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Breeding Aspects of Freeze-dry Processing in *Fragaria* L.

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Abbreviations

ANOVA	Analysis of variance
asl	Above sea level
°C	Degree Celsius
cc	cubic centimeter
CI	Confidence interval
cm	Centimeter(s)
CTAB	Cetyltrimethylammoniumbromide (CTAB)
CV	Coefficient of variance
DIN	German Institute for Standardization
DM	Dry matter
DNA	Deoxyribonucleicacid
EDTA	Ethylenediaminetetraaceticacid
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	FAO Statistical Division
FAS	Foreign Agriculture Service
FW	Fresh weight
g	Gram(s)
GLM	General linear model
h	Hour(s)
IOZ	Institute of Fruit Breeding
IQF	Individual Quick Frozen
ISS	International Space Station
l	Liter(s)
LRRP	Long Range Reconnaissance Patrols
M	Molar mass
mbar	Milibar
µg	Microgram(s)
µl	Microliter(s)
min	Minute(s)
ml	Milliliter
mm	Millimeter
n	number of
NASA	National Aeronautics and Space Administration
ng	Nanogram(s)
NIR	Near infrared
NMR	Nuclear magnetic resonance
no.	number
PCR	Polymerase chain reaction
pH	Potential of hydrogen

pmol	Picomol
p-value	Probability-value
PVP	Polyvinylpyrrolidone
RAPD	Random amplification of polymorphic DNA
RI	Refractive index
rpm	Revolutions per minute
SD	Standard deviation
sec	Second(s)
SSR	Simple Sequence Repeats
TAE	Tris-acetate
TBE	Tris-borate-EDTA
TE	Tris-EDTA
TEMED	Tetramethylethylenediamine
TM	Fruchttrockenmasse
Tris	Tris(hydroxymethyl)-aminomethane
TSW	Thousand seed weight
U	Unit(s)
USDA	United States Department of Agriculture
v	Version
vs.	*versus*

A Objectives

The main objectives of the present work were the scientific elaboration and the establishment of a breeding program for processing strawberries suitable for freeze-drying. Worldwide no specially bred cultivar is present and the few available processing cultivars do not meet today's horticultural or industrial demands. The following *modus operandi* was chosen: basic research regarding the important parameters of a freeze-drying cultivar and selections according to these traits were simultaneously conducted. The knowledge gained, was directly incorporated into the on-going selection process and steadily improved.

A 1 Scientific and Social Significance

In Europe, two countries are mainly involved in the strawberry processing sector: Germany as the major European fruit processor and Poland as the predominant European producer of processing strawberries. However, it is remarkable that since decades the entire sector is based on only one cultivar: 'Senga Sengana'. This is highly risky and the Polish growers as well as the freeze-dry industry are currently the first which have to notice the negative consequences, since competitors in overseas are flooding the market with low-priced and low-quality frozen and freeze-dried berries. The import of such frozen strawberries from third countries for processing is no good option for Germany, because it has to keep its high quality standards. This can only be assured by high quality frozen strawberries (the quality of Chinese strawberries for jam preparation is for example expected to be 20 to 40% below Polish 'Senga Sengana'). To this, a European grower can assure, due to the topological und social vicinity a checkable quality, hygiene, labelling, food safety as well as environmental-friendliness of their production.

There are two potential scenarios: the European strawberry processing and production industry decrease, starting with the freeze-dryers and followed by other branches, or the production at lower cost but at same high or even higher quality level is assured. The latter scenario could be reached by an overdue new cultivar, which is expected by the EU Commission to impact the sector strongly (COMMISSION EUROPEAN COMMUNITIES 2006).

Already in 1939, SENGBUSCH the breeder of ‘Senga Sengana’ and one of the most famous German breeding researcher stated that it is necessary to realize the results of breeding research into applied breeding programs in order to be “veritably fruitful” (SENGBUSCH 1939a). The presented work is seeking to fulfill these requirements. Further, the participation of public and private institutions in funding the establishment of fruit breeding program could also act as a model for other processed strawberry products as well as other processed fruits.

B Introduction

B 1 History of Freeze-Drying

The processing of food played a decisive role in the history of man. In ancient times the primary purpose was the preservation of food for later usage - a crucial advantage, since the processed food could be stored for hard times and its availability independent of season or natural catastrophe. Additionally, the products become more portable and tradable by increasing the value-to-weight ratio (CONNOR and SCHIEK 1997). In the course of time other properties like enhanced palatability, digestibility and in particular the sensory appeal, gained by altered and often refined flavor grew in importance.

One of the oldest processing methods is drying by sun or air, since no lengthy experiments were needed for the development of this method and dried figs, dates or grapes, fallen from the tree or vine provided the paradigm (TANNAHILL 1988). Evidences exist that in 12,000 B.C. Egyptian tribes at the lower River Nile were already drying food (SHEPARD 2000) and also the Bible has various mentions of raisins. A special and more ingenious modification of drying was carried out by the ancient Peruvian Incas of the Andes (HALL 2001). They discovered that a better preservation was reached if the food was dried at high altitudes above Machu Picchu (2360 m, asl). The coldness froze the product and it contained water, then the low air pressure together with high radiation sublimated the water: an instantaneous transformation of the solid to the gaseous state, which is feasible by the physical property that water has a vapor pressure also at low temperatures.

The additional advantages in contrast to drying by air or sun are:

- Deterioration of the color and nutritional value (carbohydrates, fruit acids, phenolic and other non-volatile compounds) on a low level
- Shrinkage does almost not occur, the product maintains its texture and shape
- Quick and easy rehydration, due to a high hygroscopy

Disadvantages are:

- Hermetically sealed storage needed, due to the high hygroscopy
- Today, one of the most expensive methods due to high capital and energy costs

This freeze-dry technology was developed and is still used in South America in the described manner to produce mainly chuño, a preservable potato product (figure 1a), (COURIEL, 1980). Freeze-drying was either not observed by the Conquistadores, who were too busy with plundering treasures or the technology was buried in oblivion in Europe, until its reinvention in 1890 by ALTMANN in Leipzig, Germany. The first use of an equipment with a pump for freeze-drying was described by BENEDICT and MANNING (1905). SHACKELL (1909) at the US Missouri Agricultural Experiment Station was the first who used machinery with a mechanical pump and the three main, current components: drying chamber, condenser chamber, and a vacuum system (JENNINGS 1999).

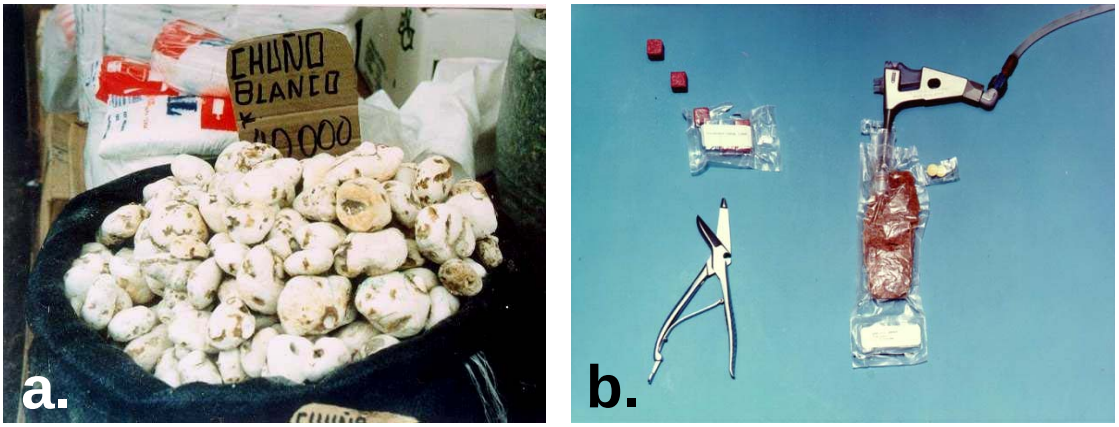


Figure 1 a and b: 1 a Chuño at a local South-American market (Picture of FAO http//www.fao.org/inpho/). 1 b Freeze-dried meals of the GEMINI missions. Left: Meal cubes. Right: The meal could be re-hydrated by inserting a “cold water gun” into the meal package. (Picture of NASA http://www.nasm.si.edu/exhibitions/attm/nojs/food.1.html)

The primary utilization of this method began as a tool for scientific research, but the two major fields of application emerged in the 1930s: freeze-drying of pharmaceutics

and food. The freeze-drying of pharmaceutics gained enormous importance and experienced technological innovation by military demand. It began during the Second World War. Many units of penicillin and human blood plasma were needed in a preserved form and freeze-drying was carried out for the first time in industrial scale in the US (RUPPRECHT 1993). GREAVES and ADAIR (1939) provided the basis of the process by a scientific and engineering investigation in Cambridge UK and FLOSDORF (1945) and FLOSDORF *et al*. (1940) were experimenting already in 1935 on freeze-drying blood products and penicillin. FLOSDORF (1949) also envisaged the usage for food. However, the freeze-drying of food came up after the Brazilian government approached the Swiss company NESTLE with the request to provide an opportunity for preservation of their coffee surpluses in the year 1930. Freeze-drying solved not only this problem of preservation, but also improved the thitherto used instant coffee which was produced by air drying. This coffee was first presented by KATO 1891 at the Pan-American World Exhibition. The first freeze-dried coffee was brought to the market in 1938 in Switzerland under the trademark of Nescafe. In the early 1960s the US Army Soldier Systems Center at Natick started to develop freeze-dried food for combat feeding. The first freeze-dried ration of the US Army was introduced during the Vietnam War for Long Range Reconnaissance Patrols (LRRP). Later these products became part of the common Meals Ready to Eat. The NASA space programs benefited enormously from this military research into feeding (figure 1 b). Since water is produced as a by-product by the fuel cells of the used space ships, it is abundantly available. Freeze-dried food can be easily rehydrated in space, leads to a significant weight reduction and is thus the perfect space food (NASA 1975). Currently the International Space Station (ISS) uses solar arrays and water is not anymore plentiful on hand. Consequently, the importance of rehydrateable food is reduced even though it is still a common food in space (NASA 1986). The adverse global power of the Cold War and the Space Race, the USSR and the Eastern Block states, as well as their succession states were also and still are using freeze-dried food for military and spaceflight purposes. One of the centers of technology development was the Institute for Cryobiology and Lyophilization in Sofia, Bulgaria (ICL 1999).

However, compared to the world wide civilian freeze-dry industry the military and aeronautic application is today negligible. The greatest monetary impact subsists in the pharmaceutical industry, with a proportion of 8 to 10% of the total US health care

costs (8 Billions US Dollar in 1999) and the quantitative - in the food industry (JENNINGS, 1999). The main freeze-dried food products are coffee and fruit as food ingredients. Among fruit, the cultivated strawberry (*Fragaria* ×*ananassa* Duch.) reigns supreme due to their general popularity.

B 2 History of Strawberry Processing

The genus *Fragaria* belongs to the family *Rosaceae* and comprises several species which are common to the temperate zones of the world. While the American and European species have been characterized by STAUDT (1962, 1989, 1999) the definition of the Asian species is still on its way (STAUDT and OLBRICHT 2007). An overview of the strawberry species and their distribution was published by HANCOCK (1999). Strawberries are herbaceous perennials that are pollinated by insects, predominantly by bees. Perfect flowering and dioecism occurs with self-compatible, self-incompatible, dioecious and trioecious breeding systems. The cultivated strawberry is trioecious. In botanical terms the strawberry fruit is an aggregate accessory fruit. The actual fruitlets are called achenes and are embedded in the receptacle. In terms of common usage and for easier reading the aggregated nut fruit of *Fragaria* is called a fruit, a berry or a strawberry in the presented work. Strawberries are non-climacteric fruits. It is referred to the standard literature for further information of the botany of strawberries (BAUER 1960, HONDELMANN 1976, BRINGHURST and VOTH 1984, HANCOCK 1999).

Due to its wide habitat, the consumption of strawberries by humans is entrenched in different cultures of the world. In contrast to the domestication of the main grain crops 10,000 years ago (HANCOCK 1992), the cultivation of strawberries reaches only 2000 years back in the history of men (HANCOCK 1999). Reason for that was the locally available abundance of wild berries, which made cultivation unnecessary. Romans, Greeks and the Indians of South America were the first who cultivated strawberries.

The triumphal procession of the strawberry begun in the 17th century in Brest, France with the contemporaneous cultivation of the octoploid *F. chiloensis* (L.) Miller from South America and the octoploid North American *F. virginiana* Miller (STAUDT 1961, WILHELM and SAGAN 1972 and references within). The species *F. virginiana* was already introduced to the Old World in 1586 by survivors of the first English colony

Virginia, which were taken back by a fleet of Sir Francis Drake. Later on new colonies were established on the North American East Coast and therewith further strawberry seeds and plants came to Europe. The Indians of Northern America were cultivating maize and other crops but no strawberries. Therefore, the Europeans introduced wild species of *F. virginiana* and not cultivars to Europe.

In the 16th and 17th centuries several educated men also explored South America and reported, besides, of large-fruiting strawberry of Chile and Peru (WILHELM and SAGAN 1972). It is remarkable that in contrast to the history of *F. virginiana* no one tried to bring these strawberries to Europe. This lasted until 1714. Two years before the 30 year old military engineer and mathematician Amedee Francois FREZIER embarked on the 36 guns and 135 men strong man-of-war Saint Joseph (WILHELM and SAGAN 1972). FREZIER was a spy traveling undercover as a merchant with the secret mission to spy on all militarily relevant information of the Spanish at the coasts of Chile and Peru. In Conception, Chile his attention was attracted by the large-fruiting strawberries, which were cultivated by the local Indians and called quelghen. This advertence was completely justified from a horticultural point of view. The quelghen was a giga type with large pale red fruit with white pulp selected from wild *F. chiloensis* by the Mapuches or Huilliches tribe which had a highly developed agriculture. The strawberries of the wild were called lahuene or lahueni. Such a giga form was never selected by Europeans, Northern Americans or Asians in their native strawberry species.

From a military point of view this quelghen and strawberries in common were also interesting. Wild strawberries played an exceptional role in the warfare of the Mapuches. They planted strawberries on clearings to allure conquistadores. After Spanish soldiers dropped their arms and pleasurably ate the berries the Mapuches ambushed the soldiers and killed them (GONZALES de NAJERA 1866 cited by HANCOCK 1999). However, the highest effect on French military power could have been reached by curing scurvy, an ultimately fatal disease and the bane of long sea journeys. Later in 1747, after having made several experiments, the British naval surgeon LIND recommended that concentrated lemon juice syrup should be served throughout the Royal Navy (DAVIES *et al*. 1991). The naval authorities were unwilling to take notice of this medical advice and in 1780 1600 of 12,000 men in the fleet still died mostly by scurvy; only 60 of these died by battle. Finally in the year 1793 an experiment was conducted on the persuasion of BLANE: The HMS Suffolk set sail for

a 19 week long voyage without touching any port and every man on board was given the lemon juice as suggested by LINDT. On arrival at Madras, India there had been not a single case of scurvy and thenceforth lemons were a regular issue to the British Navy. Taking in consideration that strawberries have a higher level of ascorbic acid (average 70 mg/ml) than lemons (average 52 mg/ml) (HERRMANN 2001) and that strawberries can also be cultivated in the European homelands, strawberries could have also been a very powerful “weapon”. FREZIER did most likely not know about this possible impact on military but still chose some of the plants with the largest fruit to take back to France. The value he attached to these plants is highlighted by the use of very rare drinking water for watering these plants during the crossing of the Atlantic. In 1714 FREZIER arrived in France together with five living plants of *F. chiloensis*. At this time, *F. virginiana* was cultivated already for a century in Europe and several selections were known. Huge expectations were raised in the new strawberry species with the reported large fruit. But this exact feature did not appear and the plants were largely barren. It is most likely that by choosing the largest fruiting plants FREZIER also picked the pistillate plants and in the Old World the pollinator was missing. This aspect was observed consciously or unconsciously by farmers of Brittany. They solved the problem by cultivating the new *F. chiloensis* together with *F. virginiana* and *F. moschata* as pollinator and yielded fruit from all three species. Another result of this co-cultivation was coincidental hybridizations leading to an octoploid hybrid (2n = 56). Most likely *F. virginiana* was the successful pollinator STAUDT (1961). The hybrid status was first recognized by DUCHESNE 1766 at the age of 18 or 19, at a time when sexuality in plants had only recently been discovered. He specified the hybrid as *F.* ×*ananassa*. Since the fruit tasted good and were superior in consideration of fruit size and total yield, *F.* ×*ananassa* replaced more and more the thitherto-cultivated forms of the native European strawberry species as well as the two introduced parental species from the New World.

The main contribution to the success of *F.* ×*ananassa* was the beginning of systematic and accidental breeding work. The regular breeding strategy was and still is a pedigree breeding: elite parents are chosen and crossed and clones are selected out of the resulting F_1 population and tested over several years. The reason for this simple and successful strategy is the high variability by high heterozygoty in the F_1 and maintenance of this heterozygotic status by vegetative propagation. Despite this standard breeding program, accidental seedlings were also important in the

beginning of the breeding history and special breeding strategies occurred. Due to the octoploid set of chromosomes the two progenitors of the cultivated strawberry were and are still used for further addition of genetic variation into *F.* ×*ananassa*. Accessions of the native beach strawberry of California *F. chiloensis* with the two important traits, glossy leaves and lengthening the bearing season, played a certain role. The legendary California breeder ETTER used this local accession for breeding work. GLOEDE a nurserymen of Sablons, France, used the Californian strawberry for crossings and in 1858 released the cultivar '*Fragaria lucida perfecta*' from the cross 'The Californian' x 'British Queen'. The '*Fragaria lucida perfecta*' occurs later in the pedigree of the famous German cultivar 'Mieze Schindler' selected in 1925 by SCHINDLER. BRINGHURST and VOTH (1978) used accessions of *F. virginiana* spp. *glauca* for subsequent backcrosses with *F.* ×*ananassa* to transmit day-neutrality characteristic to *F.* ×*ananassa*. Backcrossing was also applied by BARRITT and SHANKS (1980) to transfer aphid resistance of a *F. chiloensis* accession to *F.* ×*ananassa*.

In this context, *F. iturupensis* STAUDT is also interesting as it is the third natural species which, with its eight sets of chromosomes, has the same ploidy level as the cultivated strawberry. *F. iturupensis* is common to the Southern Kurile Island Iturup. Because Iturup is one of the disputed islands of the lasting 1945 Kurile conflict between Japan and Russia, plants or seeds of *F. iturupensis* were not available for a long time (VILLAFRANCA 1993). The octoploid status defined by STAUDT (1973) was even mistrusted (STAUDT G. pers. comm. 2005). In 2003 an US American plant collection expedition was ventured and the unfamiliar species was collected at the Eastern slope of Atsonupuri Volcano on Iturup Island (HUMMER *et al*. 2005). These plants and their seeds are now available for direct incrossing on the octoploid level into *F.* ×*ananassa*. The crossability to *F.* ×*ananassa* is proved resulting in high germination rates using common cultivars (STAUDT G. and K. OLBRICHT pers. comm. 2007).

The hybridization with *Fragaria* species of a lower chromosome level occurs in nature and often results in interspecific hybrids with altered sets of chromosomes (BRINGHURST and KHAN 1963, BRINGHURST and GILL 1970). Also, diverse approaches were made to cross other *Fragaria* species directed into *F.* ×*ananassa* to broaden its gene pool (FEDEROVA 1934, SCOTT 1951, BAUER 1960, STAUDT 1967). The approach to elevate the cultivated strawberry on the decaploid level is

especially interesting. The first strawberry plants with such a level of chromosomes (2n = 70) naturally occurred (BAUER 1969). BAUER and BAUER (1979) obtained decaploid plants through open pollination of the hexaploid F_1 of the cross *F.* ×*ananassa* 'Sparkle' x *F. vesca* var. *semperflorens* L. (tetraploid, 2n = 28) by *F.* ×*ananassa*. The decaploid cultivar 'Spadeka' was selected from the resulting F_2 and introduced in 1977 (BAUER and BAUER, 1979). A following decaploid is 'Florika' released in 1989. The tenth fold chromosomes level was also reported by ULRICH (1972) and explained by unreduced gametes. Decaploid strawberries were also investigated and reported by SPIEGLER *et al*. (1986). One breeding objective of the decaploid strawberries was the suitability for mechanical harvest. The calyx of the berries were indeed easier to remove and the infructescences were upright and over the foliage, but other problems occurred and are reason for the moderate success of these cultivars. Far distance hybrids were also obtained, mostly by crossing species of the near genera *Potentilla* or *Duchesnea* into *Fragaria* species (ELLIS 1962). The most successful intergeneric strawberry cultivar so far is 'Pink Panda' of the Canadian breeder ELLIS. The hybrid between *F.* ×*ananassa* and *Potentilla palustris* L. is a successful cultivar of the ornamental market due to its attractive pink flowers. However, the intention of this intergeneric breeding program is the transfer of winter hardiness trait of *Potentilla* to the gene pool of *F.* ×*ananassa*.

The history of the industrial strawberry processing is closely connected to the development of appropriate cultivars for processing and started with the canning industry. Canning was particularly strong in the US and breeders like ETTER had already selected cultivars like 'Ettersburg 80' or 'Ettersburg 121' for this usage at the beginning of the 20th century (WILHELM and SAGAN 1972). Canning was superseded by the freezing industry because of the rationing of cans during the Second World War and the resulting introduction of freezer compartments as standard, in house hold refrigerators (CONNOR and SCHIEK 1997). Since processing strawberries are normally traded today as frozen ones, the freezing industry is still the starting point for downstream processing. The for-canning suitable cultivar 'Marshall' was one of the first also used for freezing. 'Marshall' had already been introduced in 1893 after it was accidentally found as a seedling just a short distance south of Boston (DARROW 1966, NOTES 1894). It was also known for its good canning suitability. The freezing of the berries was carried out by rolling barrels

with ‘Marshall’ back and forth to ensure a synchronous freezing of all berries (Oregon Strawberry Commission 2001). This cautious practice and the deliberate choice of a cultivar exemplifies that high product quality with a special character was in demand for processing strawberries even back then. According to the slogan: “Quality cannot be gained from processing, but it certainly can be lost” (DeANCOS *et al*. 2006). ‘Marshall’ retained its importance until the 1960s and was, for example, in 1962 still the seventh most cultivated strawberry in the Northwest, the main US processing region (DARROW 1966). Nevertheless, other special cultivars with good processing quality occurred, like the cultivar ‘Northwest’ bred by SCHWARTZE of the Western Washington Experiment Station in 1949, ‘Hood’ introduced 1965, the Canadian ‘Totem’ from DAUBENY 1979 or ‘Puget Reliance’ (Oregon Strawberry Commission, 2001). ‘Totem’ is still the most grown processing cultivar in the Northwest with 34% of all commercial sold plants (6.6 million plants) in 2005 (MOORE, 2005). It is interesting to notice the appearance of the new cultivar ‘Tillamook’ with 2.2 million sold plants (11.4%) in the year 2005, introduced in 2002 by FINN (FINN 2004). It sent ‘Puget Reliance’ (1.8 million sold plants) off to the third place (MOORE, 2005). It remains to be seen if ‘Tillamook’ will surpass ‘Totem’ in the future.

In Europe, the development of the strawberry processing industry was similar. Canning was also an important sales market for strawberries before the Second World War. MACHERAU (1929) reports that strawberries were the most important fruit for canning in the Weimar Republic. Each year “thousands of hundredweights” of ‘Jucunda’ were supplied from Holland to the German processors. Due to the strained situation of the German agriculture, MACHERAU (1929) recommended also the German cultivars ‘Sieger’ of BÖTTNER and ‘Hohenzollern’ for this usage. Both cultivars have the right traits of firm pulp and uniform, not too large fruit. The most legendary European processing cultivar is the German cultivar ‘Senga Sengana’, introduced by SENGBUSCH in 1954. The deep-freeze procedure of food was introduced in the late 1930s by the “Reichsnährstand” (Reich Food Administration) of the Third Reich for securing of the national feeding and the establishment of an autarchy in preparation for war. SENGBUSCH started an evaluation of the present strawberry genotypes for freezing suitability in 1941, but concluded that none was applicative. A special cultivar had to be bred. Crosses were done in 1943 with the in the canning industry used US American cultivar ‘Markee’ and European cultivars, *inter alia* the already mentioned cultivar ‘Sieger’ (JORDAN *et al*. 1950). In the year

1944, 10,000 seedlings were selected and tested for freezing performance from populations of 40,000 F_1 plants. For further selection 1500 chosen genotypes were planted as clones and tested in 1945 under the war and post-war confusion for freezing and thawing performance. Albeit, genotypes were still selected and transferred in 1948 from under Soviet-Russian administration standing Luckenwalde to Hamburg. There they passed through different yield- and processing-tests, until in 1954 the cultivar 'Senga Sengana', from the cross 'Markee' x 'Sieger', was launched as the first cultivar in the world selected for freezing (SENGBUSCH 1954). Due to the excellent fruit processing parameters (deep red pulp and skin color, uniform fruit size, good clasping of the calyx, good freezing/thawing performance) and the extraordinary adaptability to different environments, 'Senga Sengana' got the most successful processing cultivar in Europe. Remarkable is that it has kept this position for over 50 years until present. It is almost exclusively used in Poland, even though in Poland today 'Senga Sengana' is characterized by low yields (average 3 to 4 t/ha), small fruits and a low resistance to diseases (MAACK 2005). The single largest importer of Polish strawberries is Germany, whose self sufficiency of processing strawberries amounts to only 1 to 5% of the total (MAACK and SCHMIDT 2002). Germany is also the world's largest frozen strawberry importer with 73,294 t (USDA, FAS 2007). German processed products based on all berries are valued approximately to 0.6 billion Euros in 2002 and a total supply need of approximately 52,000 t of strawberries per year (MAACK 2005).

Who freeze-dried the first strawberry or where it occurred is unknown. But it is known that the LRRP rations and the rations of the early Mercury missions contained already freeze-dried strawberries (LACHANCE 2006). More remarkable is that as early as in the 1960s experiments were started by the private industry to add freeze-dried strawberry slices in cereals, which is today a major use (JOHNS P. pers. comm. 2006). It happened in Watsonville California USA, one of the main frozen strawberry production areas of that time and today a very important city for strawberry production and development. The Californian strawberry frozen food packer OLIVER cooperated with POST CEREALS (JOHNS P. pers. comm. 2006). They found out at what temperature the zero degree berries had to be raised so that they could be sliced without shattering. Further, special centrifugal spinning machinery was developed and a small freeze-drier was build at the National Ice and Cold Storage Company in Watsonville. Unfortunately for them, they were ahead of

the times and there was no consumer acceptance of freeze-dried berries in cereals. As a consequence, the expensive process was shelved. Today, these freeze-dried strawberries are an established product on the market with growing importance.

The special breeding objectives for processing cultivars were, so far, the good coloring of the pulp, the uniformity of the fruits in size and form, the juice retaining quality after thawing, and easy calyx removal (HONDELMANN and SENGBUSCH 1963, BARRITT 1976, POPOVA *et al.* 1979, MAZHOROV 1991). Moreover, the breeding goal of suitability for mechanical harvest is closely connected with the processing application. Several strawberry harvest machines were developed in North America and Europe with similar systems (MORRIS *et al*. 1978, FIEDLER 1983). The strawberries were cut off at ground level or were stripped off by a comb system and foliage and residuals were separated from the fruit. Since the harvesters could not distinguish between ripe and unripe fruit, no profitable yields could be reached with the common processing cultivars or any other (RUFF and HOLMES 1976). Therefore, the additional breeding goals were simultaneous ripening and long and strong pedicles which, at best, present the fruit above the foliage. In the German Democratic Republic the strawberry harvester (E840) was developed and at the same time the special cultivars ‘Fratina’ and ‘Fracunda’ were bred (FIEDLER 1987, FISCHER and ULRICH 1989). Also in Denmark, an once-over harvester was developed together with the special cultivars ‘Mimek’ and ‘Primek’ (THUESEN 1989).

Today, the cultivated strawberry is one of the most consumed fruit with approximately a 3.7 million t world production in the year 2005 (FAOSTAT 2007). Thereby, the processing industry is a significant market. The world leading strawberry producer is the US with nearly 1 million t of fruit (USDA, FAS 2007). In the US approximately 25% of the annual yield is used for frozen and processed production. Therefore, the US is also the world leading processing strawberry producer. The main processing cultivars are US or Canadian cultivars like ‘Totem’, ‘Hood’ and ‘Tillamook’. Poland is the world’s largest exporter of frozen strawberries. About 60 to 70% (70 to 125,000 t) of their overall strawberry crop is sold to the fruit industry (FAOSTAT 2007, SKUPIEN and JAKUBOWSKA 2004). The main processing cultivar of Poland is the German cultivar ‘Senga Sengana’. An emerging competitor on the strawberry processing market is China with its major processing strawberry producing provinces Hebei, Shandong and Liaoning. After a period of a dramatically rising production of frozen strawberries (exports from 2001 to 2003: 21,153 t, 34,968 t and 77,972 t

respectively), the predicted total production of frozen berries in 2006 is 10% less in comparison to 2005, i.e. 82,000 t (BUTTERWORTH and LEI 2005) The main reason for the stagnation is the higher price of Chinese strawberries as well as an initiation of a safeguard investigation of the EU, lodged by the contestant Poland. The result was a temporary anti-dumping protective implemented by the EU in October 2006 (JF 2006). However, the decrease of the Chinese frozen strawberry production is just minimal and proceeds on a high level. Furthermore, the Polish frozen strawberry production felt in 2006 as well. The leading export destination of Chinese frozen strawberries was the EU-25 (45% market share by volume), followed by Japan (16%), the US (15%) and Canada (5%) (USDA, FAS 2007). China already captured the Japanese frozen strawberry market with 64% in terms of volume, which valued approximately US Dollar 18.4 million on a Cost, Insurance, Freight basis (ITO 2005). These Chinese frozen strawberries are mostly processed in Japan into jam and yogurt, products in which cheaper ingredients can be used with a lower grade of quality. The first cultivars cultivated in China were Japanese cultivars like ‘Tonoyoka’ and ‘Hokowase’ followed by US American cultivars like ‘Chandler’, ‘Selva’, ‘Allstar’ or ‘Honeoye’ and then European cultivars like ‘Elsanta’ (ROUDEILLAC 2007). The German processing cultivar ‘Senga Sengana’ is also cultivated in China generally for the processing market as well as unknown cultivars with trade names like ‘American No. 3, 6 or 13’. In 1985, China started its own strawberry breeding program and to a small extent Chinese cultivars are cultivated today (GIFFORD and LEI 2004, ROUDEILLAC 2007). ROUDEILLAC (2007) lists several new Chinese strawberry cultivars and their breeding background. The cultivars ‘Shuo Xiang’ and ‘Shimei No.1 to 4’ were specially bred for the processing industry. It is assumed that the importance of Chinese cultivars will rise, due to a better adaptability of these genotypes to local climates. China will be an important competitor on the frozen and processed strawberry world market in the future and should not be underestimated (CARTER *et al*. 2005).

The predominantly cultural system worldwide for processing strawberries is the matted row culture. Due to the lower price of processing strawberries in comparison to the fresh market, more intensive systems are unprofitable.

Most strawberries for processing are traded without calyx and as frozen blocks or individually quick frozen (IQF), because of the high perishability of the product and associated technical and logistical consequences. For IQF the freshly harvested,

preferably fully ripe but still firm strawberries are washed, sorted, and immediately frozen at the field in a blast air freeze tunnel (flow-freezer) at -40°C and 2.5 to 5.0 m/s air speed. This temperature assures a freezing rate of 5 to 10 cm/h according to the definitions of the Institute International of Refrigeration (IIR, 1986). The freezing process, the frozen storage and the thawing process are critical for the later structural and physical characteristics of the processed product (CASTRO *et al*. 2002). The developing ice crystals destroy the structural integrity of the cell walls, whereas the size, form and status of the ice crystals and therefore the damage can be regulated by the freezing temperature. But that is limited by technical and economical boundaries and the effect can get lost by recrystallization or cracking of whole fruits during storage (DELGADO and RUBIOLO 2005, RAHMAN 1999). Further, the frozen strawberries are packed for retail sales, stored in cold storage houses or directly transported to processing plants. Many processed strawberry products are known, but the widespread ones are: jelly or jam, puree, juice, concentrate or syrup, and various dried strawberries, in particular freeze-dried strawberries.

Figure 2: Freeze-dried strawberry products of the cultivar ‘Senga Sengana’. From left to right: whole, sliced, cubed and smashed.

