Van Rijn BV and Wolf & Wolf.

Cover illustration: Mirjam van Buuren

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Hutten, R.C.B.

Basic aspects of potato breeding via the diploid level / R.C.B. Hutten. - [S.l: s.n.]
Thesis Wageningen - With references - With summaries in English and Dutch
ISBN 90-5485-292-5
Subject headings: breeding, diploids, potato

STELLINGEN

1. De veel gepredikte superioriteit van FDR 2n-gameten over SDR 2n-gameten gaat niet voor alle eigenschappen op.
   Dit proefschrift

2. Het heeft weinig zin om diploïde aardappelgeniteurs direct op opbrengst te beselecteren.
   Dit proefschrift

3. Bij het opsporen van pseudogame dihaploïden van tetraploïde aardappel voegen zaadgroottemetingen of iso-enzymanalyses nauwelijks iets toe aan de efficiëntie van de algemeen toegepaste detectie via de merker kiemstip.
   Caligari en medewerkers (1988) Ann. Appl. Biol. 112: 323-328
   Liu & Douches (1993) Euphytica 70: 113-126

4. Het kruisen van twee tetraploïde rassen of geniteurs biedt meer kans op het succesvol selecteren van een nieuw aardappelras dan het paarsgewijs somatisch fuseren van dihaploïden uit die twee rassen of geniteurs.

5. Incompatibiliteit in diploïde Solanumgenotypen vormt geen belangrijk obstakel bij aardappelveredeling op het diploïde niveau.

6. Bij het kweken van amylosevrije aardappelrassen verdient het aanbeveling tevens te selecteren op een anthocyaanhoudende schil.

7. Het gebruik van de aanduiding "gifpieper" voor rassen als Bintje, Eigenheimer en Bildtstar is een vorm van misleiding van de consument en dient derhalve te worden verboden.

8. Het is onrechtvaardig dat spaarloonregelingen meer opleveren voor personen met een hoger salaris.

9. Vergroting van het doel zal de agressie onder het voetbalpubliek doen afnemen.

Stellingen behorende bij het proefschrift "Basic aspects of potato breeding via the diploid level" door R.C.B. Hutten.

Wageningen, 28 september 1994.

ABSTRACT

In this thesis research is presented on all steps in a potato breeding program via the diploid level: dihaploid induction, selection at the diploid level and sexual polyploidization. In spite of the significant seed parent x pollinator interaction estimated, IVP 101 was found to have a significant higher dihaploid induction ability than the widely used pollinators IVP 35 and IVP 48. Significant differences in dihaploid production ability between seed parents were also found. Dihaploid populations from 31 varieties or breeding lines were evaluated for occurrence and frequency of mutant phenotypes, for tuberization, flowering, pollen stainability, 2n-pollen production and resistance to Ro-1. The expression of yield, yield components, vine maturity, under water weight and chip colour at the diploid and tetraploid level and the parental effects on these characters of 4x.2x progenies were investigated to find an answer to the question whether at the diploid level other selection criteria should be employed than at the tetraploid level when selecting tetraploid breeding lines. For yield direct selection at the diploid level had no effect on the performance of tetraploid progeny. For under water weight more stringent and for vine maturity less stringent selection criteria were required at the diploid level than at the tetraploid level. The frequently reported superiority of FDR 2n-gametes versus SDR 2n-gametes could be confirmed for yield only. For vine maturity, under water weight and chip colour no considerable differences were found between means of FDR and SDR progenies from reciprocal 4x-2x crosses.

VOORWOORD

Het in dit proefschrift beschreven onderzoek vormde samen met een aardappelveredelingsprogramma op diploïd niveau het project getiteld "Onderzoek naar de strategie en haalbaarheid van aardappelveredeling op diploïd niveau". Dit project werd grotendeels uitgevoerd bij de vakgroep Plantenveredeling van de LUW. Daarnaast werd o.a. gebruik gemaakt van proefvelden en proefruimten van C. Meijer BV, Hettema Zonen Kweekbedrijf BV, Stet en Slot BV, Handelmaatschappij Van Rijn BV, Wolf & Wolf en CPRO/DLO. Marja Schippers en verder alle medewerkers en oud-medewerkers van de vakgroep Plantenveredeling, genoemde bedrijven en CPRO/DLO, en studenten van de LUW die op enigerlei wijze een bijdrage hebben geleverd aan het project wil ik bij deze bedanken voor hun hulp.

Nu dit proefschrift is voltooid hoop ik naast de onvermijdelijke aardappelen ook weer voldoende tijd te hebben voor Jacqueline, Annick en Rick, en een paar van mijn hobbies.

CONTENTS

CHAPTER 1

INTRODUCTION

The advantages of potato breeding via the diploid level

The detection of dihaploids (2n = 2x = 24) in progenies obtained by pollinating the tetraploid (2n = 4x = 48) potato (*Solanum tuberosum* L.) with selected clones of *Solanum phureja* Juz. et Buk. has turned the breeders' attention towards potato breeding via the diploid level. Hougas & Peloquin (1958) emphasized two major advantages of this new approach: (1) direct gene transfer from the wild and cultivated diploid *Solanum* species to *S. tuberosum*, and (2) disomic instead of tetrasomic inheritance of characters, a potential improvement of breeding efficiency.

Most tuber-bearing *Solanum* species are diploid. These species are valuable to potato breeding as sources of resistance genes and of allelic diversity to improve quantitative characters. Most of these species can be crossed only with diploid forms of *S. tuberosum*.

The phenotypic variation in a segregating diploid potato population is larger than in a genetically equivalent tetraploid population and so there will be more individuals at each extreme in diploid populations (Stebbins, 1956). As a result of this, considerably smaller progeny sizes and lower numbers of backcross generations are needed in breeding at the diploid level.

The breeding scheme

Chase (1963) outlined a hypothetical breeding scheme to show how manipulation of the ploidy level may be useful in a potato breeding program. This so called 'analytic breeding' is a three step process involving, 1) analysis of the tetraploid potato to diploid lines via its gametes, 2) intensive breeding and selection at the diploid level, and 3) recovery of the tetraploid level and further selection.

Tetraploid potato can be reduced to the diploid level through its dihaploid female gametes (pseudogamy) or male gametes (anther culture). Dihaploids can be obtained routinely by pollinating tetraploid potatoes with diploid *S. phureja* clones selected for high dihaploid inducing ability and homozygosity for embryo spot (Hermsen & Verdenius, 1973), whereas the production of dihaploids by means of anther culture (Dunwell & Sunderland, 1973; Mix, 1983; Johansson, 1986) is more laborious and hardly successful.

In fact breeding potato at the diploid level is breeding of parental lines. This requires another approach than direct breeding of potato varieties, because lacking agronomic characters such as resistances or quality characters can be contributed to the offspring by the other parent and biological characters such as fertility and 2n-gamete production or plant regeneration ability are primarily important for parental lines.

Recovery of the tetraploid level can be achieved by somatic chromosome doubling, by somatic hybridization or via 2n-gametes. Somatic doubling and somatic hybridization have unfavourable genetic effects on the tetraploid potato clones produced. Somatic doubling of diploid potatoes is approximately equivalent to two generations of selfing a tetraploid (Mendoza & Haynes, 1973) and the resulting inbreeding depression may affect the agronomic value of the recovered tetraploids considerably. With somatic hybridization there is no genetic recombination, and undesirable genetic information will be maintained in the tetraploid hybrids. Furthermore, the routine production of tetraploid somatic hybrids is still hampered by technical imperfections and by recalcitrance of many diploid parents. A widely advocated method to return to the tetraploid level is sexual polyploidization by means of numerically unreduced (2n) gametes. Sexual polyploidization can be either unilateral (4x-2x crosses) or bilateral (2x-2x crosses). There are many reports on promising high yields of tetraploid progenies derived from 4x-2x crosses (Mok & Peloquin, 1975; De Jong & Tai, 1977; Mendiburu & Peloquin, 1977; Veilleux & Lauer, 1981; Sanford & Hanneman, 1982; Yerk & Peloquin, 1990; Darmo & Peloquin, 1991). As far as the author is aware two varieties were derived from such crosses. These varieties are Yukon Gold (Johnston & Rowberry, 1981) and Krantz (Lauer et al., 1988). Promising high yields of tetraploid progenies derived from 2x-2x crosses were reported by Werner & Peloquin (1991). For practical breeding purposes, unilateral sexual polyploidization (USP) by means of 4x.2x crosses seems most favourable when compared to USP by 2x.4x crosses and to bilateral sexual polyploidization (BSP). This is, because 2n-pollen occurs more commonly and at higher frequencies than 2n-eggs, and selection for 2n-pollen is less laborious than selection for 2n eggs. Furthermore, in contrast to BSP, USP provides breeders the opportunity to use well known and successful tetraploid parents.

The practical scheme for potato breeding via the diploid level, as advocated by the author, is presented in figure 1 which is self-explanatory after the preceding introductory considerations.

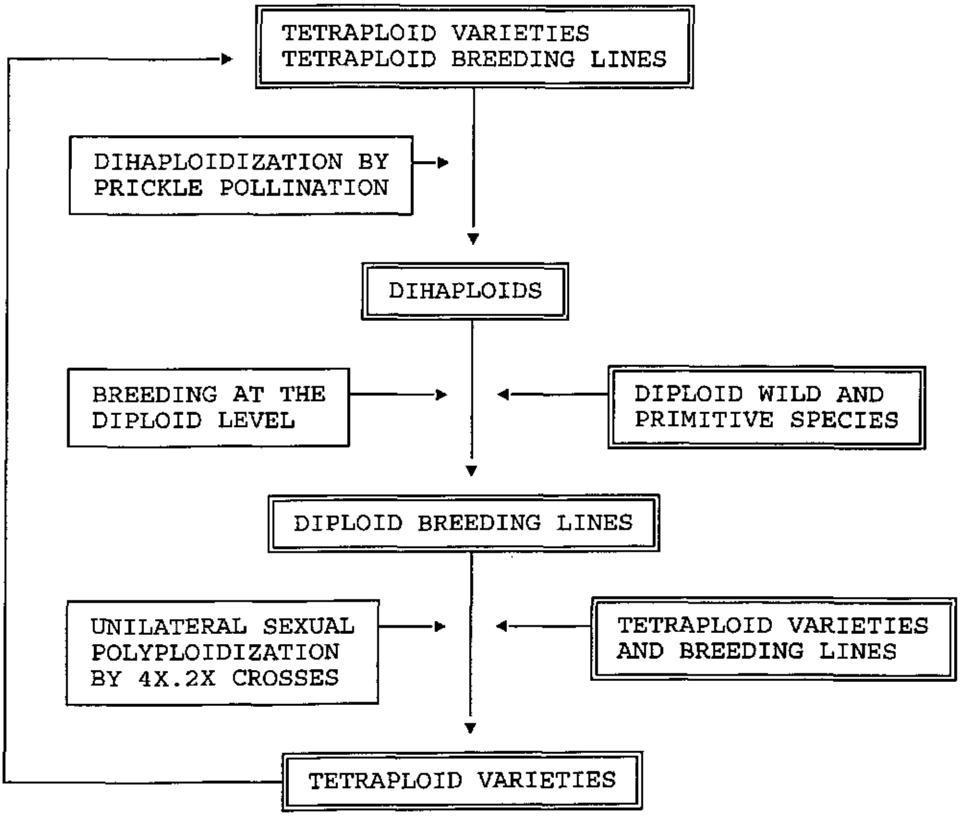

Figure 1. Schematic representation of the advocated scheme for potato breeding via the diploid level. Boxes with double lines indicate plant material, whereas boxes with single lines indicate steps in the breeding scheme.

The investigations

The investigations reported in this thesis are related to different steps in the breeding scheme presented in figure 1.

In chapter 2 dihaploid induction ability of selected *S. phureja* pollinators and dihaploid production ability of various tetraploid potato varieties and breeding lines are compared. Furthermore, seed parent x pollinator interaction is investigated. Chapter 3 describes various dihaploid populations for characters, which are important for the further use of these dihaploids in the breeding program.

An important question for a potato breeding program via the diploid level is wether selection criteria to be used at the diploid level should be the same as those applied when selecting tetraploid breeding lines. Different selection criteria are obvious when the expression of characters is different at both ploidy levels, and/or when the effect of diploid and tetraploid breeding lines on characters of tetraploid offspring is different. In chapter 4 the performance of diploid and tetraploid progenies from 2x.2x crosses are compared for a range of agronomic characters. In the chapters 5 and 6, parental effects on these agronomic characters are analyzed in progenies from 4x.2x matings. Combining the results obtained in the chapters 4, 5 and 6, conclusions are drawn concerning the adaptation of selection criteria for selection of diploid breeding lines.

The 2n-gametes in potato result from meiotic nuclear restitution mechanisms, which may occur during the first or second meiotic division. These 2n-gametes are called first division restitution (FDR) and second division restitution (SDR) gametes respectively. FDR 2n-gametes are expected to preserve a larger proportion of the parental heterozygosity and epistasis than SDR 2n-gametes (Hermsen, 1984). As multi-allelism and epistasis may contribute considerably to the performance of potato, FDR 2n-gametes are considered to be superior to SDR 2n-gametes. This superiority was demonstrated for yield by Mok & Peloquin (1975). Chapter 7 reports on the comparison of FDR and SDR progenies from reciprocal 4x-2x crosses for a range of agronomic characters.

Finally some remarks are added to give a more adequate view on potato breeding via the diploid level.

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CHAPTER 2

ANALYSIS OF DIHAPLOID INDUCTION AND PRODUCTION ABILITY AND SEED PARENT x POLLINATOR INTERACTION IN POTATO

R.C.B. HUTTEN, E.J.M.M. SCHOLBERG, D.J. HUIGEN,
J.G.TH.HERMSEN & E. JACOBSEN

(EUPHYTICA 72: 61-64, 1994)

Summary

The production of dihaploids is the first step in a potato breeding program at the diploid level. Dihaploid induction ability, dihaploid production ability and seed parent x pollinator interaction were analyzed for 28 x 3, seed parent x pollinator combinations. This is the first report on significant interaction between seed parents and pollinators. Despite this interaction, IVP 101 had a significantly higher dihaploid induction ability than the widely used pollinators IVP 35 and IVP 48. Previous findings on significant differences in dihaploid production ability between seed parents were clearly confirmed.

Introduction

The first step in a potato (*Solanum tuberosum* L.) breeding program at the diploid level is the production of dihaploids (Chase, 1963; Hermsen et al., 1987). Dihaploids can be obtained routinely from unfertilized egg cells via pseudogamy, i.e. through prickle pollination of tetraploid potatoes by selected clones, in particular from the diploid species *Solanum phureja* Juz. et Buk. The efficiency of dihaploid production is determined by both, production ability of the tetraploid seed parent and induction ability of the diploid pollinator (Hougas et al., 1964; Frandsen, 1967; Hermsen & Verdenius, 1973).

The efficiency of dihaploid selection in progenies from *S. tuberosum* x *S. phureja* crosses is greatly increased by incorporating a dominant seed marker (embryo-spot) into the pollinators which allows the removal of unwanted hybrid seeds as suggested by Peloquin & Hougas (1959) and realized by Hermsen & Verdenius (1973).

Hougas et al. (1964) stated that the most successful pollinator produced the largest number of dihaploids per berry on all seed parents. However, in some publications there are indications of interaction between seed parents and pollinators. Frandsen (1967), for instance, estimated that P.I. 225682.1 in general induced more dihaploids per berry than P.I. 225682.22, although on some seed parents P.I. 225682.22 induced more dihaploids per berry. Hermsen & Verdenius (1973) estimated that the general dihaploid production ability of variety Gineke was lower than that of variety Radosa, although with some pollinators Gineke produced more dihaploids per berry than Radosa.

The present investigation was carried out to estimate:

1. Dihaploid induction ability of IVP 101, a new pollinator, which proved to have a higher monohaploid induction ability than IVP 35 and IVP 48 (Uijtewaal et al., 1987).
2. Dihaploid production ability of a large number of varieties and breeding lines used in tetraploid breeding programmes by Dutch potato breeders.
3. Interaction between seed parents and pollinators.

Materials and methods

The pollinators used for comparison in this investigation were IVP 35, IVP 48 and IVP 101. All of them are homozygous for the seed marker 'embryo-spot'. IVP 101 has been derived from the cross (G609 x IVP 48) x (IVP 10 x IVP 1). Parentage, 'embryo-spot' genotype, and dihaploid induction ability of the pure *S. phureja* siblings IVP 1, IVP 10, IVP 35 and IVP 48 were presented by Hermsen & Verdenius (1973). G609 is a dihaploid from pure *S. tuberosum* variety Gineke and was included in the parentage of IVP 101 because G609 combines its own dihaploid induction ability with a high degree of male fertility, profuse flowering and vigour.

The seed parents are divided into three groups in Table 3, group 1 comprising seventeen varieties and two clones of unknown origin introduced as X1 and X2, group 2 consisting of three breeding lines with resistance to PVX and PVY and group 3 consisting of six breeding lines with resistance to several pathotypes of *Globodera rostochiensis* Woll. and *Globodera pallida* Stone.

Seed parents and pollinators were grafted onto tomato rootstock (variety Virosa). With few exceptions, five grafts per seed parent and eight grafts per pollinator were planted in the greenhouse. From the first inflorescence onwards, two stems per graft were allowed to develop. All flowers appearing between May 18th and August 28th 1987 were emasculated and prickle pollinated. The number of berries produced ranged from 12 to 241 per combination (average 78).

Seeds with spotless embryos were selected and sown in 1988 with a maximum of 100 seeds per combination. Non-emerging seeds were kept for three months. Seedlings showing nodal bands (homologous to embryo-spot) were removed as being hybrids between seed parent and pollinator (Hermsen & Verdenius, 1973). Seedlings without nodal bands were checked for ploidy level by stomatal guard cell chloroplast counts (Frandsen, 1968). However, among these seedlings non-viable plants with white or yellow cotyledons occurred in which chloroplasts could not be counted. Such recessive mutant segregants were considered to be dihaploids.

Results are presented as number of emerged dihaploids per 100 berries.