Freeze-dried strawberries on their part are traded whole, sliced, cubed/smashed or powdered (figure 2). For the sliced and cubed form, the frozen berries are thawed and cut. For the smashed form the frozen strawberries are smashed in special barrels. The strawberry powder occurs as a by-product during the fabrication process of the other trading types. The freeze-drying process retains the typically bright red

color, structuring and form of strawberries and produces a crisp texture with a low bulk density (approximately about 0.1 g/cc) (SINHA 2006). Due to the highly value-adding process, freeze-dried strawberries are exclusively used as ingredients in high price products like ready-to-eat cereals, snack bars, or sweets like pralines.

B 2.2 The Parameter Dry Matter

A high product Dry Matter (DM) is a remarkable new demand of the processing industry. The importance of this request is reflected in the customary payment of the crops according to the DM content. The interest of the processing industry in high DM is mostly based upon the financial reward and the simple conclusion that a fruit with high DM contains less water and thus more of the end-product substance. Certainly, the importance of this parameter depends on the technological process and its intensity, as well as the financial value of the processed fruit. It is the highest in the drying industry, moderate in the puree industry and relative low in the jam industry. In addition, the DM is often linked to quality traits which influence the taste preference of the consumer. Moreover, DM is easier to quantify than other important characteristics, which are correlated with it, like oil content or soluble solids.

High DM as a breeding goal is well known for processed field crops like potatoes (*Solanum tuberosum* L.), carrots (*Daucus carota* L. ssp. *sativus* Hoffm.) or onions (*Allium cepa* L.) (SCANLON *et al*. 1999, SMITH and DAVIS 1977, LISINSKA 1989, KELLER and GUHL 1981, NIEWHOF *et al*. 1973, HENRIKSEN and HANSEN 2001). Special cultivars are grown for the processing industry, since they combine particular quality parameters with a high DM. In contrast to the field crop processing industry, the fruit processing industry is smaller, thus intensive processing procedures like drying or juice concentrate production are relatively new. Therefore, the relevance of DM has not been fully considered in orcharding so far. Nevertheless, the DM is of special interest for some fruit and their applications:

DM of kiwi fruit (*Actinidia chinensis* Planch.) correlates positively with the soluble solid content (McGLONE and KAWANO 1998, FENTON and KENNEDY 1998, McGLONE *et al.* 2002). Thus, DM is often used as an indicator for harvest maturity and internal quality. It was shown by BURDON *et al.* (2004) that this quality trait can actually be perceived by the consumers. Test persons were able to discriminate between kiwi fruit of different DM. They preferred fruit with higher DM. OSBORNE *et*

al. (1999) suggested even the grading and sorting of kiwi fruit according to this parameter. For the same purpose, WALSH *et al.* (2004) tested the Near Infrared Spectroscopy technique on various fruit. Further, a positive correlation of DM and vitamin C content was reported in kiwi fruit by CHENG *et al.* (2004). A similar predictive model for the storage quality was described for the apple (*Malus domestica* Borkh.) cultivar 'Royal Gala' (McGLONE *et al.* 2003). It is based on the correlation between the DM at harvest time and the post-storage soluble solid content. Furthermore, the correlation to soluble solid content in freshly picked fruit was described for mangos (*Mangifera indica* L.), peaches (*Prunus persica* L.) and mandarin fruit (*Citrus reticulata* Blanco) (LECHAUDEL *et al.* 2002, JACKMAN *et al.* 2004, GUTHRIE *et al.* 2005). A correlation of DM with the oil content was demonstrated for avocado fruit (*Persea americana* Mill.) and olives (*Olea europea* L.) (LEE *et al.* 1983, MICKELBART and JAMES 2003). The correlation in avocados is even so strong that the determination of DM is established as the worldwide standard for the harvest time appointment (WOOLF *et al.* 2003). For black currant fruit (*Ribes nigrum* L.), the parameter DM was evaluated as important and an inheritance analysis was carried out by FRANCHUK and MANAENKOVA (1971).

Despite its economical importance, the DM of strawberry has not been in the centre of comprehensive particular investigation. The research in processing strawberries was mostly done in chemical, physical or sensory analyses of frozen fruit or processed products (KÖHLER 1954, BAUMUNK and HONDELMANN 1968, WILLIAMS 1977, SKREDE 1982, GARCIA-VIGUERA *et al*. 1999, STRALSJÖ *et al*. 2003, LEFEVER *et al*. 2004, DELGADO and RUBIOLO 2005), or in the performance of the strawberry fruit and their optimization during the technological process (EVANS *et al*. 2002, KHALLOUFI and RATTI 2003, MORAGA *et al*. 2004).

The majority of publications were published in the former Eastern Block States, predominantly in the USSR. They are general fruit evaluations with DM as one among other fruit parameters under investigation (SEDOVA and OSIPOVA 1975, NIKOLOV 1983, SUKHOIVAN 1986, PRICHKO *et al.* 2005). Most authors investigated the parameter DM over several years and at several locations. For example, SAMORODOVA-BIANKI (1972) published DM values from 1950 to 1967. These efforts enabled the authors to draw conclusions about the stability of the traits and the environmental influences on the parameter (LATYPOVA and TATAUROVA 1972, IVANOV and STAMBOLIEV 1973, MAZHOROV and SAMORODOVA-BIANKI

1985, MAZHOROV 1991). MAZHOROV and SAMORODOVA-BIANKI (1985) chose parents with high DM values for crossing based on data collected over five years. Additional, MAZHOROV (1991) ascertained the pedigrees of promising cultivars and combined these with the gained fruit parameters in advices for cross combinations. Unfortunately, no data is published about the results of these crosses. Polish publications are also dealing with DM comparisons of cultivars and the influences of the years (LENARTOWICZ *et al.* 1986), fertilization (LENARTOWICZ 1973), or cold storage (SKUPIEN and JAKUBOWSKA 2004). DM values collected over 24 years were listed and analyzed by PLOCHARSKI (1989) and include the European standard processing cultivar 'Senga Sengana'. GEGOV *et al.* (1982) mentioned DM as one of the most important traits for freeze-drying suitability.

On the contrary, Western publications have drawn little attention to the parameter fruit DM. Most of the reports are part of general fruit trait evaluations under sometimes altered environment or production conditions (HARDH and HARDH 1977, HANCOCK *et al.* 1984, THUESEN 1985, KALT and McDONALD 1997, HOPPULA and KARHU 2006, KAMPERIDOU and VASILAKAKIS 2006). It is interesting to note an evaluation conducted by SELVARAJ *et al.* (1976) who published the DM values from European and North-American cultivars grown in Bangalore. The DM as an important trait for processed strawberry products is mentioned in publications of STIEGER (1975) and SKREDE (1980). DM was additionally used as an indicator for assimilate partitioning or the sink-source relations by FORNEY and BREEN (1985a, 1985b) or HANSEN (1995).

A special strawberry breeding program as well as a selection method for a high DM cultivar was only mentioned by HEMPHILL *et al.* (1992) and HEMPHILL and MARTIN (1992). MASNY *et al.* (2001) reported new Polish strawberry selections with high DM and good processing and freezing suitability, but none of the named selections prevailed on the market so far.

Consequently, comprehensive basic research regarding the parameter DM in fruit of *Fragaria* has not been performed or published. However, it is strongly needed.

B 3 Breeding Parameters

A specification of the required breeding parameters is the basis for the establishment of each breeding program. Regarding a freeze-dry cultivar, general parameters and specific known as well as new processing parameters are demanded. A detailed disquisition of the various general strawberry parameters for clone selection, like yield, firmness, resistance against diseases or possibility of propagation, is set aside. It is referred to the known literature (BAUER 1960, HONDELMANN 1976 and references within). Nevertheless, these parameters represent the basis for further specialized selections and still are very important. Due to the three freeze-dried strawberry product types, whole, sliced, smashed or cubed, and their different applications, a separate consideration of the requirements to the fruit is advisable.

B 3.1 Fruit Dry Matter

By far, the most important trait for the drying industry is the DM of the fruit. Estimations of the industry assume, that starting from a 10% DM level an absolute enhancement of 1% DM leads to a decrease of 10% processing costs. The DM is for all three product forms of equal importance. This trait was determined as the major breeding goal, as well as the major object of investigation.

B 3.2 Harvest Performance

Since strawberries are still individually picked by hand, the labor cost for the harvest entails a great part of the total costs of strawberries. In general, clearly lay out and easy to detach berries are demanded for the fresh as well as the processing market. In contrast to the strawberries for the fresh market, the calyxes of the industrial strawberries are directly removed on the field by a little blade attached at a finger of the picker. This procedure is called capping. Consequently, the detachability of the calyx from the berry is a very important time factor and therefore a main cause of the harvest cost. A cultivar with a hardly removable calyx has no chances to become a cultivar for processing.

B 3.3 Fruit Parameters

B 3.3.1 Color

The fruit color is of high importance for all strawberry processing cultivars. The pulp as well as the fruit skin should be red to dark red and in this regard ‘Senga Sengana’ is considered as an ideal colored fruit for the European market.
Due to their high value, freeze-dried strawberries are used as food ingredients normally in a smaller proportion than the other components. Thus, the consumer perceives and identifies the strawberry part predominantly by the appearance and not by the taste. The color is important for all three freeze-dried products. However, the smaller the strawberry product is the more important the color gets for the identification. Certainly, most consumers would not recognize a white 1 cm cube swimming in fruit cereal as a strawberry. Contrary, a 1 cm red cube would most people suggest a tasty strawberry.
Besides the pulp and the fruit skin, the color of the achenes plays also a role. After the processing, the color of the achenes of the standard cultivar ‘Senga Sengana’ is yellowish to greenish as in the fresh fruit. This is preferred by the consumer but it is expected that another achenes color could also be accepted depending on the overall impression.

B 3.3.2 Color Pattern

Strawberries as aggregated fruits are composed of a receptacle with the components epidermal layer, cortex and pith (HANCOCK 1999). At the fruit skin numerous achenes are present, which are supplied with nutrients by vascular bundles. These are drawing through the receptacle in a typical pattern and are often colored white, which silhouetted them against the often red pulp (figure 3). This pattern is a significant factor for the consumer product recognition besides the pulp color (see B 3.3.1). It is very important for whole fruit and fruit slices, but just of lower importance for the smashed or cubed form.

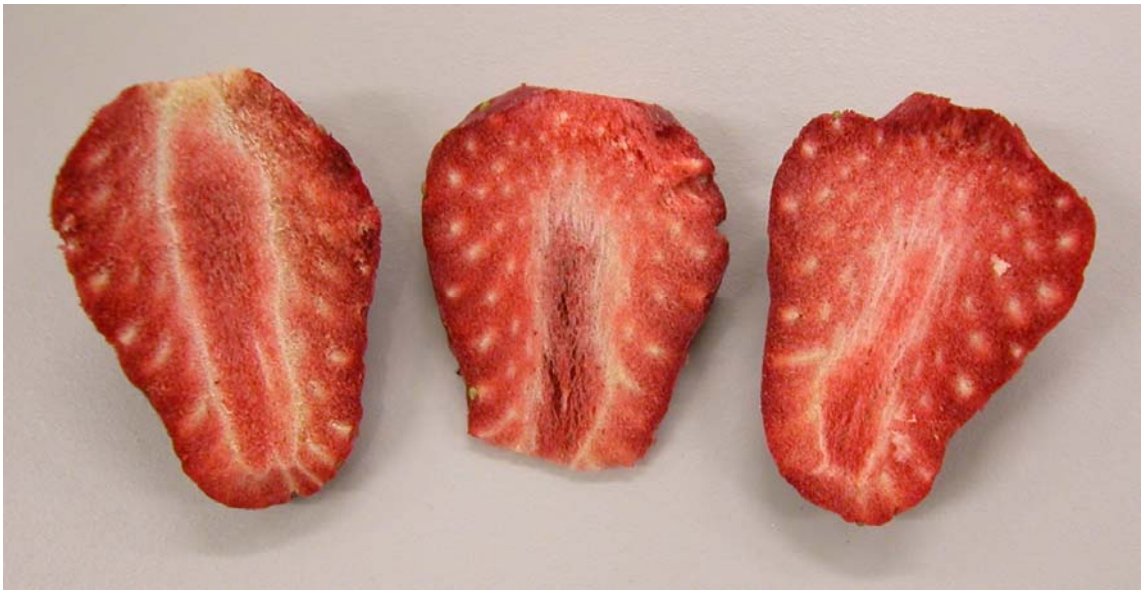


Figure 3: Three cuts through a strawberry fruit of a not termed selection.

B 3.3.3 Technological Freeze-Dry Suitability

Each strawberry charge does not perform equally under industrial processing conditions. Sometimes the phenomenon occurs that charges need to be freeze-dried discriminative longer, independently from their DM or other evident reasons. The cause for this is unknown so far, but selections of higher selection stages have to be checked for their reliable performance during the technological process.

B 3.3.4 Size and Uniformity

The size and the uniformity are known parameters for a processing cultivar. The ideal fruit size is smaller than that of the berries for fresh market. However, the freeze-dry process makes special demands on fruit size and uniformity.

As for general processing cultivars, the uniformity of the berries is important for a homogeneous appearing and standardizable end-product. Additional, uniformity is needed for the technological process: before cutting or smashing the berries are thawed and large differences in fruit size results in hard frozen cores in large berries which damage the cutting edges and smaller fruits get pulpy which produces too much waste.

A special small fruit size is demanded for the whole fruit product. Currently, this size is sorted out by hand on the field. Because, by logistic reasons, no parallel usage of

larger and smaller fruit can be done, this procedure is extremely labor and time intensive.

B 3.3.5 Cavity

Two types of cavities have to be distinguished: the naturally occurring cavity which is formed during the growth of the fruit (KADER 1991) (figure 4a) and the cavity which results by removal of the pith with the calyx (figure 4b).

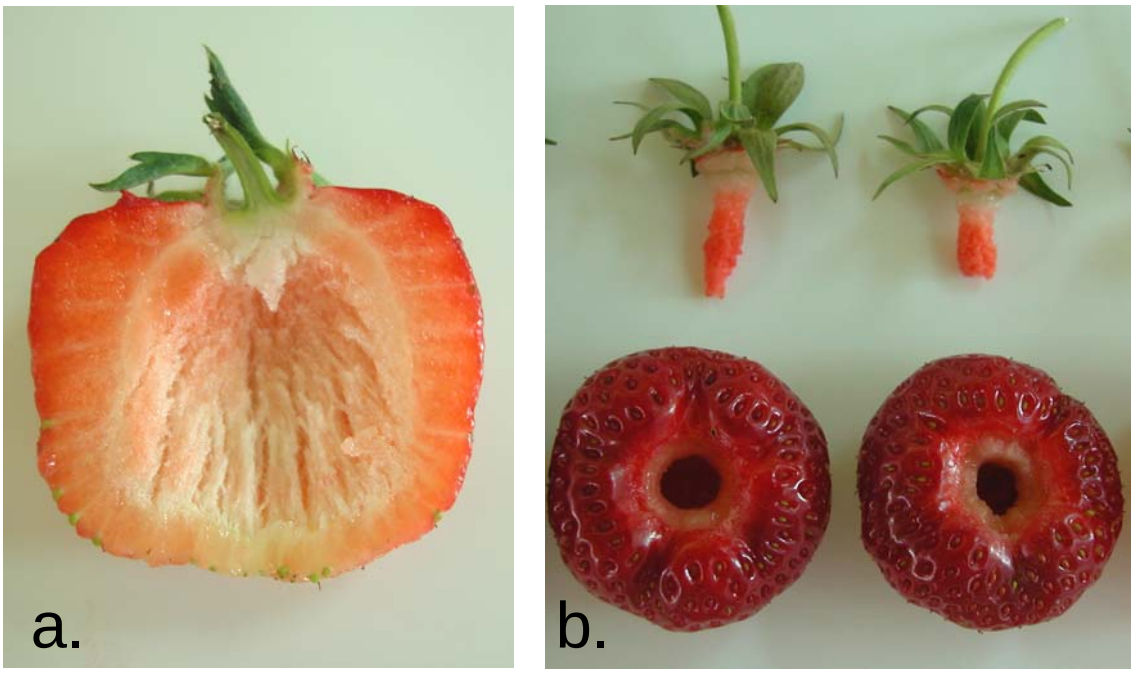


Figure 4 a and b: The two different types of cavities in strawberries. 4 a. shows the cavity formed during the growth of a strawberry fruit. 4 b. displays cavities which resulted by the removal of the pith with the calyx. Both figures show fruit of not termed seedlings.

A cavity formed by rapid growing can be tolerated for fruit processed to cubed or smashed products but is undesirable for the whole or sliced fruit form. Strawberries which have a cavity in the fruit after detaching the calyx are unacceptable for all product forms. Through this cavity washing water can enter and lower the total DM dramatically.

B 3.3.6 Aroma and Taste

The taste is one of the major breeding goals of current fresh fruit breeding programs and the objective of extensive research. However, as mentioned in B 3.3.4 the appearance of the fruit is of higher importance for the product recognition. The taste is of less importance for freeze dried strawberries. Nevertheless, the taste can not be excluded, as a typical strawberry taste is still desired and no off-flavors should appear.

It has also to be considered, that the taste of the fresh and processed berries can vary by a cogitable conversion of substances, the loss or gain of volatile aroma compounds. These lost compounds can be re-extracted by distillation of the condensed water-volatile alloy and added again to the product. But this procedure would increase the product costs unnecessarily and is not operated in the processing industry in a large scale. An alternative is the sale as natural aroma components.

B 3.3.7 Abrasion

During processing of strawberries to freeze-dried products, up to 10% of DM is lost by abrasion.

Two factors are most promising for an aspired lowering of this loss: the firmness of the fruit skin and the achenes which in a raised position could act as a buffer-bar.

C Material and Methods

C 1 Material

C 1.1 Plant Material

The plant material used in the present work comprised cultivars, species, selections and seedlings. All selections coded by a P followed by a number are crosses of *F.* ×ananassa with its North American parent *F. virginiana*. All selections coded by a D and followed by a number (with the exception of D7/19) as well as the selections 97/362 and 97/369 are backcrosses of *F.* ×ananassa with the South American species *F. chiloensis*.
The plants derived from purchase, breeding work or germplasm collection.

C 1.2 Instruments

Table 1: Instruments.

Instruments	**Producers**
Ball mill MM 300	Retsch
Centrifuges:	
Eppendorf Centrifuge 5415 C, rotor no. F45-18-11	Eppendorf
Laborfuge 400R	Heraeus
Drying ovens:	
Drying oven UT 6420	Heraeus
Drying oven, not termed	Memmert
Electrophoresis EC-105l	EC Apperatus Cooperation
FirmTech firmness tester	Bioworks
Freeze Dryer:	
Alpha 1-2 LD	Christ
Pilot plant, not termed	
Nuclear Magnetic Resonance (NMR) Pilot plant, not termed	Dr. Blümler
pH meter 691	Metrohm
Refractometer PR-100	Atago
Scales:	
Analyze scale EW 2200-2NM	Kern
Precision scale FA-110-4i	Faust
Analyze scale LA230 S	Satorius
Sequencer Li-cor Clobal Edition IR2 DNA Sequencer NENJ+M	Li-cor
Spectral photometer: Spectronic 601	Milton Roy
Thermomixer, comfort, compact and Stat plus	Eppendorf
Thermocycler Mastercycler Gradient	Eppendorf
Thermocycler iCycler	Biorad
Water bath	Julabo
Water bath	Koettermann

C 1.3 Chemicals and Disposable Material

Table 2: Chemicals and disposable material.

Substances/products	**Producers**
Ammonium persulphate	Sigma
Agarose	FMC Bioproducts
Boric Acid	Sigma
Chloroform	Merk
CTAB	Merk
dNTPs:	
dATP, 100 mM	MBI Fermentas
dCTP, 100 mM	MBI Fermentas
dGTP, 100 mM	MBI Fermentas
dTTP, 100 mM	MBI Fermentas
EDTA	Merk
Ethanol (EtOH)	J.T. Baker
Ethidium bromide (EtBr)	Sigma
Ficoll 400	Fluka
Gelatin	Merk
Hydrochloric acid (HCl)	Riedel-deHaen
Isoamyl alcohol	J.T. Baker
λ DNA/Eco 471	MBI Fermentas
Magnesium chloride ($MgCl_2$)	Sigma
ß-Mercaptoethanol	Merk
Octanol	J.T. Baker
Phenol	Merk
Polyvinylpyrrolidone (PVP)	Merk
Potassium chloride (KCl)	Merk
Potassium hexacyanoferrate (II)	Merk
Reaction cups, 0.2 ml	MBI Fermentas
Reaction cups, 1.5 ml	Eppendorf
Water (H_2O), ultra filtrated and UV-treated with a TKA High Purity Water System (TKA Lab HP 6 UV/UF, 08.1104)	TKA
Sea sand, extra pure	Merk
Sea sand, size: 0.1-0.3 mm	AppliChem
Sodium hydroxide 0.5 mol/l	Biesterfeld
Sodium acetate (NaAc)	Merk
Sodium chloride (NaCl)	Fluka
Sodium hydroxide (NaOH)	Merk
Tetramethyl-ethylenediamine (TEMED)	Merk
Tris	Pharmacia Biotech
Urea	Sigma
Zinc sulfate	Merk

C 1.4 Enzymes

Table 3: Enzymes.

Enzymes	**Producers**
Rnase A, 100 U/mg	Sigma
Taq DNA Polymerase, 1 U/µl	MBI Fermentas

C 1.5 Kits

Table 4: Kits.

Name of the kit	Producers
DNeasy Plant Kit	Quiagen
Multiplex Kit	Quiagen
Testing Combination: Sucrose/D-glucose	Boehringer

C 1.6 Buffers and Solutions

Table 5: Buffers and solutions.

Buffers and solutions	Compounds
Carrez I:	36 g Potassium hexacyanoferrate (II) *3H_2O / 1000 ml
Carrez II:	72 g Zinc sulfate *7H_2O / 1000 ml
dNTPs (10 mM)	10 µl 100 mM dATP 10 µl 100 mM dCTP 10 µl 100 mM dGTP 10 µl 100 mM dTTP 60 µl water
Ethanol (70%)	70 ml ethanol (100 %) 30ml water
HEUN extraction buffer according to HEUN *et al*. (1991)	100 ml 1M Tris, pH:7.5 140 ml 5M NaCl 20 ml 0.5M EDTA 740 ml H_2O 10g/l CTAB 10g/l ß-Mercaptoethanol
Loading dye buffer (L-buffer)	15 g Ficoll 400 0.25 g bromphenol blue buffer TE to a final volume of 100 ml was added
Primers	degenerated primers: 25 µM
Solution 1: Solution 2: Solution 3: Solution 4:	Triethanolamin-buffer, Boehringer Enzymatic solution (HK/G6P-DH), Boehringer Citrate-buffer / ß-Fructosidase, Boehringer Phosphoglucose-Isomerase, Boehringer

Tris acetate EDTA (TAE) buffer 50x	50x 242 g Tris 57.1 ml/l acetic acid 18.61 g EDTA the pH was adjusted to 8.0 Water to a final volume of 1000 ml was added 1 x 2 ml 50 x TAE 98 ml water
Tris borate EDTA (TBE) buffer 10x	108 g Tris 55 g Boric Acid 20 ml 0.5M EDTA water to a final volume of 1000 ml was added
TE buffer	10 mM Tris-Cl pH 7.5 1 mM EDTA
Tris-HCl pH 8.3	12.11 g/l Tris the pH was adjusted to 8.3
WILLIAMS buffer:	10 mM Tris-HCl pH 8.3 50 mM KCl 2.0 mM $MgCl_2$ 0.001% gelatin

C 1.7 Special Software

Table 6: Special software.

Software	**Producers**
Fruitsoft 1.5v	BioWorks
Minitab 14.1v	Minitab Inc.
Quiamult 60	Li-cor

C 1.8 Companies

Table 7: Companies.

Company's name	**Principle office**
AppliChem	Gatersleben, Germany
Atago	Tokyo, Japan
Biesterfeld	Hamburg, Germany
Biorad	Munich, Germany
Bioworks	NY, USA
Boehringer	Ingelheim, Germany
Christ	Osterode, Germany
EC Apperatus Cooperation	St. Petersburg-Florida, USA
Eppendorf	Cologne, Germany
Faust	Cologne, Germany
Fluka	Neu-Ulm, Germany
Heraeus	Hanau, Germany
Invitrogen	Groningen, The Netherlands
J.T. Baker	NJ, USA

Julabo	Seelbach, Germany
Kern	Balingen-Frommern, Germany
Li-cor	NB, USA
MBI Fermentas	Vilnius, Lithuania
Memmert	Schwabach, Germany
Merck	Darmstadt, Germany
Metrohm	Herisau, Swiss
Milton Roy	PA, USA
Minitab Inc.	PA, USA
Pharmacia Biotech	Vienna, Austria
Quiagen	Hilden, Germany
Retsch	Haan, Germany
Riedel-de Haen	Seelze, Germany
Roth	Karlsruhe, Germany
Satorius	Goettingen, Germany
Sigma	Deisenhofen, Germany
TKA	Niederelbert, Germany

C 2 Methods

C 2.1 Plant Material

C 2.1.1 Standard Cultivation

All plants, if not mentioned different, were cultivated in 2004, 2005 or 2006 at the test field of the Institute of Fruit Breeding (IOZ) in Dresden-Pillnitz (113 m asl). Dresden-Pillnitz is located in the Lowland Elbe River valley in the East of Germany. The rainfall in June was 67.5 mm in 2004, 59.0 mm in 2005 and 78.0 mm in 2006. The average temperature in June was 16.9 °C in 2004, 18.0 °C in 2005 and 18.2 °C in 2006. Detailed average per day climate data for June and July of each year was received by the meteorological office of the Saxon State Institute of Agriculture (LFL). The soil type of the test field was sandy loam to loamy sand on a gravel ground. Standard commercial cultural practices and irrigation, if required, were used. The intertillage was oat (*Avena sativa* L.) in a three year annual rhythm. Vegetative propagated material of the IOZ or from a nursery was used, or breeding material was established by crossing and seed starting.

C 2.1.1.1 Vegetative Propagation

Vegetative propagation by runners is still the standard method of the practice, even if F_1 hybrid seed stocks are available (BENTVELSEN *et al*. 1997, BENTVELSEN and STERK, 1996). Due to their negligible importance in fruit culture, these F_1 hybrids were not included in the present work.
The runners of the to propagate plants were cut off in July and planted into multi-plates with a peat-sand substrate (2:1) and kept in the greenhouse until the planting. Planting material from commercial nurseries or other party was planted at the same time, to exclude an influence of the planting season.

C 2.1.1.2 Establishment of Breeding Material

For crossing, vegetative propagated plants cultivated frost-protected in pots were transferred into a heated (18.0°C day / 15°C night) part of the greenhouse at the end

of January. To assure a good formation of flowers and their organs, additional light exposure (16 h long day conditions) had to be given. The genotypes chosen as a male breeding partner were relocated some weeks earlier for harvesting pollen. The pollen was stored in 1.5 ml plastic reaction tubes standing upright and not closed in an exsiccator at room temperature until usage. Alternatively, pollen from last year which was stored in closed 1.5 ml reaction tubes or glass petri dish at 4°C were used. The flowers of the plants chosen as the female cross partner were carefully emasculated with tweezers before the flower bud was totally opened and pollinated with the stored and defined pollen of the male cross partner by a soft brush. The pollination was repeated for several days, whereby after each pollination the emasculated plants were directly isolated under a small meshed frame against uncontrolled pollination.

The berries were harvested when fully ripe and the fruit skin with the seeds was peeled cautiously with a knife and dried on filter paper at room temperature. The seeds could be easily rubbed of from the filter paper and stored in glass vessels or were directly sowed in a peat-sand substrate (2:1) and stratificated at 2°C in the dark for two weeks. The number of sowed seeds was adapted to the purpose of the cross and the by experience expected germinability.

The stratificated seeds were transferred to the greenhouse and kept moistly at a temperature of 18 to 20°C. The germination happened in a period of 3 to 4 weeks in which the seedlings were transplanted into pots with a peat-sand substrate (2:1). Depending on the objective of the cross, the seedlings were left unselected or selected according their habit.

C 2.1.1.3 Plantation

All plantations at the IOZ were carried out in the year before harvest as three row blocks with 80 cm space between the rows and 25 cm distance between each plant. The distance between different selections or cultivars was 50 cm. *F. ×ananassa* cultivars and selections were planted in blocks of 9 to 42 clones and the other *Fragaria* species with a minimum of 12 plants per genotype. The number of seedling populations amounted normally from 50 to 300 plants. Selected genotypes were vegetative propagated (C 2.1.1.1) and three plants were planted as A-selections and at least nine plants as B-selections.

C 2.1.1.4 Harvest

The first date of harvest for each genotype was individually set when approximately 20% of the strawberries of a block showed full color. Only fully red strawberries were picked for the standard practice and are referred in this work also as ripe. All further pickings of a genotype were carried out if enough fruits were obtained. Normally after two to three days. This procedure is consistent with the harvest practice of the local strawberry growers.

C 2.1.2 Specific Cultivation

All cultivation methods which were deviating from the standard cultivation are listed in this chapter.

C 2.1.2.1 Ripening Stage

For the evaluation of the influence of the ripening stage, all strawberries of the four cultivars ‘Avalon Classic’, ‘Dover’, ‘Elsanta’ and ‘Lambada’ were picked at the same picking date (June 16th 2005) out of the cultivar blocks. The berries were sorted according to the ripening stages of color development: unripe (total green), half-ripe (color change with green tip), ripe (fully colored) and overripe (dark red and loss of firmness). In the case of ‘Lambada’ not enough overripe fruit were present. The samples of each cultivar and ripening stage were divided in three equal repetitions of at least 50 g, the number of fruit and total weight were recorded and the DM was determined according to C 2.2.2.1.

C 2.1.2.2 Single Fruit Analysis

For single fruit analysis, six plants, each of the cultivars ‘Ciflorette’, ‘Elsanta’ and ‘Senga Sengana’, were randomly chosen out of the cultivar blocks in the year 2005. The fruit from these plants were picked in a fully ripe stage. Fruit of ‘Ciflorette’ were picked on June 8th, 10th, 13th, 16th and 21st, fruit of ‘Elsanta’ and ‘Senga Sengana’ on June 16th, 18th and 21st. The cultivar, date, plant number and rank order of fruit were recorded. The rank orders of a fruit truss of the cultivar ‘Mieze Schindler’ is shown in

figure 5. In the following work the fruit rank A is considered as the highest or primary and the fruit rank D as the lowest or tertiary.

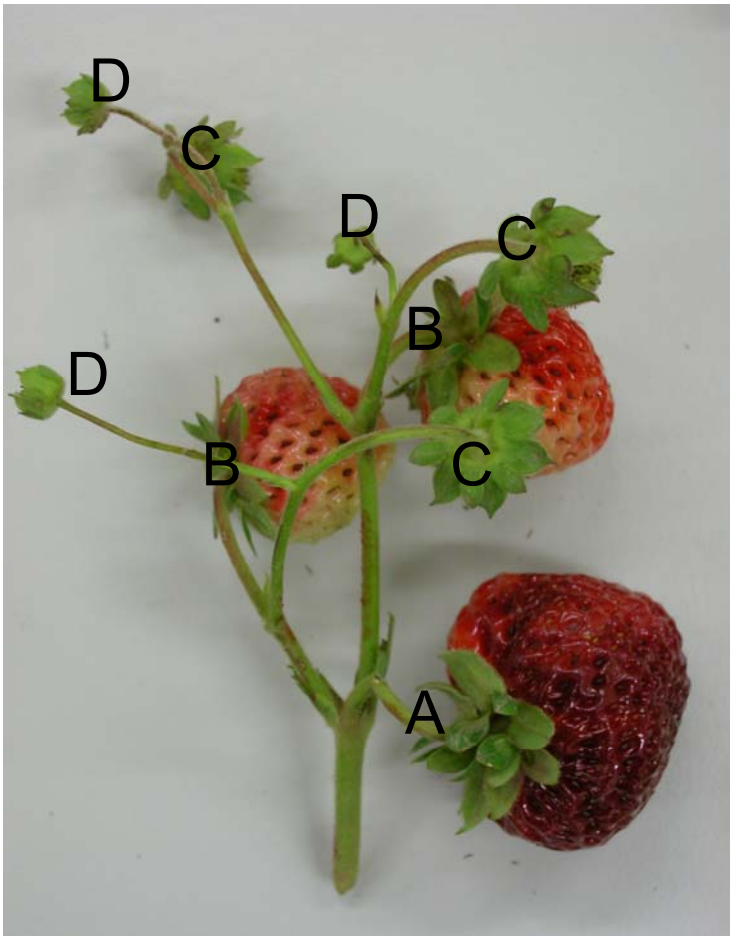


Figure 5: Infructescence of the cultivar ‘Mieze Schindler’. The fruit ranks are indicated by alphabetic characters.

In the case of ‘Ciflorette’ one plant was randomly chosen and the trusses were numbered and recorded. The DM and fruit weight of each single fruit was determined according to C 2.2.2.1. The DM of berries with less than 10 g was determined according to a modified protocol. The berry was cut in two halves and these were homogenized separately in two beakers filled with sea sand. The samples were dried until weight constancy.

C 2.1.2.3 Location

In 2005 plants of the cultivars ‘Mieze Schindler’ and ‘Senga Sengana’ were purchased from HUMMEL Stuttgart Germany and ‘Roxana’ from NEW FRUITS Cesena Italy. The plant material was directly sent from the propagators to the test stations of the University of Natural Resources and Applied Life Science (BOKU) in Vienna Austria, Geisenheim Research Center Germany, Research Institute of Pomology and Floriculture in Skiernievice Poland and the IOZ in Dresden Germany. The plantation and the further cultural practice were carried out according the local standard practice. Each cultivar was planted in triple replication blocks of 15 plants each. The sequence of the cultivar blocks at Dresden, Skiernievice and Vienna was: ‘Roxana’, ‘Mieze Schindler’, ‘Senga Sengana’, ‘Roxana’, ‘Mieze Schindler’, ‘Senga Sengana’, ‘Roxana’, ‘Mieze Schindler’, ‘Senga Sengana’. Due to a deviating row system at Geisenheim, at this location the sequence of the blocks was different. The first date of harvest for each cultivar was set when approximately 300 to 500 g of fruit were ripe in each replication block. The two subsequent pickings were also carried out if this amount of berries was ripe. Diseased or deformed fruit were discarded. The calyx was removed and the berries were frozen and stored in sealed plastic bags at -20°C. The analysis of all samples was carried out at the location Dresden.
The DM, Brix, citric acid and average fruit weight were determined according to methods C 2.2.2.1, C 2.2.3, C 2.2.4.

C 2.1.2.4 F_1 Clone Populations

In 2004 every fifth plant of a seedling population of the cross ‘Mieze Schindler’ x ‘Elsanta’ were propagated as tripe clones and planted in three row blocks. In total 200 genotypes as tripe clones were present in 2005 and the second picking of these clones were analyzed for DM according to C 2.2.2.1. Some of the picking charges were smaller than 200 g but still investigated. Every genotype was again propagated and planted in new three row blocks. Additionally, 168 plants of the planting of 2004 persisted on the field, for a second harvest year. In 2006, the fruit of the second pickings of the one year old and two year old planting were investigated for average fruit weight and DM according to C 2.2.2.1. Due to capacity restrictions, not all genotypes could be analyzed. Therefore, every third genotype and the genotypes

with a DM higher than 11.9% and lower than 9.7% DM in the investigation of 2005 were chosen.

C 2.1.2.5 Bi-Parental Diallel

Based on the results of the gene pool screening of 2004 (D 2.1), a diallel with two parental sets of different DM levels were planned. The crosses between these sets were designed to gain knowledge about the inheritance of the trait DM. The genotypes ‘Ciflorette’ and 97/369 constituted parental Set “High DM”, the cultivars ‘Korona’ and ‘Roxana’ formed parental Set “Low DM”. In 2005, crosses between genotypes of both sets were performed in a reciprocal mating design without selfings (figure 6).

			♀			
			High DM		Low DM	
			‘Ciflorette’	97/362	‘Korona’	‘Roxana’
♂	High DM	‘Ciflorette’			18	19
		97/369			16	17
	Low DM	‘Korona’	13	12		
		‘Roxana’	15	14		

Figure 6: Incomplete diallel bases on parental sets. The numbers specify the population number.

In 2005 the unselected seedlings were planted at the test field of the IOZ in two block rows as displayed in figure 7.

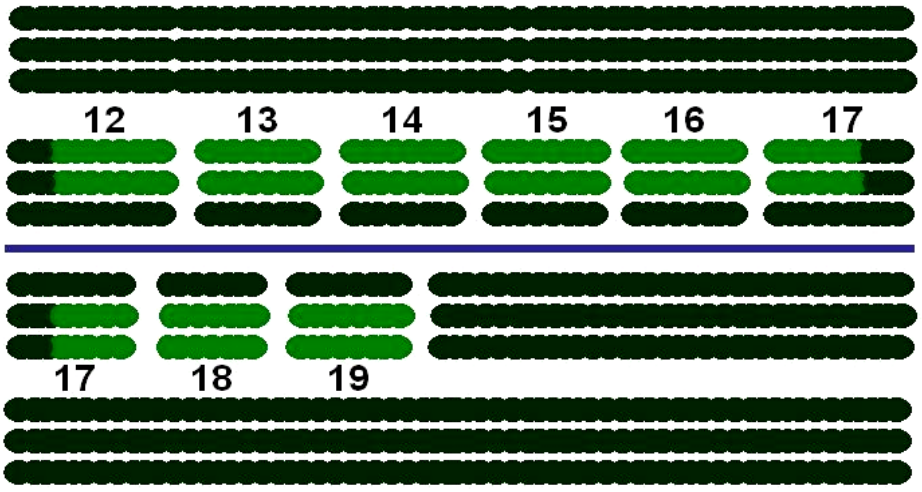


Figure 7: Planting of the bi-parental diallel. Dark green indicate other plantings or buffer plants. Bright green are the rows of diallel populations and the number specifies the population number. The blue line represents the location of the irrigation pipe.

The fruit of the seedlings were picked in this manner that no ripe fruit had to be discarded. The first two pickings of the plants of the diallel populations were unified and stored at -20°C. Only diseased fruit were discarded; no fruit due to its size. Sometimes this procedure resulted in small samples in regard to fruit number or weight. The DM and the average fruit weight of this unified sample were determined according to C 2.2.2.1. Several randomly chosen genotypes were freeze-dried according to C 2.2.2.2 and analyzed according to C 2.2.4.1 and C 2.2.5. Additionally, the yield of the first two pickings was recorded.

Figure 8: Different appearances of chlorophyll defects on different seedlings.

At June 28th, each single planting position of the populations was evaluated for presents of a plant. The mortality rate of each population was calculated according to the formula: (number of not present plants at June 28th)* 100 / number of planted plants, and recorded as percentage. The rate of analyzable plants was calculated according the formula: (number of plants with fruit at June 28th / number of planted plants)* 100. Further, each single plant was evaluated qualitative for the genetic defects dwarfism and chlorophyll defects as well as for the rate of mildew (*Sphaerotheca macularis* Wallr.:Fr.) affection. The category chlorophyll defects

included the common known defect June yellows or leaf variegation (PLAKIDAS 1932, DEMAREE and DARROW 1937). Different intensities are presented in figure 8. The percentages of dwarfism and chlorophyll defects were calculated based on all present plants. The following simple rating was used for the mildew affection: 0: No, 1: Weak, 2: Average, 3: Severe (figure 9). After the last picking, the percentage of the plants with no fruit was recorded by calculating on the basis of present plants.

Figure 9: Illustration of the mildew affection rating. 0: No, 1: Weak, 2: Average, 3: Severe.

Additionally, selection work was conducted. At June 7th a pre-selection was done on the basis of habitus and flower. At June 28th the second selection level was carried out by means of the fruit.

C 2.1.2.6 Pollen mixture *vs*. Parental Cross

A comparative cross experiment between a parental cross with defined parents and a pollen mixture with one defined mother was started in 2003 by OLBRICHT. Figure 10 explicates the crossing scheme. The pollen fertility of each paternal parent was ensured by a test of pollen germination capacity (method not shown). The same crossing partners were used for both approaches. In the case of the parental crosses

four different crosses were done with ‘Fraroma’ as maternal and ‘Elsanta’, ‘Honeoye’, ‘Korona’ and ‘Senga Sengana’ as paternal parent. The pollen mixture was created by adding the same numbers of anthers of each paternal parent in a 1.5 ml reaction tube. Then, the maternal parent ‘Fraroma’ was pollenized by this pollen mixture.

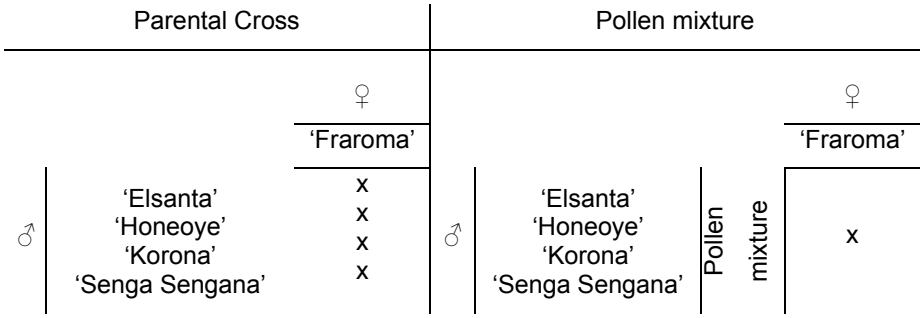


Figure 10: Crossing scheme of the comparative experiment between parental cross and pollen mixture.

The seedlings were planted according the standard procedure and passed through three selection levels for fresh market.

C 2.2 Determinations of Fruit Quality Parameters

C 2.2.1 Firmness

The FirmTech instrument and Fruitsoft 1.5 software of BIOWORKS was used for the firmness determination of selected genotypes in the year 2004. The instrument squeezes gently the fruit with a probe. Either the depth of compression at defined compression or the used compression at fixed depth of compression is recorded in g/mm.

A turntable with 12 oval shaped indentures and a round probe were used. The following parameters were set: force threshold: 100 to 250 g/mm (compression limited), Speed: Load Cell 12 mm/s and Table 1.18 rpm.

For analyzes, 30 strawberries were chosen out of the sample. The measurements were carried out according the manufacture protocol.

C 2.2.2 Dry Matter Determination

At least 200 g of strawberries were taken for a regular DM analysis. Since the average fruit size is much smaller in the case of *Fragaria* species than for *F.* ×ananassa, only 50 to 100 g of strawberries of those species was used.
The analysis was carried out either immediately after harvest or the sample was frozen and stored in a sealed plastic bag at -20°C until analysis. The samples were not stored longer than four months, because of significant changes on the DM by frozen storage (SKUPIEN and JAKUBOWSKA 2004).

C 2.2.2.1 Drying Oven

The described method has the highest sample throughput and was carried out in most of the cases. A modified protocol according to the German norm DIN 10764 (Determination of moisture content of soluble coffee) was used.
In preparation for the DM determination, beakers were filled with 20 to 25 g sea sand and a glass bar was given in each beaker. The beakers were placed into the drying oven for at least 15 min at 70°C. After that time the beakers were put into an exsiccator for cooling down to room temperature and weighed afterwards.
For analysis, the fruit sample of one genotype was purred and an aliquot of approximately 3 to 4 g was transferred into each of three sea sand filled 100 ml beaker. The beakers with the sample were weighed again. The strawberry puree was ground with the sea sand by the glass bar and the beaker was placed into a drying oven, where the sample was dried at 70°C for 24 h. In all cases weight constancy occurred up to this time. The DM percentage was calculated for each beaker according to the formula: output-weight [g]* 100 / initial weight [g], and recorded as percentage of fresh weight. The total DM of a sample was calculated by averaging over the three DM values.
For single fruit analysis frozen samples were used and smashed directly into the beakers after the defrosting. Fruit smaller than 12 g were placed into one with 35 to 40 g sea sand filled and weighed beaker. Fruit bigger than 12 g were cut in two or three equal parts and each part was transferred separately into prepared beakers. In this case the initial weights as well as the output-weights of the parts were summed and the DM was calculated according the above mentioned formula.

A modified protocol was used in order to calculate the proportion of the strawberry achenes on the dry and fresh weight basis. For this purpose, the fruits were cut into discs of approximately 0.5 cm and dried onto a petri dish with a filter paper in a drying oven. The achenes were rubbed off and weighted additionally separately, after drying. This method caused considerable additional work and was just carried out for selected genotypes.

C 2.2.2.2 Freeze-Dryer

If further investigations should be conducted (C 2.2.4, C 2.2.5) or a strawberry genotype should be evaluated for its appearance after the freeze-dry process a freeze-dryer was operated for DM determination. A disadvantage was the limited sample throughput. Either a laboratory freeze-dryer or a pilot plant freeze-dryer was used.

The whole fruit samples were laid onto a weighed petri dish or an aluminum bowl and placed into the freeze-dryer. In the case of the laboratory freeze-dryer (Alpha 1-2 LD) the sample stayed for at least 72 h in the machine. No adjustments could be made on this freeze-dryer. The freeze-dryer conditions of the pilot plant were 1 mbar and 50°C for 72 h.

After weight constancy the sample was weighed again and the DM was determined according the above mentioned formula.

The DM proportion of the achenes could be determined by weighting single freeze-dried berries, removing the achenes of these fruit and weighting only the achenes. The percentage of the achenes per fruit was calculated according the formula: achenes [g] / (fruit with achenes [g] / 100), and recorded as percentage of DM. Three fruit of the crossing parents of the diallel were investigated and their values averaged.

C 2.2.3 Refractometry

The index of refraction or refractive index (RI) is a fundamental physical property of a substance. Based on the RI and the fact that the RI of a liquid changes against the soluble solids dissolved in the liquid, BRIX developed 1870 a calibration method to determine the sugar content of liquids. The after him named Brix value is therefore the percentage (%) of the concentration of soluble solids in an aqueous solution.

Today, the RI and the Brix value can be measured with a refractometer in a fast and sufficient way. It has to be considered that all soluble solids have an effect on the refraction and therefore all solids like sugars, salts, proteins or acids which a dissolved have a part in the calculated Brix value. Refractometers are normally calibrated by sucrose solutions (10% sucrose in water is 10% Brix), but the in the present work used digital refractometers could be calibrated by distilled water to 0.0%.

For selection work, the Brix value was determined directly on the test field. For measurement some drops of a pooled strawberry fruit solution of one genotype were applied on the prism surface of the refractometer and the calculated Brix value could be read off after 3 seconds.

All other Brix determinations were carried out in the laboratory. The puree originating from the DM determination described in (C 2.2.2) or the citric acid determination (C 2.2.4) was used. Approximately 5 ml of the purred sample was filled into centrifuge tubes and centrifuged for 5 min at 8000 rpm. The supernatant was used for Brix measurement.

C 2.2.4 Citric Acid Determinations

Since the citric acid determinations were carried out in two different labs, also two different methods had to be carried out due to logistical reasons. Since no comparative experiment between these two methods could be conducted, the results of the two methods have to be separately considered.

C 2.2.4.1 Citric Acid Determination I

This method was carried out on freeze-dried samples of C 2.2.2.2. The sample was pulverized by a coffee grinder into fine powder. 1.5 g of this powder was transferred into a 100 ml beaker and moistened with neutralized EtOH 96%. 50 ml distilled water was added and the suspension was titrated with NaOH to a pH-value of 8.2. The used quantity of NaOH was recorded and the present citric acid calculated by the formula:

$$\frac{\text{Volume NaOH [ml]} * 3.2}{\text{1.5 g (sample weight)}} = \text{Citric acid [\%]}$$

Two repetitions of each sample were carried out and the mean and standard deviation was calculated.

C 2.2.4.2 Citric Acid Determination II

This method was carried out on fresh or defrosted fruit samples.
The pureed fruit sample of the DM determination C 2.2.2 were used after 5 ml of this sample was centrifuged in centrifuge tubes for 3 min at 400 rpm. The supernatant was used for acid determination.
50 ml distilled water was added to 5 ml supernatant and the suspension was titrated with NaOH to a pH-value of 8.2. The used quantity of NaOH was recorded and the present citric acid calculated by the formula:

$$\frac{\text{Volume NaOH [ml]* 6.7}}{\text{5.0 g (sample weight)}} = \text{Citric acid [\%]}$$

One repetition per sample was carried out and the values were averaged.

C 2.2.5 Sucrose, Glucose and Fructose Determination

The determination of sucrose, glucose and fructose content of freeze-dried sample was determined with the glucose, fructose, sucrose kit of BOEHRINGER.
The sample solution was prepared by weighting out 0.5 g of freeze-dried fruit powder of C 2.2.2.2 into a volumetric flask and adding approximately 40 ml distilled water. For better solubility, the solution was incubated in a water bath for 30 min at 40°C and mixed in between. Further, the solution was cooled down to room temperature, 5 ml Carrez I solution and Carrez II solution of the BOEHRINGER kit were added and the mixture was filled up to a volume of 100 ml and mixed. After incubation at room temperature for 10 min the suspension was filtered. Since a sample solution of 0.05 to 0.80 g/l was expected, the filtrate was diluted 1:5 with distilled water according the manufactures specifications. The further steps were carried out with the BOEHRINGER solutions 1, 2, 3 and 4 and according to the protocol of the manufactures kit.

C 2.2.6 Nuclear Magnetic Resonance

The Nuclear Magnetic Resonance (NMR) technique offers inter alia a powerful tool for non-invasive visualization of the inside of living organisms. Its application for medical imaging is well known. However, the technique was also used in plant science.

All research regarding the NMR technique was carried out by BLÜMLER of the Research Centre Jülich GmbH, Germany. A prototype high magnetic field spectrometer was used (figure 11 a and b).

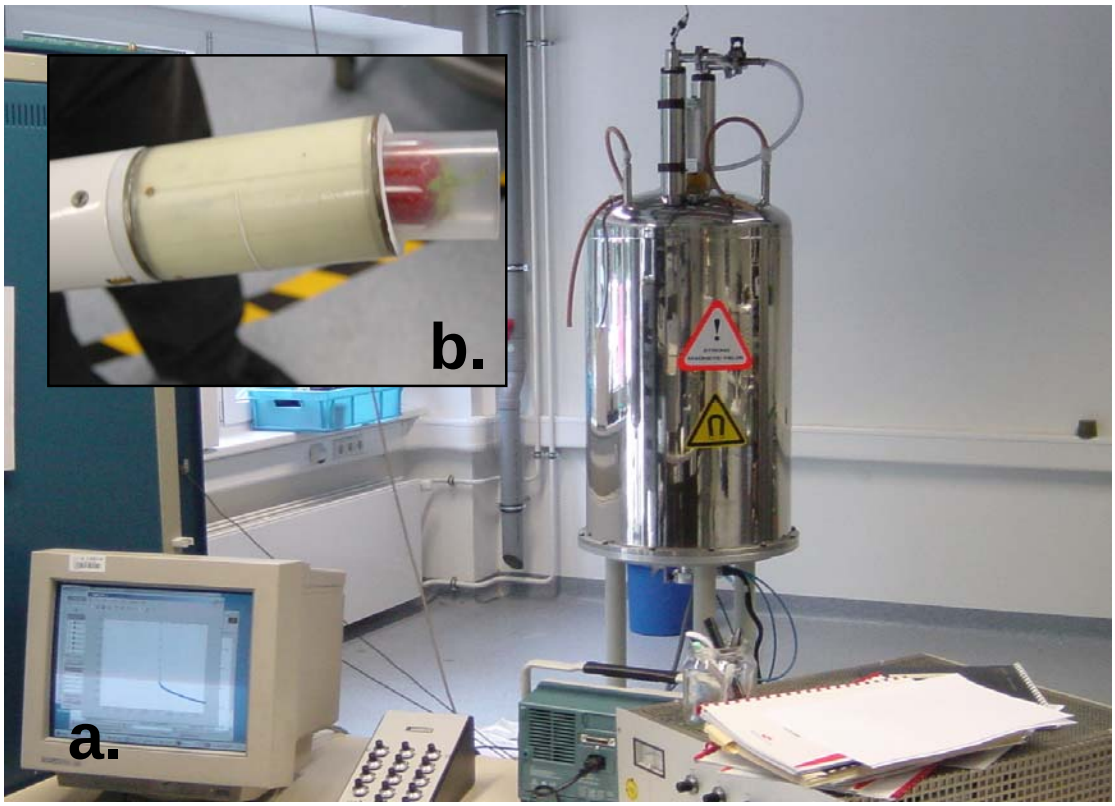


Figure 11 a and b: 11 a: The prototype high magnetic field spectrometer in the laboratory of the Research Centre Jülich GmbH. The smaller figure 11 b shows the specimen holder loaded with a strawberry fruit of 'Alba'.

The investigations were limited by the approximately 3.0 cm diameter of the specimen holder (figure 11 b). Therefore only fruit with this or a smaller diameter could be used.

The specimen holder was loaded with a fruit and inserted in the spectrometer. The instrument settings were based on the research and operating experience of

BLÜMLER. The receiving signal of the relaxation time was integrated to produce a figure in x to y and x to z coordinate area. These areas could be assembled to a three-dimensional illustration. The areas with a fast relaxation time have high free water content and thus low DM.

C 2.3 Molecular Biology

The seedlings of the pollen mixture (C 2.1.2.6) were analyzed by a molecular biological fingerprint.

C 2.3.1 DNA Extraction and Quantification

The following protocol was used for DNA extraction.
Axillary leaf buds were taken from field plants and stored in 1.5 ml reaction tubes at -20°C. Approximately 120 mg of frozen plant material was transferred to a 2.2 ml reaction tube containing a stainless steel ball and kept consequently at liquid nitrogen temperature. The plant material was grinded for 3 min by a ball mill at 25/sec. Then, 750 µl HEUN extraction buffer with 1% PVP were added on the frozen and entirely grinded sample and mixed diligently. The suspension was incubated for 15 min and 65°C in a water bath and inverted two to three times every 5 min. The mixture was centrifuged for 10 min at 13.2 thousand rpm. The supernatant and the steel ball were discarded, 375 µl chloroform: octanol (24:1) was added to the sample and mixed heavily for 5 min. After centrifugation at 13.2 rpm for 10 to 20 min, the supernatant was transferred to a new 2.2 ml reaction tube and 833 µl of -20°C cold EtOH (98%) was added. The mixture was carefully inverted and centrifuged at 13.2 rpm for 10 min. The supernatant was carefully drained and 416 µl of -20°C cold EtOH (70%) was added. Again, the mixture was carefully inverted and centrifuged at 13.2 rpm for 10 min. The supernatant was carefully drained and the remaining EtOH removed by a pipetting. It was high importance not to remove the pellet at the bottom of the reaction tube. The reaction tube with the pellet was incubated for 10 min at 37°C. Finally, 1000 µl TE buffer and 1 µl RNase were added and the mixture was gently vortexted. The mixture was incubated for 30 min at 37°C and directly used or stored at 5°C over night.

The phenol-chloroform extraction and following DNA precipitation by NaAc and EtOH was conducted according the standard protocol as described in (MÜLHARD 2006). The DNA was dissolved in 100 µl H_2O. Occasionally, the centrifugation of the precipitated DNA failed. The precipitated DNA stayed in solution even after high rpm for up to 1 h. Problematic was that a pellet occurred at the bottom of the reaction tube which did not contain DNA. It is assumed that effectively proteins were precipitated while leaving the DNA in solution. For that reason, the risk was high to drain the DNA together with the solution. Knowing this, it was easy to hook out the DNA and to continue with the protocol.
The DNA quantification was performed by gel electrophoresis at standard settings. A 1% TAE agarose gel and the λ DNA/Eco 471 marker of known concentration were used.

C 2.3.2 Analysis by Random Amplification of Polymorphic DNA

The Random Amplification of Polymorphic DNA (RAPD) primers, used in the present work, were designed according to HANCOCK and CALLOW (1994), GRAHAM *et al*. (1996) and DEGANI *et al*. (1998). In the above literature these primers were mentioned as favorably for *Fragaria*. Detailed information about the used primers is presented in annex G 1. All PCR's were proceeded in 0.2 ml PCR tubes of MBI FERMENTAS, by the use of the QUIAGEN PCR kit and by a PCR thermocycler. The standard stock solution for RAPDs was:

Table 8: PCR Stock solution for RAPDs.

Component	Volume [µl]
H_2O	15.00
10x-buffer (without Mg^{2+})	2.50
$MgCl_2$ (1mM)	1.00
dNTPs (10 mU)	1.25
Primer (25 pmol)	2.00
Template (10ng)	3.00
Taq DNA Polymerase (1 µg/ml)	0.25
Total	25.00

The standard cycle conditions for RAPDs, in the present work, were:

Stage 1:		95°C		5:00 min
Stage 2: (38x)	Step 1:	95°C	for	30 sec
	Step 2:	36°C	for	60 sec
	Step 3:	72°C	for	120 sec
Stage 3:		72°C	for	10:00 min
Hold temperature:		4°C		

The PCR products were evaluated directly by agarose gel electrophoresis according the following protocol.

A 2% gel was prepared by solubilizing 2 g agarose per 100 ml in TAE buffer. 10 µl EtBr was added per 100 ml solution. As required, the agarose solution was poured into a gel casting tray with a comp. After about 30 min the gel solidifies and is ready for usage. The PCR product was 1:10 diluted with sterile water and 15% loading buffer was added. The electrophoresis was proceeded with voltage and current conditions according to WESTERMEIER (1990) and evaluated by a transilluminator.

C 2.3.3 Analysis by Simple Sequence Repeats

In the present work, 10 Simple Sequence Repeats (SSR) primers of LEWERS *et al*. (2005) and 4 SSR primers of BASSIL *et al.* (2006) were utilized. More information about the used SSR primers and the three assorted primer sets MM1, MP1 and MP2 is listed in annex G 2. All PCR's were proceeded in 0.2 ml PCR tubes of MBI FERMENTAS, by the use of the Multiplex-Kit of QUIAGEN and by a PCR thermocycler.

The stock solution for MM1 was:

Table 9: PCR Stock solution for MM1.

Component	Volume [μl]
MM-solution	5.0
Q-solution	1.0
Primer : (4 SSR primer pairs)	
forward	0.1
reverse	0.4
Template (10 ng)	2.0
Total	10.0

The cycle conditions for MM1, in the present work, were:

Stage 1:		95°C		15:00 min
Stage 2: (30x)	Step 1:	94°C	for	00:30 min
	Step 2:	61°C	for	01:30 min
	Step 3:	72°C	for	01:30 min
Stage 3:		72°C	for	10:00 min
Hold temperature:		4°C		

The stock solution for MP1 and MP2 was:

Table 10: PCR Stock solution for MP1 and MP2.

Component	Volume [μl]
MM-solution	5.00
Q-solution	1.00
H_2O	0.75
Primer : (5 SSR primer pairs)	
forward	0.05
reverse	0.20
Template (10 ng)	2.00
Total	10.00

The cycle conditions for MP1 and MP2 were:

Stage 1:		95°C		15:00 min
Stage 2: (30x)	Step 1:	94°C	for	00:30 min
	Step 2:	56°C	for	01:30 min
	Step 3:	72°C	for	01:30 min
Stage 3:		72°C	for	10:00 min
Hold temperature:		4°C		

The PCR products were stored at -20°C or evaluated directly by polyacrylamide gel electrophoresis according the following protocol.
The gel was prepared by solubilizing 4.2 g urea for a 10 ml solution in distilled water. 3.6 ml Long Ranger solution and 3.0 ml 10x TBE buffer were added per 10 ml solution and the mixture was filled up with distilled water to a volume of 10 ml. 6.7 ml of TEMED solution was added per 10 ml mixture to initiate polymerization which was further catalyzed by addition of 66.7 ml ammonium persulphate (4 mg/ml). The above mixture was rapidly poured between tilted glass plates with spacers. After about 1:30 h at room temperature the polymerization is complete and the gel is ready for usage. 2 µl PCR product was diluted with 15 µl LICOR-buffer and loaded onto the gel. The polyacrylamide gel was run in TBE buffer for 1:50 h. The evaluation was carried out by the software Quiamult.

C 2.4 Data Analysis

Data has been subjected to analysis of variance (ANOVA) procedures, if applicable. Significant differences of unequal sample sizes were analyzed by FISHER's pairwise comparison at an individual error rate of 5%.
Due to the family error rate, the TUKEY's pairwise comparison was used for the DM value comparison of the location experiment (C 2.1.2.3). For C 2.1.2.3, the General Linear Model (GLM) was used to perform a univariate analysis of variance of the unbalanced design, because the response variables had missing values. Not all response variables had the same missing value pattern. Therefore, the command was run separately for each of the response variables. As response variables were set DM, Brix, citric acid and average fruit weight *vs*. the factors cultivar, picking, block

and location. The factors cultivar and harvest were chosen as fixed, block and location as random factors. The blocks were nested within the locations.

A classification of the DM values of the gene pool screening (D 2.1.2) was done in qualitative declarations, according to the arbitrary classification scheme for several qualitative trait values of PLOCHARSKI (1989). Since the resulting classes were too narrow, the standard deviation (SD) had to be taken instead of the standard error of the mean. The classification was not applied to the entire gene pool. The non *F.* ×ananassa genotypes and the decaploid 'Spadeka' were excluded. From the mean was subtracted the 1.5 fold respectively 0.5 fold SD value. The resulting values were taken as the upper border for the classes "very low" and "low". The adding of the 0.5 fold respectively 1.5 fold SD to the mean indicated the upper boarders of the classes "intermediate" and "high". Values higher than the 1.5 fold SD added to the mean were classified as "very high". Was a genotype investigated in more than one year and classified in more than one year the lowest and highest class was combined for classification.

For C 2.1.2.5, the bi-parental Diallel, additionally to the means of the parents, the parental means were calculated by summing the two means of the parents and dividing the product by factor 2. Because the yield data were not following a normal distribution the KRUSKAL -WALLIS test offered a nonparametric alternative to the one-way analysis of variance. The KRUSKAL-WALLIS test hypotheses are: H0: the population medians are all equal vs. H1: the medians are not all equal.

The MOODS median test was used for the comparison of the DM medians of the A-selections (D 3.2.1), because the data were not following a normal distribution and this nonparametric test is robust against outliers and errors in data.

The MINITAB Software version 14.1 was used.

D Results

According to the *ceteris-paribus* principle, all presented results strictly refer to the mentioned cultivation methods, locations and genotypes or hybridizations.

D 1 Aspects of Dry Matter Determination

D 1.1 Accuracy

The accuracy of results has two important aspects: the trueness and the precision (MENDITTO *et al.* 2007). The trueness was tried to assure by an exact technical realization for all methods as described in chapter B. In this regard, the three applied DM determination methods (sea sand, filter paper and freeze-dryer) were tested for significant differences. In 2004 fruit of the cultivar ‘Korona’ were dried with the mentioned methods and at two different temperatures (60°C and 70°C) of the drying oven. The methods or temperature modifications differed not at a 5% level of significance. The p-value was 0.329 (annex G 3). The precision was attempted to effect by exact executions of all tests. The sample drawing was considered as the most crucial factor. Therefore, several tests were carried out to determine adequate sample structure and quantity.

D 1.1.1 Ripening Stages

The DM content of different ripening stages of the cultivars ‘Avalon Classic’, ‘Dover’, ‘Elsanta’ and ‘Lambada’ are listed in annex G 4. The SD of the three repetitions per cultivar ranged between 0.1% and 0.9% DM. The replications of the overripe samples of all investigated cultivars had the highest SD. Significant differences between the DM of the ripening stages were present at a 5% level of significance in each cultivar (annex G 4). A one-way multiple comparison test of FISHER demonstrated that the DM content of the overripe fruit were significant higher than for all other ripening stages for the cultivars ‘Avalon classic’, ‘Dover’ and ‘Elsanta’. No overripe fruit of ‘Lambada’ were present. The unripe fruit of ‘Elsanta’ varied also from the half-ripe and ripe fruit. The mean of the unripe as well as the half-ripe fruit of ‘Lambada’ were significant smaller than the mean of the ripe fruit. Since unripe and half-ripe berries

are easily to identify, high importance was attached not to pick overripe fruit for analysis. In case of doubt, the respective fruit was discarded.

D 1.1.2 Samples out of Blocks

The actual precision of the standard block design was tested by determination of the SD and coefficient of variation (CV) for the DM of different blocks with the same genotypes in 2004. The first three picking dates were investigated. For the cultivar ‘Elsanta’ the blocks located in row number 11, 12 and 13 of the IOZ test field with 24, 48 and 36 plants per block and samples from the same picking dates of a nearby LFL test field were analyzed. For the cultivar ‘Yamaska’ one block in row number 11 of the IOZ test field with 12 plants and samples from the same picking dates of a LFL test field were compared. The results are listed in table 11. Since the CV’s of each picking of the two cultivars are smaller or around 5% and the SD is not higher than 0.5% DM, the precision of the sample taking out of blocks was defined as high according to THOMAS (2006).
This rating was confirmed by the results of several selections each with two blocks with 24 plants. The CVs of those blocks were also not higher than 5% in 2004 (data not shown).