Results and discussion

Overall berry production, percentage berry set and number of hybrid seeds (spotted) per 100 berries are given separately for each pollinator in Table 1. The pollinators have turned out to be equally effective in producing berries. IVP 101 produced a significantly lower number of hybrid seeds than IVP 35 and IVP 48. This difference is of minor importance because it takes little effort to remove hybrid seeds from progenies.

Table 1. Pooled data per pollinator on number of berries produced, percentage berry set and mean number of spotted seeds per 100 berries. a and b indicate significant differences for $p<0,05$.

CHARACTERS	IVP 35	IVP 48	IVP 101
NUMBER OF BERRIES	2081	2673	1770
PERCENTAGE BERRY SET	76 %	78 %	74 %
HYBRID SEEDS/100 BERRIES	1861 b	1414 b	584 a

In Table 2, overall percentages of dihaploids, plants with nodal bands (hybrids), tetraploids without nodal bands, and non-emerging seeds after sowing of selected spotless seeds are shown. Tetraploids without nodal bands may originate from unreduced egg cells or from undesired self or cross pollination. In total, 29

Table 2. Pooled data per pollinator on percentage of dihaploids, plants with nodal bands, tetraploids without nodal bands and non-emerging seeds after sowing of selected spotless seeds.

CLASSES	IVP 35	IVP 48	IVP 101
DIHAPLOIDS	70 %	71 %	70 %
PLANTS WITH NODAL BANDS	7 %	6 %	5 %
TETRAPLOIDS WITHOUT NODAL BANDS	0.5 %	0.3 %	0.5 %
NON-EMERGING SEEDS	23 %	23 %	25 %

tetraploids without nodal bands were formed out of 16 different seed parents, and no indication about their origin was found. It is clear from Table 2, that after sowing of the spotless seeds similar results were obtained from the pollinators.

Because no differences were found in respect to the results mentioned in Tables 1 and 2, the three pollinators may be distinguished on the basis of induced number of dihaploids per 100 berries. These data are presented in Table 3.

There were no replications in this experiment. Therefore, variance for seed parent x pollinator interaction could only be analyzed indirectly by estimating non-additivity which is part of the interaction. For estimating non-additivity, Tukey's test of additivity is used (Snedecor & Cochran, 1967). Non-additivity and thus seed parent x pollinator interaction was highly significant ($p << 0.01$).

Despite the seed parent x pollinator interaction, the analysis of variance still revealed a highly significant effect of both the seed parents and the pollinators on dihaploid production ($p << 0,01$). The LSD (5 %) for pollinators is 41 dihaploids per 100 berries and for seed parents 126 dihaploids per 100 berries.

It is apparent from the data that IVP 101, besides the higher monohaploid induction ability (Uijtewaal et al., 1987), has a significantly higher dihaploid induction ability than IVP 35 and IVP 48. In contrast to results by Hougas et al. (1964), a high dihaploid induction ability is not associated with a high frequency of hybrid production. The correlation coefficient of number of dihaploids per 100 berries and number of spotted seeds per 100 berries amounts to 0.05 and is apparently not significant. In fact, the number of spotted seeds per 100 berries is significantly lower for IVP 101 (the best pollinator) than for IVP 35 and IVP 48 (Table 1).

As expected from previous reports there are also significant differences in dihaploid production ability between seed parents within each group. These differences must certainly be taken into account, along with all other characters,

Table 3: Number of dihaploids per 100 berries for 28 x 3, seed parent x pollinator, combinations. The LSD (5 %) for pollinators is 41 dihaploids per 100 berries and for seed parents 126 dihaploids per 100 berries.

TETRAPLOID	IVP 35	IVP 48	IVP 101	MEAN
group 1:				
AGRIA	50	23	24	32
MATILDA	29	11	58	33
HERTHA	39	41	61	47
ESCORT	66	59	79	68
AMANDA	58	64	135	86
BRIGHT	147	88	74	103
LADY ROSETTA	114	68	184	122
AMINCA	112	189	110	137
X1	124	155	156	145
VAN GOGH	170	153	196	173
CALGARY	138	218	284	213
MORENE	116	254	279	216
GALINA	162	229	283	225
EBA	219	194	308	240
MONDIAL	213	191	317	240
ALCMARIA	195	305	282	261
X2	314	276	351	314
TITANA	357	235	400	331
CERTA	106	224	742	357
group 2:				
W 72-22-496	24	81	71	59
V78-852/106NXY	89	168	217	158
WS 73-3-391	183	218	440	280
group 3:				
CMK 85-17-40	50	38	71	53
AM 70-2166	134	130	238	167
AM 66-42	153	173	245	190
VE 71-105	221	224	563	336
AM 78-3704	253	309	453	338
VE 66-295	421	443	525	463
MEAN	152	170	255	

when seed parents are being chosen.

In this experiment, two other seed parents, variety Barbara and breeding line AM 78-4102, were also investigated. However, results from these two seed parents are not taken into account, because an unknown number of their dihaploids were erroneously considered hybrids and removed from the progenies. This was due to 'nodal band'-like pigmentation occurring in Barbara and AM 78-4102 and in some of their dihaploids. A lot of other seed parents and their dihaploids had pigmented stems which, like the pigmentation in Barbara and AM 78-4102, originated from wild or primitive species in their ancestry. The introduction of these pigmentations into tetraploid breeding lines and varieties makes them less suitable as genetic markers for dihaploid selection.

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CHAPTER 3

EVALUATION OF DIHAPLOID POPULATIONS FROM VARIOUS POTATO VARIETIES AND BREEDING LINES

R.C.B. HUTTEN, W.J.J. SOPPE, J.G.TH. HERMSEN & E. JACOBSEN

(SUBMITTED)

Summary

The production of dihaploids from tetraploid varieties and breeding lines is the first step in a potato breeding program via the diploid level. There are large differences in usefulness between tetraploids as starting material for such a breeding program. These differences are not only caused by differences in dihaploid production ability of the tetraploids but also by differences in frequency of useless dihaploids appearing in the dihaploid populations. Large numbers of dihaploids are useless in a breeding program either because they are (sub)lethal or do not tuberize or flower. Dihaploid populations from 20 varieties and 11 breeding lines were evaluated for occurrence and frequency of mutant phenotypes, tuberization, flowering, pollen stainability, 2n-pollen production and resistance to Ro-1. Dihaploid populations were found to differ greatly for all these characters. One population contained even up to 62 % lethal mutants. Overall results showed that 40 % of 5377 dihaploids obtained did not tuberize and 32 % of 825 vigorous dihaploids did not form flowers. Only 27 and 4 % of 825 vigorous dihaploids had a pollen stainability higher than 20 and 60 % respectively. Among the 222 dihaploids with a pollen stainability of 20 % and higher, 23 % produced some amount of 2n-pollen. Not one of these 2n-pollen producing dihaploids had a pollen stainability higher than 60 %. Based on segregation ratios found in the dihaploid populations, assumptions were made on the genotype for Ro-1 resistance of each resistant tetraploid. Among the investigated dihaploid populations the number of resistant versus susceptible dihaploids was found to range from 8:26 to 62:8.

Introduction

The first step in a potato (*Solanum tuberosum* L.) breeding program via the diploid level is reducing the ploidy of autotetraploid potato to the diploid level (Chase, 1963; Hermsen et al., 1987). Dihaploids can be obtained routinely by pollinating tetraploid potatoes with diploid *Solanum phureja* clones selected for high haploid induction ability and homozygosity for embryo spot (Hermsen & Verdenius, 1973). Tetraploids may differ largely in the ability to produce suitable dihaploids as starting material in a diploid potato breeding program. There are significant differences between tetraploids in dihaploid production ability (Hougas et al., 1964; Frandsen, 1967; Hermsen & Verdenius, 1973; Hutten et al., 1994), in tuberization ability of dihaploids (Van Suchtelen, 1966; Neele & Louwes, 1986), and in flowering behaviour, pollen stainability and seed set of dihaploids (Peloquin & Hougas, 1960; Bender, 1963; Van Suchtelen, 1966; Gorea, 1970; Carroll & Low, 1975; Carroll & Low, 1976). Varieties and tetraploid breeding lines should be selected as starting material in a potato breeding program via the diploid level on the basis of their characters and the ability to produce suitable dihaploids.

In the present investigation dihaploid populations from 20 varieties and 11 breeding lines were evaluated for the appearance of mutants, tuberization ability, flowering behaviour, pollen stainability and 2n pollen production. In addition dihaploid populations from those tetraploids that are resistant to pathotype 1 of *Globodera rostochiensis* Woll. (Ro-1), were evaluated for this trait. The dihaploid production ability of most of the investigated tetraploids was analyzed by Hutten et al. (1994).

Materials and methods

The dihaploid populations evaluated in this investigation were derived from the tetraploid parents listed in table 1. These tetraploid parents consist of 20 varieties, three breeding lines with resistance to PVX and PVY (V78-852/106NXY, W 72-22-496 and WS 73-3-391), and eight breeding lines with resistance to several pathotypes of *Globodera pallida* Stone and *G. rostochiensis* (AM 66-42, AM 70-2166, AM 78-3704, AM 78-3778, CMK 85-17-40, VE 66-295, VE 70-86 and VE 71-105). Most of these tetraploids are widely being used by Dutch potato breeders in breeding programs at the tetraploid level. The dihaploids were obtained by prickle pollination with the haploid inducers IVP 35, IVP 48 (Hermsen & Verdenius, 1973) and IVP 101 (Hutten et al., 1994). The dihaploids were checked for ploidy level by stomatal guard cell chloroplast counts (Frandsen, 1968). Vigorous dihaploid seedlings were planted in the field, whereas non-vigorous ones were grown in 13 x 13 cm pots in a greenhouse. Among the non-vigorous dihaploids two distinct types of mutants were found: dwarfs, and plants with white or yellow cotyledons. The observed dwarf mutants were dark green and reached a maximum height of about 10 cm. These dwarf mutants seem similar to the gibberellin mutants (ga_1ga_1) found by Bamberg & Hanneman (1991). Mutants with white cotyledons were previously described by Simmonds (1965) and named 'albino' (*alal*). Mutants with yellow cotyledons (*ycyc*) were described by Jongedijk et al. (1990). The dwarf mutants could be grown in pots and part of them was able to produce tubers, whereas the mutants with white or yellow cotyledons were unable to form true leaves and died soon after emergence.

Tuberization, flowering behaviour and pollen stainability

All dihaploid seedlings that produced at least one tuber, big enough to maintain the dihaploid, were scored as tuberizing.

Flowering behaviour and pollen stainability were evaluated using dihaploids with a fair vigour and tuberization. One tuber of each dihaploid was grown in a greenhouse. One stem per plant was allowed to develop and all newly formed tubers were removed at regular intervals to promote flowering. Table 2 shows the dihaploid populations grown during period 1 (February 1 - May 31) and period 2 (June 1 - September 30). All dihaploids were investigated for their ability to produce flower buds, opened flowers and pollen grains. When dihaploids produced pollen grains a maximum per dihaploid of three pollen preparations stained with lactophenol acid fuchsin was made. The frequency of deeply stained round pollen grains was estimated in each pollen preparation. Per dihaploid the pollen preparation with the highest frequency of stainable pollen grains was used for the evaluation of the dihaploid populations. When unreduced (2n) pollen was produced its frequency was estimated using pollen size to discriminate 2n- from n-pollen (Quinn et al., 1974). The dihaploid populations were split up into six classes on the basis of flower and pollen development. These six classes are:

1. no flower buds;
2. deciduous flower buds;
3. flowers but no pollen;
4. stainable pollen 0-20 %;
5. stainable pollen 21-60 %;
6. stainable pollen 61-100 %.

The number of 2n-pollen producing dihaploids with a pollen stainability of more than 20 % was also estimated.

Screening for resistance to Ro-1

Dihaploids were screened for resistance to pathotype Ro-1 of the potato cyst nematode using closed containers (Phillips et al., 1980). When two tubers were available the screening was done in duplicate. The transparent containers had a volume of 125 ml. About 30 cysts of isolate Mierenbos A (MB^a) were added to each container. After being stored in the dark for seven weeks the number of newly formed cysts visible through the container wall was counted. When none or only very few cysts were visible the dihaploid was considered resistant. When more than 10 cysts were visible the dihaploid was considered susceptible. All populations showed a clear-cut segregation for resistant and susceptible individuals. However, results obtained from some poorly rooting dihaploids were questionable, and therefore discarded.

The tuberization of dihaploids derived from CMK 85-17-40 was so poor that tests for nematode resistance could not be performed.

Chi square (χ^2) tests were applied to detect significance of differences in tuberization between dihaploid populations (table 1) and to test the goodness of fit of observed and theoretical segregation ratios for resistance to Ro-1 within dihaploid populations (table 3). The tests were performed with Statgrafics 4.0.

Results

Mutants

Dwarf plants were observed in seven dihaploid populations (table 1). The observed frequencies of dwarf plants were very low (0.4-2.9 %), except in the dihaploid population from Titana (37.4 %).

Mutants with white or yellow cotyledons were observed in nine dihaploid populations (table 1). The observed frequencies of these mutants were very low (0.4-1.5 %) in five populations. Higher frequencies (6.1-61.7 %) were observed in dihaploid populations from Amanda, W 72-22-496, AM 66-42 and AM 70-2166.

Tuberization

The estimated frequencies of tuberizing dihaploids are presented in table 1. Significant differences were found between dihaploid populations. The overall frequency of tuberizing dihaploids was 60 %. The frequencies in individual dihaploid populations ranged from 22 % (AM 66-42) to 81 % (Eba).

Flowering behaviour and pollen stainability

The numbers of dihaploids in the phenotypic classes of each dihaploid population are presented in table 2. In total 32 % of the investigated vigorous dihaploids did

Table 1. Total number of dihaploids evaluated and the estimated frequencies (%) of dwarfs, dihaploids with white (or yellow) cotyledons, and tuberizing dihaploids in dihaploid populations from 20 varieties and 11 breeding lines. The frequency of dwarfs is given as the frequency among dihaploids with green cotyledons. The frequency of tuberizing dihaploids includes the tuberizing dwarfs. Frequencies of tuberizing dihaploids followed by a common letter are not significantly different at $P<0.05$.

TETRAPLOID PARENT	TOTAL NUMBER OF DIHAPLOIDS	FREQUENCY (%) OF		
		DWARFS	WHITE COTYLEDONS	TUBERIZING DIHAPLOIDS
ADRETTA	51	0	0	71^{i-o}
AGRIA	33	0	0	52^{c-i}
ALCMARIA	168	0	0	65^{f-k}
AMANDA	162	0	8.0	44^{c}
AMINCA	228	0	0	64^{f-k}
BARBARA	123	2.4	0	48^{cd}
BRIGHT	124	0	0	52^{c-e}
CALGARY	226	0	0	77^{m-o}
CERTA	230	0	0	76^{l-o}
EBA	245	0	0	81^{o}
ESCORT	163	0	1.2	45^{c}
GALINA	245	0.8	0	31^{b}
GLORIA	55	0	0	$53^{c-g,i}$
HERTHA	154	0.6	0	44^{c}
LADY ROSETTA	241	0	0	$56^{d-f,i}$
MATILDA	89	0	1.1	66^{f-l}
MONDIAL	246	0	0	61^{e-i}
MORENE	197	0	0	73^{j-n}
TITANA	195	37.4	0	68^{h-l}
VAN GOGH	235	0	0	66^{g-k}
V78-852/106NXY	202	0	0	$56^{d-f,i}$
W 72-22-496	33	0	6.1	45^{b-e}
WS 73-3-391	225	0	0	79^{no}
AM 66-42	180	2.9	61.7	22^{a}
AM 70-2166	184	0.6	17.4	45^{c}
AM 78-3704	232	0	0.4	64^{f-j}
AM 78-3778	99	0	0	47^{cd}
CMK 85-17-40	66	0	1.5	39^{bc}
VE 66-295	261	0	0	73^{k-n}
VE 70-86	226	0.4	0	64^{f-j}
VE 71-105	259	0	0.7	71^{j-m}
GRAND TOTAL	5377			60

Table 2. Flowering behaviour and pollen stainability of diha-ploid populations. Period 1 = February 1 - May 31, and period 2 = June 1 - September 30. The total number of dihaploids was split up into six classes according to flowering behaviour and pollen stainability. These six classes are: no flower buds (NB), deciduous flower buds (DB), flowers but no pollen (NP), stainable pollen 0-20 % (0-20), stainable pollen 21-60 % (21-60) and stainable pollen 61-100 % (61-100). 2n gives the number of 2n-pollen producing dihaploids with a pollen stainability of more than 20 %.

TETRAPLOID PARENTS	PERIOD	NUMBER OF DIHAPLOIDS							
		TOTAL	NB	DB	NP	0-20	21-60	61-100	2n
ADRETTA	2	6	1	1	0	0	3	1	1
AGRIA	2	8	0	3	2	3	0	0	0
ALCMARIA	1	44	5	4	7	21	6	1	1
AMANDA	2	21	1	2	1	7	9	1	2
AMINCA	2	49	8	23	17	0	1	0	1
BARBARA	2	5	0	0	0	5	0	0	0
BRIGHT	2	21	6	6	2	4	3	0	1
CALGARY	2	12	1	4	0	4	3	0	0
CERTA	2	14	0	0	0	0	5	9	0
EBA	1	31	4	14	5	6	2	0	2
ESCORT	2	30	5	1	0	21	3	0	1
GALINA	2	15	1	2	0	4	3	5	1
GLORIA	1	13	2	4	0	2	4	1	0
HERTHA	2	20	4	6	2	7	1	0	0
LADY ROSETTA	2	46	4	7	16	10	8	1	4
MATILDA	2	24	4	5	0	11	4	0	1
MONDIAL	2	20	1	8	5	2	4	0	0
MORENE	1	5	0	3	0	2	0	0	0
TITANA	1	39	4	3	0	9	21	2	6
VAN GOGH	1	51	5	3	4	12	23	4	1
V78-852/106NXY	2	46	3	7	2	33	1	0	1
W 72-22-496	2	8	0	1	2	4	1	0	0
WS 73-3-391	2	24	1	9	9	3	2	0	2
AM 66-42	1	19	1	3	0	10	5	0	0
AM 70-2166	1	25	3	9	0	1	10	2	0
AM 78-3704	1	42	3	13	7	13	6	0	5
AM 78-3778	1	24	4	10	2	3	3	2	1
CMK 85-17-40	2	2	0	2	0	0	0	0	0
VE 66-295	1	65	2	23	8	11	21	0	9
VE 70-86	1	40	3	7	7	16	6	1	4
VE 71-105	1	56	6	2	0	14	32	2	7
GRAND TOTAL		825	82	185	98	238	190	32	51
% TOTAL			10	22	12	29	23	4	6

Table 3. Number of resistant (R) and susceptible (S) dihaploids from different tetraploid parents with resistance to pathotype Ro-1 of *G. rostochiensis*, possible sources of resistance, and χ^2 tests for goodness of fit to the theoretical ratios indicated.
adg = *S. andigena*, opl = *S. oplocense*, spg = *S. spegazzinii* and vrn = *S. vernei*.
? = source species not (fully) known.
* = observed ratio deviating significantly from theoretical ratio.
x = χ^2 test not performed (one or two theoretical classes <5 individuals).