Table 11: The DM in [%] of the different blocks of ‘Elsanta’ and ‘Yamaska’ as well as the mean, standard deviation and CV of the same picking dates. The symbol ‘-‘ indicates a missing value.

	DM [%]						
‘Elsanta’	Block Nr.						
Picking date	11	12	13	LFL	Mean	SD	CV [%]
11.06.04	11.4	10.9	10.5	11.0	11.0	0.4	3.4
14.06.04	10.0	10.4	-	9.6	10.0	0.4	4.0
16.06.04	-	10.6	9.7	9.7	10.0	0.5	5.2
‘Yamaska’	Block Nr.						
Picking date	11			LFL	Mean	SD	CV [%]
28.06.04	10.3			10.8	10.6	0.4	3.4
30.06.04	10.6			9.9	10.3	0.5	4.8
02.07.04	10.0			9.9	10.0	0.1	0.7

Samples with higher DM values, evaluated according the CV, appear to have a smaller variation than samples with a smaller DM, even if same SDs are given. Therefore, the SD is used in the further work for comparison of the precision of results with the same units and the CV for comparisons of the precisions of results with different units. It is referred to the respective chapters.

D 1.2 Single Fruit Analysis

In 2005, the fruit of the cultivars ‘Elsanta’, ‘Ciflorette’ and ‘Senga Sengana’ were picked and investigated separately as described in C 2.1.2.2. This offered a separate consideration of the DM and fruit weight of the single fruit, plants and picking dates. The complete data are presented in annex G 5.

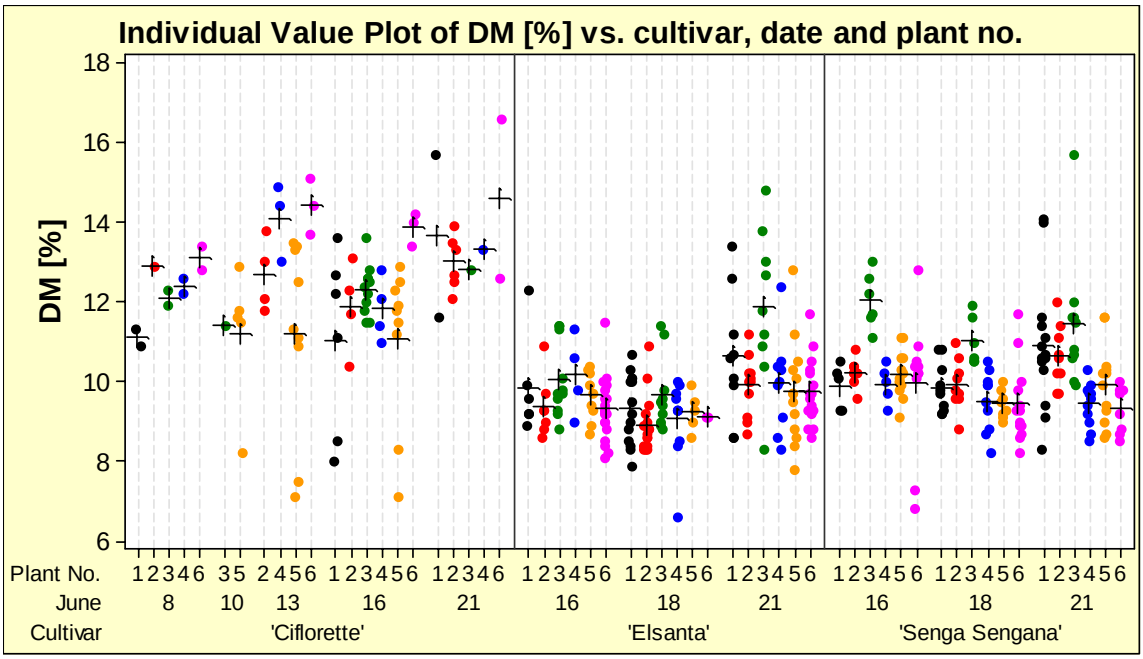


Figure 12: Individual value plot of DM [%] grouped according plant number, picking date and cultivar. The crosses are indicating the means.

Figure 12 shows an individual value plot with the DM of single fruit as variable. The data are grouped according the plant number, picking date and cultivar. A high DM variability existed in each cultivar and their single plants. The inclusion of the picking dates shows that this variability was present at all picking dates and that the pickings itself are not the cause of variability. The presented means of the single plants differed from each other, even at same picking dates. The plant No. 6 of ‘Ciflorette’ and the plant No. 3 of ‘Senga Sengana’ had at all picking dates the highest mean, in comparison to the other plants of the respective cultivar. This individual variance is well known for horticultural crops and it is an important factor for sample drawing (THOMAS 2006).

For comparison, in figure 13, an individual value plot of the fruit weight is presented for the same single fruit of the same plants, cultivars and picking dates. The

variability over all picking dates and plants is high and caused by a decline of fruit weight from the first to the last picking date. This decline of fruit weight is clearly shown by the decreasing means. In contrast, the variability of the single plants is smaller and varied between the different picking dates.

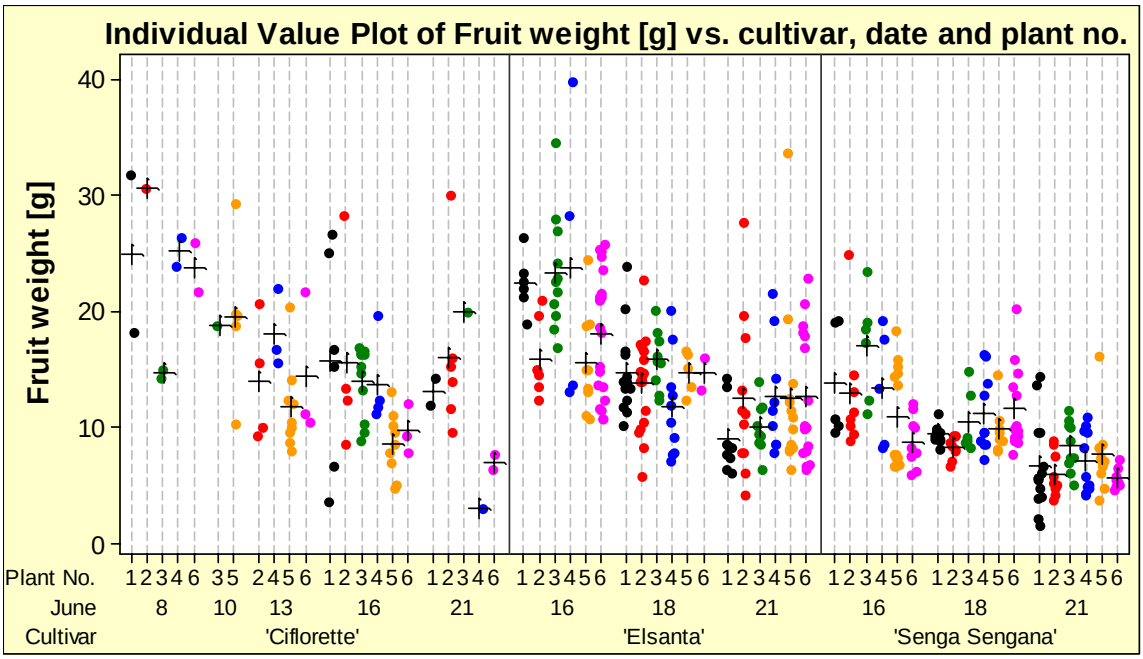


Figure 13: Individual value plot of fruit weight [g] grouped according plant number, picking date and cultivar. The crosses are indicating the means.

In figure 14 a and b, the individual value plots of ‘Elsanta’ and ‘Senga Sengana’ are shown sorted according the rank of the fruit, picking date and plant number. Due to a deficiency in number of fruit, no data are obtained for ‘Ciflorette’. The fruit of one plant and one picking date of ‘Elsanta’ had with lower rank orders also lower DM means. Exceptions were the pickings at June 21st of the plant No. 3 and at June16th of the plant No. 5. Such an evident decrease in DM with the rank order was not present in the cultivar ‘Senga Sengana’. Only the fruit of the plant No. 5 showed a decrease of DM mean with a lower rank order in all three picking dates. The fruit of the other plants showed both, increases and decreases of DM mean by rank orders.

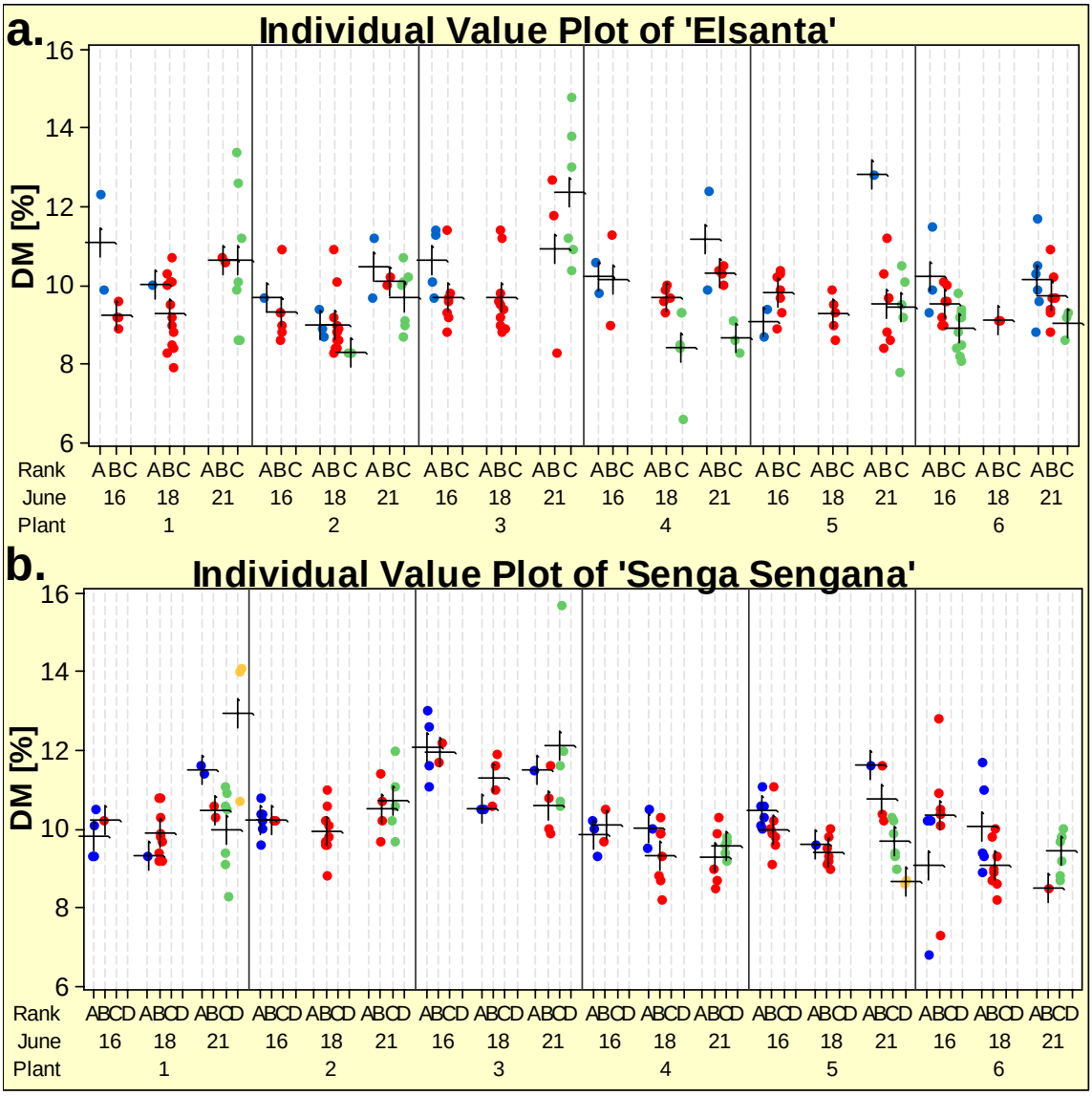


Figure 14 a and b: Individual value plot of DM [%] grouped according to rank, picking date and plant number. The crosses are indicating the means.

Figure 15 shows the plot of DM *vs.* fruit weight for all three investigated cultivars. No correlation was found between these traits for any of the cultivars in all fruit or fruit of a certain rank. The range of the fruit weight decreased and the range of the DM increased from fruit rank A to C, respectively D. Only the B and D fruit of ‘Senga Sengana’ had a smaller range than the next higher fruit rank. The seven very low values minor than 9.0% DM of the cultivar ‘Ciflorette’ are remarkable (figure 15).

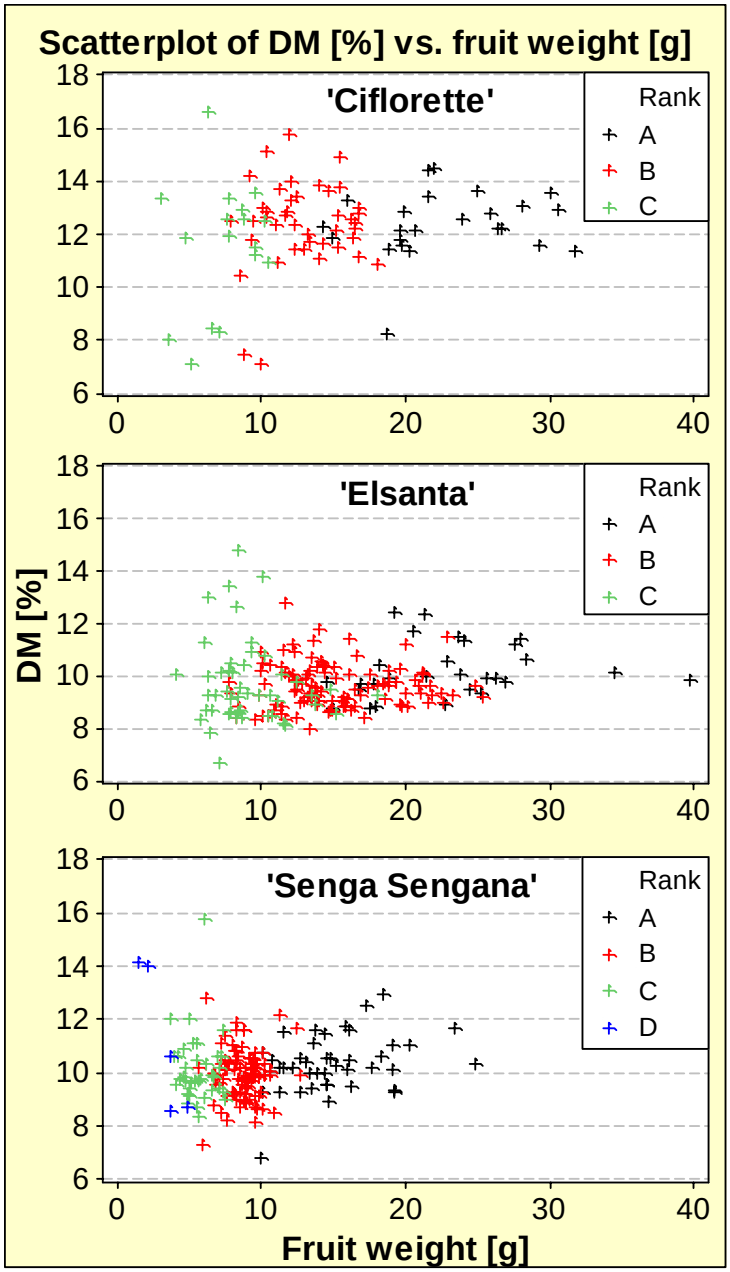


Figure 15: Scatterplot of DM [%] *vs.* fruit weight [g] sorted by the rank of 'Ciflorette', 'Elsanta' and 'Senga Sengana'.

They belonged to two C rank berries of the plant No. 1 and to five fruit of the plant No. 5. Since the infructescences of plant No. 5 were arbitrarily numbered, a more detailed illustration according the infructescence numbers of plant No. 5 can be presented in figure 16. The five berries of the plant No. 5 with a DM less than 9.0% derived all from one infructescence.

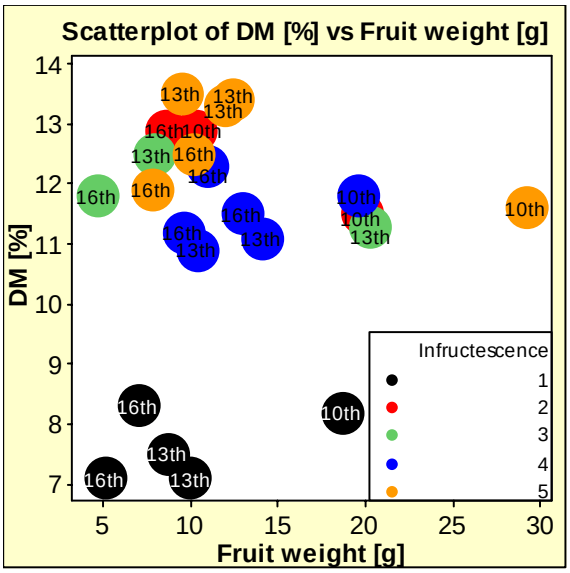


Figure 16: Scatterplot of DM [%] *vs.* fruit weight [g] for plant No. 5 of 'Ciflorette'. The infructescence number is marked by color and the picking date of each fruit is indicated.

They comprised one fruit of rank A picked at June 10th, two rank B fruit picked at June 13th and two rank C fruit picked at June 16th.

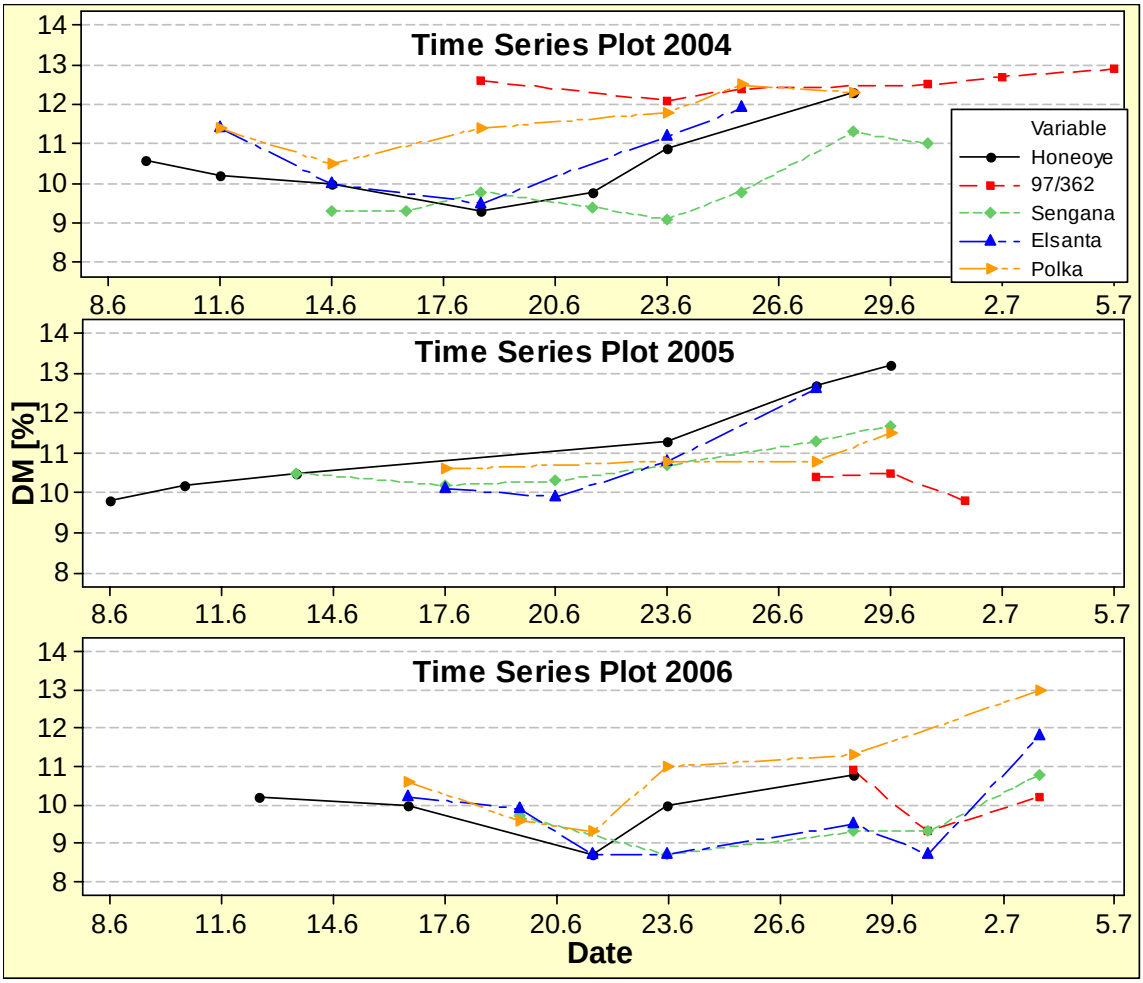
The mean of all fruit of 'Ciflorette' was 12.2% DM ± 1.7%, of 'Elsanta' 9.7% DM ± 1.2% and 'Senga Sengana' 10.1% DM ± 1.2%. After discarding the seven very low values of 'Ciflorette' the mean was 12.6% DM ± 1.2%.

D 1.3 Harvest

In practice, strawberries are usually picked several times during a harvest period. Thereby, the individual pickings are regarded as a source of variation. During the present work, some genotypes of the gene pool screening D 2.1 were picked up to three times during a harvest. However, to gain enhanced knowledge about the DM during harvest, the cultivars 'Senga Sengana', 'Elsanta', 'Honeoye' 'Polka' and the selection 97/362 were picked more than three times in three consecutive years, if this was possible.

The data shown in figure 17 display the DM values of the mentioned genotypes during the harvest in 2004, 2005 and 2006. In all years, the cultivar 'Honeoye' had the first ripe fruit, followed by 'Elsanta' and Polka' in 2004 and 2006. 'Senga Sengana' was the second earliest cultivar in 2005 and the selection 97/362 was in all years the latest genotype to pick. In 2005 and 2006, the first picking date of 97/362 was 8 respectively 9 days later compared to the first picking of 2004. The DM of most genotypes varied from lower values during the first picking dates and increased after several pickings. This variation occurred in all three investigated years. However, this

increase in DM during the picking season did not take place continuously. Variation of the DM values occurred between the first and the last picking dates.



Figures 17: Times series plots of DM [%] for the years 2004, 2005 and 2006.

The values of the selection 97/362 varied highly between the years. The DM values of the six individual pickings in 2004 varied between 12.1% and 12.9% DM. In contrast, in the years 2005 and 2006, only three pickings could be done at very late picking dates and the DM values of these years were dramatically decreased.

D 1.4 Climate Data

Figure 18 presents the DM during the harvest of 2006 in comparison to the climate data precipitation, global radiation, air temperature and air humidity. The data of 2006

was chosen as an example due to the significant drops and subsequent increase of DM at June 21st and June 30th.

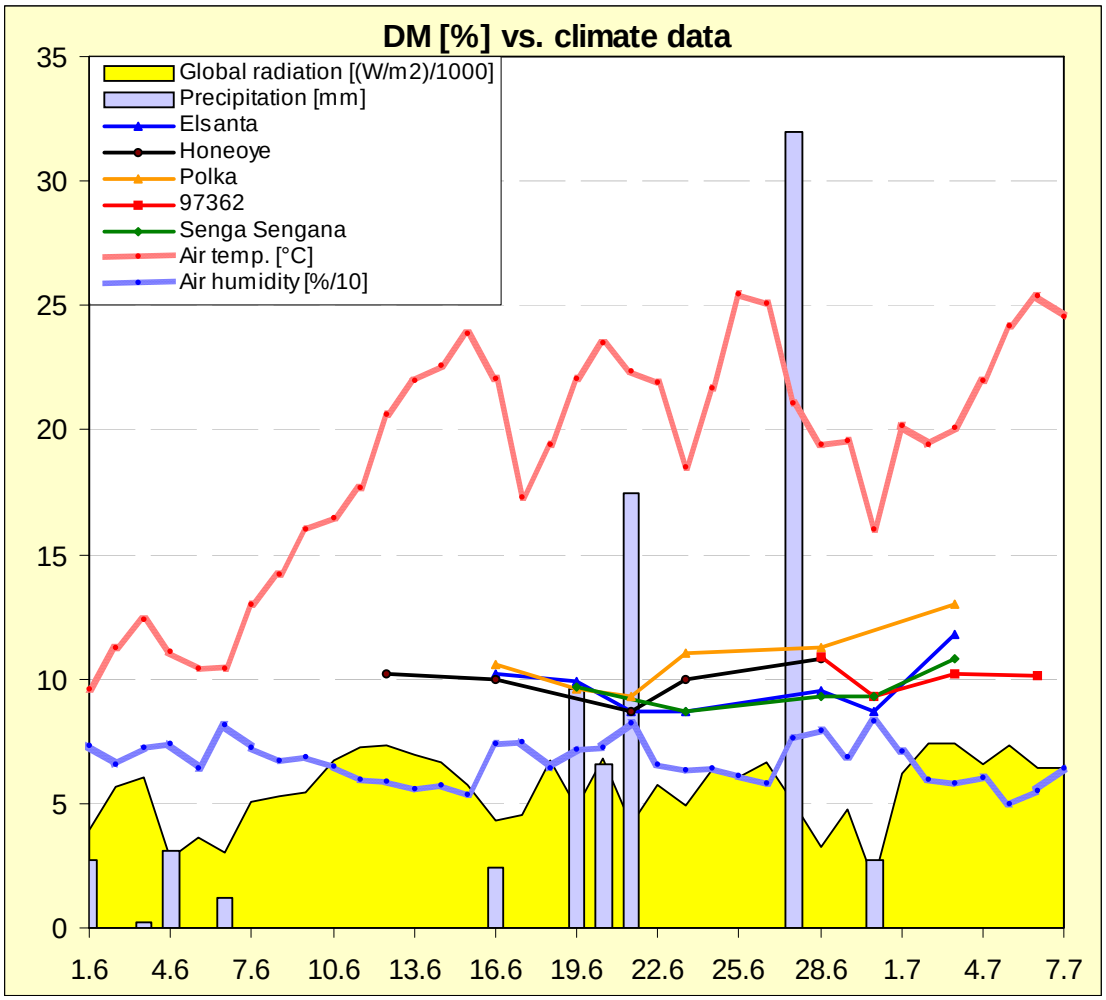


Figure 18: DM [%] during picking season in comparison to climate data.

The harvest season of 2006 preceded a period without precipitation and rapidly increasing air temperature from 10.4°C at June 6th to 23.9°C at June 15th. The global radiation increased and the air humidity decreased in this period. At June 17th, the air temperature declined back to 17.3°C and also the global radiation decreased. The air humidity increased. A rainfall period followed from June 19th to 21st with 9.6, 6.6 and 17.5 mm. The air temperature and air humidity during this period was again higher but decreased afterwards. At June 27th, a major rainfall caused precipitation of 32.0 mm. This was the highest day value measured during June and July of all three years. The air temperature dropped to 16.0°C three days after this event. Also the global radiation felt drastically after June 27th while the air humidity stayed at a higher

level of 70 to 80%. After June 30th no precipitation is recorded and the global radiation and air temperature increased to the former level.

D 1.5 Location

In 2006, the cultivars ‘Mieze Schindler’ (intermediate-high DM), ‘Elsanta’ and ‘Roxana’ (both low DM) were harvested at four different locations as described in C 2.1.2.3. The complete data of DM, Brix, citric acid and average fruit weight of all pickings and locations are listed in annex G 6. The mean, SD and CV of each picking date, location and trait are shown in annex G 7. The average SD of the DM of all cultivars and picking dates was 0.4% DM for the location Skiernievice, 0.7% DM for Vienna, 0.4% DM for Geisenheim and 0.4% DM for Dresden. The sampling of the location Vienna is still in the range of a satisfactory precision. However, at Vienna a SD of 1.5% DM occurred and at Geisenheim of 1.1% DM, both in the third picking of ‘Roxana’.

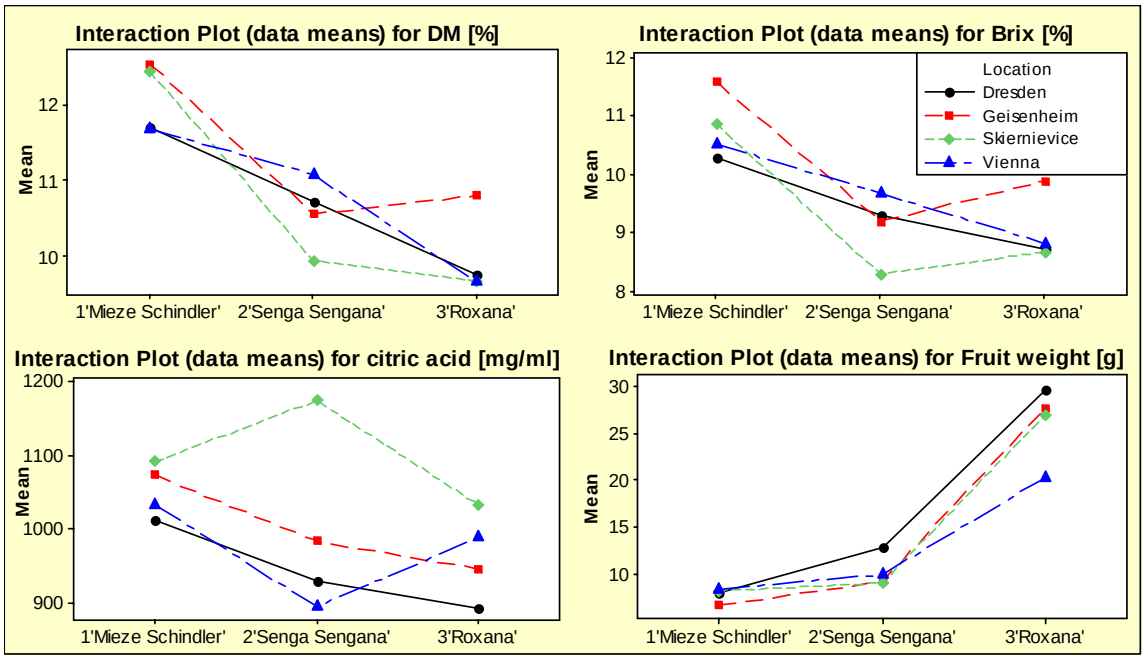
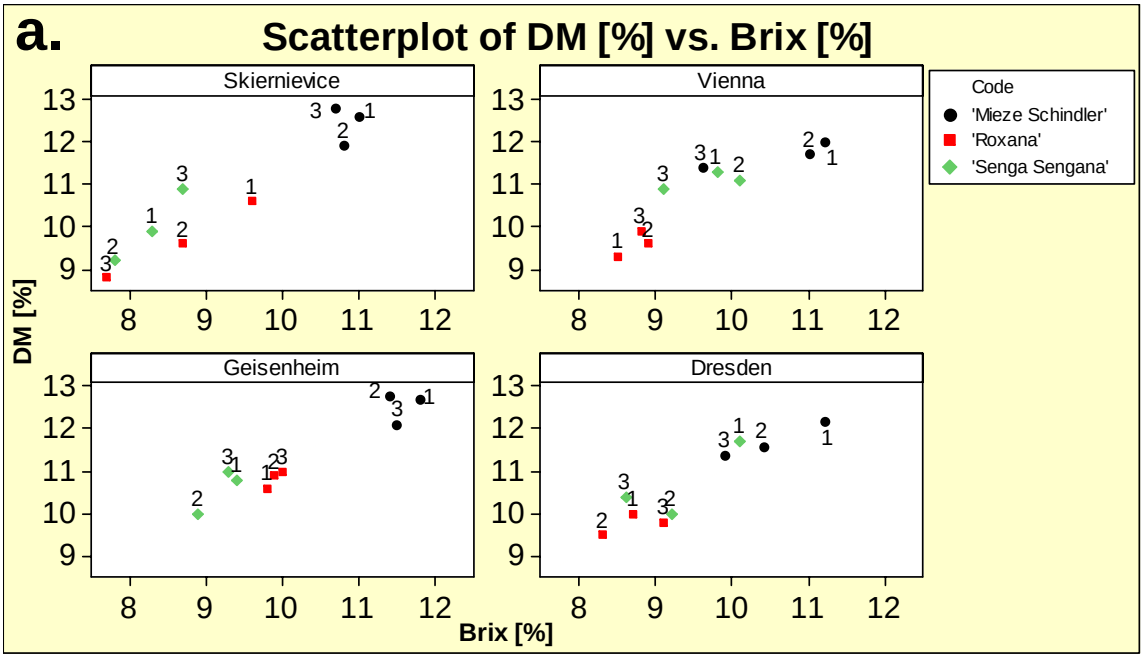


Figure 19: Interaction plots for DM [%], Brix [%], citric acid [mg/ml] and average fruit weight [g].

A GLM analysis is shown for DM, Brix, citric acid and average fruit weight *vs.* factors in annex G 8. For DM, the ANOVA table indicates that there is a significant evidence for a cultivar effect (p-value= 0.003) and an effect of the interaction between cultivar by picking by location (p-value= 0.003) at F-test p-values 0.05. There is no significant

evidence for an effect of the picking date, location, block or their remaining possible interactions. In comparison, the ANOVA table for Brix shows also a significant effect of the cultivar and the interaction between cultivar by picking by location. Different results were achieved for the response variables citric acid and average fruit weight. The ANOVA table of citric acid indicates that there is only a significant evidence for an effect of the interaction cultivar by picking by location (p-value= 0.005). No significant evidence is present for an effect of cultivar, picking, block, location or the remaining interactions. The ANOVA table of average fruit weight specifies a significant effect of cultivar (p-value< 0.001), picking (p-value= 0.001) and the interactions cultivar by picking (p-value= 0.013) and cultivar by location (p-value= 0.027). Other effects were not significant at p-value > 0.05. Figure 19 shows interaction plots for the data means of the mentioned traits with the factors location and cultivar. The order of the DM means of the cultivars is the same for the locations Skiernievice, Vienna and Dresden. Geisenheim was the only location where the DM mean of ‘Roxana’ was higher than ‘Senga Sengana’. The interaction plot for Brix is similar, only the lower Brix mean of ‘Senga Sengana’ than ‘Roxana’ at the location Skiernievice is deviating. The order of the citric acid means is the same at the location Geisenheim and Dresden. The other locations showed a different order. All cultivars had at the location Skiernievice the highest citric acid mean. The order of the fruit weight means of the cultivars was the same at all locations.

A high positive correlation between DM and Brix is evidently presented in figure 20 a. The calculated PEARSON correlation coefficients r of each location confirms this assessment. For the data of Skiernievice, r is 0.95, 0.89 for Vienna, 0.95 for Geisenheim and 0.93 for Dresden. In all cases the p-values are < 0.001. Significant differences between the average DM of the cultivars occurred at each location (annex G 9). A one-way multiple comparison test of TUKEY showed that the DM content of ‘Mieze Schindler’ differed significantly from the DM contents of all other cultivars at the locations Skiernievice and Geisenheim. The cultivar ‘Roxana’ was distinguishable from all the others in Vienna. In Dresden the DM content of the cultivar ‘Mieze Schindler’ differed from the DM content of ‘Roxana’. As seen in figure 20 b, no obvious significant correlation between DM and citric acid is identifiable for the locations Skiernievice and Vienna. Compared to the clusters of the locations Geisenheim and Dresden, the collective as well as particular cultivar values of citric acid and DM had a wide range and do not result in concentrated clusters.



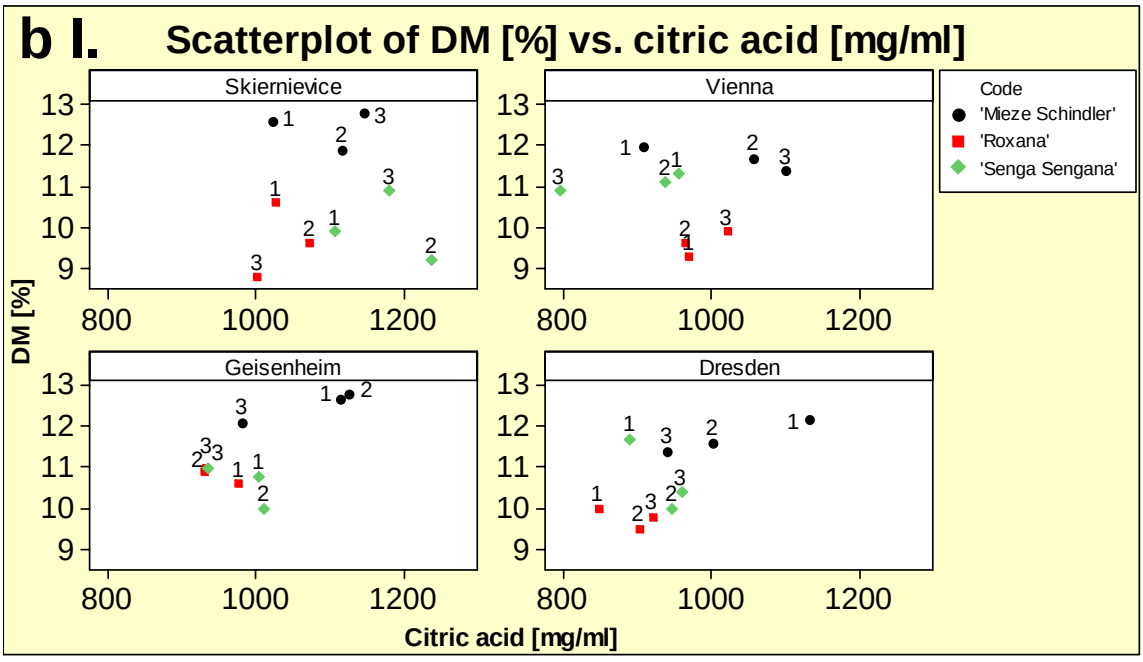
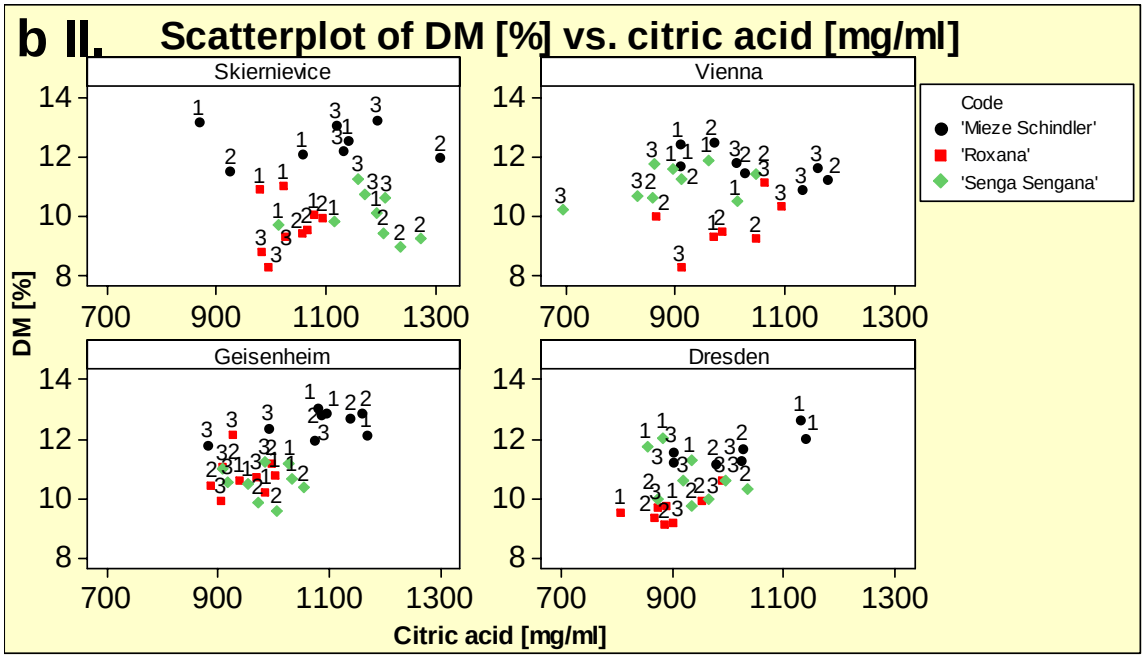


Figure 20 a and b I: The plots of DM [%] *vs.* Brix [%] and DM [%] *vs.* citric acid [mg/ml] for all locations, cultivars and the first three pickings are displayed. Cultivars are merged in colored groups and the order of picking is exhibit as numbered label.



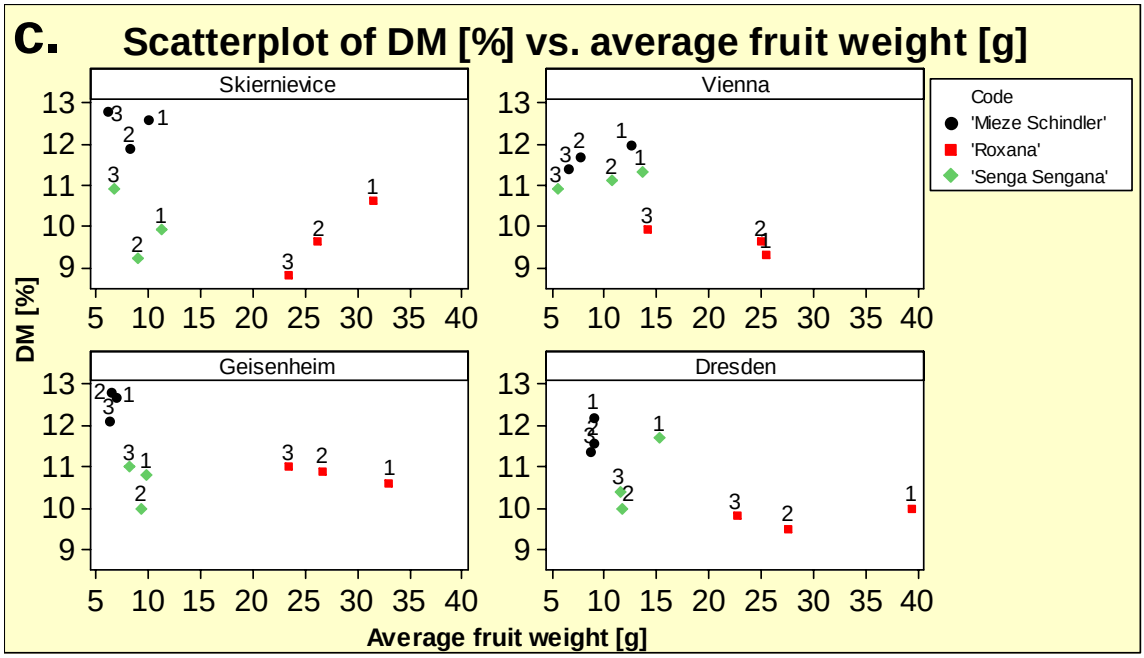


Figure 20 b II and c: The plots of DM [%] *vs.* citric acid [mg/ml] and DM [%] *vs.* average fruit weight [g] for all locations, cultivars and the first three pickings are displayed. Cultivars are merged in colored groups and the order of picking is exhibit as numbered label. Figure 20 b II shows the values of the replications.

For example, the citric acid of ‘Mieze Schindler’ from Vienna ranged about 139.4 mg/ml and the DM about 0.6% DM in an absolute scale. A low positive correlation between DM and citric acid was found for the locations Geisenheim (r= 0.70,

p-value= 0.037) and Dresden (r= 0.63, p-value= 0.069). The figure 20 b II shows exemplarily for all figures 20 the same plots of b I with all repetitions and at different scale ranges for x and y. The conclusions drawn from figure b I are not altered by the diagrams with the repetitions. This was the case for all plots of figure 20. Figure 20 c shows that a low negative correlation between the DM and the average fruit weight of all samples exists at all locations. The clusters of the ‘Mieze Schindler’ and ‘Senga Sengana’ are concentrated, while those of ‘Roxana’ are wider due to a higher variation in the average fruit weight. If the cluster of ‘Roxana’, from the diagram of Skiernievice, is considered individually, it displays even a low positive correlation.

D 1.6 Variability within Fruit

The variability within fruit was investigated by NMR technique as described in C 2.2.6.

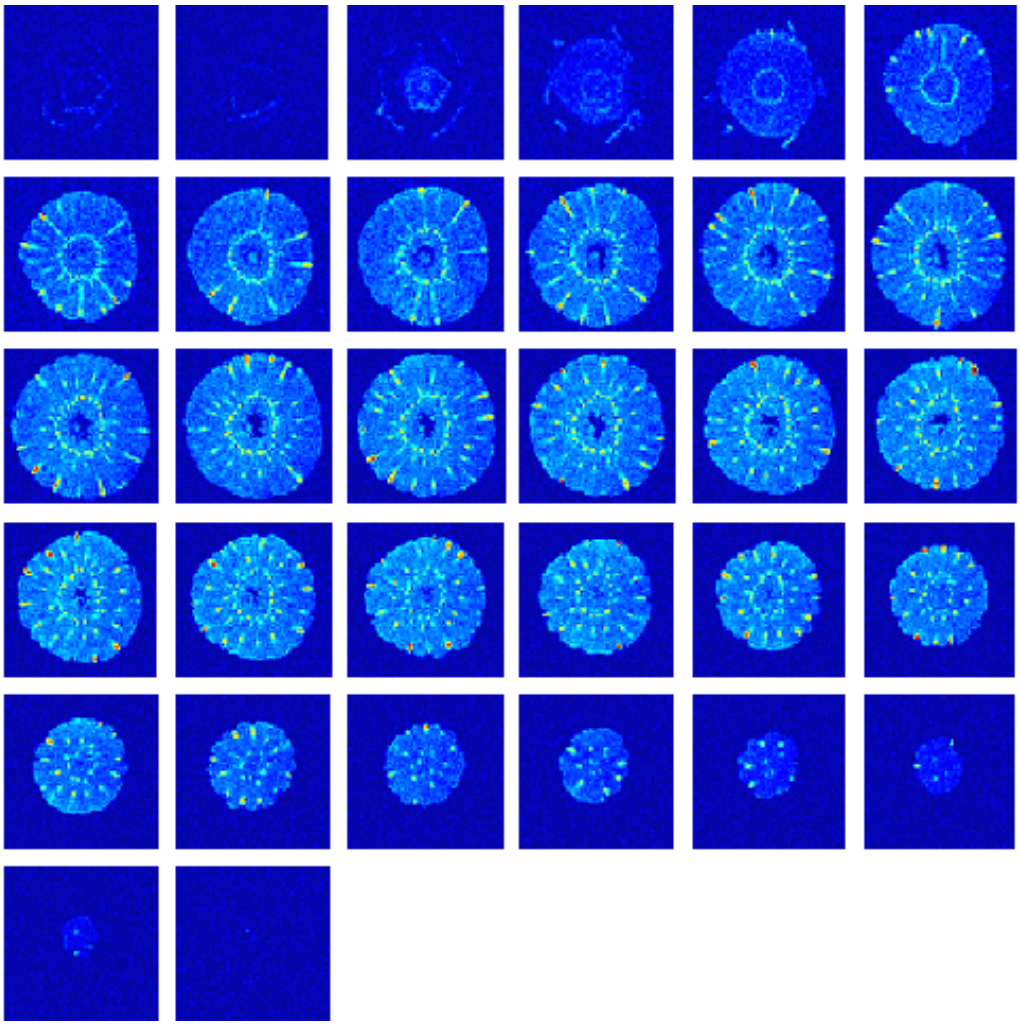


Figure 21: Sequence of NMR scans of the cultivar ‘Alba’. xy area.

Figure 21 illustrates, in reading direction, a sequence of scans from the base to the top of a fruit of the cultivar ‘Alba’. This area was defined as xy. The figure 22 shows the xz area of the same fruit. All scans are presented in pseudocolor for better demonstration. The colors indicate the relaxation time, which can be seen as the availability of free water: Red means higher free water content, blue lower free water content.

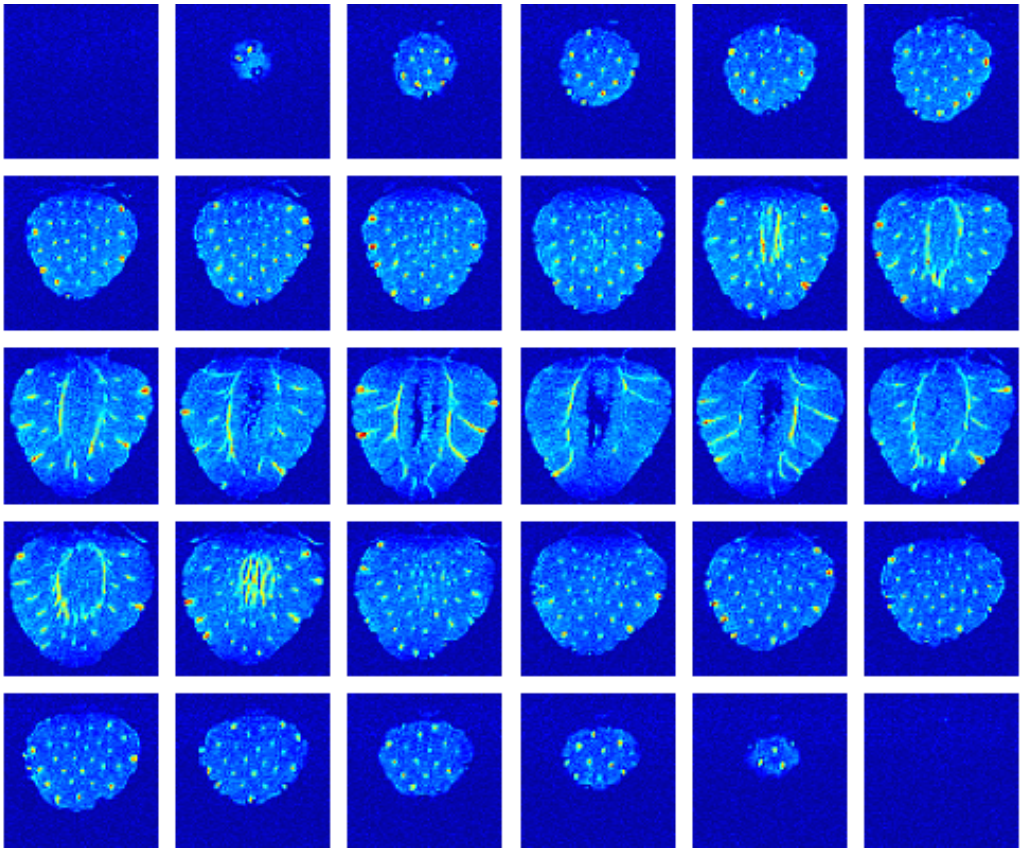
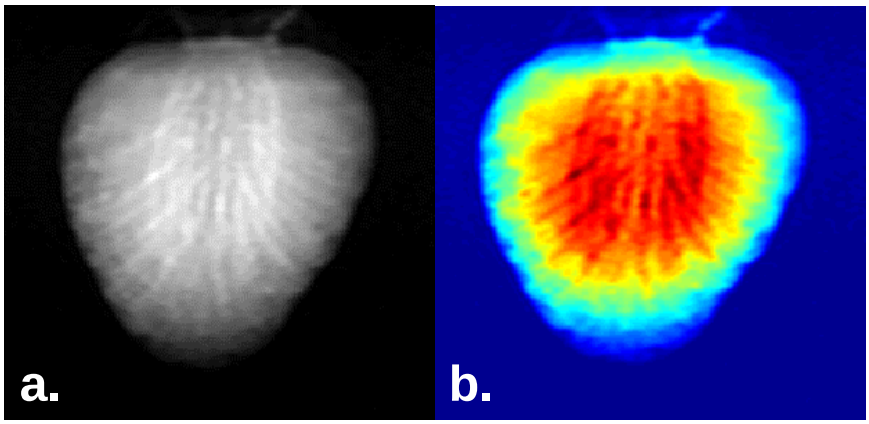


Figure 22: Sequence of NMR scans of the cultivar ‘Alba’. xz area.

Due to the high contrast of the scans, the figures depict the water respectively DM distribution inside a *F. ×ananassa* fruit. The vascular bundles show a higher content of free water than the surrounding tissues. The dark blue area in the middle scans (figure 21 and 22) was caused by a cavity, as they often occur.



Figures 23 a and b: 3D-projection of NMR scans of the cultivar ‘Alba’.

Further, the two area scans can be pieced together to a three-dimensional projection as illustrated in figures 23 a and b. The vascular bundles are again silhouetted against the surrounding tissue, but the cavity is not visible in this display format. These figures can be deceptive. They suggest that the freest water was present in the centre of the fruit and decreased to the skin. However, this effect results from the overlay of several scans and not from a concentration of free water in the centre of the fruit body. The figures 23 a and b demonstrate that the free water was distributed homogenously in all areas. Only the vascular bundles which are enclosing the pith and diverging from it through the pulp tissue to the achenes had higher free water content.

D 1.7 Composition

The determinations of the DM composition were concentrated to glucose, fructose, sucrose and citric acid, the main components according to the literature (HERRMANN 2001). Other compounds are referred as residues. In 2006, the DM fruit composition was quantified from crossing partners of an incomplete diallel (C 2.1.2.5) and some of their seedling plants. These analyzes were conducted by SUNDERMANN (2006) within the scope of a supervised diploma work. The percentage of the achenes was also determined for the crossing partners according to C 2.2.2.1.

Table 12: Average percentages and SD of sugars, citric acid and DM of the first pickings in 2006.

Genotype, Picking		Sugar [%] DM				Citric acid	Residues	DM
		Fructose	Glucose	Sucrose	Total	[%] DM	[%] DM	[%]
Korona, 1st	Mean	**24.4**	**21.0**	**10.1**	**55.5**	**7.1**	**37.4**	**10.8**
	SD	-	-	-	-	-		
Korona, 2nd	Mean	**19.5**	**17.1**	**10.2**	**46.7**	**7.9**	**45.4**	**10.3**
	SD	0.3	0.3	0.3	0.5	0.0		
Korona, 3rd	Mean	**11.8**	**10.2**	**6.2**	**28.1**	**7.8**	**64.1**	**9.6**
	SD	0.2	0.1	1.0	1.1	0.0		
Roxana, 1st	Mean	**24.0**	**23.1**	**15.4**	**62.4**	**8.0**	**29.6**	**10.8**
	SD	0.0	0.2	0.3	0.6	0.1		
Roxana, 2nd	Mean	**15.9**	**14.6**	**7.1**	**37.6**	**9.2**	**53.2**	**9.3**
	SD	0.3	0.4	0.3	1.0	0.1		
Ciflorette, 1st	Mean	**26.6**	**25.8**	**14.5**	**67.0**	**5.6**	**27.4**	**14.1**
	SD	0.2	0.5	0.1	0.7	0.1		
Ciflorette, 2nd	Mean	**21.8**	**20.1**	**14.0**	**55.8**	**6.5**	**37.7**	**13.3**
	SD	0.4	0.2	0.2	0.7	0.1		
97/369, 1st	Mean	**21.5**	**19.6**	**12.7**	**53.7**	**7.5**	**38.8**	**10.9**
	SD	0.3	0.2	0.3	0.8	0.1		
97/369, 2nd	Mean	**19.30**	**18.1**	**8.4**	**45.9**	**7.7**	**53.6**	**9.8**
	SD	0.2	0.3	0.4	0.6	0.2		

The table 12 lists the average percentages and SD of sugars, citric acid and DM of the first pickings of specific genotypes. The complete table is listed in SUNDERMANN (2006) together with a corresponding ANOVA. Significant differences between picking dates of a genotype were present for the total sugar and citric acid levels. In all four genotypes, the DM and total sugar content was decreasing from the first to the second picking date. In the case of ‘Korona’, these parameters were lower also at the third picking date than at the second. In contrast, the citric acid of all genotypes increased from the first to the second picking date. The citric acid level of the third picking date of ‘Korona’ averaged between the first and second picking date. The cultivar ‘Ciflorette’ had the significant highest DM levels with 14.1% DM in the first and 13.3% DM in the second picking. The fruit of those samples had also the significant lowest citric acid levels, 5.6% and 6.5%. The other genotypes had similar DM values at the first picking date but different levels of total sugar. The ratio of the three main sugars fructose, glucose and sucrose is to find in all genotypes with around 2:2:1.

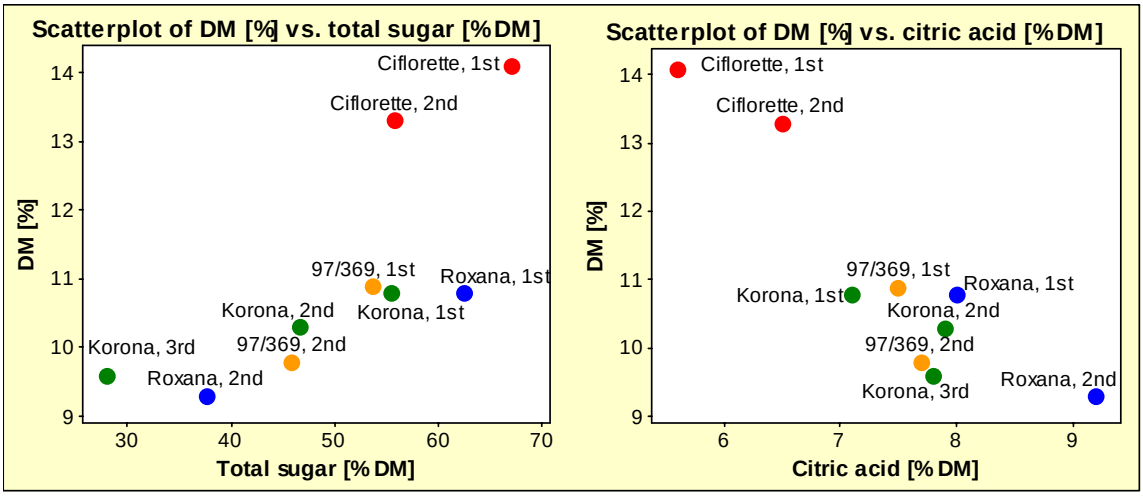


Figure 24: Scatterplots of DM [%] *vs.* total sugar [% DM] and citric acid [% DM] for cultivars and a selection.

The scatterplots shown in figure 24 revealed a positive correlation of r= 0.755 (p-value= 0.019) between DM and total sugar content and a negative correlation of r= -0.902 (p-value< 0.001) for DM *vs.* citric acid content. This connection exists between the investigated cultivars and between the pickings of the cultivars.

The pie charts in figure 25 show the average DM composition of the first two pickings of the crossing partners. The displayed achenes proportion is referred to the table listed in annex G 10. The DM compositions of the genotypes ‘Korona’, ‘Roxana’ and 97/369 are comparable. The total sugars represented around 50% of the total DM, the citric acid around 8% and the residues around 32%. The DM composition of ‘Ciflorette’ was deviating. The percentage of total sugar was higher (61.5%) and the citric acid (6.1%) and the residues (22.9%) proportion lower. The percentages of the achenes ranged between all genotypes from 8.2% to 10.3%. The cultivar ‘Ciflorette’, with the highest DM value, had an average achenes proportion of 9.5%, which is in between the range of all investigated genotypes.

The table in annex G 11 lists the DM composition of the two, according C 2.1.2.5 selected, seedlings per population with the highest and the lowest DM value. Deviating ratios from the common 2:2:1 ratio between the main sugars fructose, glucose and sucrose were present. In the most seedlings the sugars fructose and glucose were higher than sucrose, only in the seedlings 12/87, 12/84, 17/42, 18/49 sucrose had a higher level. In 12/87 and 18/49 the sucrose level was much higher.

The lowest sucrose level, also in comparison to the two other sugars, occurred in seedling 19/109.

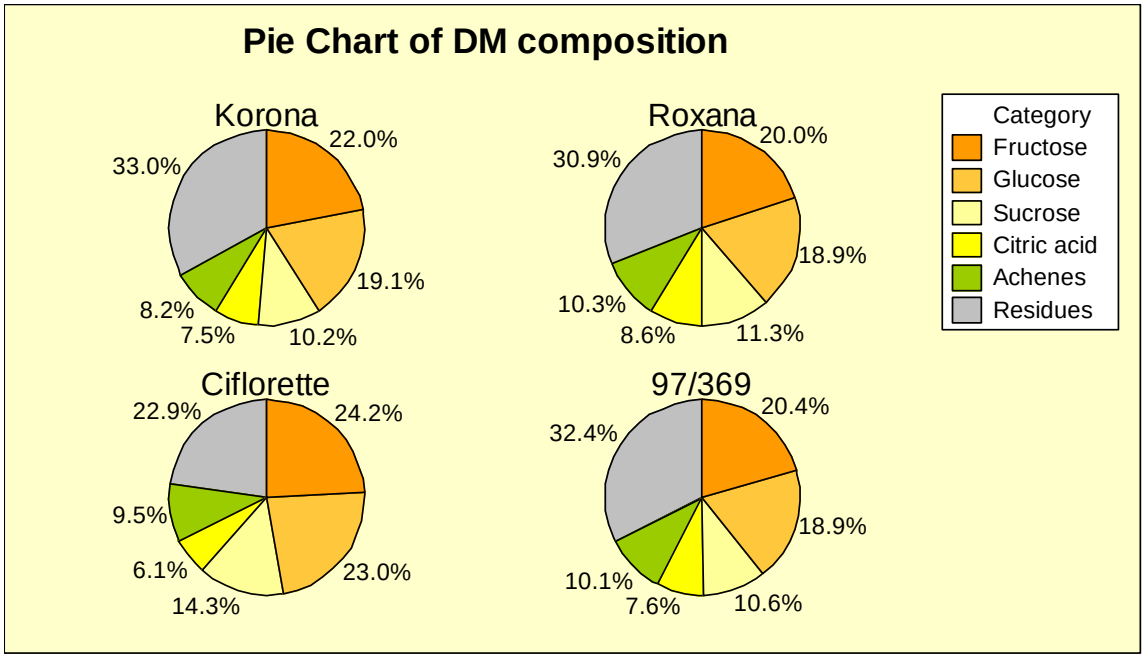


Figure 25: Pie chart of average DM composition of the first two pickings of the four crossing partners.

Considering single populations, the seedling with the higher DM value mostly had, in comparison with the seedling of lower DM, also a higher total sugar level and a lower citric acid level. This is clearly shown in the plots of figure 26.

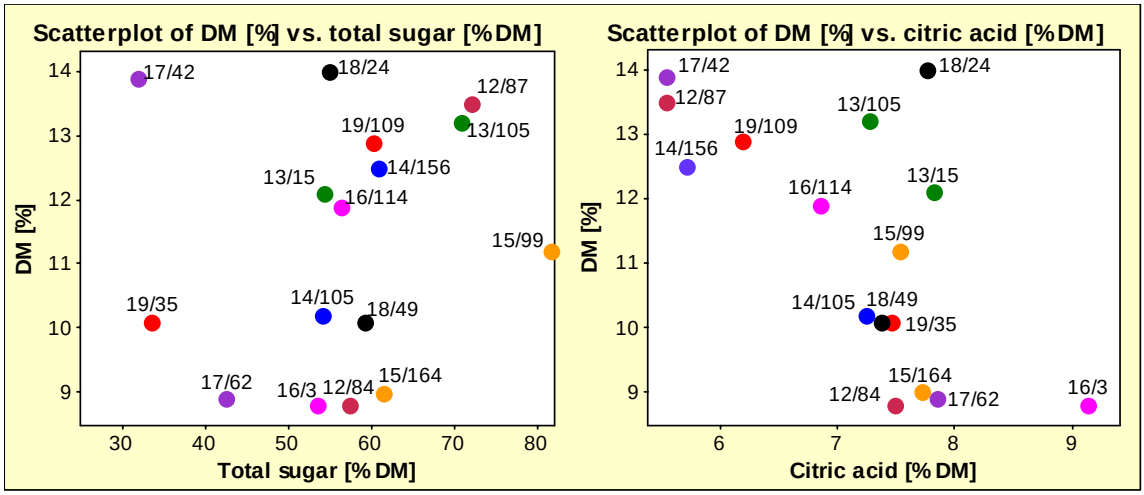


Figure 26: Scatterplots of DM [%] *vs.* total sugar [% DM] and citric acid [% DM] for selected seedlings.

Exceptions were the seedlings of population 17 and 18; they had a lower total sugar level in the fruit of the seedling with the higher DM. No significant correlation was present between the DM and the total sugar level (r= 0.145, p-value= 0.593) of all seedlings. A certain negative correlation occurred between DM and the citric level of all seedlings (r= -0.653, p-value= 0.006).