TETRAPLOID PARENT	NUMBER OF DIHAPLOIDS		SOURCES OF RESISTANCE	χ^2			
	R	S		1:1	3:1	5:1	7:1
AGRIA	2	4	?	x	-	-	-
ALCMARIA	38	37	adg	0.01	23.68*	-	-
AMANDA	15	11	adg	0.62	4.15*	-	-
AMINCA	36	31	adg	0.37	16.16*	-	-
BARBARA	22	4	adg spg vrn	12.46*	1.28	x	-
CALGARY	33	11	adg vrn	11.00*	0.00	2.20	6.29*
CERTA	36	41	?	0.32	32.77*	-	-
GLORIA	5	7	adg	0.33	x	-	-
HERTHA	8	26	adg	9.53*	-	-	-
LADY ROSETTA	39	10	adg vrn	17.16*	0.55	0.49	2.80
MONDIAL	16	16	adg vrn	0.00	10.67*	-	-
MORENE	33	13	vrn	8.70*	0.26	4.45*	-
VAN GOGH	45	38	adg + ?	0.59	19.12*	-	-
V78-852/106NXY	30	12	?	7.71*	0.29	4.29*	-
W 72-22-496	7	3	adg	1.60	x	-	-
WS 73-3-391	18	9	adg	3.00	1.00	x	-
AM 66-42	8	2	vrn	3.60	x	-	-
AM 70-2166	17	4	opl vrn	8.05*	0.40	x	-
AM 78-3704	16	15	adg opl spg vrn	0.03	9.04*	-	-
AM 78-3778	8	3	adg opl vrn	2.27	x	-	-
VE 66-295	61	43	adg vrn	3.12	14.82*	-	-
VE 70-86	29	13	vrn	6.10*	0.79	6.17*	-
VE 71-105	62	8	vrn	41.66*	6.88*	1.39	0.07

not produce open flowers and can therefore not be used in a breeding program. The pollen stainability of the remaining dihaploids was generally poor. Some dihaploids were detected that combined a pollen stainability of over 20 % with the production of 2n-pollen.

Resistance to Ro-1

The numbers of dihaploids resistant and susceptible to Ro-1 are presented in table 3. The *Solanum* species from which resistance of the tetraploid parents to various pathotypes of the potato cyst nematode were or could have been derived are also presented as far as known to the authors. The known species involved are *Solanum tuberosum* ssp. *andigena* Juz. et Buk. (adg), *Solanum oplocense* Hawkes (opl), *Solanum spegazzinii* Bitt. (spg) and *Solanum vernei* Bitt. et Wittm. (vrn). In these species at least five monogenic dominant genes conferring resistance to Ro-1 have been reported: H_1 in *S. tuberosum* ssp. *andigena* (Toxopeus & Huijsman, 1953; Huijsman, 1955), *Fa* and *Fb* in *S. spegazzinii* (Ross, 1962), and *B* and *C* in *S. vernei* (Plaisted et al., 1962). χ^2 tests reveal that in several dihaploid populations the observed ratios resistant:susceptible deviate significantly from the 1:1 ratio expected from monogenic inheritance and simplex condition in the tetraploid.

Discussion

Mutants

Haploidization of tetraploid potato causes a substantial increase of homozygosity, which corresponds to the effect of three generations selfing of the tetraploid (Hougas & Peloquin, 1958). So a dihaploid population is expected to display most of the inferior recessive alleles present in the tetraploid. When many of these inferior alleles appear in a double dose in the dihaploids or when the frequency of some of these inferior homozygotes in the dihaploid population is high, the tetraploid parent is less useful as starting material in a breeding program via the diploid level. In some of the dihaploid populations under investigation dwarf plants and/or plants with white or yellow cotyledons appeared. These mutants were numerous in dihaploid populations from Titana, AM 66-42 and AM 70-2166. Genetic analysis revealed a monogenic recessive inheritance of the characters dwarfing (Bamberg & Hanneman, 1991) and white (Simmonds, 1965) and yellow cotyledons (Jongedijk et al., 1990). It is not sure, whether white and yellow cotyledons are based on different genes. Unfortunately the mutant(s) were pooled in the present investigation.

The frequencies in which the mutants appeared were rather small in most of the dihaploid populations. In these populations the mutants likely are products of double reduction because their frequencies are similar to frequencies of double reduction found for isozyme alleles in potato by Haynes & Douches (1993). The segregation ratio normal:dwarf plants observed in the dihaploid population from Titana (122:73) does not fit ratios expected on the basis of a recessive monogenic inheritance of this mutant. The same applies for the observed ratios green: white/yellow cotyledons in dihaploid populations from Amanda (149:13), W 72-22-496 (31:2) and AM 66-42 (69:111). Only the ratio green:white/yellow cotyledons found in the dihaploid population from AM 70-2166 (152:32) fits a 5:1 ratio indicating a duplex condition in AM 70-2166 if the mutant is monogenic recessive. The segregation ratios observed in the dihaploid populations from Amanda, Titana, W 72-22-496 and AM 66-42, do not fit a monogenic recessive genetic model. Here the inheritance of the mutant character may be more complicated or the non-fitting ratios may be caused by distortion of monogenic ratios due to genetic or environmental factors not yet analyzed.

Tuberization

Significant differences between frequencies of tuberizing dihaploids were found within each of the three groups of tetraploids (table 1). So besides the dihaploid production ability of the tetraploid parent, the frequency of tuberizing dihaploids in the dihaploid population is a point to take into account, when selecting tetraploids as starting material in a potato breeding program via the diploid level. In this context it is notewhorty that (sub)lethal alleles at a single locus can reduce the produced dihaploid population by 17 % ((sub)lethal allele in the tetraploid in double dose) or 50 % ((sub)lethal allele in triple dose).

Flowering behaviour and pollen stainability

All dihaploids that fail to flower can obviously not be used in a potato breeding program. In the dihaploid populations which we investigated, an overall percentage of 32 % of the vigorous dihaploids did not flower. This percentage is lower than those reported by Gorea (1970) and Carroll & Low (1975) who found overall percentages of 37 % and 47 % respectively. That our estimated percentage of non-flowering dihaploids was only 32 %, may be due to the fact that we investigated only vigorous dihaploids.

Peloquin & Hougas (1960) found pollen stainability to range from 60 to 80 % in their male fertile dihaploids and from 0 to 20 % in their male sterile dihaploids. When we use these percentages as criteria for male fertility and male sterility, only 4 % of the investigated dihaploids were male fertile. However, 23 % of our dihaploids had a pollen stainability ranging from 20 to 60 % and a number of them may turn out to be male fertile. So 32 % of all the vigorous dihaploids that we obtained, could not be used in a diploid potato breeding program and 41-64 %

could be used as female parent only.

When we compare flowering behaviour and pollen stainability in individual dihaploid populations, there are large differences in frequency of dihaploids that produce open flowers and of dihaploids that are male sterile (pollen stainability < 20 %). High frequencies of dihaploids producing open flowers were found in dihaploid populations from Amanda, Certa, Van Gogh and VE 71-105, whereas low frequencies were found in dihaploid populations from Aminca, Eba and AM 78-3778. The tetraploid parents Aminca, Eba and AM 78-3778 produce a reasonable amount of flowers. So there seems to be no relation between the frequency of flowering dihaploids in the dihaploid population and the amount of flowers produced by the tetraploid parent. High frequencies of dihaploids with > 20 % stainable pollen grains were found in dihaploid populations from Certa, Galina, Titana, Van Gogh and VE 71-105, whereas low frequencies of such dihaploids were found in dihaploid populations of Aminca, Hertha and V78-852/106NXY. As far as male fertility of the tetraploids is known to the authors, there seems to be little association, if any, between the male fertility of the tetraploid parent and the frequency of male fertile dihaploids in the corresponding dihaploid population.

The last step in a potato breeding program via the diploid level is the recovery of the tetraploid level (Chase, 1963; Hermsen et al., 1987). A widely advocated method to return to the tetraploid level is sexual polyploidization by means of 2n-gametes. So the production of 2n-gametes is an important character to select for at the diploid level. A total number of 51 vigorous dihaploids with a pollen stainability of >20 % was found also to produce 2n-pollen. However, none of them had a pollen stainability of more than 60 %. In 10 of these 51 dihaploids the estimated percentage of 2n-pollen exceeded 5 % of the stainable pollen.

Resistance to Ro-1

All observed ratios resistant:susceptible were analyzed by the χ^2 test to determine the goodness of fit to the theoretical ratios 1:1, 3:1, 5:1 and 7:1 (table 3). When one, two and three different resistance genes are present in a simplex condition in the tetraploid parent, the expected ratios are 1:1, 3:1 and 7:1 respectively. A 5:1 ratio is expected when one resistance gene is present in a duplex condition. In several dihaploid populations not all appropriate χ^2 tests could be performed because the expected number of susceptible dihaploids was lower than five.

The tetraploid parents Alcmaria, Amanda, Aminca, Gloria, Hertha, W 72-22-

496 and WS 73-3-391 are known to have their Ro-1 resistance derived from the H_1 gene only. So we may expect to find 1:1 and 5:1 ratios for resistant:susceptible in their dihaploid populations. Segregation ratios in the dihaploid populations from Alcmaria, Amanda and Aminca fit the 1:1 ratio only, indicating a simplex condition of the dominant resistance allele in the tetraploid parent. The ratios observed in dihaploid populations from Gloria, W 72-22-496 and WS 73-3-391 also fit the 1:1 ratio but these populations are too small to test the goodness of fit to 5:1. However, only for the dihaploid population from WS 73-3-391 there is a fair probability that the observed ratio fits 5:1 (Warwick, 1932), indicating that the resistance gene is present in a duplex condition in the tetraploid. In the dihaploid population from Hertha there is no fit to 1:1, but a shortage of resistant dihaploids.

The tetraploid parents Morene, AM 66-42, VE 70-86 and VE 71-105 are known to have their Ro-1 resistance derived from *S. vernei* only. In *S.vernei* two independent resistance genes *B* and *C* are assumed to control resistance (Plaisted et al., 1962). Segregation ratios in the dihaploid populations from Morene and VE 70-86 fit the digenic 3:1 ratio only, indicating two resistance genes in a simplex condition in the tetraploid parents. The dihaploid population from AM 66-42 is too small to test a fit to other ratios than 1:1. However, because AM 66-42 is the parent of variety Morene which brought in the Ro-1 resistance, AM 66-42 must at least contain two resistance genes in a simplex condition. The ratio observed in the dihaploid population from VE 71-105 does not fit 1:1 or 3:1, but fits 5:1 and 11:1 (not presented in table 3). Assuming maximally two independent and dominant resistance genes, this indicates that at least one resistance gene in the tetraploid parent is present in a duplex condition. The other resistance gene may be present additionally in a simplex condition.

In the other dihaploid populations the origin of the Ro-1 resistance is not or not exactly known. According to the observed ratios resistant:susceptible we can only state how many genes are likely to be involved but we do not know for sure from which species they originate. The dihaploid population from Agria is too small to perform any χ^2 test. The dihaploid population from AM 78-3778 is too small to test for other ratios than 1:1 to which the observed ratio fits, though poorly. However, there is a fair probability that the observed ratio fits 3:1, 5:1 and 7:1 (Warwick, 1932). Segregation ratios in the dihaploid populations from Certa, Mondial, Van Gogh, AM 78-3704 and VE 66-295 fit 1:1 only, indicating that one resistance gene is present in a simplex condition in the tetraploid parents. The ratio in the dihaploid population from V78-852/106NXY only fits 3:1 pointing to two resistance genes in simplex condition in the parent. The ratio in the dihaploid population from Calgary fits 3:1 and 5:1, indicating that in the tetraploid parent either two independent resistance genes are present in simplex condition or one resistance gene in duplex condition. The segregation ratio observed in the dihaploid population from Lady Rosetta fits 3:1, 5:1 and 7:1. So it is possible that Lady Rosetta carries two or three independent resistance genes in simplex condition or one gene in duplex condition. The dihaploid population from Lady Rosetta was too small to test for goodness of fit to 11:1 but the probability that the observed ratio fits is negligible (Warwick, 1932). The ratios in the dihaploid populations from Barbara and AM 70-2166 do not fit 1:1. Segregation ratios in these dihaploid populations do fit 3:1. Further χ^2 tests can not be performed because the dihaploid populations are too small. However, there is a fair probability that observed ratios in both dihaploid populations fit 5:1, 7:1 and 11:1 (Warwick, 1932). So the genetic basis of Ro-1 resistance in Barbara and AM 70-2166 still remains unknown.

The use of dihaploid populations for genetic research is hampered by lack of randomness of the gametic samples due to linkage relations of alleles from unclassifiable plants. Although in this investigation the observed segregation ratios for resistance to Ro-1 fit the theoretical ratios in all dihaploid populations except one (from Hertha), segregation ratios may be distorted by genetic linkage to loci with inferior alleles. Frequent distortion of ratios in potato dihaploid populations is reported by El-Kharbotly et al. (In press) for R genes conferring resistance to *Phytophthora infestans* (Mont.) de Bary.

Conclusions

The dihaploid production ability of autotetraploid varieties and breeding lines is not the only important character defining their usefulness as starting material in a potato breeding program via the diploid level. This investigation revealed large differences between dihaploid populations in frequency of (sub)lethal mutants, of non-tuberizing dihaploids and of non-flowering dihaploids. When high frequencies of such dihaploids appear in a dihaploid population the usefulness of the corresponding tetraploid parent is highly reduced. Large differences between dihaploid populations were also found for the frequency of dihaploids that have a good pollen stainability. The presence of some diploid potato clones with good male fertility is a necessity for the success of a potato breeding program via the diploid level.

Besides the characters already mentioned, the frequency of dihaploids displaying the desired agronomic characters of the tetraploid parent, such as resistance to Ro-1, is also of importance for the selection of varieties and breeding lines as starting material in a potato breeding program via the diploid level.

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CHAPTER 4

COMPARATIVE PERFORMANCE OF DIPLOID AND TETRAPLOID PROGENIES FROM 2X.2X CROSSES IN POTATO

R.C.B. HUTTEN, M.G.M. SCHIPPERS, J.G.TH. HERMSEN &
E. JACOBSEN

(SUBMITTED)

Summary

Selection criteria for agronomic characters in a potato breeding program at the diploid (2x) level may differ from selection criteria which are in use when selecting breeding lines at the tetraploid (4x) level. Differences between selection criteria are expected, (1) when expression of the characters is different at both ploidy levels and/or (2) when the effect of diploid breeding lines on agronomic characters of tetraploid progenies is different from the effect of tetraploid breeding lines. In this investigation sets of diploid and tetraploid progenies, each set derived from the same 2x.2x cross, were compared as to the expression of six agronomic characters. Diploid progenies had significantly lower yields (due to smaller tubers) and significantly higher under water weights than tetraploid progenies. Vine maturity and chip colour were similarly expressed at both ploidy levels. Correlations between yield and yield components, and between under water weight and chip colour were similar at both ploidy levels. The lower yields and higher under water weights found in diploids point to the need of different selection criteria for selecting diploid and tetraploid breeding lines.

Introduction

The first step in a potato breeding program via the diploid level is reducing the ploidy of autotetraploid potato (*Solanum tuberosum* L.) to the diploid level and collecting diploid *Solanum* species to create a broadly based diploid gene pool. The next step is that of breeding and selecting within the diploid potato germplasm (Chase, 1963; Hermsen et al., 1987). The final step is returning to the tetraploid level and selection of varieties. Breeding tetraploid potato varieties via the diploid level raises two questions: (1) Is the expression of the desired characters similar at both ploidy levels? (2) To what degree are the desired characters transferred intact to the tetraploid level during the final step in the breeding program?

To compare the expression of agronomic characters at both ploidy levels, diploid and tetraploid potatoes with equivalent genotypes are needed. Such investigations have already been reported by several authors. Rowe (1967) intercrossed diploid hybrids between *Solanum phureja* Juz. et Buk. and dihaploids of *S. tuberosum*, and also their vegetatively doubled counterparts. When grown from true seed the tetraploid progenies had superior vegetative vigour and were higher yielding than the corresponding diploid progenies. Tetraploid superiority for yield was less evident when the diploid and tetraploid progenies were grown from tubers. Maris (1990) also intermated diploid *S. phureja*-dihaploid *S. tuberosum* hybrids and their vegetatively doubled counterparts. In addition to the F1's several sib and backcross progenies were investigated at both ploidy levels. The diploid progenies showed a mean yield reduction of 23 % relative to the tetraploid progenies. Mendiburu & Peloquin (1977), in three separate investigations, estimated yield reductions of 25, 29 and 34 % for diploids from 2x.2x (*S. phureja*-dihaploid hybrids) crosses relative to tetraploids derived from the same crosses (bilateral sexual polyploidization). In a similar investigation at two locations, Sanford & Hanneman Jr. (1982) estimated relative yield reductions for diploids of 12 and 50 %. Concerning yield components Rowe (1967) found the higher yields of tetraploid progenies grown from true seed to be due to more and bigger tubers, whereas Maris (1990) found the higher yields of tetraploid progenies to be due to bigger tubers alone. Maris (1990) found the tuber number of tetraploid progenies to be even lower than that of diploid progenies.

Maris (1990) also compared both ploidy levels for vine maturity and specific gravity. Tetraploid progenies were later maturing than diploid progenies, whereas specific gravity was the same at both ploidy levels.