D 1.7.1 Proportion of Achenes

Because the achenes are a significant proportion of the total DM (figure 25), a separate consideration of the achenes proportion of the fruit is appropriate. Table 13 lists some fruit and achenes parameter for several *Fragaria* accessions investigated in 2004 and 2005. The lower fruit weight and higher DM of the diploid genotypes *F. nilgerrensis*, *F. vesca* 'Mignonette' and *F. vesca* 'Rügen' in comparison to *F. ×ananassa* genotypes is evident. The higher achenes proportion of the fresh weight and DM is interesting. In a relative scale, the achenes of the accessions of *F. nilgerrensis*, *F. vesca* 'Mignonette' and *F. vesca* 'Rügen' had a 191.4%, 243.8% respectively 207.6% higher proportion of DM than the achenes of 'Senga Sengana'. Since the investigated diploid genotypes had not a higher number of achenes per fruit and even a lower TSW, this effect can only be ascribed to the smaller fruit of the diploid species.

Table 13: The DM [%], Brix [%], TSW [g], average fruit weight [g], number of seeds/fruit, g seeds/fruit, % seed/fresh weight (FW), % seed/DM of several genotypes.

Genotype	'Elsanta'	'Senga Sengana'	D 3/2	D 3/5	97/362	*F. nilgerrensis*	*F. vesca* 'Mignonette'	*F. vesca* 'Rügen'
Date	21.06.04	21.06.04	21.06.04	21.06.04	21.06.04	06.06.05	01.07.05	01.07.05
DM [%]	10.4	9.3	12.5	13.2	12.1	14.8	17.5	18.4
Brix [%]	9.9	8.5	11	11.3	10.5	9.4	11.9	12.4
TSW [g]	0.68	0.6	0.84	0.95	0.67	0.3	0.35	0.39
Fruit weight [g]	28.4	11.8	10.2	12.7	9.7	0.9	0.8	1.1
n seeds/fruit	231	193.5	177.8	231.5	161	128.5	147.3	168.8
g seeds/fruit	0.16	0.12	0.15	0.22	0.11	0.04	0.05	0.07
% seed/FW	0.55	0.98	1.47	1.74	1.11	4.52	6.32	5.93
% seed/DM	5.3	10.5	11.8	13.1	9.2	30.6	36.1	32.3

D 2 Breeding Aspects

D 2.1 *Fragaria* Gene Pool

D 2.1.1 *Genus*

Several accessions of *Fragaria* were screened for their DM level in 2004, 2005 and 2006. Annex G 12 lists the DM values of 98 *Fragaria* accessions according the year of investigation. The values of the first three pickings as well as the mean of these pickings are shown. In some cases only one or two pickings could be carried out. The investigations were focused on *F.* ×*ananassa* and its cultivars, since these are the strawberries of worldwide economical importance. However, also different other *Fragaria* species and backcrosses of *F.* ×*ananassa* with one of its parental species were investigated.

The overall DM distribution in all accessions over the three investigated years ranged from a mean of 7.5% to 18.4% DM (annex G 12). The vast majority of high mean values over 14.0% belong to the accessions of *F. nilgerrensis* Schlecht, *F. virginiana*, *F. viridis* Duch. and *F. vesca*. In 2004 *F. virginiana* had a smaller DM mean of 12.8 % with a range of 12.5 to 13.2% DM. The DM value of the analyzed *F. moschata* Duch. was with 12.3% in 2005 also smaller than the other species accessions. The range in the harvest period of 2006 of the decaploid interspecific hybrid ‘Spadeka’ was 9.7 to 10.2% DM.

D 2.1.2 *Fragaria* ×*ananassa*

The DM distribution of the *F.* ×*ananassa* accessions in all three investigated years ranged from 7.5% to 14.9% DM. Higher values above 15.0% DM were reached by accessions of *F. vesca* or *F. viridis*. The values of *F. moschata* (12.3% DM in 2005), *F. virginiana* (12.8% DM in 2004), *F. nilgerrensis* (14.8% DM in 2005), the decaploid ‘Spadeka’ and values of the backcrosses of *F.* ×*ananassa* with one of its parental species are found within the range of *F.* ×*ananassa*. If the values of *F. moschata*, *F. virginiana*, *F. nilgerrensis* and ‘Spadeka’ are excluded, the remaining genotypes present the medium-term available gene pool for the development of a *F.* ×*ananassa* cultivar. A qualitative graduation of the quantitative DM values is necessary for a

further nominal communication about the DM of genotypes. A modified classification according to the arbitrary scheme of Plocharski (1989), as described in C 2.4, was applied to the own data. The range of the classes “very low”, “low”, “intermediate”, “high” and “very high” is listed in annex G 12 and the resulting classes for each genotype are tagged by color.

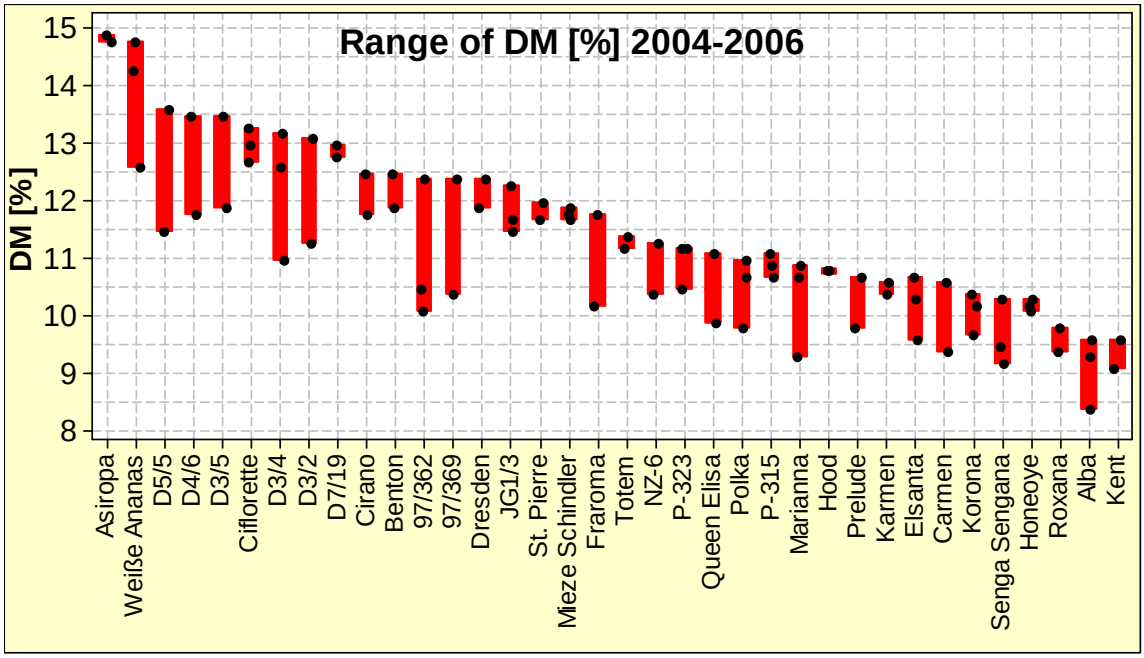


Figure 27: Range of DM [%] of 36 genotypes which were analyzed in more than one year. The black dots mark the means per investigation year.

The range between the years of the DM means of 36 genotypes (around 25% of all genotypes is displayed in figure 27. Especially, the backcrosses with *F. chiloensis* and the cultivar ‘Weiße Ananas’ had a wide range of DM over the years. For example, the selections 97/362 and 97/369 had both a DM mean of 12.4% in 2004 and the mean of the D-numbers were even higher than 13.0% DM in the same year. On the other hand, these high values were not reached in 2005 and 2006. Consequently, the classification of some of the investigated genotypes differed between the years.

D 2.1.2.1 Correlations with other Quality Traits

Annex G 13 lists the firmness, citric acid and average fruit weight for the first three pickings and the mean of genotypes analyzed in 2004. This allows comparisons of

DM *vs.* these important quality traits. The data is grouped according to the breeding background. The backcrosses with *F. chiloensis* and *F. virginiana* had at least one common parent. Figure 28 illustrates a plot of the averaged DM *vs.* the average fruit weight.

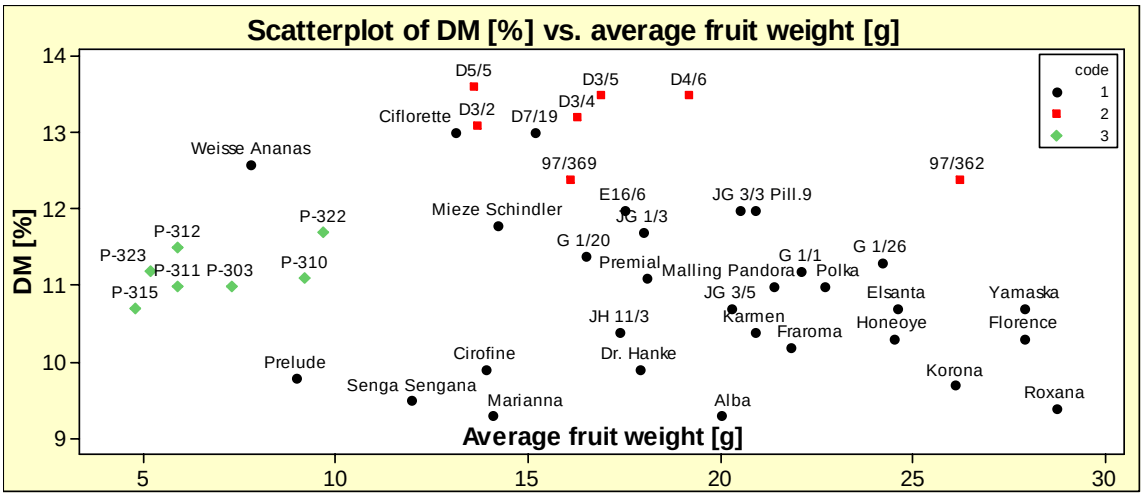


Fig 28: Scatterplot of averaged DM [%] *vs.* average fruit weight [g]. Coding: 1: *F.* ×*ananassa*, 2: Backcrosses with *F. chiloensis*, 3: Backcrosses with *F. virginiana*. Data from 2004. N=44

No significant correlation is present for these two traits (r= -0.187, p-value= 0.224). The grouping according to the breeding background shows that also in one group no significant correlation occurs. Figure 29 illustrates a plot of the averaged DM *vs.* the means of firmness.

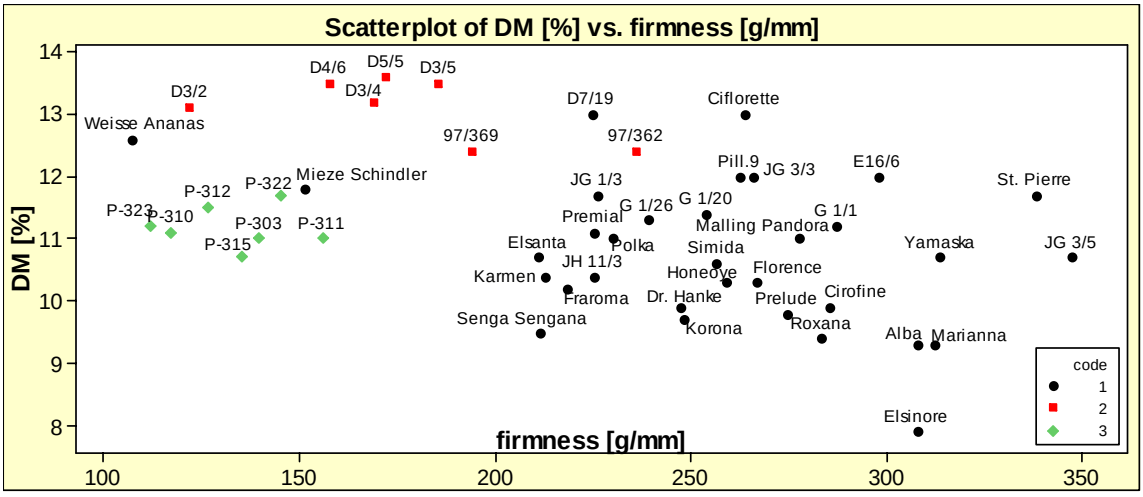


Fig 29: Scatterplot of averaged DM [%] *vs.* firmness [g/mm]. Coding: 1: *F.* ×*ananassa*, 2: Backcrosses with *F. chiloensis*, 3: Backcrosses with *F. virginiana*. Data from 2004. N=47

A low negative correlation occurs with r= -0.448, p-value= 0.002. However, grouping the genotypes according their genetic background reveals three separate clusters with no obvious correlation within each cluster. The backcrosses of *F.* ×*ananassa* with its North and South American parent were characterized by lower firmness. The old cultivars 'Mieze Schindler' and 'Weiße Ananas' are found within this low range of firmness. The DM of the backcrosses with *F. chiloensis* had higher DM levels over 12.0%. The clusters of the referred groups looked similar to the plot of DM *vs.* average fruit weight (figure 28). However, figure 30 shows that this similarity is based on a correlation of r= 0.643, p-value< 0.001. If the three breeding backgrounds considered independently, only a correlation of the backcrosses with *F. chiloensis* persists (r= 0.760, p-value= 0.047).

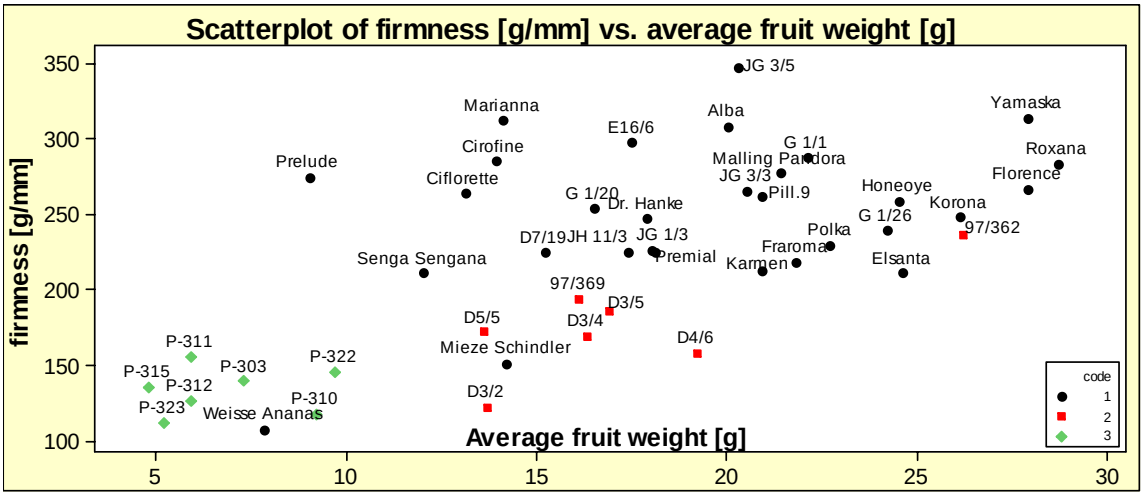


Fig 30: Scatterplot of averaged firmness [g/mm] *vs.* averaged average fruit weight [g]. Coding: 1: *F.* ×*ananassa*, 2: Backcrosses with *F. chiloensis*, 3: Backcrosses with *F. virginiana*. Data of 2004. N=44

The plot of averaged DM *vs.* citric acid showed no correlation between these two characteristics (figure 31). Almost all genotypes with more than 1100 mg/ml were backcrosses with one of the parental species of *F.* ×*ananassa*. The remontant cultivar 'Elsinore' had the lowest average DM as well as citric acid value.

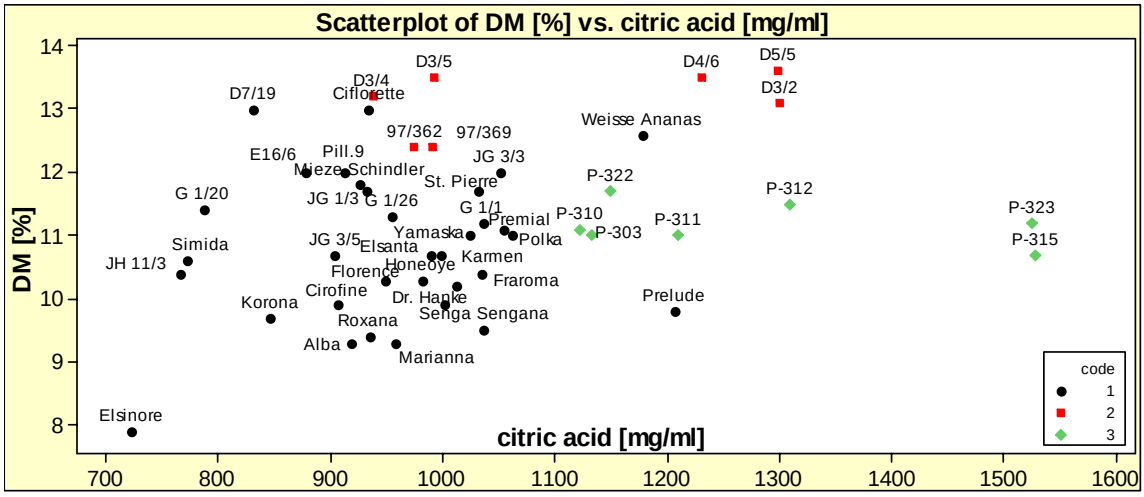


Fig 31: Scatterplot of averaged DM [%] *vs.* citric acid [mg/ml]. Coding: 1: *F.* ×*ananassa*, 2: Backcrosses with *F. chiloensis*, 3: Backcrosses with *F. virginiana*. Data from 2004. N=47

Figure 32 shows the plot of averaged DM *vs.* Brix. A clear positive correlation (r= 0.932, p-value< 0.001) occurred. The most of the genotypes higher than 12.0% DM originated from backcrosses with *F. chiloensis*. The genotypes 'Ciflorette' and D7/19 had also DM values above 12.0%. The correlation between all single measurements of DM *vs.* the corresponding Brix values was r= 0.862, p-value< 0.001. The regression was DM [%] = 1.420 + 1.043 Brix [%].

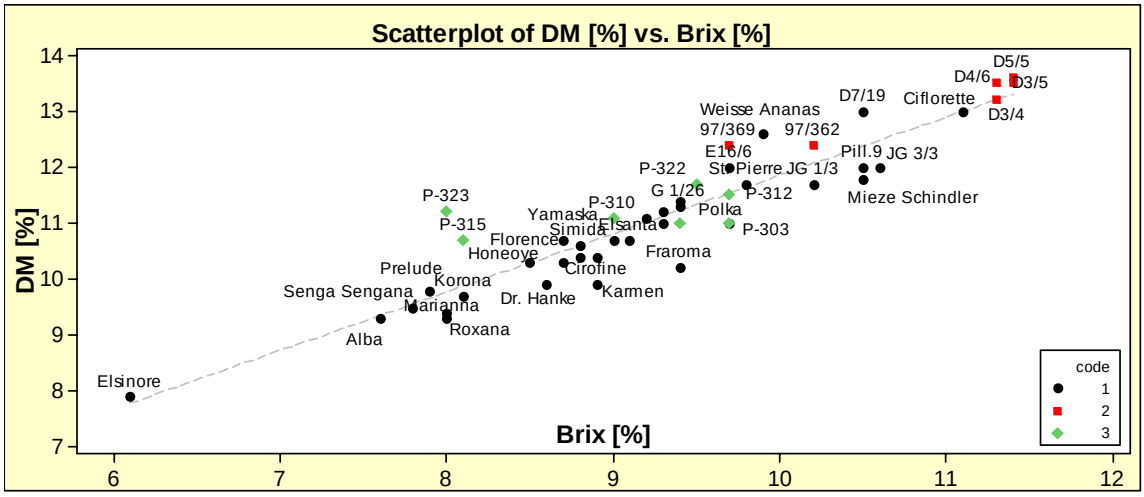


Fig 32: Scatterplot of averaged DM [%] *vs.* Brix [%]. Coding: 1: *F.* ×*ananassa*, 2: Backcrosses with *F. chiloensis*, 3: Backcrosses with *F. virginiana*. The dashed grey line indicates the linear regression fit. Data from 2004. N=46

D 2.2 Inheritance

D 2.2.1 F_1 Clone Populations

The F_1 population ‘Mieze Schindler’ x ‘Elsanta’ planted as clones of three plants each offered the possibility to study distributions of the traits independently of an influence of the physiology of the seedling stage. The number of investigated genotypes was 184 in 2005 and 78, in 2006. Of these 78 samples 27 were not randomly chosen, due to the selection explained in C 2.1.2.4. Therefore, the number of randomly chosen samples was 51.

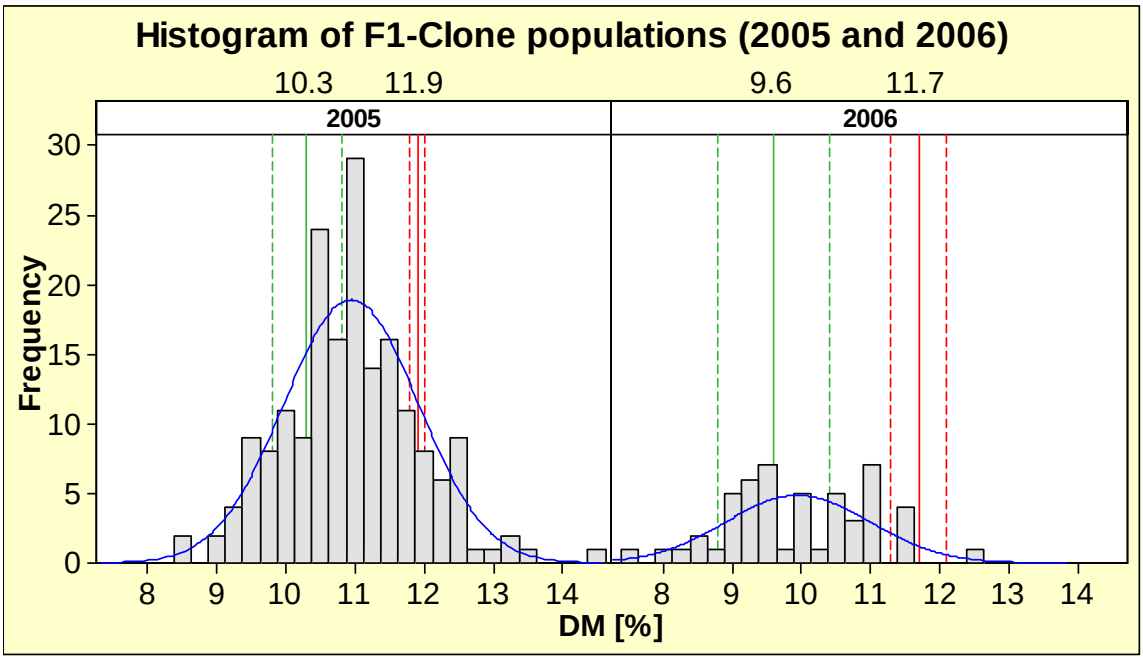


Figure 33: Frequency distribution of DM of the F_1 population ‘Mieze Schindler’ x ‘Elsanta’ in the years 2005 and 2006. A one year old planting was investigated in both years. The green and red continuous lines mark the means of the DM of ‘Elsanta’ respectively ‘Mieze Schindler’ in the respective year. The dashed lines indicate the corresponding SD.

Figure 33 illustrate the frequency distributions of DM of one year old plantings in 2005 as well as in 2006. The DM of the population ranged from 8.5% to 14.5% DM in 2005 and from 7.4% to 12.4% in 2006. The mean was 11.0% $\pm$ 1.0% DM in 2005 and 9.9% $\pm$ 1.1% DM in 2006. In comparison, the range of all investigated 78 genotypes in 2006 ranged from 6.3% to 14.5% DM. The mean was 9.8% $\pm$ 1.3% DM. The distribution in both histograms correspond approximately a GAUSSIAN distribution.

Further, the mean of the populations were located between the means of the parents in both years. In 2006, the mean of the population were found in the range of the mean plus SD of the father ‘Elsanta’.

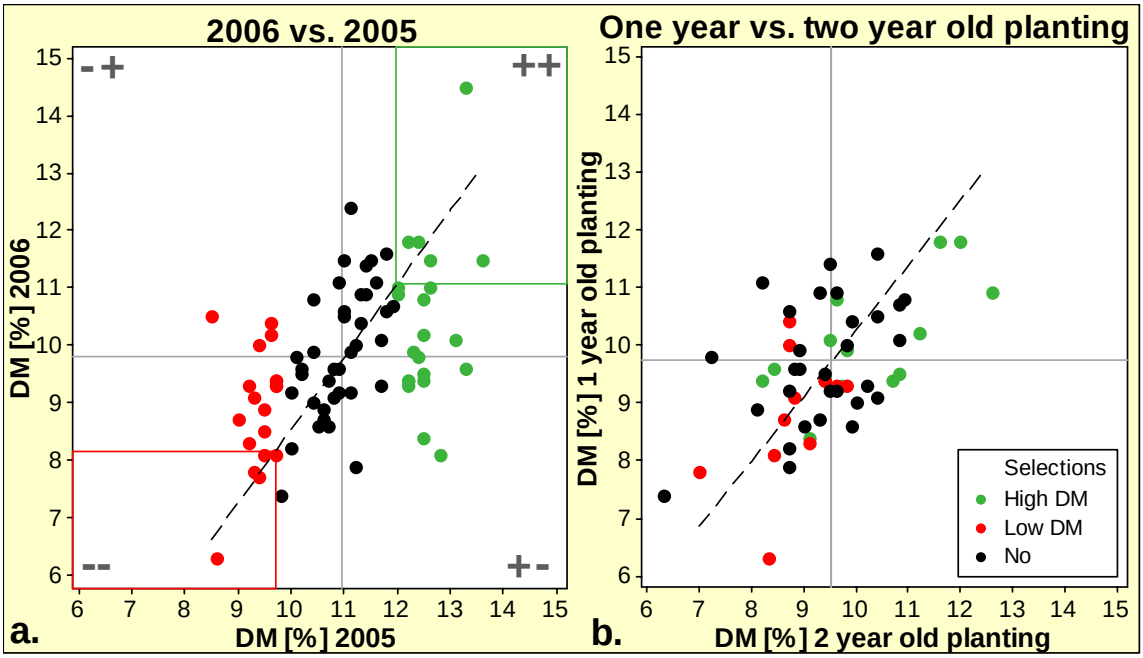


Figure 34 a and b: Scatterplot of DM [%] values from 2005 *vs.* DM values of 2006 (a) and of a one year *vs.* two year old planting of the F_1 clone population (b). The grey lines indicate the means and the dotted lines represent the main axis of correlation. The red and green rectangles are explained in the text.

Figure 34 a illustrates a plot between all 78 sample values of 2006 *vs.* the corresponding values of 2005. The grey lines in the figures mark the means. These lines are segmenting the coordinate system in four quadrants. The genotypes in a certain quadrant had a higher or lower value in 2005 and 2006 than the mean of the relevant population. For example, the genotypes in the upper left quadrant of figure 34 a had a lower value than the mean of the population in 2005 and a higher value in 2006. This is indicated by the symbol -+. The dotted black line is the main axis of correlation and its equation was y= -4.6836 + 1.2806x. Since no certain pattern of the values occurred, a systematic lack-of-fit can be excluded. The correlation between the values of these two years was r= 0.517, p-value< 0.001. Due to the single measurements of a genotype in each year, the variation of the genotypes during harvest is unknown. The values marked by a red and green dot are the genotypes selected for low respectively high DM in 2005 (C 2.1.2.4). The selection limits of

≤ 9.7% and ≥ 12.0% DM were deliberately chosen. In 2006, the DM of 18 low and 21 high DM genotypes in 2005 could be determined.

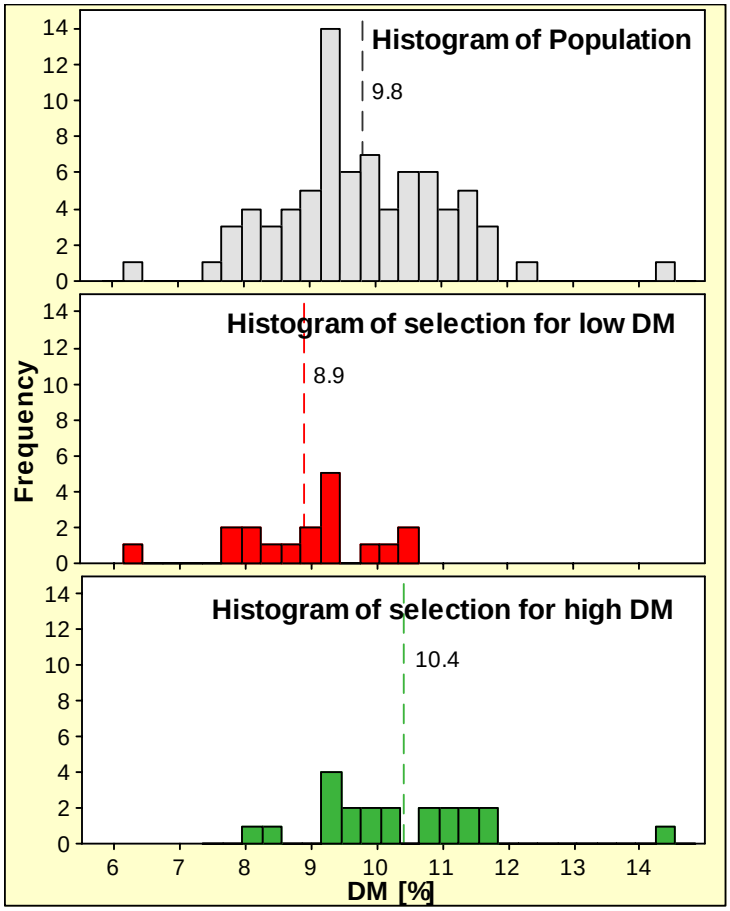


Figure 35: DM [%] distribution of clones, selected for low and high DM in 2005, in comparison to the population including the selected clones. All values are from the one year old planting of 2006. The dotted lines mark the mean of the distribution. The mean value is presented beside the line.

In figure 35, the DM distributions of these selected clones are displayed in comparison to the DM distribution of the whole population in the year 2006. The means of all distributions differed from each other. Significant differences existed only between the mean of the selections for low DM and the selections for high DM as well as the mean of the population. No significant differences were present between the mean of the selections for high DM and the mean of the population. The corresponding ANOVA and multiple comparison test of FISHER are listed in annex G 14.

The corresponding selection limits of the year 2006 were ≤ 8.2% and ≥ 11.1% DM and can be calculated by using the equation mentioned above. The red and green

rectangles of figure 34 a comprises all genotypes with a DM values fitting in the low respectively high DM selection limits of 2005 and 2006.
Figure 34 b displays a plot between the one year old and the two year old planting analyzed in 2006. The grey lines in the figure are again the means of both plantings. The dotted black line is again the main axis of correlation. The equation was y= -1.0216 + 1.1256x. The correlation coefficient was r = 0.564, p-value< 0.001. The values marked by a red or green dot are again the genotypes selected for high respectively low DM in 2005.

D 2.2.2 Bi-Parental Diallel

Some parts of these results were achieved within the scope of a supervised diploma work of SUNDERMANN (2006).
The raw data of the picking date, fruit number, average fruit weight and DM of the eight populations of the progenies are listed in SUNDERMANN (2006). The DM values of the first three pickings of the crossing partners are listed in table 12. According to the classification scheme of annex G 12, the DM of the cultivar ‘Ciflorette’ was categorized in 2006 as “very high” and “low” for 97/369, ‘Korona’ and ‘Roxana’. In 2004, the year of the planning stage of the diallel design, the DM level of ‘Ciflorette’ and 97/369 were classified as “high” and “low” for ‘Korona’ and ‘Roxana’. Since the first two pickings of the progenies were unified to a single sample, also the mean and SD of the first two pickings of the crossing partners were used for further comparisons. The mean and SD of ‘Ciflorette’ was 13.7% $\pm$ 0.5% DM, 10.6% $\pm$ 0.4% DM for ‘Korona’, 10.1% $\pm$ 1.1% DM for ‘Roxana’ and 10.4% $\pm$ 0.8% DM for 97/369.
Outliers were calculated by the statistic program Minitab. The outliers which were deriving from a sample with only one fruit, an average fruit weight smaller than 4 g or from a plant of a dwarf habitus were excluded from further calculations. Figure 36 shows the frequency distributions of all eight populations and the corresponding Mean, SD and sample number N. KOLMOGOROV-SMIRNOV-tests, at the 8% level of significance, confirmed that the trait DM follows in all populations a Gaussian distribution (not shown).