The present investigation was carried out to compare the expression of yield, yield components, vine maturity, under water weight (like specific gravity an indirect estimate of starch content), and chip colour at both ploidy levels. Only Maris (1990) reported comparative data on expression of vine maturity and under water weight at the diploid and at the tetraploid level. Data on chip colour have not been reported so far. Equivalent diploid and tetraploid genotypes are needed for comparison. They were obtained by producing sets of diploid and tetraploid progenies from 2x.2x crosses.

Materials and methods

Sets of diploid and tetraploid progenies could be obtained from seven 2x.2x crosses. The diploid clones used as parents in these 2x.2x crosses were USW 5295.7 (B), USW 7589.2 (D), 77.2102.37 (E), and clones derived from crosses between the clones B, D, E, and USW 5337.3 (C). The pedigrees of these clones have been summarized by Jongedijk & Ramanna (1988). The parental clones were selected for 2n-eggs or 2n-pollen production, in addition to their normal production of n gametes. Progenies from crosses between these selected diploid clones are expected to consist of diploid and tetraploid plants only. The frequency of triploid plants from such matings is expected to be very low due to the 'triploid block' (Marks, 1966). Seedlings from 2x.2x crosses were checked for ploidy level by counting chloroplasts in stomatal guard cells (Frandsen, 1968). Besides vigorous diploid and tetraploid seedlings a large number of various growth deficient seedlings was obtained. In addition a few vigorous seedlings occurred of which the ploidy level could not be estimated unambiguously. Growth deficient seedlings and seedlings with an unknown ploidy level were not included in the investigation.

From the total number of seedlings obtained (table 1) a random sample of maximally 84 seedlings of each ploidy level was grown in 11 x 11 cm pots in a greenhouse. All seedlings which produced two fairly sized seed tubers were

Table 1. Total number of vigorous diploid (2x) and tetraploid (4x) seedlings obtained from 2x.2x crosses, and total number of diploid and tetraploid clones used in the field trial, selected as described in materials and methods.

CROSS	TOTAL NUMBER			
	VIGOROUS SEEDLINGS		CLONES IN FIELD TRIAL	
	2x	4x	2x	4x
D x E	65	85	43	48
B x E	581	74	42	37
CD1015 x CE1006	92	115	50	55
CD1015 x ED1045	64	92	26	47
CD1045 x ED1045	443	134	42	47
BE1050 x CE69	190	170	39	36
BE1050 x CE1006	270	256	20	36
GRAND TOTAL OF CLONES			262	306

selected for the field trial. In this way the numbers of clones in table 1 were obtained for the field trial. The field trial consisted of two randomized blocks per ploidy level, each block containing half of the total number of genotypes of each of the progenies. All measurements were done on a two plants/clone basis. The field trial was conducted in 1991 near Wageningen on a clay soil. Plant distances were 40 cm within and 75 cm between ridges. The two seed tubers of each clone were placed in the same ridge. Seed tubers were planted on April 18th and the foliage was destroyed by spraying 'Reglone' on September 18th. Parents were not included in the field trial because some were infected with PVY and/or PLRV so no reliable estimates of their agronomic characters could be made.

The characters estimated were:

1. yield in g for all tubers bigger than 2 cm in diameter;
2. number of tubers bigger than 2 cm in diameter;
3. tuber size on a 1 - 9 scale (1 = very small);
4. vine maturity on a 1 - 9 scale (1 = very late);
5. under water weight converted to the under water weight of 5000 g potatoes;
6. chip colour on a 1 - 9 scale (1 = very dark).

Clones were stored at about 9 °C until they were chipped in the first week of February. To estimate chip colour of a clone three 1.5 mm slices from each of three tubers were chipped and fried in 180 °C peanut oil until bubbling stopped.

Results

Mean yield, tuber number, and tuber size in progenies at both ploidy levels are presented in table 2. The overall mean yield of the diploids is 25 % lower than that of the tetraploids. The results for tuber number and tuber size show that the higher yields of the tetraploid progenies are due to bigger tubers alone. The overall mean number of tubers in the tetraploid progenies is significantly lower than in the diploid progenies.

Data on vine maturity, under water weight, and chip colour in the seven sets of diploid and tetraploid progenies are presented in table 3 together with the overall means.

Regarding vine maturity there is no significant difference between diploid and tetraploid progenies in five out of seven sets. The earlier maturity of diploids found in two sets of progenies is probably due to drought stress (see discussion). Also the overall mean for vine maturity does not show a significant difference between

Table 2. Mean yield (g), tuber number, and tuber size (1 - 9 scale, 1 = very small) of two clonal plants in diploid (2x) and tetraploid (4x) progenies from 2x.2x crosses. * and ** indicate significant differences between ploidy levels at $p<0.05$ and $p<0.01$ respectively.

CROSS	YIELD		TUBER NUMBER		TUBER SIZE	
	2x	4x	2x	4x	2x	4x
D x E	1449	2200**	34,6	38,8	4,79	5,50**
B x E	1072	1878**	34,6	32,6	4,24	5,22**
CD1015 x CE1006	1594	1426	46,4	44,4	4,66	4,40
CD1015 x ED1045	1493	1792	47,5	38,7	4,46	5,28**
CD1045 x ED1045	1092	1590**	40,4	31,6*	3,98	4,83**
BE1050 x CE69	1481	2368**	37,3	28,2*	4,90	5,97**
BE1050 x CE1006	1478	1772	27,1	26,8	5,45	5,44
OVERALL MEAN	1370	1835**	38,9	35,3*	4,58	5,18**

Table 3. Mean vine maturity (scale 1 - 9, 1 = very late), under water weight (converted to the under water weight of 5000 g potatoes) and chip colour (scale 1 - 9, 1 = very dark) in diploid (2x) and tetraploid (4x) progenies from 2x.2x crosses. * and ** indicate significant differences between ploidy levels at $p<0.05$ and $p<0.01$ respectively.

CROSS	VINE MATURITY		UNDER WATER WEIGHT		CHIP COLOUR	
	2x	4x	2x	4x	2x	4x
D x E	5,58	5,96	420	394	4,93	5,13
B x E	6,50	6,43	457	417*	6,02	5,72
CD1015 x CE1006	5,54	6,18	407	397	5,63	5,84
CD1015 x ED1045	5,88	6,19	425	401	5,73	6,04
CD1045 x ED1045	6,74	5,91*	449	428	5,93	6,21
BE1050 x CE69	7,00	5,81**	404	368*	5,69	5,58
BE1050 x CE1006	6,60	6,22	413	393	5,80	6,00
OVERALL MEAN	6,23	6,10	426	400**	5,66	5,79

diploid and tetraploid progenies. So it may be concluded that diploid and tetraploid progenies from the seven 2x.2x crosses have a similar vine maturity.

Regarding under water weight all seven diploid progenies have higher under water weights than their tetraploid counterparts, although the differences are significant in two sets only. The overall mean for under water weight shows a highly significant superiority of the diploids.

The results for chip colour do not show any significant differences between diploid and tetraploid progenies, neither in the separate sets nor in the overall

Table 4. Correlation coefficients concerning yield and yield components in diploid (2x) and tetraploid (4x) progenies from the crosses indicated, together with references, in the notes (1), (2), (3), (4), and (5). All correlation coefficients estimated in the present paper are significant except the one between tuber number and tuber size for 2x progenies.

CHARACTER	NOTE	TUBER NUMBER		MEAN TUBER WEIGHT		TUBER SIZE	
		2x	4x	2x	4x	2x	4x
YIELD	(1)	-	0.32	-	0.60	-	-
	(2)	-	0.30↔0.49	-	0.48↔0.64	-	-
	(3)	0.61	0.54	0.40	0.43	-	-
	(4)	-	0.42↔0.64	-	0.23↔0.56	-	-
	(5)	0.51	0.33	-	-	0.64	0.66
TUBER NUMBER	(1)			-	-0.47	-	-
	(2)			-	-0.40↔-0.55	-	-
	(3)			-0.32	-0.38	-	-
	(4)			-	-0.21↔-0.49	-	-
	(5)			-	-	-0.05	-0.17

(1) Killick (1977); in tetraploid progenies from 4x.4x crosses within *S. tuberosum* L.
(2) Maris (1969); in tetraploid progenies from 4x.4x crosses within *S. tuberosum* L.
(3) Maris (1990); in diploid and tetraploid progenies from crosses between diploid *S. tuberosum* x *S.phureja* hybrids and between their vegetatively doubled counterparts respectively.
(4) Tarn & Tai (1977); in tetraploid progenies from 4x.4x crosses within *S. tuberosum* L.
(5) The present investigation; in diploid and tetraploid progenies from 2x.2x crosses.

mean. Hence the expression of chip colour is the same at both ploidy levels.

Some of the agronomic characters studied herein are known to be correlated at the tetraploid level. It is of interest to know if these correlations also appear at the diploid level, and if so, to what extent.

Table 4 summarizes the correlation data for yield and yield components from the literature and from the present investigation, together with the provenance and ploidy level of the progenies in which the correlations were determined. The data show that correlations concerning yield and yield components are similar at both ploidy levels.

Besides the actual chip colour, the specific gravity of a clone is also an important character to take into account in a selection program for good chipping breeding lines. The time it takes to fry chips is decreasing with an increasing specific gravity and the yield of fried chips increases with higher specific gravity. In addition, absorption of frying oils is greater when potatoes with low specific gravity are processed. Chips with high oil contents are poor in quality, are more costly to produce, and have a shorter shelf-life. Cunningham & Stevenson (1963) estimated correlation coefficients between chip colour and specific gravity in progenies of 4x.4x crosses to be 0.26 and 0.32 in different years. In the present investigation the correlation coefficient in diploid progenies was 0.34 and in tetraploid progenies 0.28. They are highly significant and similar to those found by Cunningham & Stevenson for 4x.4x crosses. Hence also the correlation between chip colour and specific gravity is similar at both ploidy levels.

Discussion

The overall mean yield (table 2) of the diploids is 25 % lower than that of the tetraploids. This figure is similar to the ones presented in all previous reports (Mendiburu & Peloquin, 1977; Sanford & Hanneman Jr., 1982; Maris, 1990), and mentioned in the introduction to this paper. However, the diploid progeny from CD1015 x CE1006 is higher yielding, though not significantly, than its tetraploid counterpart. This exception is likely due to an inbreeding effect being more severe in this set of progenies than in others, because the parents are closely related (Jongedijk & Ramanna, 1988; this paper).

The results from the analysis of yield components (table 2) are in accordance with those obtained by Maris (1990). However, they are conflicting with the finding by Rowe (1967) in true seed progenies that the higher yields of tetraploid progenies are due to both more and bigger tubers. Considering that the results by Rowe (l.c.) were obtained with true seed progenies and could not be reproduced with progenies grown from tubers, the results concerning yield components presented in this paper and those by Maris (l.c.) likely give the more accurate picture of yield components at both ploidy levels.

Regarding vine maturity, Maris (1990) estimated tetraploid progenies to be later maturing than diploid progenies. This conclusion is inconsistent with our observations. The earlier maturity of diploids found in two out of seven sets of progenies (table 3) may be caused by the extremely low rainfall (11.5 mm) between July 26th and September 15th during the field trial. The shortage of water may have caused a stress induced maturity of diploids, especially because in general these are less vigorous and have a smaller root system than tetraploids. Inconsistence of results by Maris (1990) and results presented in this paper (table

3) was also demonstrated for under water weight, because Maris did not find differences between the ploidy levels. Maybe these inconsistences are due to the different origin of the progenies as explained in the notes (3) and (5) under table 4.

Rothacker & Schäfer (1961) compared dihaploids of the variety Apta with a tetraploid selfed progeny of this variety. Although these types of progenies can not be designated as genetically equivalent, because dihaploids are extreme inbreds (Hougas & Peloquin, 1958), the results are of interest. The dihaploids were estimated to have a lower starch content than the selfed progeny of the variety. So various investigations of diploid and tetraploid potatoes concerning under water weight show contradictory results.

Besides progeny mean, within progeny variation is important in plant breeding. A higher within progeny variation will enlarge the number of selected clones when the same selection criteria are applied. So it would be of interest to compare variances of progenies in diploid and tetraploid breeding programmes. However, this is not feasible with the progenies obtained in this investigation. Our tetraploid progenies are derived by bilateral sexual polyploidization and within progeny variation of these progenies is expected to differ from the within progeny variation of 4x.4x cross progenies, normal progenies in a potato breeding program at the tetraploid level.

Conclusions

Summarizing, it can be stated that this investigation concerning the expression of agronomic characters of potato at the diploid and the tetraploid level revealed significant differences for yield, yield components and under water weight between both ploidy levels. For vine maturity and chip colour no differences between ploidy levels were found. The results for under water weight and vine maturity are inconsistent with those reported by other authors. Correlations between yield, tuber number and tuber size (or mean tuber weight) and between chip colour and under water weight are similar at both ploidy levels.

These results have the following implications for selection criteria at the diploid and tetraploid level. A different expression of characters at the diploid level implies that other criteria for selection have to be applied in a diploid potato breeding program. A similar expression of characters at both ploidy levels is not a guarantee that selection can be carried out in the same way as at the tetraploid level. The transfer of characters in the last step of a diploid potato breeding program, returning to the tetraploid level, is also of major importance for selection criteria. Diploid breeding lines can have an effect on characters of their tetraploid progenies that differs from the effect of tetraploid breeding lines (De Jong & Tai, 1991).

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CHAPTER 5

ANALYSIS OF THE PARENTAL EFFECTS ON MEAN YIELD AND UNDER WATER WEIGHT OF POTATO 4X.2X PROGENIES

R.C.B. HUTTEN, M.G.M. SCHIPPERS, W.J. WERKMAN,
J.P.M. VERGROESEN, J.G.TH. HERMSEN & E. JACOBSEN

(SUBMITTED)

Summary

The parental effects on progenies from 4x.2x(FDR) crosses were analyzed to determine whether and to what extent the selection criteria used for selecting breeding lines at the tetraploid level have to be modified when selecting breeding lines at the diploid level. For yield and yield components multiple regression of progeny means on means of diploid and tetraploid parents revealed low coefficients of determination and low or even negative slopes for the diploid parents. This indicates that the yield of tetraploid progenies from 4x.2x crosses can not be predicted from the yield of their diploid parents. Consequently, direct selection for yield among diploid breeding lines is ineffective. Multiple regression for under water weight revealed a high coefficient of determination coupled with a greater effect of the diploid parents on the progenies. Deviations of progeny means from the midparent values were towards the diploid parents. This indicates that for under water weight selection of diploid breeding lines has to be conducted more stringently than selection of tetraploid breeding lines.

Introduction

Selection criteria for agronomic characters in a potato breeding program at the diploid level may differ from selection criteria employed when selecting breeding lines at the tetraploid level. Differences between selection criteria are expected (1) when expression of the characters is different at both ploidy levels and/or (2) when the effect of diploid breeding lines on the characters of tetraploid progenies is different from the effect of tetraploid breeding lines.

Yield is not expressed equally at both ploidy levels. Diploid potatoes have yield reductions ranging from 12 to 50 % relative to tetraploid potatoes (Mendiburu & Peloquin, 1977b; Sanford & Hanneman Jr., 1982; Maris, 1990; Hutten et al., submitted). This yield reduction is due to smaller tubers only (Maris, 1990; Hutten et al., submitted).

Different results from comparative investigations at both ploidy levels are reported for under water weight (or specific gravity). Maris (1990) found that specific gravity was the same at both ploidy levels, whereas Hutten et al. (submitted) estimated the under water weight of diploids to be about 6 % higher than that of tetraploids.

In 4x.4x crosses within *S. tuberosum* ssp. *tuberosum*, progeny means for yield and mean tuber weight are lower, and for tuber number higher than the midparent (MP) values (Maris, 1969; Tarn & Tai, 1983; Maris, 1989). When one of the parents used in 4x.4x crosses is a 'primitive' species such as *S. tuberosum* ssp. *andigena*, progeny means for yield may be higher than MP values (Tarn & Tai, 1977; Tarn & Tai 1983; Maris, 1989). In addition tuber numbers in such crosses are higher and mean tuber weights lower than MP values (Maris, 1989).

In 4x.4x crosses, progeny means for specific gravity are generally equal to MP values (Engel, 1956; Cunningham & Stevenson, 1963; Johansen et al., 1967; Maris, 1969; Mendoza & Haynes, 1977; Tai & Tarn, 1980; Tarn & Tai, 1983; Maris, 1989).

The last step in a potato breeding program at the diploid level is returning to the tetraploid level (Chase, 1963; Hermsen et al., 1987). One way to reach this goal is unilateral sexual polyploidization, in which tetraploid varieties or breeding lines are crossed as females with synaptic diploid males producing 2n-pollen preferably by first division restitution (FDR). The effects of diploid and tetraploid parents on the characters of 4x.2x progenies can be evaluated by comparing multiple regression slopes of the parents, and by comparing progeny means with MP values.

There are reports of progenies from 4x.2x(FDR) crosses out-yielding their tetraploid parents (De Jong & Tai, 1977; Mendiburu & Peloquin, 1977a; Darmo & Peloquin, 1991; De Jong & Tai, 1991). These higher yields coincide with more but smaller tubers (De Jong & Tai, 1977; De Jong & Tai, 1991). The diploid parents used in these crosses are either dihaploid-wild species hybrids or dihaploid-primitive species hybrids. The heterosis observed for yield may be associated with newly introduced genes and late maturity, such as in crosses between *S. tuberosum* ssp. *tuberosum* and ssp. *andigena*.

De Jong & Tai (1991) found the mean specific gravity for all 4x.2x progenies to be equal to the mean of the diploid and tetraploid parental means. Comparisons between progeny means and MP values for individual 4x.2x crosses were not presented.

Multiple regression analysis of progeny means on means of diploid and tetraploid parents revealed poor predicting power of the performance of diploid and tetraploid parents on their progenies. Coefficients of determination (R^2) found for yield, tuber number, mean tuber weight, and specific gravity were 0.18, 0.19, 0.30, and 0.25 respectively. All slopes were positive. Diploid parents had a greater effect (larger slope) on tuber number, mean tuber weight, and specific gravity. Tetraploid parents had a greater effect on yield (De Jong & Tai, 1991).

The present investigations were carried out to estimate the relative effects of diploid and tetraploid parents on yield, tuber number, tuber size, and under water weight of 4x.2x progenies. Combining the present results with those reported from investigations concerning the expression of these characters at both ploidy levels, an indication will be obtained about selection criteria for these characters at the diploid level.

Materials and methods

The parental effects on yield and yield components, and on under water weight of 4x.2x progenies were investigated in separate experiments. Crosses were made between varieties and diploid F1 hybrids of the clones USW 5293.3 (A), USW 5295.7 (B), USW 5337.3 (C), USW 7589.2 (D), and 77.2102.37 (E). The pedigrees of these diploid clones have been summarized by Jongedijk & Ramanna (1988). All diploids used produce 2n-pollen predominantly through fusion of metaphase II spindles, which leads to FDR (Ramanna, 1979).

For studying the parental effects on yield, tuber number, and tuber size of 4x.2x progenies six varieties with variable yields were crossed with two high and two low yielding diploids. All 24 cross combinations were successful. A maximum of 100 seeds per cross was sown and seedlings were grown in 9 x 9 cm pots in a greenhouse. A maximum of 40 clones per progeny with two fairly sized seed tubers per clone was used for the field trial. The actual progeny size ranged from 38 to 40 clones, except from Lutetia x EA1006 with 31 and Radosa x EA1006 with 29 clones. The field trial consisted of two randomized blocks. Each block contained a random half of the progenies. The field trial was conducted in 1991 near Uithuizen on a light clay soil. Plant distances were 35 cm within and 75 cm between ridges. The two seed tubers of each clone were placed in the same ridge. Seed tubers were planted on April 12th and clones were harvested on September 2nd. All measurements were taken on a 2 plants/clone basis. Tuber size was expressed on a 1 - 9 scale (1 = very small).

For studying the parental effects on under water weight of 4x.2x progenies three varieties with a high and three with a low under water weight were crossed with two diploids with a high and four with a low under water weight. Out of the 36 possible cross combinations 27 were successful. A maximum of 100 seeds per cross was sown and seedlings were grown in 10 x 10 cm pots in a greenhouse. The seedlings tuberized too poorly to conduct a field trial with two seed tubers per clone. So from each progeny a maximum of 40 clones with one fairly sized seed tuber was used for the field trial. The actual progeny size ranged from 37 to 40. The field trial consisted of two randomized blocks. Each block contained a random half of the progenies. The field trial was conducted in 1991 near Nagele on a light clay soil. Plant distances were 50 cm within and 75 cm between ridges. Seed tubers were planted on April 12th and plants were harvested on September 3rd. All measurements were taken on a single plant basis. The under water weight was converted to the under water weight of 5000 g potatoes.

Parents were multiplied under the same conditions as the seedlings were grown. Alongside the field trials the diploid and tetraploid parents were grown separately in six randomized replications per ploidy level. Measurements were taken on the same number of plants as in the corresponding field trial.

Results

Field trial Uithuizen

The means of parents and 4x.2x progenies for yield, tuber number, and tuber size are presented in the tables 1, 2, and 3 respectively. The overall progeny means for yield of the diploid parents (table 1) reveal significant differences between all diploid parents. The overall progeny mean for yield of ED1048, one of the low yielding diploid parents, being the highest, whereas the overall progeny mean for yield of EA1006, one of the high yielding diploid parents, is the lowest. Results of multiple regression analyses of progeny means on means of tetraploid and diploid parents are presented in table 6. For yield, tuber number, and tuber size the estimated coefficients of determination (R^2) are 0.08, 0.22, and 0.23 respectively.

Table 1. Field trial Uithuizen. Mean yield (kg per two plants) of parents and 4x.2x progenies. Letters a, b, c, and d indicate significant differences ($P<0.05$) between means in the same row or column.

	BC1063 (2.72)^{b}	EA1006 (2.45)^{b}	ED1048 (0.93)^{a}	BC1007 (0.82)^{a}	OVERALL MEAN
RADOSA (4.30)^{c}	2.78	1.17	3.07	2.08	2.36^{ab}
LUTETIA (3.13)^{b}	2.50	1.29	3.64	1.98	2.41^{b}
BARAKA (2.87)^{b}	2.44	1.81	2.79	2.17	2.30^{ab}
JAERLA (2.87)^{b}	2.86	1.58	3.46	1.78	2.43^{b}
SATURNA (2.72)^{ab}	1.94	1.44	2.17	2.64	2.05^{a}
ASTARTE (1.77)^{a}	2.47	1.62	3.21	2.45	2.44^{b}
OVERALL MEAN	2.50^{c}	1.51^{a}	3.05^{d}	2.18^{b}	

Table 2. Field trial Uithuizen. Mean tuber number (per two plants) of parents and 4x.2x progenies. Letters a, b, and c indicate significant differences ($P<0.05$) between means in the same row or column. For each progeny mean the deviation from the MP value is also presented. * Indicates that the MP value is not within the 95% confidence interval of the progeny mean.

	BC1063 (58.5)^{b}	EA1006 (28.8)^{a}	ED1048 (18.8)^{a}	BC1007 (33.2)^{a}	OVERALL MEAN
RADOSA (26.5)^{bc}	42.7 +0.2	22.0 -5.7	32.9 +10.3*	35.1 +5.3	34.0^{ab}
LUTETIA (22.8)^{bc}	39.1 -1.6	21.6 -4.2	38.4 +17.6*	39.2 +11.2*	35.3^{ab}
BARAKA (21.3)^{b}	43.8 +3.9	24.4 -0.7	31.8 +11.8*	46.9 +19.7*	36.8^{b}
JAERLA (10.7)^{a}	37.4 +2.8	19.4 -0.4	32.5 +17.8*	29.9 +8.0*	29.8^{a}
SATURNA (30.7)^{c}	51.1 +6.5*	34.5 +4.8	52.5 +27.8*	64.2 +32.3*	50.7^{c}
ASTARTE (22.8)^{bc}	56.3 +15.7*	35.4 +9.6*	50.4 +29.6*	51.7 +23.7*	48.4^{c}
OVERALL MEAN	45.1^{c}	26.6^{a}	39.8^{b}	44.5^{c}	

Table 3. Field trial Uithuizen. Mean tuber size (scale 1 - 9, 1 = very small) of parents and 4x.2x progenies. Letters a, b, and c indicate significant differences between means in the same row or column.

	BC1063 (5.00)^{b}	EA1006 (6.67)^{c}	ED1048 (5.83)^{bc}	BC1007 (3.00)^{a}	OVERALL MEAN
RADOSA (7.50)^{b}	6.03	4.86	6.98	5.55	5.93^{c}
LUTETIA (7.83)^{b}	6.05	4.97	7.31	5.21	5.93^{c}
BARAKA (7.17)^{b}	5.63	5.87	7.03	5.15	5.91^{c}
JAERLA (8.83)^{c}	6.45	5.80	7.48	5.18	6.23^{c}
SATURNA (5.67)^{a}	4.55	4.50	4.90	4.62	4.64^{a}
ASTARTE (5.17)^{a}	5.03	4.93	6.30	5.15	5.35^{b}
OVERALL MEAN	5.62^{b}	5.18^{a}	6.66^{c}	5.14^{a}	

Table 4. Field trial Nagele. Mean under water weight (converted to the under water weight of 5000 g potatoes) of parents and 4x.2x progenies. Letters a, b, and c indicate significant differences ($P<0.05$) between parents of the same ploidy level. For each progeny the deviation of the progeny mean from the MP value is also presented. * Indicates that the MP value is not within the 95% confidence interval of the progeny mean.

	CE1002 (532)^{c}	EB1047 (470)^{b}	BC1019 (372)^{a}	BC1078 (354)^{a}	BC1022 (350)^{a}	DB1005 (325)^{a}
ASTARTE (538)^{b}	514 -21*	515 +11	438 -17*	425 -21*	431 -13	---
CERTA (525)^{b}	507 -22*	518 +21*	450 +2	450 +11	---	---
GALINA (504)^{b}	494 -24*	485 -2	403 -35*	421 -8	407 -20*	399 -16*
SIRTEMA (392)^{a}	449 -13	443 +12	392 +10	392 +19*	---	364 +6
MIRKA (375)^{a}	446 -8	456 +34*	---	370 +6	---	---
KONDOR (356)^{a}	433 -11	439 +26*	413 +49*	---	---	367 +27*

Table 5. Field trial Nagele. Mean yield (kg per plant) of parents and 4x.2x progenies. Letters a and b indicate significant differences ($P<0.05$) between parents of the same ploidy level.

	DB1005 (1.61)^{b}	BC1078 (1.20)^{ab}	CE1002 (1.10)^{ab}	EB1047 (1.04)^{ab}	BC1022 (0.89)^{a}	BC1019 (0.75)^{a}
KONDOR (1.69)^{a}	1.71	--	1.37	1.26	--	1.05
SIRTEMA (1.48)^{a}	1.21	0.94	0.79	1.04	--	1.78
ASTARTE (1.35)^{a}	--	1.01	1.40	1.37	1.28	1.33
MIRKA (1.22)^{a}	--	1.41	1.54	1.47	--	--
GALINA (0.99)^{a}	1.83	1.36	1.36	1.46	1.54	1.39
CERTA (0.96)^{a}	--	1.71	1.49	1.46	--	1.47

Table 6. Relationships for yield, tuber number, tuber size, and under water weight between progeny means (y) and tetraploid (x_1) and diploid (x_2) parents. * Indicate significant differences ($P<0.05$) between slopes of x_1 and x_2 (NS = not significantly different).

Character	$y = a + bx_1 + cx_2$	SLOPES	R^2
Field trial Uithuizen			
Yield	$y = 2.95 - 0.04x_1 - 0.30x_2$	NS	0.08
Tuber number	$y = 12.3 + 0.82x_1 + 0.23x_2$	*	0.22
Tuber size	$y = 2.49 + 0.35x_1 + 0.14x_2$	NS	0.23
Field trial Nagele			
Yield	$y = 1.69 - 0.41x_1 + 0.18x_2$	*	0.14
Under water weight	$y = 93 + 0.34x_1 + 0.45x_2$	*	0.86

These low coefficients of determination indicate a poor predicting power of the performance of parents on yield and yield components of 4x.2x progenies. Tetraploid parents had a greater effect (larger slopes; parameters b and c in table 6) on tuber number and tuber size of their 4x.2x progenies than the diploid parents. Slopes for yield were negative for both diploid and tetraploid parents.

It has been demonstrated that tetraploids have higher yields than diploids due to bigger tubers (Maris, 1990; Hutten et al., submitted). Because yield and tuber size are expressed differently at both ploidy levels it is of no use to compare progeny means with MP values for these characters. Therefore such comparisons have been made for tuber number only (table 2). As in 4x.4x crosses, mean tuber numbers of the progenies are mostly higher than the MP values. Mean tuber sizes (table 3) are generally smaller than tuber sizes of the corresponding tetraploid parents.

Field trial Nagele

The field trial at Nagele primarily aimed at estimating the parental effects on under water weight of 4x.2x progenies. The results are presented in table 4. Because for estimating under water weights also yields have to be assessed, parental effects on yield could also be analyzed from this field trial (table 5).

The coefficient of determination (R^2) for under water weight was found to be 0.86 (table 6). This high coefficient of determination indicates a good predicting power of the performance of both parents on under water weight of 4x.2x progenies. The slope found for the diploid parents is significantly larger than that found for the tetraploid parents. This indicates a greater effect of the diploid parents on under water weight of 4x.2x progenies. The MP value is not within the 95 % confidence interval of the progeny mean (table 4) of 14 progenies. In these 14 progenies deviations of the progeny means from the MP values are positive in eight and negative in six progenies. Further analysis of these results reveals both negative and positive deviations in 4x(high under water weight).2x(high under water weight), only negative ones in 4x(high).2x(low), and only positive ones in 4x(low).2x(high) and 4x(low).2x(low). In 4x(high).2x(high) the deviations are three times negative, and positive only once. These negative deviations as well as the positive deviations in 4x(low).2x(low) are expected because both parents are near to the maximium and minimum levels of expression respectively. In 4x(high).2x(low) and 4x(low).2x(high) crosses progeny means are deviating from the MP values towards the diploid parents.

The R^2 estimated for yield was 0.14 (table 6). This indicates a poor predicting power of the performance of both parents on yield of 4x.2x progenies. The slopes for diploid and tetraploid parents differed significantly. The slope for the tetraploid parents was significantly negative ($P<0.05$) whereas the slope for the diploid parents was positive.

Discussion

Yield and yield components

In both field trials the coefficients of determination estimated for yield, tuber number and tuber size are similar to the coefficients of determination found by De Jong & Tai (1991). However, the slopes estimated here for tetraploid and diploid parents are very different from those estimated by De Jong & Tai (l.c.). In this investigation the tetraploids had a greater effect on tuber number and tuber size of their 4x.2x progenies than the diploids, whereas in the investigation by De Jong & Tai (l.c.) the diploids had the greater effect. However, one should notice that tuber size is not exactly the same as mean tuber weight. The estimated slopes concerning yield in our two field trials were different. They were negative in three out of four cases. They differ from the slopes 0.21 for tetraploids and 0.12 for diploids found by De Jong & Tai (1991). These results all indicate that neither in a diploid nor in a tetraploid potato breeding program aimed at producing breeding lines, direct selection for yield is of any use. For tuber number we estimated a significantly larger slope for the tetraploid parents. This indicates that tuber number could be the only yield component which is worthwhile to select for in tetraploid parents. However, this statement is not in line with the results by De Jong & Tai (1991).

When direct selection for yield among parents is not effective one has to rely upon testcross results for estimating the breeding value of clones for this character. There are many reports on the combining ability for yield of tetraploid and diploid parents in 4x.2x(FDR) crosses. Some authors found a significant general combining ability of both tetraploid and diploid parents (GCA(4x) and GCA(2x)) and a non-significant specific combining ability (SCA) (Mendiburu & Peloquin, 1977a; McHale & Lauer, 1981; Veilleux & Lauer, 1981; Keijzer-van der Stoel et al., 1991). However, there are also reports of significance of GCA(4x), GCA(2x), and SCA (Ortiz et al., 1988), significance of only GCA(4x) (De Jong & Tai, 1977), significance of only SCA (Mok & Peloquin, 1975), and non-significance of GCA(4x), GCA(2x), and SCA (Darmo & Peloquin, 1991; Ortiz et al., 1991). There are two groups of authors reporting that GCA(4x) was greater than GCA(2x) (Darmo & Peloquin, 1991; Keijzer-van der Stoel et al., 1991), whereas the reverse was reported by Ortiz et al. (1988). Evaluation of our field trial near Uithuizen, a complete factorial design, revealed significant GCA(4x), GCA(2x), and SCA. GCA(2x) was much greater than GCA(4x), the ratio GCA(2x)/GCA(4x) being

28.6. It appears that the results from the analysis of combining ability for yield in 4x.2x crosses are highly divergent. In our view this must be due to the choice of parents.

GCA(2x) is estimated to be significant in several investigations. This means that selection for yield in a diploid breeding program can be carried out indirectly by making 4x.2x testcrosses. Unfortunately most of the diploids do not produce 2n pollen and 4x.2x testcrosses can not be made. The GCA of diploids in 4x.2x crosses might be predicted by analyzing combining abilities in 2x.2x crosses as results by Keijzer-van der Stoel et al. (1991) suggest. However, a comparison made between seven sets of diploid and tetraploid progenies from 2x.2x crosses revealed no correlation between rankings for yield of diploid and corresponding tetraploid progenies (Hutten et al., submitted).

In only five out of 24 progenies in table 1 the mean yield was higher than the yield of the tetraploid parent. Three of these five progenies are from Astarte, a late maturing variety, for which yield may have been underestimated in this investigation because it was harvested too early for this variety. Also remarkable are the yields of progenies with EA1006, a high yielding diploid, as a common parent. All progeny means for yield of this clone are lower than that of the parental clone itself. The high yields of 4x.2x(FDR) crosses reported by others are not found in this investigation. A reason for this might be that BC1063, EA1006 and ED1048 are fairly early maturing clones. Consequently, late maturity which is one of the causes of the reported high yields in 4x.2x(FDR) progenies, is not involved in the progenies.

Under water weight

The coefficient of determination estimated for under water weight (0.86) is much higher than the coefficient of determination found by De Jong & Tai (1991). The greater effect (larger slope) of diploid parents on under water weight of 4x.2x progenies was also found by De Jong & Tai (l.c.). Because selection among diploid parents has more effect on 4x.2x progenies, and because 4x.2x progeny means are closer to the diploid parental mean, diploid parents have a greater contribution to the inheritance of under water weight in 4x.2x crosses than tetraploid parents. If under water weight in diploids is expressed at a higher level than in tetraploids (Hutten et al., submitted), MP values should be corrected and reduced by about 3 %. As a result of this reduction the deviations of the progeny mean from the MP value of 4x(high).2x(high) and 4x(high).2x(low) crosses undergo a significant change. The deviations become positive for 4x(high).2x(high) and almost zero for 4x(high).2x(low). The positive deviations found for 4x(high).2x(high) are hard to explain. The MP values found for progeny means of 4x(high).2x(low) are similar to normal progeny means of 4x.4x crosses but they disharmonize with the progeny means of 4x(low).2x(high) crosses which now show a very strong deviation towards the diploid parents. For multiple regression analysis this correction only influences the slope for the diploid parents, which will change by about 6%. When an equal expression of under water weights at both ploidy levels is assumed the obtained results can easily be explained. This is not the case when a higher expression of the under water weight at the diploid level is assumed.

Conclusions

Yield and yield components

Some conclusions can be drawn about selection criteria for yield and under water weight in potato breeding programs at the diploid level and at the tetraploid level, aimed at breeding lines.

Multiple regression for yield revealed low coefficients of determination along with highly variable slopes for both diploid and tetraploid parents. This indicates that selection for yield in diploid as well as in tetraploid breeding lines does not make sense. The low coefficients of determination found for tuber number and tuber size (or mean tuber weight) indicate that selection for one of these yield components can not be used as an alternative approach, at least not in diploids. In this investigation, the slope for tuber number of the tetraploid parents indicates that selection in tetraploid parents is feasible. However, this result was not corroborated by the results reported by De Jong & Tai (1991).