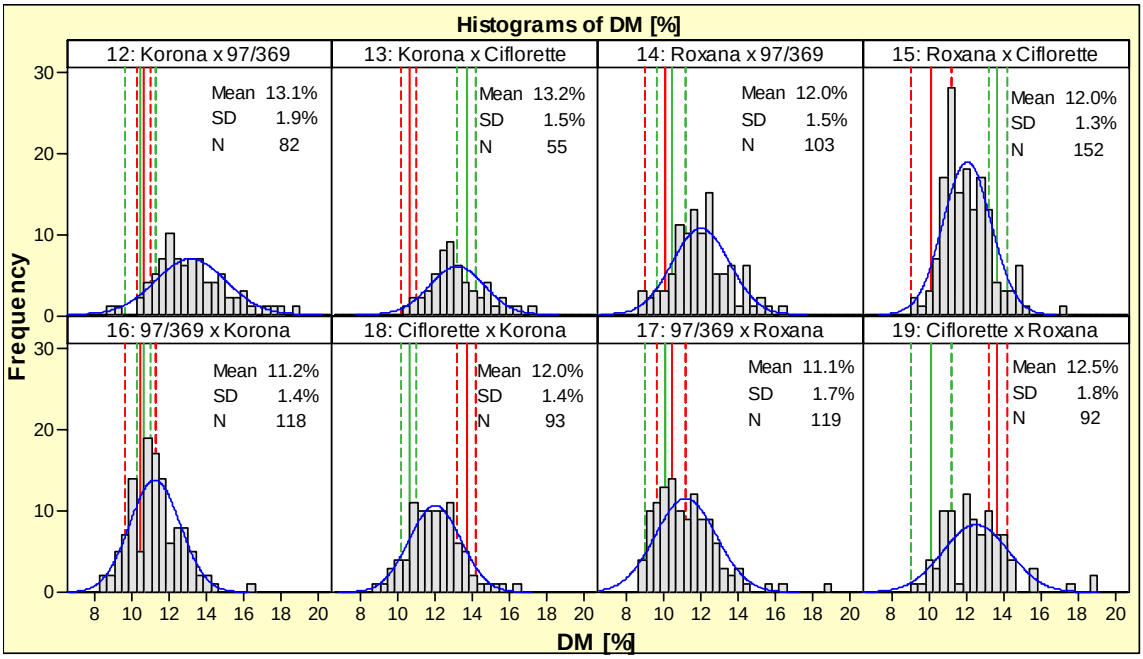


Figure 36: Histograms of DM [%]. The second row shows the reciprocal crosses. The blue lines are indicating the Gaussian distribution. The red and green continuous lines mark the means of mother and father in the respective population. The dashed lines indicate the corresponding SD. The Mean, SD and number of observations is stated.

The means of the populations were located between the means of the parents in all populations which have ‘Ciflorette’ as a crossing partner. In all cases, the means of the remaining populations were higher than the means of any parent. However, the means of the populations 97/369 x ’Korona’ and 97/369 x ‘Roxana’ were located in the range of the mean + SD of the mother 97/369. This is also displayed in table 14. Further, it can be seen that only in the population 18 (‘Ciflorette’ x ‘Korona’) the mean of the F_1 was lower than the parental mean (Table 14). In this population there were also more seedling values below the lowest parent than above the highest parents. The distributions in the progenies of all other population were vice versa. In the populations 12 and 14 (‘Korona’ x ‘97/369 and ‘Roxana’ x 97/369), there were 95% respectively 86% of all seedling values above the value of the highest parent.

Table 14. Comparison of the DM [%] of the populations and their parents.

Cross combination	Code	N	DM [%]					% F_1 above highest parent	% F_1 Below Lowest Parent
			Mean of Parents	Parental means	F_1 Means	F_1 ranges x_{max} - x_{min}	F_1 interquartile ranges $x_{0.75} - x_{0.25}$		
Korona x 97/369	12	82	10.6 10.4	10.5	13.1	8.8-18.8	11.8-14.3	95	2
Korona x Ciflorette	13	55	10.6 13.7	12.2	13.2	10.2-17.1	12.2-13.9	29	2
Roxana x 97/369	14	103	10.1 10.4	10.3	12.0	8.7-16.6	11.0-12.8	86	11
Roxana x Ciflorette	15	152	10.1 13.7	11.9	12.0	9.1-17.2	11.1-12.9	11	3
97/369 x Korona	16	118	10.4 10.6	10.5	11.2	8.3-16.3	10.1-12.1	66	30
97/369 x Roxana	17	119	10.4 10.1	10.3	11.1	8.7-19.0	9.9-12.1	60	29
Ciflorette x Korona	18	93	13.7 10.6	12.2	12.0	8.8-16.6	10.9-12.8	8	14
Ciflorette x Roxana	19	92	13.7 10.1	11.9	12.5	9.1-18.9	11.2-13.5	20	4

Significant differences occurred between the DM level means of the populations at a 5% level of significance (annex G 15). The multiple comparison test of FISCHER is listed in annex G 15 and the confidence intervals for DM level means of the population are illustrated in figure 37.

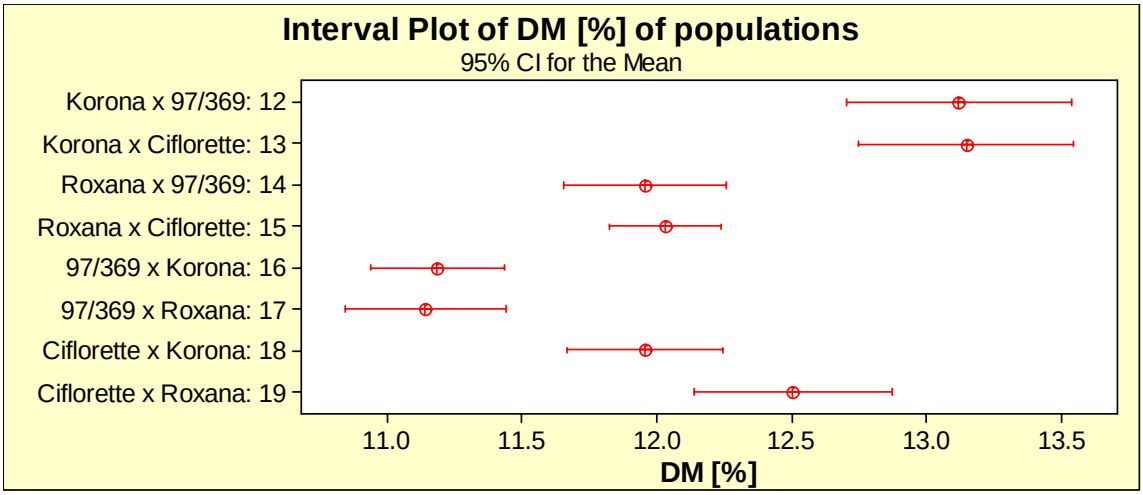


Figure 37: 95% confidence interval (CI) plots of the DM [%] of the populations. The crosses are indicating the means.

A grouping according the maternal crossing partner becomes obvious in six of the eight populations. The DM level means of the populations 12 and 13 could not be distinguished from each other but were significantly higher than all other populations (annex G 15). The lowest significant DM level means occurred in the populations 16 and 17. The confidence intervals of the remaining populations 14, 15, 18 and 19 were located between these groups. From these populations, only the population 19 differed from the other three populations significantly. The populations 18 and 19 were therewith the only investigated populations with equal mother which differed from each other. In contrast, all populations with the same father, as crossing partner, as well as all reciprocal crosses differed significantly.

D 2.2.2.1 Dry Matter *versus* Yield

KOLMOGOROV-SMIRNOV tests revealed that not in all populations the means of the average fruit weight, the number of fruits and the resulting yield followed a normal distribution (data not shown). The distribution of the yield was skewed to the left. Since the determination of these variables has a low measurement error, outliers could not be excluded by logical reasons. Therefore, figure 38 displays the boxplots of the data. The box endpoints were the 37.5% and 62.5% percentiles, resulting in an interquartile range expected to include about 25% of the data.

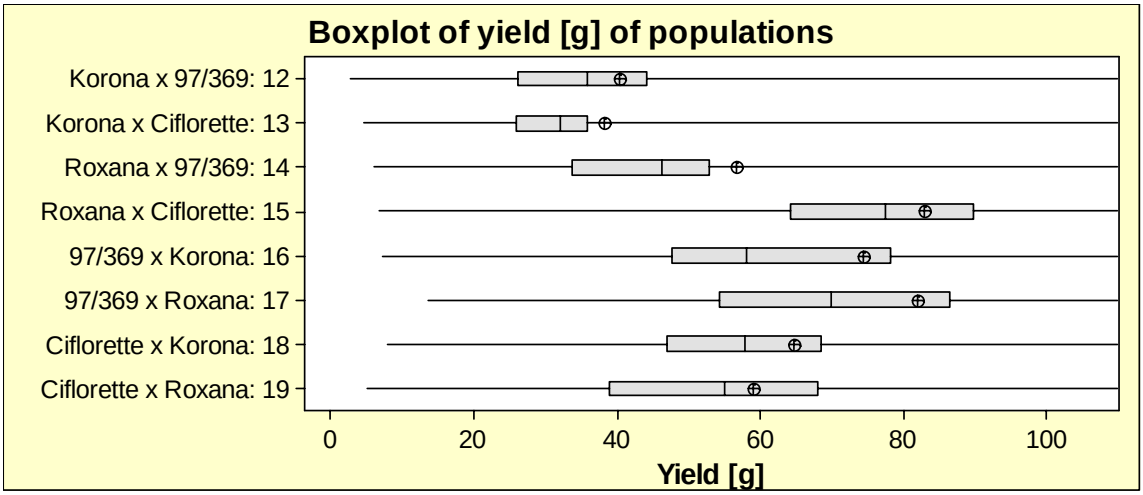


Figure 38: Boxplots of yield [g] of the populations. The interquartile range is expected to include 25% of the values. The cross indicates the mean of the distribution.

In all populations, the left skewness of the distributions can obviously be seen by the mean which is higher than the median. Annex G 16 shows a corresponding KRUSKAL-WALLIS test for yield. The z-values of the first two populations with the maternal parent ‘Korona’ indicate that the mean ranks were much lower than the mean rank of all populations. The medians of the populations with ‘Ciflorette’ as maternal parents are also similar. Despite these two groupings according to one crossing partner, no other evident grouping occurred. The mean rank for the population 15 (‘Roxana’ x ‘Ciflorette’) was much higher than the mean rank for all other observations.

Figure 39 shows the plot of the DM mean of the populations versus the yield median. A negative correlation is obviously present (r= -0.727, p-value= 0.041).

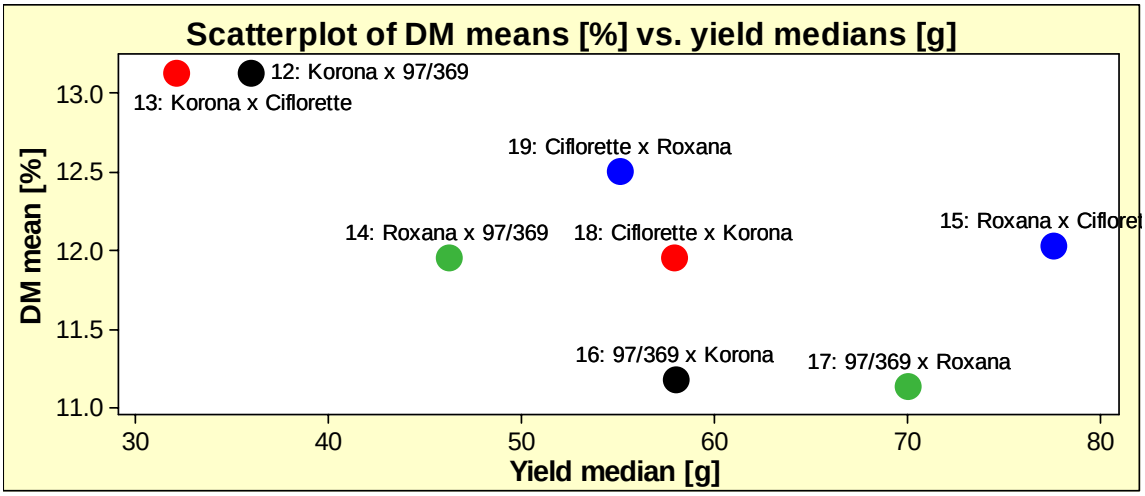


Figure 39: Scatterplot of DM means [%] *vs.* yield medians [g]. Values of populations with the same crossing partners have the same color.

The plots of figure 40 illustrate the association of DM *vs.* yield of the first two pickings of the seedlings sorted by population. No genotypes were present in the upper right corner of any plot. In the lower left corner there were several combinations realized. For each population, a diagonal from above left to down right could be drawn which separated these two areas. The clusters of the populations differed also in their shape. The plot of the populations 12, 13 and 19 showed a limit in yield at around 150g. In comparison, the clusters of the populations 15, 16 and 17 exceeded this limit evidently, but their clusters were much more flat. The shape of the clusters of the populations 14 and 18 were in between these two types.

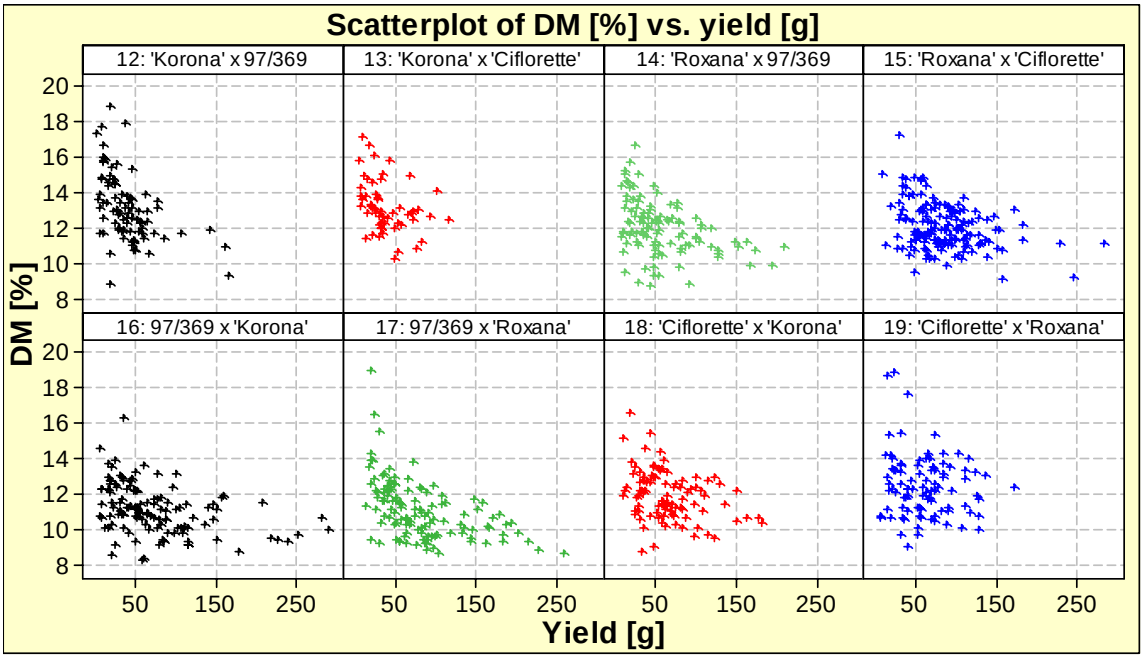


Figure 40: Scatterplot of DM [%] *vs.* average yield [g] of the first to pickings of the seedling. The plots are sorted according the maternal parent.

D 2.2.2.2 Combining Ability and Combination Effects

The combining ability in regard to DM is displayed in table 15. The means of the populations of 'Ciflorette' and 97/369 as paternal parents were higher than the means of the populations of 'Korona' and 'Roxana' as paternal parent. Considering the maternal parents, the highest population mean resulted from 'Korona' and the lowest DM mean from 97/369 as maternal parent. The mean of 'Ciflorette' and 'Roxana' differed highly from each other but resulted in populations of similar average DM means.

Table 15: Average DM of the parents.

			Father (DM %)				
			Korona (10,6)	**Roxana** (10,1)	**Ciflorette** (13,7)	**97/369** (10,4)	**Mean of mother:**
Mother (DM %)	**Korona**	(10,6)	-	-	13.2	13.1	**13.2**
	Roxana	(10,1)	-	-	12.0	12.0	**12.0**
	Ciflorette	(13,7)	12.0	12.5	-	-	**12.1**
	97/369	(10,4)	11.2	11.1	-	-	**11.1**
	Mean of father:		**11.5**	**11.7**	**12.6**	**12.5**	**12.1**

In applied plant breeding programs, the combining ability of a certain quality trait like DM can not be evaluated separately from other characteristics. The traits which reflect the vigor of the plants of a cross combination are of essential importance. They provide the basis for a successful selection.

As crucial factors, indicating the vigor, the mortality rate and the number of plants without fruit were considered. The observations are listed in table 16. It has to be remembered, that no selection was applied during the seedling stage in the greenhouse.

Table 16: Comparison of the populations.

Cross combination	Code	N	Analyzable Plants [%]	Mortality rate [%]	Observations [% of present plants]				
					No fruit	Dwarfism	Chlorophyll -defects	Selected	
								pre	end
Korona x 97/369	12	162	51.2	14.8	39.9	0.7	-	1.4	0.7
Korona x Ciflorette	13	130	43.8	26.9	40.0	8.4	-	-	-
Roxana x 97/369	14	160	65.6	3.8	31.8	1.3	4.5	1.9	-
Roxana x Ciflorette	15	176	87.5	4.5	8.3	3.0	2.4	6.5	6.5
97/369 x Korona	16	163	72.4	11.7	18.1	0.7	-	5.6	4.9
97/369 x Roxana	17	158	75.3	5.1	20.7	3.3	0.7	6.7	4.0
Ciflorette x Korona	18	162	57.4	14.8	32.6	2.2	-	3.6	2.9
Ciflorette x Roxana	19	174	53.4	16.7	35.9	3.4	1.4	-	-

From all planted seedlings of the cross combinations ‘Korona’ x 97/369 and ‘Korona’ x ‘Ciflorette’ only 51.2% respectively 43.8% were analyzable. The reasons for that were high mortality rates and high rates of plants without fruit. The population 13 (‘Korona’ x ‘Ciflorette’) had, with 40.0% plants without fruit, the highest rate of all populations and additionally also the highest mortality rate of 26.9%. The lowest mortality of 3.8% rate was shown by the population 14 (‘Roxana’ x 97/369). However, this population had still a rate of 31.8% plants without fruit and therefore a medium rate of analyzed plants of 65.6%. The highest rate of analyzable plants was 87.5% and was realized in the population 15, a cross combination of ‘Roxana’ x ‘Ciflorette’. The second and third best rates were found in populations 16 and 17.

Further, in table 16, the occurrence rate of the genetically based defects dwarfism and chlorophyll defects are listed. No correlation occurred to the chosen vigor characteristics. The highest rate of dwarfism was found in the population 13 which had also the lowest rate of analyzable plants. However, the population 12 ('Korona' x 97/369) had one of the lowest dwarfism rates and the second lowest rate of analyzed plants. The population 15 had the highest rate of analyzable plants, but still a medium rate of dwarfism. Chlorophyll defects occurred with the rates of 4.5%, 2.4%, 0.7% and 1.4% only in the populations 14, 15, 17 and 19; all cross combinations with 'Roxana'. In the other populations none of these defects were observed.

In the last column of table 16 the percentages of the selected genotypes out of all present plants are listed. The column is split in percentages of selections before the harvest (06/07/2006) and at the end of the harvest (06/28/2006). With the exception of the population 15, all end-selection rates were smaller than the pre-selection rates. No seedlings in the populations 13 and 19 were pre-selected. The end-selection rates ranged from 6.5% in the population 15 to 0.7% in the population 12. The selection rates of the populations 16, 17 and 18 were between those. Not any single plant was selected in the populations 13, 14 or 19 at the end of harvest.

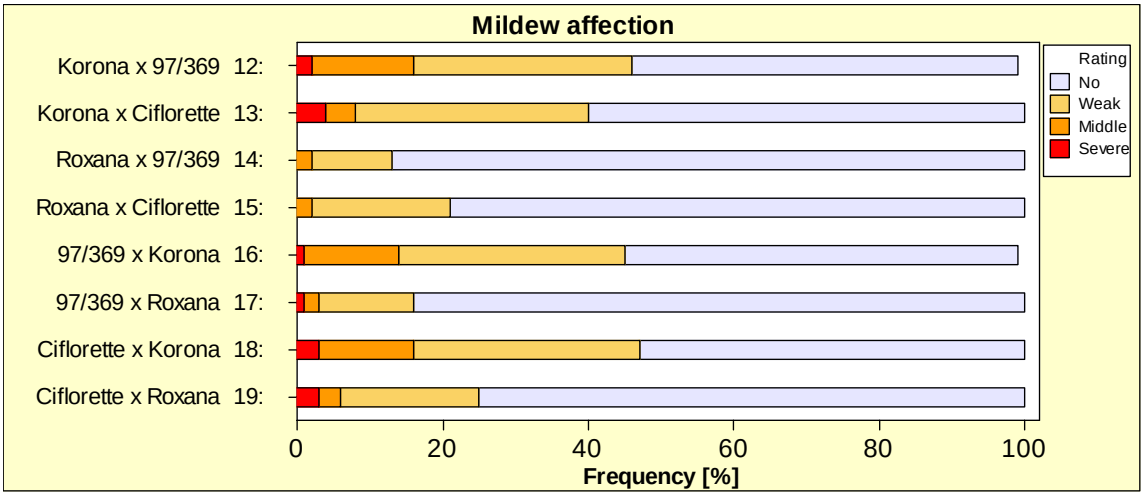


Figure 41: Frequency of the rating of mildew susceptibility in the eight populations.

Additionally, the rate of mildew susceptibility was rated (C 2.1.2.5). The results are illustrated in figure 41. The lowest total susceptibility rates were present in the populations 14 and 15 (13% and 21%) as well as 17 and 19 (16% and 25%). The first two mentioned populations have 'Roxana' as maternal parent and the last two populations as paternal parent. The populations 14 and 15 were also the only

populations in which no rating of severe affection occurred. The highest total susceptibility rates occurred in the populations 12, 13, 16 and 18, were ‘Korona’ was one crossing partner.

D 2.2.2.3 Color and Color Pattern

Figures 42 and 43 illustrate randomly conducted assortments of fruit of all eight populations of the diallel. The corresponding crossing partners are located at the sides of the populations. The maternal partner is found above the paternal parent. Figure 42 presents the outsides of the fruits and figures 43 sections through the same fruits.

All cross combination resulted in populations of high variability in regard to skin and pulp color, color pattern, size or shape. The skin color ranged from light-red to dark-red (figure 42). The examples for dark-red skin color are the first fruit in the second row of the population 14 or the forth fruit in the first row of the population 16. Some fruits had orange skin color, like the third fruit in the first row of the population 16 or the second fruit in the second row of the population 17. The pulp color displayed in figure 43 had also a wide range from white to dark-red. Sometimes a yellowish pulp color occurred, as shown by the fruit of the last fruit in the third row of the population 19. The pulp color was never darker than the skin color. In the case of the both mentioned examples, for dark-red fruit different pulp colors occurred. The fruit of the population 14 had a white to red colored pulp, while the color of the fruit of the population 17 was dark-red.

The color pattern of the fruit varied also widely in all populations from missing to intense pattern. The reason for missing color pattern can be a white pulp color together with white colored vascular bundles and pith, like the first strawberry in the second row of the population 12, or a colored pulp together with vascular bundles and pith in the same color, like for example the first fruit in the third row of the population 19. An illustration for intense color pattern is the third fruit in the third row of the population 18. The forth fruit in the first row of the population 17 had also intense white colored vascular bundles. However, due to the light-red pulp color the contrast is lower and therefore this fruit is not as striking as the other one. A lot of strawberries occurred which pattern intensities were difficult to rate. For example, the white frame of the pith of the first fruit in the first row of the population 16 silhouetted

clearly against the red pulp color. On the other hand, the color of the vascular bundles of this fruit did not differ from the surrounding tissue and no pattern was present.

In all the populations, fruit were present with different formed centers. Some fruit had no cavity in the centre. Also, fruit occurred where piths looked like torn apart, like the first fruit in the first row of the population 15. Others had cavities in the centre with a pith only connected to the upper part of the surrounding tissue, like the first fruit in the first row of the population 14. If a cavity occurred without a pith, for example like at the second fruit in second row of the population 16, the corresponding pith was found in the other not presented half of the respective fruit.

Differences occurred also between the appearances of the populations. The pulp color and color pattern looked more variable in the populations with 97/369 as a crossing partner. In comparison to the other populations, the fruit of these populations were also brighter and the occurrence of the hollowed fruit was more frequent. The frequency of oblong shaped fruit seems to had been higher in the populations with ‘Ciflorette’ as a crossing partner. This shape type occurred also in the populations of other combinations but it was much less frequent. Especially the fruit of ‘Ciflorette’ x ‘Roxana’ and the reciprocal cross showed this oblong shape.

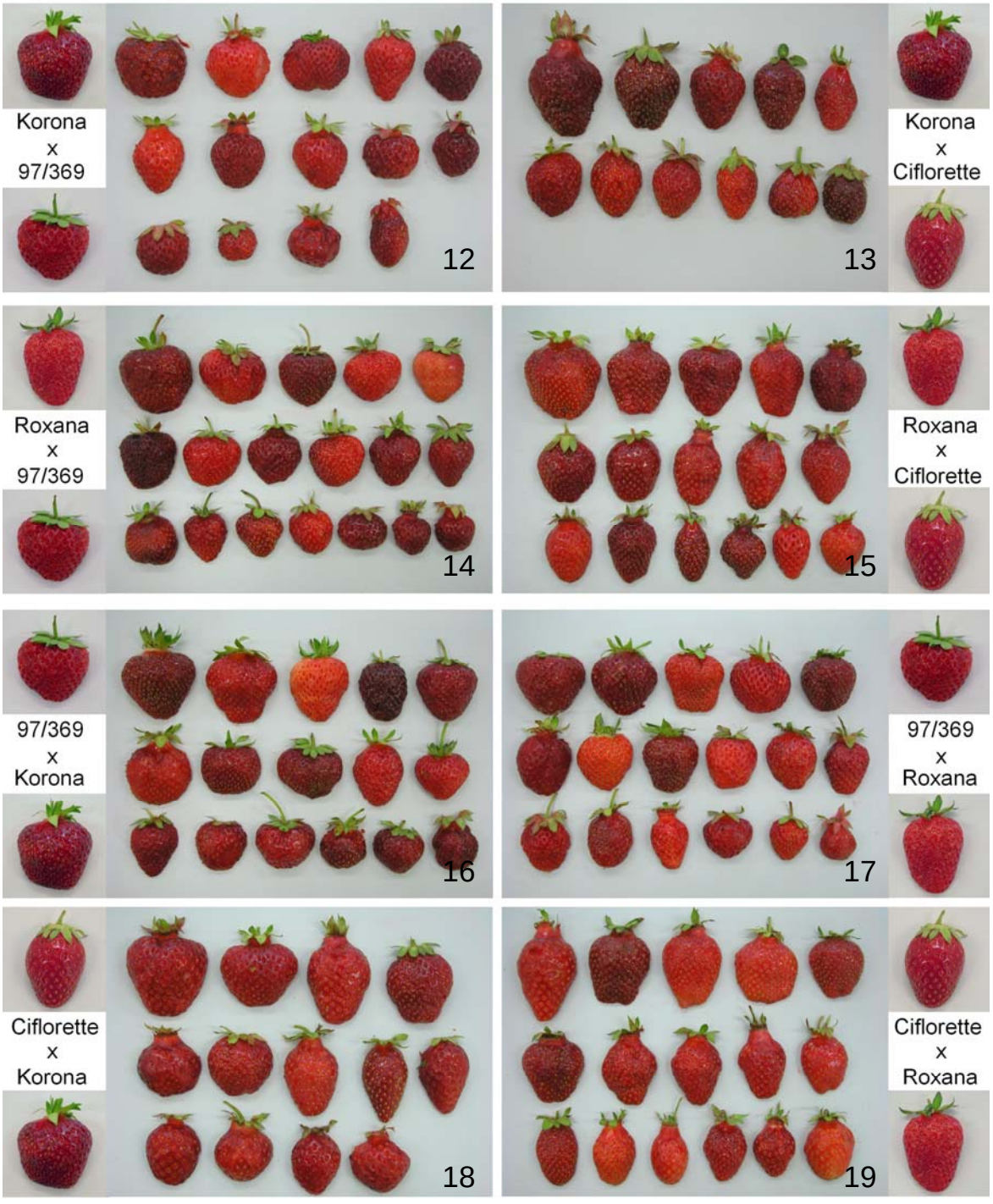


Figure 42: External appearance of the populations 12 to 19. The numbers indicate the populations' number.

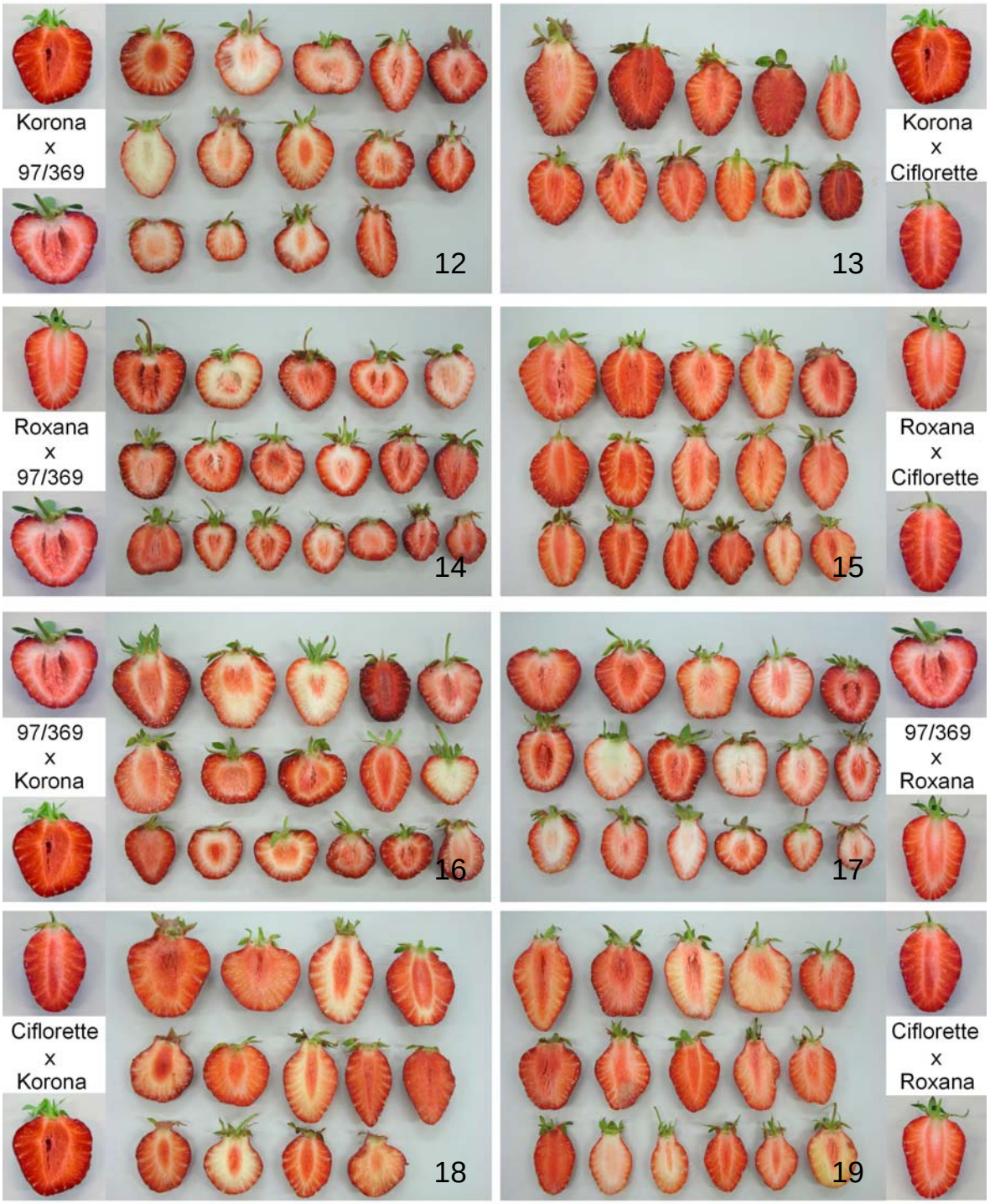


Fig 43: Internal appearance of the populations. The numbers indicate the population' number.