Under water weight

A high coefficient of determination was found for under water weight. The effect of the diploid parents on the progenies was significantly greater than the effect of the tetraploid parents. Assuming equal expression at both ploidy levels, the progeny means are deviating from the MP values towards the diploid parents. Assuming a higher expression of under water weight at the diploid level, only in 4x(low).2x(high) crosses the progeny means are deviating from the MP values towards the diploid parents. Regardless of the possibility of a different expression of under water weight at both ploidy levels, selection criteria for under water weight in a diploid potato breeding program have to be more stringent than in a tetraploid potato breeding program.

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CHAPTER 6

ANALYSIS OF THE PARENTAL EFFECTS ON MEAN VINE MATURITY AND CHIP COLOUR OF POTATO 4X.2X PROGENIES

R.C.B. HUTTEN, M.G.M. SCHIPPERS, J. EISING, P.M. VAN TIL,
J.G.TH. HERMSEN & E. JACOBSEN

(SUBMITTED)

Summary

The parental effects on progenies from potato 4x.2x(FDR) crosses were analyzed to determine whether and to what extent the selection criteria used for selecting tetraploid breeding lines have to be adjusted when selecting diploid breeding lines. For vine maturity and chip colour multiple regression analysis of progeny means on means of diploid and tetraploid parents revealed high coefficients of determination (R^2) for both characters. This indicates good predicting power of the performance of both diploid and tetraploid parents on the performance of their 4x.2x progenies. For vine maturity the multiple regression slope for the tetraploid parents was significantly larger than for the diploid parents, and progeny means were towards the tetraploid parents. This indicates that selection criteria for vine maturity may be less stringent at the diploid level. However, diploids may be earlier maturing than tetraploids and therefore selection criteria have to be similar at both ploidy levels. For chip colour, multiple regression slopes for diploid and tetraploid parents were similar and progeny means were towards the darkest coloured parent no matter of its ploidy level. This indicates that selection of breeding lines for chip colour can be conducted similarly at both ploidy levels.

Introduction

Selection criteria for agronomic characters in a potato breeding program at the diploid level may differ from selection criteria employed when selecting breeding lines at the tetraploid level. Differences between selection criteria are expected (1) when expression of agronomic characters is different at both ploidy levels, and/or (2) when the effect of diploid breeding lines on agronomic characters of tetraploid progenies is different from the effect of tetraploid breeding lines.

Different results from comparative investigations at both ploidy levels are reported for vine maturity. Maris (1990) found diploids to mature earlier than tetraploids, whereas Hutten et al. (submitted) found no difference for vine maturity between both ploidy levels. Chip colour is similarly expressed at both ploidy levels (Hutten et al., submitted).

In 4x.4x crosses progeny means for vine maturity generally are equal to the midparent (MP) values (Maris, 1969; Mendoza & Haynes, 1977; Tai & Tarn, 1980; Tarn & Tai, 1983; Maris, 1989). However, incidentally progeny means were found to be earlier (Johansen et al., 1967) or later (Tarn & Tai, 1983) than the MP values. Progeny means for chip colour were found to be lower (darker colour) than MP values (Cunningham & Stevenson, 1963).

The last step in a potato breeding program at the diploid level is recovery of the tetraploid level (Chase, 1963; Hermsen et al., 1987). One way of tetraploidization is unilateral sexual polyploidization crossing tetraploid varieties or breeding lines as females with synaptic diploid males producing 2n-pollen, preferably by first division restitution (FDR). The parental effects of diploids and tetraploids on characters of 4x.2x progenies can be estimated by comparing multiple regression slopes of diploid and tetraploid parents, and by comparing progeny means with MP values.

De Jong & Tai (1991) found mean chip scores of 4x.2x progenies to be lower than MP values. Multiple regression analysis of progeny means on means of diploid and tetraploid parents revealed a coefficient of determination (R^2) of 0.22. This indicates a poor predicting power of both diploid and tetraploid parents on their progenies. The tetraploid parents had a greater effect (larger slope) on chip colour of 4x.2x progenies than the diploid parents.

The present investigations were carried out to estimate the relative effects of diploid and tetraploid parents on vine maturity and chip colour of 4x.2x progenies. Combining the present results with the reported ones on the expression of vine maturity and chip colour at both ploidy levels, an indication will be obtained about selection criteria for these characters at the diploid level.

Materials and Methods

The parental effects on vine maturity and chip colour of 4x.2x progenies were investigated in separate field trials. Crosses were made between varieties and diploid F1 hybrids of the clones USW 5295.7 (B), USW 5337.3 (C), USW 7589.2

(D), and 77.2102.37 (E). The pedigrees of these diploid clones have been summarized by Jongedijk & Ramanna (1988). All mentioned diploids produce 2n-pollen predominantly through fusion of metaphase II spindles, which leads to FDR (Ramanna, 1979).

For studying the parental effects on vine maturity three early maturing varieties (Aminca, Arkula, and Sirtema), and three late maturing varieties (Astarte, Atrela, and Kardal) were crossed with three early maturing diploids (BE1014, CE1062, and CE182), and three late maturing diploids (BC1032, BC1069, and CD1042). All 36 cross combinations were successful. A maximum of 100 seeds per cross was sown and seedlings were grown in 10 x 10 cm pots in a greenhouse. A maximum of 40 clones per progeny with two fairly sized seed tubers per clone was used for the field trial. The actual progeny size ranged from 30 to 40 clones, except from Atrela x CD1042 with 25 clones. The field trial consisted of two randomized blocks. Each block contained a random half of the progenies. The field trial was conducted in 1991 near Bant on a sandy clay soil. Plant distances were 33 cm within and 75 cm between ridges. The two seed tubers of each clone were placed in the same ridge. Seed tubers were planted on April 11th and vine maturity was scored in the first week of August. Vine maturity scores were taken on a two plants/clone basis and expressed on a 1 - 9 scale (1 = very late).

For studying the parental effects on chip colour of 4x.2x progenies three varieties with a light chip colour (Hertha, Lady Rosetta, and Saturna), and three varieties with a dark chip colour (Ajax, Estima, and Nicola) were crossed with two diploids with a very light chip colour (BE1059 and EB1005), and four diploids with a dark chip colour (BC1078, BC1083, BE1021, and DB1005). Out of the 36 possible cross combinations 32 were successful. A maximum of 100 seeds per cross was sown and seedlings were grown in 9 x 9 cm pots in a greenhouse. A maximum of 40 clones per progeny with two fairly sized seed tubers per clone was used for the field trial. The actual progeny size ranged from 31 to 40 clones. The field trial consisted of two randomized blocks. Each block contained a random half of the progenies. The field trial was conducted in 1991 near Rilland on a clay soil. Plant distances were 33 cm within and 75 cm between ridges. The two seed tubers of each clone were placed in the same ridge. Seed tubers were planted on April 18th. The foliage was destroyed on September 2nd and clones were harvested on September 17th. Clones were stored at 8 °C until they were chipped in the last week of October. To estimate chip colour of a clone three 1.5 mm slices from each of three tubers were chipped and fried in 180 °C peanut oil until bubbling stopped.

Table 1. Field trial Bant. Mean vine maturity of parents and 4x.2x progenies (1 - 9 scale, 1 = very late). Letters a, b, c, d, and e indicate significant differences ($P<0.05$) between means in the same row or column. For each progeny the deviation of the progeny mean from the MP value is also presented. * Indicates that the MP value is not within the 95% confidence interval of the progeny mean.

	CE182 (9.00)^{c}	CE1062 (8.20)^{c}	BE1014 (8.17)^{c}	BC1069 (4.20)^{b}	BC1032 (3.40)^{b}	CD1042 (2.00)^{a}	OVERALL MEAN
AMINCA	6.97	6.81	6.53	5.91	5.55	4.57	6.13^{c}
(8.17)^{b}	-1.62*	-1.38*	-1.64*	-0.28	-0.24	-0.52	
ARKULA	6.92	7.63	6.25	6.26	5.70	5.38	6.35^{c}
(8.17)^{b}	-1.67*	-0.56*	-1.92*	+0.08	-0.09	+0.30	
SIRTEMA	7.18	6.97	6.45	5.33	6.45	4.91	6.22^{c}
(8.00)^{b}	-1.32*	-1.13*	-1.64*	-0.77*	+0.75*	-0.09	
ASTARTE	5.58	6.18	4.69	3.97	3.00	2.87	4.44^{b}
(3.50)^{a}	-0.67*	+0.33	-1.15*	+0.12	-0.45	+0.12	
KARDAL	5.10	6.00	4.89	3.67	3.85	3.08	4.45^{b}
(2.83)^{a}	-0.82*	+0.49*	-0.61*	+0.16	+0.74*	+0.67*	
ATRELA	4.48	5.39	4.25	3.03	3.19	3.36	4.01^{a}
(2.67)^{a}	-1.36*	-0.05	-1.17*	-0.41	+0.16	+1.03*	
OVERALL MEAN	6.03^{d}	6.52^{e}	5.52^{c}	4.75^{b}	4.64^{b}	4.09^{a}	

Chip colour was expressed on a 1 - 9 scale (1 = very dark).

Diploid and tetraploid parents used in the 4x.2x crosses were multiplied under the same conditions as the seedlings were grown. Alongside the field trials the diploid and tetraploid parents were grown separately in six randomized replications per ploidy level. Diploid parents BC1032, BC1069, CD1042, and CE1062 were replicated only 5 times, because their number of seed tubers was limited. Measurements were taken on a two plants/clone basis.

Results

Field trial Bant

The means of parents and 4x.2x progenies for vine maturity together with the deviations of the progeny mean from the MP value are presented in table 1. The

Table 2. Field trial Rilland. Mean chip colour of parents and 4x.2x progenies (1 - 9 scale, 1 = very dark). Letters a, b, c, and d indicate significant differences ($p<0.05$) between parents of the same ploidy level. For each progeny the deviation of the progeny mean from the MP value is also presented. * Indicates that the MP value is not within the 95% confidence interval of the progeny mean.

	BE1059 (8.00)^{d}	EB1005 (7.16)^{d}	BE1021 (4.50)^{c}	BC1083 (3.17)^{bc}	BC1078 (2.50)^{ab}	DB1005 (1.16)^{a}
SATURNA (6.33)^{b}	6.03 -1.14*	5.68 -1.07*	5.24 -0.18	3.95 -0.80*	4.43 +0.02	3.06 -0.69*
LADY ROSETTA (5.83)^{b}	6.26 -0.66*	6.22 -0.28	3.41 -1.76*	3.06 -1.44*	4.50 +0.34	2.97 -0.53*
HERTHA (5.83)^{b}	5.98 -0.94*	--	--	3.60 -0.90*	3.60 -0.57*	--
AJAX (2.50)^{a}	4.33 -0.92*	4.46 -0.37	3.30 -0.20	2.65 -0.19	2.90 +0.40	1.87 +0.04
ESTIMA (2.00)^{a}	4.35 -0.65*	4.33 -0.25	2.44 -0.81*	2.70 +0.12	2.93 +0.68*	1.72 +0.14
NICOLA (2.00)^{a}	3.20 -1.80*	3.73 -0.85*	3.25 0.00	1.95 -0.64*	2.48 +0.23	--

Table 3. Relationships for vine maturity and chip colour between progeny means (y) and tetraploid (x_1) and diploid (x_2) parents. * Indicates a significant difference ($P<0.05$) between slopes of x_1 and x_2 (NS = not significantly different).

Character	$y = a + bx_1 + cx_2$	SLOPES	R^2
Field trial Bant			
Vine maturity	$y = 1.38 + 0.38x_1 + 0.30x_2$	*	0.87
Field trial Rilland			
Chip colour	$y = 0.48 + 0.39x_1 + 0.38x_2$	NS	0.85

MP value is not within the 95 % confidence interval of the progeny mean for 21 progenies. Further analysis of these 21 progenies reveals both positive and negative deviations in 4x(early).2x(late) and 4x(late).2x(early), only negative ones in

4x(early).2x(early), and only positive ones in 4x(late).2x(late).

Results from multiple regression analysis are shown in table 3. The coefficient of determination (R^2) found for vine maturity was 0.87 and the slope for the tetraploid parents was significantly larger than the slope for the diploid parents.

Field trial Rilland

The means of parents and 4x.2x progenies for chip colour together with the deviations of the progeny mean from the MP value are presented in table 2. The MP value is not within the 95 % confidence interval of the progeny mean for 18 progenies. Further analysis of these 18 progenies reveals both negative and positive deviations in 4x(dark).2x(dark), and only negative ones in 4x(light).2x(light), 4x(light).2x(dark) and 4x(dark).2x(light).

Results from multiple regression analysis are shown in table 3. The coefficient of determination for chip colour was 0.85 and the slopes for diploid and tetraploid parents were nearly equal.

Discussion

High coefficients of determination were estimated for both vine maturity and chip colour. This indicates that vine maturity and chip colour of 4x.2x progenies can well be predicted from the performance of the parents regarding these characters. De Jong & Tai (1991) estimated a low coefficient of determination for chip colour. These different results are probably due to the choice of parents used in the investigation. The more divergent the parents are for the character to be investigated, the higher the expected coefficient of determination. This indicates that for investigating the coefficient of determination, parents have to be chosen which differ greatly for the character to be investigated. Otherwise no conclusions can be drawn from the estimated value for the coefficient of determination.

For vine maturity the multiple regression slope for the tetraploid parents was significantly larger than that of the diploid parents. This indicates a greater effect of the tetraploid parents on vine maturity of 4x.2x progenies. The negative deviations of the progeny mean from the MP value in 4x(early).2x(early) and the positive deviations in 4x(late).2x(late) are expected because both parents are near to the maximum and minimum levels of expression respectively. In 4x(early).2x(late) the MP values generally are within the 95 % confidence interval of the progeny mean. In 4x(late).2x(early), deviations of the progeny mean from the MP value generally are towards the tetraploid parents. Maris (1990) found diploids to mature earlier than tetraploids. If corrections are made for vine maturity scores of the diploid parents, the MP values will become lower. As a result negative deviations in 4x(late).2x(early) will become less negative, and deviations in 4x(early).2x(late) will become slightly positive. In both types of progenies the deviations of the progeny mean from the MP value are now towards the tetraploid parents. No matter whether or not diploids are earlier maturing than tetraploids, the conclusion must be that tetraploid parents have a greater effect on vine maturity of 4x.2x progenies than diploid parents, while progeny means are towards the tetraploid parents.

For chip colour the multiple regression slopes for diploid and tetraploid parents were similar. This indicates that diploid and tetraploid parents have an equal effect on chip colour of 4x.2x progenies. This result is not in agreement with results reported by De Jong & Tai (1991). They found tetraploids to have a greater effect on chip colour of 4x.2x progenies. The negative deviations of the progeny mean from the MP value found for 4x(light).2x(light) crosses are expected because both parents are near the maximum level of expression. For 4x(light).2x(dark) and 4x(dark).2x(light) crosses the deviations from the MP values are also negative. This indicates that progeny means are towards the parents with the darker chip colour no matter of their ploidy level. For 4x(dark).2x(dark) crosses the MP values generally are within the 95 % confidence interval of the progeny mean, whereas positive deviations of the progeny mean from the MP value were expected. A dark chip colour seems to be a relatively dominant inherited character. This is in agreement with results found by De Jong & Tai (1991) and with results found in 4x.4x progenies (Cunningham & Stevenson, 1963).

Conclusions

A greater effect of tetraploid parents on vine maturity of 4x.2x progenies indicates that selection criteria at the diploid level can be less stringent than at the tetraploid level. However, diploids are perhaps earlier maturing than tetraploids (Maris, 1990). This indicates that selection criteria for vine maturity at the diploid level have to be more stringent than at the tetraploid level. These opposite effects on selection criteria may well neutralize each other. This would imply similar selection criteria for vine maturity at both ploidy levels.

A similar expression of chip colour at both ploidy levels (Hutten et al., submitted) combined with an equal effect of diploid and tetraploid parents on chip colour of 4x.2x progenies implies similar selection criteria for chip colour in potato breeding programs at both ploidy levels.

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CHAPTER 7

COMPARATIVE PERFORMANCE OF FDR AND SDR PROGENIES FROM RECIPROCAL 4X-2X CROSSES IN POTATO

R.C.B. HUTTEN, M.G.M. SCHIPPERS, J.G.TH. HERMSEN &
M.S. RAMANNA

(THEORETICAL AND APPLIED GENETICS, IN PRESS)

Summary

Numerically unreduced (2n) gametes from first division restitution (FDR) are considered to be superior to 2n-gametes from second division restitution (SDR) because they transfer a larger proportion of the total parental heterozygosity and epistasis intact to the tetraploid progeny. This supposed superiority was investigated by comparing 12 sets of reciprocal 4x-2x crosses. Each diploid parent used in a reciprocal set produced 2n-pollen by FDR and 2n-eggs by SDR. The investigation comprised six agronomic characters. FDR progenies (from 4x.2x) were found to have higher mean yields due to more and bigger tubers. The overall progeny mean for under water weight of FDR progenies was significantly higher than the overall progeny mean of SDR progenies (from 2x.4x). However, the absolute difference found between both overall progeny means is too small to be of practical significance. No differences between FDR and SDR progeny means were found for vine maturity and chip colour. Besides the progeny mean, within-progeny variation is important in potato breeding. For vine maturity a higher within-progeny variation was detected in SDR progenies, whereas within-progeny variations for yield, under water weight and chip colour were not different in FDR and SDR progenies. For vine maturity SDR 2n-gametes are concluded to be superior to FDR 2n-gametes, because with the same progeny means of FDR and SDR progenies the within-progeny variation was higher in SDR progenies. So the assumed superiority of FDR 2n-gametes was confirmed for yield but was not observed for vine maturity, under water weight and chip colour.

Introduction

The final step in a potato breeding program via the diploid level is recovery of the tetraploid level (Chase, 1963; Hermsen et al., 1987). A widely advocated method to return to the tetraploid level is sexual polyploidization by means of 2n-gametes, that result from meiotic nuclear restitution mechanisms. Nuclear restitution may occur either during the first or during the second meiotic division and the resulting

2n-gametes are called first division restitution (FDR) and second division restitution (SDR) 2n-gametes respectively. The genetic implications of these two types of 2n-gametes are very different. All parental heterozygous loci from centromere to first crossover and 50 % of those that are present distally will be heterozygous in FDR 2n-gametes. On the other hand, all parental heterozygous loci from centromere to first crossover will be homozygous and all those that are present distally will be heterozygous in SDR 2n-gametes. Assuming a random distribution of heterozygous loci along the parental chromosomes, the retention of heterozygosity in 2n-gametes of synaptic diploids is expected to be about 80 % in FDR 2n-gametes and nearly 40 % in SDR 2n-gametes (Hermsen, 1984). Experimental data on retained heterozygosity come very close to these theoretically estimated percentages (Douches & Quiros, 1988a; Douches & Quiros, 1988b; Jongedijk et al., 1991a). Besides heterozygosity, also epistatic gene interactions are largely maintained in FDR 2n-gametes and can be transmitted to tetraploid progenies via 2x.2x or 4x.2x crosses. As heterozygosity and epistatic interactions may greatly contribute to a good performance of potato, FDR 2n-gametes are considered to be superior to SDR 2n-gametes. For yield this superiority of FDR 2n-gametes in 4x.2x crosses was demonstrated by Mok & Peloquin (1975). Analogous investigations involving other agronomic characters have not been reported, as far as the authors are aware.

In comparing the effects of FDR and SDR Mok & Peloquin (1975) used different parents. So genotypic differences between parents may have influenced their results. Because 2n-pollen in synaptic diploids mostly result from FDR (Watanabe & Peloquin, 1992) and 2n-eggs almost exclusively from SDR (Jongedijk, 1985; Stelly & Peloquin, 1986; Werner & Peloquin, 1987; Douches & Quiros, 1988b; Jongedijk et al., 1991b; Werner & Peloquin, 1991), a better way of testing the assumed superiority of FDR 2n-gametes is to compare the performance of reciprocal 4x-2x crosses. However, such a comparison is only feasible when cytoplasmic effects on agronomic characters and sex differences in genetic recombination are absent.

For yield, differences between progenies from reciprocal potato crosses have been observed and may even be large. These reciprocal differences seem to result from divergent parental maturity rather than from differences between the cytoplasms involved (Sanford & Hanneman, 1982; Staub et al., 1982), although results by Maris (1989) do not point to such conclusion. In addition investigations on SDR progenies from reciprocal 4x-2x crosses involving tetraploid parents with cytoplasm of *Solanum tuberosum* L. and diploid parents with cytoplasm of *Solanum phureja* Juz. et Buk., did not display any reciprocal differences for yield (Kidane-Mariam & Peloquin, 1974; Kidane-Mariam & Peloquin, 1975). In reciprocal cross progenies between long-day adapted parents no consistent reciprocal differences were found for maturity (Engel, 1956; Tarn & Tai, 1977; Maris, 1989) and specific gravity (Engel, 1956; Hoopes et al., 1980; Maris, 1989). So cytoplasmic effects are not expected to interfere with the comparative research of FDR and SDR by means of reciprocal 4x-2x crosses.

Sex differences in chiasma and bivalent frequencies (Jongedijk & Ramanna, 1989), and in genetic recombination (Douches & Quiros, 1988b; Jongedijk et al., 1991a) were not observed in diploid potato clones. So the comparison of reciprocal 4x-2x progenies to estimate differences between FDR and SDR 2n-gametes is a correct approach.

Results on reciprocal 4x-2x progenies have been reported before (Kidane-Mariam & Peloquin, 1974; Kidane-Mariam & Peloquin, 1975; Mendiburu & Peloquin, 1977). The 4x.2x(FDR) progenies were higher yielding than their reciprocals. These reciprocal differences were not associated by the authors with different restitution mechanisms in micro- and megasporogenesis because at that time it was not known that 2n-egg formation in synaptic diploid potatoes is almost exclusively through SDR.

The present investigation was carried out to compare the performance of tetraploid progenies from sets of reciprocal 4x-2x crosses involving FDR and SDR 2n-gametes from the diploid parent in each set. Comparisons between FDR and SDR progenies were made for the agronomic characters yield, yield components, vine maturity, chip colour, and under water weight. Results indicate whether the superior contribution to yield of FDR 2n-gametes compared to SDR 2n-gametes also applies for other agronomic characters. A good comparison between FDR and SDR progenies for more than yield alone may answer the question wether it is justified to select only for FDR 2n-gametes in a diploid potato breeding program.

Materials and Methods

In order to obtain equivalent tetraploid progenies from FDR and SDR 2n-gametes, reciprocal 4x-2x crosses were made. Diploid parents were selected among F1 hybrids of the clones USW 5295.7 (B), USW 5337.3 (C), USW 7589.2 (D), and 77.2102.37 (E). The pedigrees of these clones have been summarized by Jongedijk & Ramanna (1988). Meiosis was studied in the diploid F1 hybrids producing high frequencies of 2n-pollen. Synaptic clones producing 2n-pollen predominantly through fusion of metaphase II spindles, which leads to FDR (Ramanna, 1979), were selected. For meiotic studies, flower buds were fixed in a 1:3:6 solution of formalin, proponic acid (saturated with ferric acetate), and ethanol respectively. Small parts of the anthers were squashed in 1 % aceto-carmine. The diploid F1 hybrids were then screened for 2n-egg production using seed set in 2x.4x test crosses as a criterion. Nine F1 hybrids were found to set berries with seeds in the test crosses.

These nine selected diploids were reciprocally crossed with the varieties Alcmaria, Certa, Escort, and Hertha. All 36 reciprocal sets of 4x-2x crosses were successful. Seed set in all 36 4x.2x crosses was comparible to seed set in 4x.4x crosses, whereas seed set was limited in several 2x.4x cross combinations. Total numbers of seeds and numbers of seeds per berry obtained from 2x.4x crosses are presented in table 1. The frequency of triploid plants in such progenies is expected to be very low due to the 'triploid block' (Marks, 1966). However, in 2x.4x cross combinations with a low seed set per berry these few triploid plants may form a rather large proportion of the progeny. Therefore we decided to restrict this investigation to the reciprocal progenies involving the diploid clones BE1050, CD1015, and CD1045 which gave a relatively high seed set in both directions of the 4x-2x crosses. In all 2x.4x cross combinations these three diploids gave more than 15 seeds per berry (table 1).

A maximum of 150 seeds per cross was sown and maximally 100 seedlings were grown in 11 x 11 cm pots in a screenhouse. All seedlings which formed two fairly sized seed tubers were included in the field trial. The actual number of clones per progeny included in the field trial is shown in table 2. The field trial consisted of two randomized blocks. Each block contained a random half of the progenies. All measurements were made on a two plants/clone basis. The field trial was conducted in 1991 near Wageningen on a clay soil. Plant distances were 40 cm within and 75 cm between ridges. The two seed tubers of each clone were placed in the same ridge. Seed tubers were planted on April 18th and the foliage was destroyed by spraying 'Reglone' on September 18th.

The characters estimated were:

1. yield in g of all tubers with a diameter of more than 2 cm;

Table 1. Total number of seeds (S) and number of seeds per berry (S/B) from 2x.4x crosses.

	ALCMARIA		CERTA		ESCORT		HERTHA	
	S	S/B	S	S/B	S	S/B	S	S/B
BC1082	4	0.3	16	0.8	7	0.2	21	0.7
BC1083	30	2.5	45	5.0	55	3.2	30	3.3
BE1050	345	34.5	400	21.1	345	19.2	581	29.1
BE1060	71	4.7	109	4.4	78	3.0	96	3.6
CD1015	137	15.2	320	18.8	587	16.3	790	21.9
CD1042	82	3.7	23	3.8	26	3.7	67	5.6
CD1045	201	20.1	671	23.1	529	20.3	1404	34.2
CD1047	43	2.7	12	1.2	29	1.8	81	3.9
CE69	20	1.7	27	2.3	67	3.4	46	2.6

Table 2. Total number of clones used in the field trial of FDR and SDR progenies obtained from reciprocal 4x-2x crosses.

PARENTS		TOTAL NUMBER OF CLONES	
4x	2x	FDR PROGENY FROM 4x.2x	SDR PROGENY FROM 2x.4x
ALCMARIA	BE1050	66	48
ALCMARIA	CD1015	76	70
ALCMARIA	CD1045	72	52
CERTA	BE1050	49	55
CERTA	CD1015	61	48
CERTA	CD1045	56	46
ESCORT	BE1050	41	44
ESCORT	CD1015	65	53
ESCORT	CD1045	69	53
HERTHA	BE1050	51	54
HERTHA	CD1015	70	47
HERTHA	CD1045	65	60
GRAND TOTAL OF CLONES		741	630

2. number of tubers bigger than 2 cm in diameter;
3. tuber size on a 1 - 9 scale (1 = very small);
4. vine maturity on a 1 - 9 scale (1 = very late);
5. under water weight converted to the under water weight of 5000 g potatoes;
6. chip colour on a 1 - 9 scale (1 = very dark).

Clones were stored at about 9°C until they were chipped in the second week of

February. To estimate chip colour of a clone three 1.5 mm thick slices from each of three tubers were chipped and fried in 180°C peanut oil until bubbling stopped.

Results

The means of FDR (4x.2x) and SDR (2x.4x) progenies for yield, tuber number, and tuber size are presented in table 3. All FDR progenies are higher yielding than their SDR reciprocals, although the differences are significant in four sets of reciprocals only. The overall mean yield shows a highly significant superiority of the FDR progenies. Overall means for tuber number and tuber size show that these higher yields of FDR progenies are due to a combination of more and bigger tubers.

Table 3. Mean yield (g), tuber number, and tuber size (1 - 9 scale, 1 = very small) of two clonal plants in FDR (4x.2x) and SDR (2x.4x) progenies. * and ** indicate significant differences between FDR and SDR progenies at $p<0.05$ and $p<0.01$ respectively.

PARENTS		YIELD		TUBER NUMBER		TUBER SIZE	
4x	2x	FDR	SDR	FDR	SDR	FDR	SDR
ALCMARIA	BE1050	2158	1791	25.5	21.2	6.97	6.77
ALCMARIA	CD1015	2404	1995*	43.3	39.6	6.66	6.30
ALCMARIA	CD1045	2321	2031	35.4	34.4	6.60	6.21
CERTA	BE1050	2060	1787	29.9	29.3	6.39	6.31
CERTA	CD1015	1980	1614	45.6	40.0	5.69	5.31
CERTA	CD1045	1921	1517*	44.6	43.0	5.39	4.89
ESCORT	BE1050	2442	2317	26.4	24.4	7.02	7.11
ESCORT	CD1015	2470	2118	33.0	34.3	6.94	6.70
ESCORT	CD1045	2720	2142**	44.1	41.3	6.32	5.94
HERTHA	BE1050	2598	2216	28.1	27.6	6.90	6.59
HERTHA	CD1015	2530	1768**	44.8	38.1*	6.41	5.70*
HERTHA	CD1045	2279	2266	43.8	44.1	5.98	6.00
OVERALL MEAN		2332	1973**	37.7	35.1*	6.43	6.16**

Progeny means for vine maturity, under water weight, and chip colour are presented in table 4. For all three agronomic characters no significant differences are found within individual sets of reciprocal progenies. A significant difference between overall means was found for under water weight only.

Besides the progeny mean, within progeny variation is important in plant

Table 4. Mean vine maturity (1 - 9 scale, 1 = very late), under water weight (converted to the under water weight of 5000 g potatoes), and chip colour (1 - 9 scale, 1 = very dark) in FDR (4x.2x) and SDR (2x.4x) progenies. * and ** indicate significant differences between FDR and SDR progenies at $p<0.05$ and $p<0.01$ respectively.

PARENTS		VINE MATURITY		UNDER WATER WEIGHT		CHIP COLOUR	
4x	2x	FDR	SDR	FDR	SDR	FDR	SDR
ALCMARIA	BE1050	6.33	6.29	388	379	4.66	4.85
ALCMARIA	CD1015	5.95	5.79	416	395	5.07	4.94
ALCMARIA	CD1045	5.56	5.62	425	411	4.99	5.10
CERTA	BE1050	4.80	5.05	449	441	5.06	5.30
CERTA	CD1015	4.07	4.06	420	410	4.56	4.81
CERTA	CD1045	4.11	4.07	441	451	4.11	4.51
ESCORT	BE1050	5.07	5.16	380	379	5.45	4.98
ESCORT	CD1015	4.72	4.72	386	368	4.73	4.94
ESCORT	CD1045	4.49	4.64	392	405	4.48	4.98
HERTHA	BE1050	5.20	5.24	444	422	5.45	5.41
HERTHA	CD1015	4.31	4.57	436	421	4.94	5.15
HERTHA	CD1045	4.38	4.50	444	437	4.40	4.78
OVERALL MEAN		4.94	5.00	418	410*	4.81	4.98

Table 5. Mean variances for yield, vine maturity, under water weight, and chip colour in FDR (4x.2x) and SDR (2x.4x) progenies. * Indicates significant differences ($P<0.05$) between FDR and SDR progenies.

CHARACTER	MEAN VARIANCE	
	FDR PROGENIES	SDR PROGENIES
YIELD	542947	432382
VINE MATURITY	1.19	1.71*
UNDER WATER WEIGHT	2047	2222
CHIP COLOUR	1.42	1.38

breeding. Mean variances for yield, vine maturity, under water weight, and chip colour of FDR and SDR progenies are presented in table 5. Mean variances of FDR and SDR progenies differ significantly for vine maturity only.

Discussion

The mean yield of the FDR (4x.2x) progenies is higher than the mean yield of the

SDR (2x.4x) progenies in all 12 sets of reciprocal 4x-2x crosses. This higher yield of FDR progenies is significant in four sets. In addition the difference between the overall mean yield of the FDR progenies and that of the SDR progenies is highly significant (Table 3). These results confirm the superiority for yield of FDR 2n-gametes vs. SDR 2n-gametes as found by Mok & Peloquin (1975), and are similar to results reported for reciprocal 4x-2x crosses wherein the diploid parents produced FDR 2n-pollen (Kidane-Mariam & Peloquin, 1974; Kidane-Mariam & Peloquin, 1975; Mendiburu & Peloquin, 1977). The higher yield of progenies obtained with FDR 2n-gametes is due to both more and bigger tubers. This is of interest because the bigger tuber size in FDR progenies indicates that the higher yields go with a good marketability.

For under water weight a significant difference between the reciprocal progenies was found for the overall mean only. However, the absolute difference between the overall means is very small. In fact it is too small to be of practical significance in a breeding program. For vine maturity and chip colour no significant differences between the reciprocal 4x-2x progenies were found. When for under water weight, vine maturity and chip colour differences between progenies derived from FDR and SDR 2n-gametes are absent or of no practical significance, FDR 2n-gametes are not superior for these characters. The results for vine maturity are important in connection with the results for yield. They show that the higher yield of FDR progenies is not due to a later maturity of these progenies.

Because the FDR and SDR progenies are only slightly different for mean vine maturity, mean under water weight and mean chip colour, a higher within-progeny variation for these characters may favour the use of SDR 2n-gametes. SDR 2n-gametes retain nearly 40 % of the parental heterozygosity, whereas FDR 2n-gametes retain about 80 %. Therefore SDR 2n-gametes may be genetically dissimilar from the parent and among each other, whereas FDR 2n-gametes are expected to be genetically more similar to the parent and each other. So FDR 2n-gametes contribute greatly to the uniformity of the progeny and lower within-progeny variations are expected. No experimental data on within-progeny variation are reported. There is only one reference (Mendiburu & Peloquin, 1977) reporting a remarkable higher degree of within-family uniformity for vegetative characters in the field in 4x.2x progenies than in 2x.4x progenies. As shown in table 5 for yield, under water weight and chip colour, no significant differences in within-progeny variation between FDR and SDR progenies were found. For vine maturity the within-progeny variation of SDR progenies was significantly higher than that of

FDR progenies. So for vine maturity SDR 2n-gametes are more desirable than FDR 2n-gametes.

The different amounts of heterozygosity retained in FDR and SDR 2n-gametes created the expectation that FDR and SDR progenies would differ both in progeny mean and within-progeny variation for all investigated characters. However, this investigation just revealed a different progeny mean only for yield and a different within-progeny variation only for vine maturity. These findings need further discussion.

For yield only a difference in progeny mean was found. Generally within equivalent progenies a higher mean yield accompanies a greater within-progeny variation. So the higher mean yield of the FDR progenies and the higher uniformity of FDR 2n-gametes have opposite effects on the within-progeny variation. That may be the reason why no difference in within-progeny variation for yield between FDR and SDR progenies was observed.

For vine maturity a higher within-progeny variation was found in SDR progenies, whereas progeny means of FDR and SDR progenies were similar. These results may be explained by assuming an additive inheritance of most of the genes involved in the expression of this character.

For under water weight and chip colour no differences were found between FDR and SDR progenies neither for progeny mean nor for the within-progeny variance. A tentative explanation might be that all clones are homozygous for most of the genes involved in the expression of these characters. Alternatively, it is likely that most of these genes are not located at very distal or proximal positions on the chromosome arms. Genes on these chromosomal positions are not affected very differently by SDR and FDR. Mendiburu & Peloquin (1979) explained that for loci at a genetic distance where 66.7 percent of the arms show a single chromatid exchange the genetic consequences of FDR and SDR are equal.

Conclusions

The superiority of FDR 2n-gametes in respect to SDR 2n-gametes was confirmed for yield. The higher yields of FDR progenies are not associated with a reduced marketability or a later vine maturity. For other important agronomic characters such as vine maturity, under water weight and chip colour no considerable differences between means of FDR and SDR progenies were found. However, for vine maturity the within-progeny variation of SDR progenies was significantly larger than that of FDR progenies. This indicates that for this character SDR 2n-gametes are more desirable than FDR 2n-gametes, perhaps due to additivity of the genes involved.

For a potato breeding program the positive effect of FDR 2n-gametes on yield is of more importance than the positive effect of SDR 2n-gametes on vine maturity. So FDR 2n-gametes are the type of 2n-gametes to be preferred in recovering the tetraploid level in the final step of a potato breeding program via the diploid level.

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CHAPTER 8

FINAL REMARKS

The advantages of potato breeding via the diploid level and reports on high yields of potato progenies derived from 4x.2x crosses have drawn breeders' attention to the profitability of using diploid stocks in potato breeding. The investigations reported in this thesis were initiated to give breeders an idea about the way in which different steps in a potato breeding program via the diploid level can be managed.

Haploidization of the autotetraploid potato, the first step in a breeding program via the diploid level, is a laborious step even when the best dihaploid inducer (IVP 101) is used. Haploidization generally results in diploid breeding lines which are little vigorous and male sterile due to severe inbreeding effects. The widespread male sterility in dihaploids greatly hampers inter-dihaploid matings.

Wild and primitive tuber-bearing *Solanum* species are invaluable sources of resistance genes, allelic diversity and male fertility. Evaluated accessions of many diploid *Solanum* species and hybrids between *Solanum* species and diploid *S. tuberosum* are available from several gene banks and research institutes in the world. The use of wild species in a potato breeding program is a long-term activity involving many successive backcrosses to diploid forms of the cultivated potato. In all backcross generations selection for desirable and against undesirable characters from the wild species has to be applied. Characteristic undesirable traits from wild species are adaptation to short days, long stolons, rapid tuber discoloration and high levels of glycoalkaloids. Selection for most agronomic characters has to be postponed to the first backcross populations because plants of the wild species and even *S. tuberosum*-wild species hybrids tuberize very poorly under Dutch field conditions.

This thesis points out that selection criteria suitable for agronomic characters at the diploid level may be different from those which are useful in selecting breeding lines at the tetraploid level. Such differences may be due to a different expression of characters at the diploid and tetraploid level, and/or to different effects of diploid and tetraploid parents on tetraploid progenies. Especially the structurally lower yield of diploids and the ineffectiveness of direct selection for yield in diploid breeding lines needs special attention of breeders, who are used to select strongly against low yields because a high marketable yield is one of the most important characters for newly bred varieties.

Sexual polyploidization to recover the tetraploid level implies selection for the additional character 2n-gamete formation. First division restitution (FDR) 2n-gametes are to be preferred. Cytological studies on mechanisms of 2n-gamete formation require great expertise and are too laborious to become a routine in potato breeding programmes. Fortunately 2n-pollen mostly results from FDR, and 2n-pollen production is easily estimated from the proportion of 'big' pollen grains. Because the total retention of heterozygosity in FDR 2n-gametes can be calculated to be about 80 %, FDR 2n-gametes are expected to be genetically similar to the parental clone. This led to the expectation that the selection of diploid parents for the use in 4x.2x crosses comes close to the selection of gametes. For monogenic and dominant characters on loci proximal to the centromere this holds true indeed. For such characters on loci distal to the centromere, up to 50 % of the offspring will lose the dominant phenotype. For polygenic characters the parental effect of diploids on 4x.2x progenies does not appear to be considerably larger than the parental effect of tetraploids. So the probability that good phenotypes of selected diploid parents are retained in the tetraploid offspring is hardly greater than it is for those of tetraploid parents.

The use of potato breeding via the diploid level as an additional tool to potato breeding programmes looks promising, even though the release of new varieties derived from diploid parents is still very limited. These limited results are mainly due to the small efforts put into diploid potato breeding by commercial breeders. A large-scale diploid breeding program based on a wide genetic diversity is needed to evaluate the true merits of potato breeding via the diploid level.

SUMMARY

The investigations reported in this thesis are related to different steps in a potato breeding program via the diploid level. They deal with haploidization by prickle pollination, selection at the diploid level and sexual polyploidization.

Haploidization

The creation of a diploid gene pool is the first step in a potato (*Solanum tuberosum* L.) breeding program via the diploid level. Dihaploids can be obtained routinely from unfertilized egg cells via pseudogamy, i.e. through prickle pollination of tetraploid potatoes by clones of the diploid species *Solanum phureja* Juz. et Buk., selected for high dihaploid induction ability and homozygosity of the dominant marker 'embryo-spot'.

In spite of the significant seed parent x pollinator interaction estimated, the new pollinator IVP 101 was found to have a significantly higher dihaploid induction ability than the well-known pollinators IVP 35 and IVP 48. The overall mean number of dihaploids induced in 28 different tetraploids by IVP 101 amounted to 255 dihaploids per 100 berries, whereas the overall means for IVP 35 and IVP 48 were 152 and 170 dihaploids per 100 berries respectively. Significant differences in dihaploid production ability between seed parents were also found. Their overall mean dihaploid production ability ranged from 32 dihaploids per 100 berries (Agria) to 463 (VE 66-295). For individual seed parent x pollinator combinations, dihaploid production ranged from 11 dihaploids per 100 berries (Matilda x IVP 48) to 742 (Certa x IVP 101).

Dihaploid populations from 31 varieties or breeding lines were evaluated for occurrence and frequency of mutant phenotypes, tuberization, flowering, pollen stainability, 2n-pollen production and resistance to Ro-1. Dihaploid populations were found to differ greatly for all these characters. Overall results showed that 60 % of 5377 dihaploids investigated tuberized, ranging from 81 % for dihaploids from Eba to 22 % for dihaploids from AM 66-42. This low frequency found for dihaploids from AM 66-42 was primarily caused by the occurrence of lethal mutants (62 %). Of 825 vigorous dihaploids investigated 68 % flowered, and only 27 and 4 % had a pollen stainability higher than 20 and 60 % respectively. Among the 222 dihaploids with over 20 % pollen stainability, 23 % produced some amount

of 2n-pollen.

The usefulness of potato varieties and tetraploid breeding lines as starting material in a potato breeding program via the diploid level is defined by the combination of their dihaploid production ability, their number and dose of (sub)lethal alleles, and the tuberizing and flowering ability and fertility of their dihaploids.

Selection at the diploid level

Selection criteria for agronomic characters in a potato breeding program at the diploid level may differ from selection criteria employed when selecting breeding lines at the tetraploid level. Differences between selection criteria are expected , (1) when expression of the characters is different at both ploidy levels and/or (2) when the parental effects of diploid breeding lines on the characters of tetraploid progenies is different from the parental effects of tetraploid breeding lines.

The expression of six agronomic characters at the diploid and tetraploid level was investigated by comparing sets of diploid and tetraploid progenies, each set derived from the same 2x.2x cross. Tetraploid progenies had significantly higher yields than diploid progenies as a result of bigger tubers only. Tuber numbers of tetraploid progenies were even smaller than those of diploid progenies. Diploid progenies had significantly higher under water weights than tetraploid progenies. Vine maturity and chip colour were similarly expressed at both ploidy levels.

Effects of diploid and tetraploid parents on tetraploid progenies were compared in progenies from 4x.2x crosses by multiple regression analysis of progeny means on parental means. For yield, tuber number and tuber size low coefficients of determination (R^2) were found. Multiple regression slopes for yield were highly variable and mostly negative for parents of both ploidy levels. The slopes for tuber number and tuber size were low except the one for tuber number of tetraploid parents. This indicates that yields of 4x.2x progenies can not be predicted from yield and/or yield components of the diploid parents. Multiple regression analysis for under water weight, vine maturity and chip colour revealed high coefficients of determination. For under water weight the diploid parents had a significantly greater effect on the progenies, and progeny means were generally towards the diploid parents. For vine maturity the tetraploid parents had a significantly larger effect on the progenies, and progeny means were towards the tetraploid parents. The parental effects of diploids and tetraploids for chip colour were similar and progeny means were towards the parent with the darker chip colour, no matter of its ploidy level.

Combining the results of these investigations on expression of agronomic characters on the diploid and the tetraploid level and parental effects on 4x.2x progenies, the following conclusions concerning selection criteria at the diploid level are drawn:

1. direct selection for yield or yield components among diploid breeding lines is ineffective;
2. selection for under water weight has to be conducted more stringently among diploid than among tetraploid breeding lines;
3. for vine maturity selection criteria may be less stringent at the diploid level;
4. selection of breeding lines for chip colour can be conducted similarly at both ploidy levels.

Sexual polyploidization

The supposed superiority of first division restitution (FDR) 2n-gametes versus second division restitution (SDR) 2n-gametes was investigated by comparing sets of reciprocal 4x-2x crosses for several agronomic characters. Each diploid parent used in a reciprocal set produced 2n-pollen by FDR and 2n-eggs by SDR. Hence the reciprocal 4x-2x crosses yielded excellent material for comparing the effects of FDR and SDR 2n-gametes.

FDR progenies were found to have higher mean yields due to more and bigger tubers. For vine maturity, under water weight and chip colour no considerable differences between means of FDR and SDR progenies were found. Besides the progeny mean, within-progeny variation is important in breeding. For vine maturity a higher within-progeny variation was detected in SDR progenies. So for yield FDR 2n-gametes may be concluded to be superior to SDR 2n-gametes, whereas for vine maturity SDR 2n-gametes are superior. For a potato breeding program the positive effect of FDR 2n-gametes on yield is more important than the positive effect of SDR 2n-gametes on vine maturity. So FDR 2n-gametes are the type of 2n-gametes to be preferred in sexual polyploidization.

SAMENVATTING

De inhoud van dit proefschrift omvat de resultaten van onderzoek, dat in directe relatie staat tot verschillende stappen van een aardappelveredelingsprogramma via het diploïde (2n = 2x = 24) niveau. Globaal bestaat het proefschrift uit drie onderdelen, namelijk: haploïdisatie door prikkelbestuiving, selectie op diploïd niveau en sexuele polyploïdisatie.

Haploïdisatie

De opbouw van diploïd basismateriaal is de eerste stap van een aardappelveredelingsprogramma via het diploïde niveau. Dihaploïden kunnen routinematig worden geproduceerd uit onbevruchte eicellen van tetraploïde (2n = 4x = 48) aardappel (*Solanum tuberosum* L.) door schijnbevruchting (pseudogamie) na prikkelbestuiving met klonen van de diploïde soort *Solanum phureja* Juz. et Buk., die zijn geselecteerd op een hoog dihaploïden-inducerend vermogen en homozygotie voor het dominante signaalkenmerk 'kiemstip'.

Ondanks de significante interactie tussen zaadouders en bestuivers bleek de nieuwe bestuiver IVP 101 een significant hoger dihaploïden-inducerend vermogen te bezitten dan de bekende bestuivers IVP 35 en IVP 48. Gemiddeld over alle 28 verschillende zaadouders werden er na prikkelbestuiving met IVP 35, IVP 48 en IVP 101 respectievelijk 152, 170 en 255 dihaploïden per 100 bessen verkregen. Ook bleken de zaadouders significant te verschillen in dihaploïden-producerend vermogen, dat uiteenliep van 32 dihaploïden per 100 bessen bij het ras Agria tot 463 bij de geniteur VE 66-295. Bij de individuele combinaties van zaadouders en bestuivers variëerde het aantal dihaploïden per 100 bessen van 11 (Matilda x IVP 48) tot 742 (Certa x IVP 101).

Dihaploïdenpopulaties van 31 rassen en geniteurs werden beoordeeld op het voorkomen van mutanten, alsook op knolzetting, bloei, pollenkleurbaarheid, 2n-pollenproductie en Ro-1-resistentie. Voor elk van deze eigenschappen werden grote verschillen gevonden. In totaal bleek van de 5377 onderzochte dihaploïden 60 % knol te zetten, variërend van 81 % van de dihaploïden van Eba tot 22 % van de dihaploïden van de geniteur AM 66-42. De lage frequentie gevonden voor dihaploïden van AM 66-42 was primair het gevolg van het hoge percentage (62 %) letale mutanten onder deze dihaploïden. Van de 825 groeikrachtige dihaploïden, die werden onderzocht op bloembiologische eigenschappen, bloeiden er 561 (68 %), terwijl een pollenkleurbaarheid boven de 20 % slechts werd waargenomen bij 222 (27 %) van de 825 dihaploïden. Van deze 222 dihaploïden hadden er maar 33 een pollenkleurbaarheid boven de 60 %, terwijl er bij 51 de productie van enig 2n-pollen werd waargenomen.

De bruikbaarheid van aardappelrassen en tetraploïde geniteurs als uitgangsmateriaal voor een veredelingsprogramma via het diploïde niveau wordt bepaald door de optelsom van het dihaploïden-producerend vermogen, het aantal en de dosis van (sub)letale genen, en knolzetting, bloei en fertiliteit van hun dihaploïden.

Selectie op diploïd niveau

Criteria voor selectie op landbouwkundige eigenschappen in diploïde aardappelpopulaties kunnen verschillen van de toegepaste criteria voor selectie van geniteurs uit tetraploïde populaties. Dergelijke verschillen zijn te verwachten voor eigenschappen waarvan de expressie verschillend is op beide ploïdieniveaus, alsook voor eigenschappen waarbij de effecten van diploïde en tetraploïde ouders op de tetraploïde nakomelingschappen verschillen.

De expressie van zes landbouwkundige eigenschappen op diploïd en op tetraploïd niveau werd onderzocht door vergelijking van diploïde en tetraploïde nakomelingschappen uit elk van zeven 2x.2x-kruisingen. De tetraploïde nakomelingschappen bleken als gevolg van grotere knollen gemiddeld significant hogere opbrengsten te hebben dan diploïde nakomelingschappen, terwijl de gemiddelde knolaantallen bij de tetraploïde nakomelingschappen juist het laagst waren. Onderwatergewichten waren bij de diploïde nakomelingschappen gemiddeld significant hoger dan bij de tetraploïde nakomelingschappen. Voor loofrijpheid en chipskleur was de expressie op beide ploïdieniveaus niet verschillend.

Effecten van diploïde en tetraploïde ouders op hun tetraploïde nakomelingschappen werden vergeleken in nakomelingschappen van 4x.2x-kruisingen door middel van multipele regressie van nakomelingschapsgemiddelden op oudergemiddelden. Lage determinatiecoëfficiënten (R^2) werden gevonden voor opbrengst, knolaantal en knolgrootte. Voor opbrengst waren de hellingen van de multipele-regressielijnen zeer variabel en overwegend negatief voor ouders van beide ploidieniveaus. De hellingen voor knolaantal en knolgrootte waren klein met uitzondering van die voor knolaantal van tetraploïde ouders. Deze resultaten betekenen, dat opbrengsten van 4x.2x-nakomelingschappen niet te voorspellen zijn uit de opbrengst en/of opbrengstcomponenten van de diploïde ouders. Multipele-regressie-analyse voor onderwatergewicht, loofrijpheid en chipskleur gaf hoge determinatiecoëfficiënten te zien. Voor onderwatergewicht hadden de diploïde ouders een significant groter effect op de nakomelingschappen dan de tetraploïde ouders, en de nakomelingschapgemiddelden lagen in het algemeen het dichtst bij de waarden van de diploïde ouders. Daarentegen hadden voor loofrijpheid juist de tetraploïde ouders een significant groter effect op de nakomelingschappen en lagen de nakomelingschapgemiddelden het dichtst bij de waarden van de tetraploïde ouders. Voor chipskleur waren de effecten van de diploïde en tetraploïde ouders niet significant verschillend en de nakomelingschapgemiddelden lagen het dichtst bij de waarde van de ouder met de donkerste chipskleur, onafhankelijk van het ploïdieniveau van die ouder.

Wanneer de resultaten van het onderzoek naar de expressie van landbouwkundige eigenschappen op diploïd en tetraploïd niveau en naar de ouderlijke effecten op 4x.2x-nakomelingschappen worden gecombineerd, kunnen de volgende conclusies worden getrokken betreffende selectiecriteria op diploïd niveau:

1. Directe selectie op opbrengst en opbrengstcomponenten van diploïde geniteurs heeft geen effect;
2. selectie op onderwatergewicht van diploïde geniteurs moet strenger zijn dan die van tetraploïde geniteurs;
3. voor loofrijpheid mogen de selectiecriteria op diploïd niveau minder streng zijn dan op tetraploïd niveau;
4. de selectie van geniteurs voor chipskleur kan op beide ploidieniveaus volgens dezelfde criteria geschieden.

Sexuele polyploïdisatie

De veronderstelde superioriteit van FDR 2n-gameten over SDR 2n-gameten werd onderzocht door de nakomelingschappen van reciproke 4x-2x-kruisingen te vergelijken voor een aantal landbouwkundige eigenschappen. De diploïde ouder van elk stel reciproke kruisingen produceerde 2n-pollen door FDR en 2n-eicellen door SDR. Deze reciproke kruisingen leverden dan ook uniek materiaal voor een zo betrouwbaar mogelijke vergelijking van de effecten van FDR en SDR 2n-gameten.

Gevonden werd, dat FDR-nakomelingschappen hogere opbrengsten leverden dan SDR-nakomelingschappen en wel als gevolg van meer en grotere knollen. Voor loofrijpheid, onderwatergewicht en chipskleur werden echter geen belangrijke verschillen tussen FDR- en SDR-nakomelingschappen gevonden. Behalve populatiegemiddelden is ook de variatie binnen de nakomelingschap belangrijk bij de veredeling. Voor loofrijpheid was deze variatie het grootst in SDR-nakomelingschappen. Er kan dus worden geconcludeerd, dat voor opbrengst FDR 2n-gameten superieur zijn over SDR 2n-gameten, terwijl voor loofrijpheid SDR 2n-gameten beter zijn. Voor een aardappelveredelingsprogramma weegt het positieve effect van SDR 2n-gameten op loofrijpheid niet op tegen het positieve effect van FDR 2n-gameten op de opbrengst. FDR 2n-gameten verdienen bij sexuele polyploïdisatie derhalve de voorkeur.

CURRICULUM VITAE

Ronaldus Cornelis Bernardus Hutten werd geboren op 4 november 1960 te Goirle. Hij behaalde in 1979 het Atheneum-B diploma aan het St. Paulus Lyceum te Tilburg en begon in dat jaar met de studie Plantenveredeling aan de Landbouwuniversiteit te Wageningen (LUW). De doctoraalfase omvatte de hoofdvakken Plantenveredeling en Plantenfysiologie, en het bijvak erfelijkheidsleer. De studie Plantenveredeling werd in januari 1988 afgerond. Van 16 februari 1987 tot 16 februari 1993 was hij werkzaam als toegevoegd onderzoeker bij de vakgroep plantenveredeling van de LUW aan het project getiteld "Onderzoek naar de strategie en haalbaarheid van aardappelveredeling op diploïd niveau". De belangrijkste onderzoeksresultaten uit dit project zijn beschreven in dit proefschrift. Vanaf 1 april 1994 is hij werkzaam als postdoc bij de vakgroep plantenveredeling van de LUW.