D 2.3 Breeding Strategies

D 2.3.1 Parental Cross *versus* Pollen Mixture

The main objective of this experiment was to investigate the proportions of the different paternal parents in a population of seedlings deriving from a pollen mixture as used in applied strawberry breeding programs. If possible, a comparison between the two approaches described in C 2.1.2.6 should be conducted in respect to the selection decisions of the breeder.

D 2.3.1.1 DNA Extraction

The extraction of *Fragaria* DNA turned out to be difficult. The first extractions with the DNeasy Plant Kit of QUIAGEN carried out according the manufactures protocol or different modifications did not result in clean enough DNA template. This could also not be remedied by different RAPD-PCR protocols (C 2.3.2). Therefore, the Cetyl trimethylammonium bromide (CTAB) procedure according DOYLE and DOYLE (1987) was tried but did also not produce reproducible clean DNA. The CTAB procedure according to HEUN *et al*. (1991) and a subsequent classical phenol-chloroform extraction resulted in reproducible clean DNA suitable for PCR. However, the method was too time-consuming and had a low sample throughput. Consequently, the CTAB procedure of HEUN was modified. The main points of modification were the adaptation of the protocol to smaller reaction tubes and higher rpm of modern table centrifuges. The protocol is presented in C 2.3.1.

D 2.3.1.2 Analysis by RAPD Markers

In the early stages, the analysis by RAPD was considered as sufficient enough for the set objective.

The not clean enough DNA templates of the first DNA isolation attempts were tried to compensate by different RAPD-PCR protocols. The 10x-buffer of QUIAGEN and the buffer according to WILLIAMS *et al*. (1990) were tested with DNA template concentrations of 10, 20 and 30 ng at different PCR conditions. No differences were present between the two buffers, but the bands of the samples, which showed

amplification, vanished with higher template concentration. However, most samples did not show amplification. Since the functioning of the PCR was proven by some samples, the DNA extraction method was modified before further analysis, as described in C 2.3.1. The extracted DNA of the modified procedure was suitable for RAPD analysis. However, the known problems of RAPDs occurred. The gained information was low and reproducibility was not always given. Therefore, SSR markers were taken instead.

D 2.3.1.3 Analysis by SSR Markers

The used SSR markers allowed a fast and reproducible assessment. In total, 111 randomly chosen seedlings of the pollen mixture population and 28 selected seedlings out of this population were analyzed. With 14 SSR markers 5 polymorphic bands, discriminating 2 paternal parents, and 14 polymorphic bands, discriminating one paternal parent, from the other possible were available. In annex G 17 the corresponding 0-1-matrix is listed. Table 17 shows the number and the proportion of the paternal parents in the 111 analyzed seedlings and 28 selected genotypes.

Table 17: Proportion of the paternal parents in the pollen mixture and their selected genotypes. K: 'Korona', H: 'Honeoye', S: 'Senga Sengana', E: 'Elsanta', X: supposable selfing.

Population	Total	K	H	S	E	X	unknown
[N]	111	61	5	26	3	6	10
[%]	-	55.0	4.5	23.4	2.7	5.4	9.0
Selections	Total	K	H	S	E	X	unknown
[N]	28	23	0	2	1	0	2
[%]	-	82.1	0.0	7.1	3.6	0.0	7.1

More than half of all analyzed plants of the population had 'Korona' as the paternal parent, followed by 'Senga Sengana' with 26 seedlings. The potential male parents 'Honeoye' and 'Elsanta' did almost not participate as partner in this particular pollen mixture. Six plants or 5.4% had bands of the maternal parent 'Fraroma' but no polymorphic band of a parent. They are most likely selfings of the self fertile mother plant. The results of 10 seedlings were not assessable by contradictory results of the markers (see also annex G 17).

From 28 selected seedlings out of the pollen mixture population, 23 plants had 'Korona' as paternal parent. Only 2 and 1 plants deriving from the cross combination

‘Fraroma’ x ‘Senga Sengana’, respectively ‘Fraroma’ x ‘Elsanta’. 2 plants were not analyzable.

D 2.3.1.4 Selection Rates

The results of D 2.3.1.3 offered the possibility to calculate the number of plants of a certain paternal parent in the pollen mixture population. Therefore, the approximate selection rates of both breeding approaches are presented also according the four cross combinations in table 18.

Table 18: Selection rates of the two breeding strategies.

	Plants [n]		
Parental cross	Total	selected	Selection rate [%]
Fraroma x Elsanta	444	2	0.5
Fraroma x Honeoye	255	6	2.4
Fraroma x Korona	213	5	2.3
Fraroma x Senga Sengana	414	1	0.2
Total:	1326	14	1.1
Pollen mixture			
Fraroma x Elsanta	25	1	3.9
Fraroma x Honeoye	42	-	-
Fraroma x Korona	516	23	4.5
Fraroma x Senga Sengana	220	2	0.9
Fraroma x Fraroma	51	-	-
Fraroma x unknown	85	2	2.4
Total:	939	28	3.0

D 3 Practical Realization

As stated already in the objectives of the present work, the research of the main breeding goal high DM and selection with regards to this trait had to be conducted at the same time. The results of the selection for high DM are presented in this chapter in chronological order.
The different technologies NMR, Near Infrared (NIR), density and conductivity were considered as selection method (methods and data not shown). However, the presented selections were carried out by the Brix value and with a digital refractometer at the test field.

D 3.1 2004

D 3.1.1 Selection

In the first year of the presented work, the selection work had to be conducted with populations of cross combinations which were not particularly created for processing or high DM selection. Two approaches were applied: All plants of chosen populations and all plants which were pre-selected for fresh market by the strawberry breeder of the IOZ were screened for high DM. The selection limit for the first approach was higher than for the second method.
The number of selected genotypes amounted 83.

D 3.1.1.1 Selection out of Populations

Three populations were screened completely and certain other populations just sporadically. 56 genotypes were selected with this approach. From a 489 seedling counting population of the cross combination ‘Fraroma’ x ‘Honeoye’, 16 genotypes were selected and 17 seedlings were selected from the population of the reciprocal cross (402 plants in total). 16 genotypes were selected from the population of ‘Fraroma’ x ‘Senga Sengana’ (414 seedlings) and seven seedlings from four different other populations.

D 3.1.1.2 Selection out of Pre-Selected Genotypes

27 individuals were selected from 199 pre-selected genotypes. From these selected genotypes, six genotypes originated from the cross combination ‘Fraroma’ x ‘Honeoye’, three from ‘Honeoye’ x ‘Fraroma’ and two from ‘Fraroma’ x ‘Senga Sengana’. Eleven genotypes were selected from the populations which were already used for D 3.1.1.1. However, due to the lower selection limit, these eleven genotypes were only selected by the second approach. No genotypes were selected by both approaches. The remaining 16 genotypes were pre-selected from populations of different other cross combinations.

D 3.2 2005

D 3.2.1 A-Selections

In 2004, 83 selected genotypes were planted as triple or sixfold clones. Some plants of the selections died or did not yield fruit. 52 selections were analyzable from the 56 selections of the selections out of populations. All plants of one selection of the cross combination ‘Fraroma’ x ‘Senga Sengana’ died during the winter and not enough fruit could be picked from three other selections of the irregular screened populations. Two of these selections had only a single plant left. From 27 genotypes of the pre-selection, 21 were analyzable.

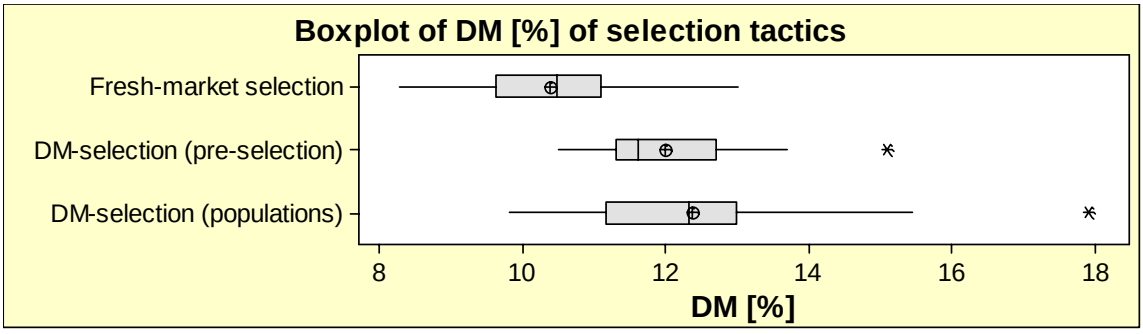


Figure 44: Boxplots of the DM distributions of the A-selections, sorted according to their selection type. The cross (circle) indicates the mean.

The DM levels of one conducted picking in the main harvest season of the 73 selections are presented in figure 44. The boxplots are sorted according to the

selection approaches. The DM level of 34 randomly chosen A-selections of a fresh market selection is also presented for comparison.
The mean as well as the median of the selections selected for high DM is higher than those of the selections selected for fresh market. A MOOD Median Test shown in annex G 18 demonstrated that this difference is significant at a p-value< 0.001.
In order to have enough genotypes for further analysis concerning the success of high DM selection, the further selection of the A-selections was conducted not very strong. Even if some selections were not suitable for processing, the only selection factor was again DM or a very small vigor. All selections with a DM <11.5% were discarded. Finally, 12 genotypes were maintained of the A-selections selected out of pre-selected genotypes and 35 genotypes of the A-selections selected by the population screening.

D 3.2.2 Selection

The selection work of the second year was focused on the populations ‘Ciflorette’ x 97/362 (142 plants) and 97/362 x ‘Ciflorette’ (271plants). The reason for this was the evaluation of both crossing partners as high DM genotypes in the year before (annex G 12). Unfortunately, both populations disappointed the high expectations. The DM of the seedlings was not obviously higher than that of other populations. To this, the color of the skin was light-red to orange and the pulp color was white to light-red. The higher frequency of yellowish pulp colored fruit in comparison to other cross combinations was also unusual. Therefore, only four genotypes were selected in total from the population of ‘Ciflorette’ x 97/362.

D 3.3 2006

D 3.3.1 A-Selections

None of the selected four genotypes of the cross combination ‘Ciflorette’ x 97/362 was selected for further propagation.

D 3.3.2 B-Selections

The genotypes of the pre-selection were planted as six- to fifteen-fold clones, the genotypes of the population screening as nine-fold clones. One genotype of each DM selection approach did not yield enough fruit for investigations. All plants of one genotype of the population screening died over the hard winter 2005/2006.
If possible, more than one picking was carried out for all B-selections in 2006. The DM, Brix, average fruit weight and average yield per plant and picking was determined. All data is detailed listed in annex G 19. For comparison of the selection success, the values of 19 available B-selections of the in 2005 randomly chosen A-selections of a fresh market selection were also investigated.

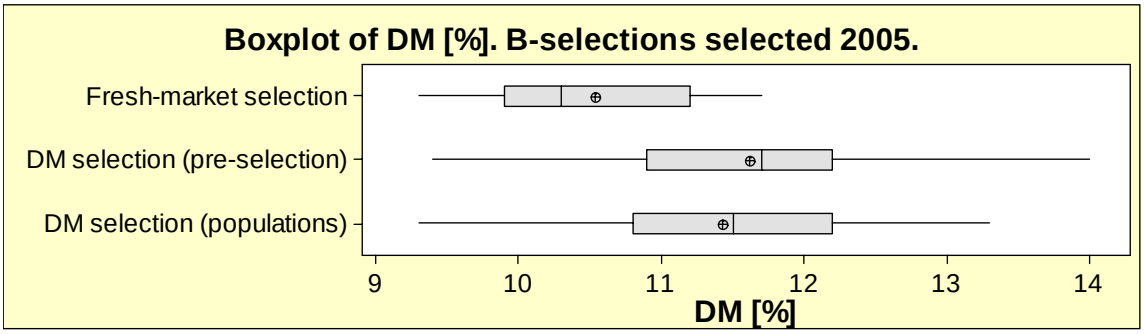


Figure 45: Boxplots of the DM distributions of the B-selections, sorted according to their selection type. The cross (circle) indicates the mean.

Figure 45 shows the boxplots of the DM means of the B-selections sorted according the three different selection approaches. Since all three variants were following a Normality Distribution, an ANOVA and FISHER's one-way multiple comparison tests were carried out at a 5% level of significance (annex G 20). Only the mean of the B-selections of the fresh market selection differed significantly from the other two groups. It can be seen by the range of the boxplots that B-selections with a lower DM occur in all selection approaches. Genotypes with a DM higher than 11.7% are only present in the high DM selection approaches.

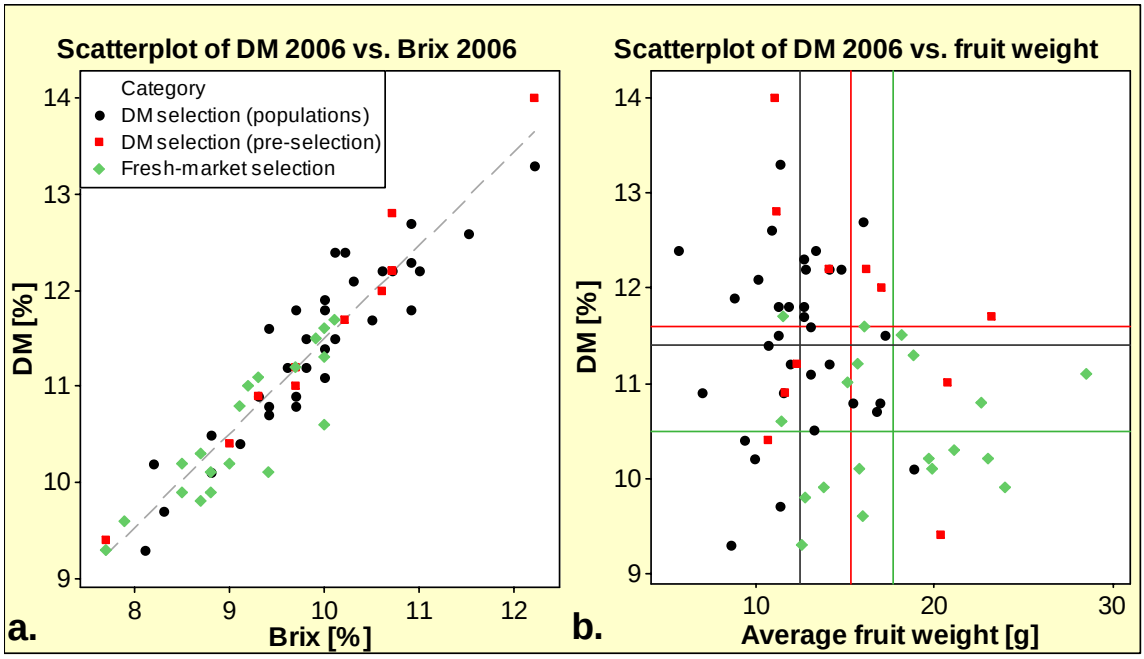


Figure 46 a and b: Scatterplots of DM *vs.* Brix (a) and DM *vs.* fruit weight (b) of the B-selections. The values are marked according to the selection approach. The dotted grey line in a indicates the regression fit. The colored lines in b represent the means of DM and average fruit weight of the corresponding colored values.

Scatterplots between DM *vs.* Brix and DM *vs.* average fruit weight are displayed in figure 46 a and b. The correlation between DM and Brix was r= 0.942, p-value< 0.001, and following a regression fit of DM [%] = 1.691 + 0.9802 Brix [%]. No high correlation is obviously present between the DM and the average fruit weight in all investigated genotypes as well as in one of the different selection approaches. The correlation coefficient r for all genotypes was -0.254, p-value= 0.045. However, figure 47 shows that the three different selection groups differ in their average fruit size.

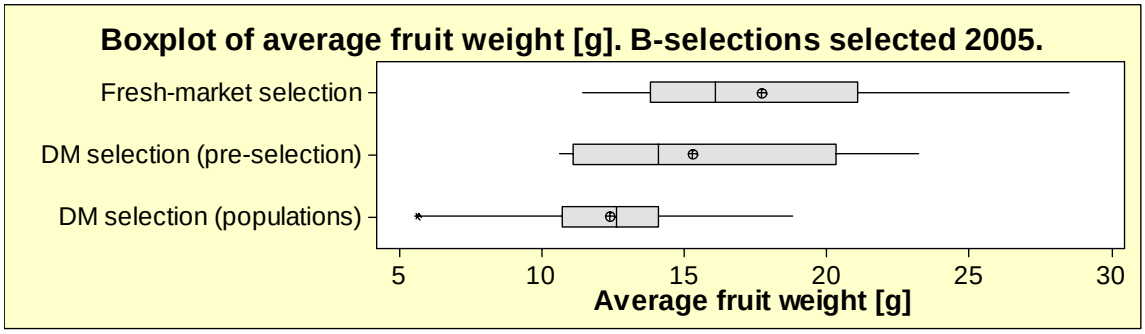


Figure 47: Boxplots of average fruit size [g] of the B-selections, sorted according to the selection approach.

An ANOVA and FISHER's one-way multiple comparison test conducted at a 5% level of significance revealed that the mean of the group "DM selection (population)" differed significantly from the two other groups (annex G 20). No difference was present between the group "DM selection (pre-selection)" and "Fresh-market selection".

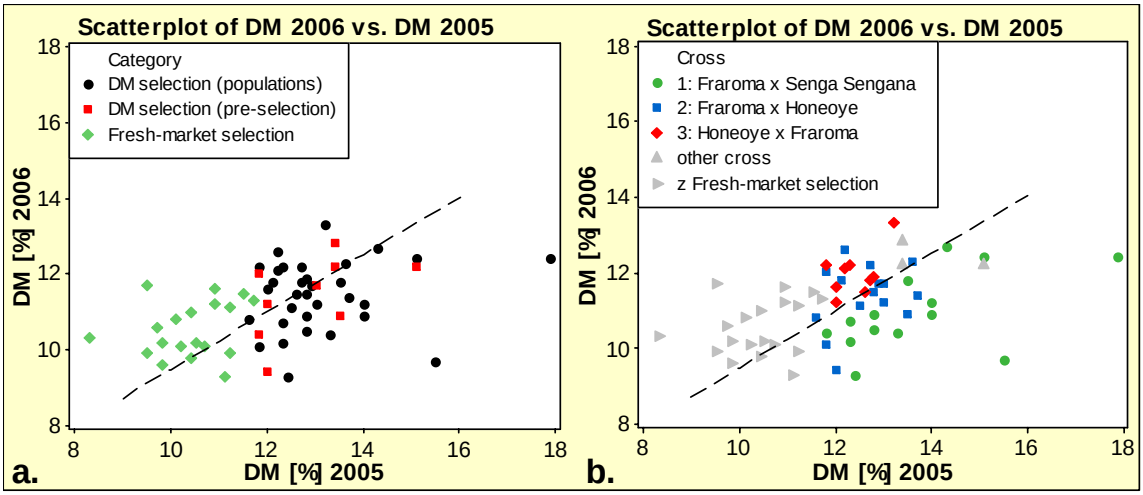


Figure 48 a and b: Scatterplot of DM [%] of 2006 *vs.* DM [%] of 2005. In figure a the different selection approaches and in figure b the genetic background of the genotypes selected for high DM are marked. Explanation is in the text.

Figure 48 a and b display the DM values of the B-selections of 2006 *vs.* the DM values of the corresponding A-selections of 2005. It has to be considered that only one measurement per plant was conducted in 2005 but more analyses per plant in 2006. The mean DM of all selections was 12.2% DM in 2005 and 11.2% in 2006. The means of the selection approaches of 2005 and 2006 are listed in table 19.

Table 19: Mean DM of the selections of the different approaches. Data of 2005 and 2006.

	Mean DM [%]		
	2005	2006	Difference
Fresh-market selection	10.4	10.5	0.1
DM-selection (pre-selection)	12.0	11.6	-0.4
DM-selection (population)	12.4	11.4	-1.0

The correlation coefficient between the DM of all investigated genotypes of 2005 *vs.* the equivalent values of 2006 was r= 0.467, p-value< 0.001. As in figure 34, the dotted black line represents the main axis of correlation and was y= 1.878 + 0.7604x. In figure 48 a, the different selection approaches are marked. Figure 48 b shows the same values, but now the genotypes of the two DM selection approaches are marked according to their cross combinations. Genotypes of the cross combination ‘Fraroma’ x ‘Senga Sengana’ are marked with a green circle, ‘Fraroma’ x ‘Honeoye’ with a blue square and ‘Honeoye’ x ‘Fraroma’ with a red rhombus. The remaining B-selections of the DM selection derived from other cross combinations and are marked with a grey triangle faced upwards. The genotypes of the fresh-market selection are represented by a grey triangle faced sidewards. The degree of deviation of the different cross combination groups from the main axis of correlation differed. The genotypes of the cross combination ‘Fraroma’ x ‘Senga Sengana’ are almost all below this axis and have higher distances to it. The values of ‘Fraroma’ x ‘Honeoye’ vary above and below the main axis, while the values of the combination ‘Honeoye’ x ‘Fraroma’ are only above it.

E Discussion

The main objective of the present work is the scientific elaboration and implementation of a breeding program for high DM processing strawberries, suitable for freeze-drying.

E 1 Aspects of Dry Matter Determination

If a new parameter like DM is defined for a crop and if the trueness of the measurement method is ensured, the next questions have to be how precise the determination of this parameter is and what the influencing factors on the parameter are. Due to different physiologies and cultivation methods, a general transfer of results from other crops is not possible. As a consequence, specific research is required.

Quantitative traits in strawberry fruit are difficult to evaluate. The cultivated strawberry has several fruit per plant which differ from each other in physiological aspects. To this, strawberries are picked several times during a harvest season and a two year harvest season is typical for extensive cultivation in the field. These circumstances led to the standard method for applied strawberry field tests; pooled samples of several clones of one genotype are planted in a block design and picked several times. Normally a sample of approximately 200 g to 500 g is analyzed and diseased or overripe fruit are discarded.

In the present work, discarding was also necessary. Especially the overripe fruit had an enormously higher DM than the ripe fruit in all investigated cultivars (D 1.1.1). Significant differences between strawberry ripening stages were also described by PEREZ *et al*. (1996) and STURM *et al.* (2003) for other fruit quality parameters as there are furanones, sugars and acids.

The sampling of 200 g strawberries out of blocks was tested and applied for evaluations of the DM of different genotypes. The results, presented in D 1.1.2, showed that a high accuracy was given for the DM determination according to this experimental design.

Further, it was shown by the SD of the repetition blocks D 1.5 (see also annex G 7) that this high accuracy also persisted at different locations and under different cultural practices, even if only nine plants per block were investigated. However, two SD

values were significantly higher at two locations. This was caused in both cases by a higher deviation of the value of only one block. In the case of the SD of 1.5% DM of the third picking date of ‘Roxana’ at the location Vienna, the third repetition, with 8.3% DM, had an analyzed sample volume of just 55.8 g (4 fruit with average 14.0 g). This small sample volume and number might have caused the higher imprecision. On the other hand, the second highest SD of 1.1% DM occurred in the third picking of ‘Roxana’ at the location Geisenheim and no repetition was smaller than 166.5 g or 8 fruit. The most deviating value of 12.2% DM even resulted from a sample volume of 216.0 g. Consequently, the reason for these high SDs of the repetitions can not be merely the smaller sample volume. A higher plant number per block could assure a sample volume of more than 200g and could possibly lower the source of error. Conversely, it has to be considered that applied breeding programs are limited in regard to time and money. In the presented experiment, an additional row of only three plants would have resulted, with three cultivars, three repetitions and four locations, in 108 additional plants to purchase and to cultivate.

A better valuation of the precision of the DM determination of the above mentioned experiment can be reached by a comparison to the other investigated traits citric acid and average fruit weight. The analysis of citric acid revealed almost ever higher CV values for the citric acid than for the corresponding DM values. An excess of a CV value of more than 10% occurred also more often. The CVs of the average fruit weight determination were even much higher and exceeded sometimes a CV of 20%. Certainly, this higher imprecision of the average fruit weight determination results from a higher variation of this trait in one picking charge. This effect is discussed in more detail in paragraph D 1.2 evaluation of the single fruit analysis. However, the consequence of these results is the need of a higher strawberry number for average fruit weight determination, while the sample volume and number are sufficient for DM determination.

The block design was also used for investigations of the DM of five genotypes during the harvest seasons of 2004, 2005 and 2006. The DM values of the first pickings of all investigated genotypes varied. Similar results were reported by SISTRUNK (1961), who described a significant effect of the harvest date on the DM level of the cultivar ‘Northwest’ and SIMS *et al*. (1997), who found variations for other fruit quality characteristics like soluble solids, citric acid, pectin, cellulose, surface color or flavor intensity. The variation of DM in the first pickings is most likely the result of altered

environmental conditions at the different picking dates. The environmental influences hereby to be considered are climate, cultural practice and the pickings themselves.

The increase of the DM of most genotypes at late picking dates is remarkable and was also described by HANSEN (1995) and AVIGDORI-AVIDOV (1986). It coincides with a dramatic decrease in yield, caused by a decline in fruit size and number (KALT and McDONALD, 1997). Because the increase of DM always took place at the late pickings of the investigated cultivars, it can be assumed that it is primary due to the decrease in yield and not due to altered environmental conditions, as discussed above. KALT and McDONALD (1997) picked three genotypes five times and also obtained higher DM values at late pickings of the cultivar 'Honeoye' but not in the cultivars 'Cavendish' and 'Kent'. Beside the deviating sampling method and location, the reason for that might be the fewer number of pickings. In direct comparison to the own values, such as those of 2004, the DM was also not increasing till the fifth picking in the genotypes 'Honeoye' and 'Senga Sengana'. The rise of DM occurred afterwards. On the other hand, the selection 97/362 picked six times showed only slightly higher DM values at late picking stages. In this case, the overall lower yield could be the cause. In practice a cultivar is not picked more than four to five times. This limit of picking dates should also not be exceeded in evaluation for breeding purposes.

Although there was sometimes the impression of faint associations between the measured DM values and the recorded climate data, it was not possible to find a correlation. Not even in the case of the two major DM drops at June 21st and June 30th of the year 2006, which were the most striking changes in all observed years. Rainfall periods predated both picking dates and it was supposed that those caused the decreases in DM. This assumption is corroborated by KIMBROUGH (1930) who reported higher moisture and lower sugar levels in strawberry fruit after rainy periods. However, the two rainfall periods differed on closer examination. The time between the precipitation and the DM drop was different. On June 21st, two days with precipitation preceded the picking date, while on June 30th a heavy rainfall was recorded three days before. Contrariwise, this heavy rainfall did not influence the DM of the picking carried out on June 28th. Also the rainfall on the 21st, which was the second highest during the harvest season 2006, had no negative influence on the DM of the following pickings at June 23rd and 28th. Furthermore, the literature reports an influence of the climate at the picking date. LATYPOVA and TATAUROVA (1972)

described an influence of the air temperature at the picking date on the sugar and DM of the cultivars ‘Mieze Schindler’ and ‘Späte Leopold’. IVANOV AND STAMBOLIEV (1973) wrote that the DM was the highest when the climate was wet and warm. However, the climate at the 21st and 30th of June 2006 differed significantly. At June 21st, the air humidity was high and the air temperature and global radiation were at an average level. In contrast, at June 30th the air humidity was also high but the air temperature and global radiation were very low. For that reason, direct comparisons of the DM and the precipitation or the climate at picking do not lead to easy causations. Consequently, other comparisons with climate data as well as a relativization of the DM values by climate data are set aside in the further course of the presented work. Such connections might be better investigated in experimental designs using climate chambers with controlled conditions.

Which climate factors influence the DM is of less interest. The cultivation of processing strawberries is, due to their low price, constricted to an extensive cultivation at fields in certain countries with access to cheap labor. These specifications do not allow big modifications of the climate by cultivation methods and the production areas are fixed to social economical reasons. The question of higher interest is therefore: do the genotypes react similarly to certain climates? The presented results of all three years show to some extent a similar response in all genotypes (figure 17). This leads to the conclusion that it has no importance for the cultivation of processing cultivars, because the climate is not manipulable.

Nevertheless, for adequate evaluation of DM of a cultivar or selection, the picking dates and the picking years have to be considered. The best impression of the DM level of a certain genotype can be reached by determination of the DM of practice-usual multiple pickings. Only one value of the first to fifth picking is imprecise, due to the present variation. However, for an applied breeding program such a roughly estimated single value can still be sufficient enough for a snapshot advice of DM. Of course only provided that impacts factors on the parameter, like on June 21st and 30th, are considered. In order to detect such impacts, it is reasonable to evaluate standard cultivars throughout the picking season. As already mentioned, the factors themselves do not have to be known. It is enough if the accuracy is assured.

The variations between the years underline the need for evaluations for more than one harvest season and the use of standard cultivars for comparison. Both are anyway standard in strawberry breeding programs. Similar conclusions were made

by SIMS *et al*. (1997) for already mentioned different fruit quality characteristics of fresh market strawberries.

E 2 Single Fruit Analysis

For strawberry field tests the block design is sufficient enough for the DM determination. However, in applied breeding programs no arbitrary numbers of plants are available for selections. In the case of seedlings, even only one single plant per genotype exists. Thus, a more detailed consideration of DM in single plants is necessary.

The conducted single fruit analysis (D 1.2) revealed a high variability between single fruit of each harvest date, single plant and cultivar. No connection between DM and the date of the three pickings was present, as it was shown between the fruit weight and the picking date (figure 13). Consequently, the variation has to be accepted. A particular variation occurred in the plant No.5 of the cultivar ‘Ciflorette’. All fruit of one infructescence but of different rank and picking dates had significantly lower DM values. It can be assumed that a certain influence affected the whole infructescence over the investigated time period and altered thereby the DM of all its berries. Unfortunately, no conspicuous observations were made. Because the most DM is transported into the fruit and not synthesized by the fruit itself (FORNEY and BREEN 1985ab), an external factor like a beginning disease or injury of the peduncle is assumable. One possibility could be for example that the labeling by a small tag, which was fixated with wire on the basis of the infructescence, injured the infructescence. In such cases the plant was not able to accumulate appropriate DM in the fruit of the particular infructescence. The reason remains unclear, but the variation between the fruit and the occurrence of inexplicable outliers underline the demand of an adequate large fruit sample for a representative DM value.

A further aggravating factor for DM determination is the variation of the DM means between single plants. The DM means of all fruit of the six single plants varied between 11.1% to 14.0% in ‘Ciflorette’, 9.3% to 10.5% in ‘Elsanta’ and 9.6% to 11.5% in ‘Senga Sengana’. This is a SD of 1.0%, 0.4% respectively 0.7%. The variation can only be reduced by a higher plant number, but exactly this can not be achieved in early stages of a selection process, were only seedling plants or few

selections are the objects of breeders decision. Therefore, this source of error for the DM determination in the early stages has to be accepted as well.

A connection between the DM and the fruit rank was shown for the cultivar ‘Elsanta’. The fruit of higher rank order had also higher DM. HANSEN (1995) also reported that fruit, picked at the same date of one infructescence, had significantly higher DM values if the ranks were higher. Furthermore, fully ripe fruit picked at the same date and grouped according the fruit weight also showed higher DM in bigger fruit. It was assumed by HANSEN (1995) that the reason for the difference in DM was the accelerated growth and development of fruit of higher rank. The growth itself is controlled by the plant hormone auxin, produced by the achenes (ARCHBOLD and DENNIS 1985, STRIK and PROCTOR 1988). The determining factor for fruit size is therefore a combination of total number of achenes and the number of achenes per surface (WEBB *et al*. 1974). The total number of achenes is fixed before enlargement occurs and is related to the rank on the infructescence, while the number of achenes per surface can be affected by the environment or competition between berries on one infructescence. JANICK and EGGERT (1968) demonstrated a significant increase in fruit weight of secondary fruit by the removal of the primary fruit. The converse removal had no effect on the fruit weight of the primary fruit. WEBB *et al*. (1978) also took into account that a similar competition could be present between different infructescences and infructescences and leaves. The presented results of ‘Elsanta’ and of HANSEN (1995) show that fruit of different ranks can not only be distinct by fruit size but also by the concentration of the incorporated assimilates, measured as DM content. This is explainable by some sort of competition for assimilates, whereas the fruit of higher rank dominate over subordinated fruit. Competition for assimilates among fruit were also described for tomato (COCKSHULL and HO 1995, HEUVELINK 1997), citrus (LENZ 1979), cucumber (MARCELIS 1993) and eggplant (LENZ 1970). However, these examples refer to a higher DM amount in grams per fruit, caused by a higher average growth rate of individual fruits, and not to the DM content. HANSEN (1993) reported that the fruit size and weight of sour cherries (*Prunus cerasus* L.) were also increased by a reduction of fruit number, but the DM content was not changed.

If a competition for assimilates exists in strawberries which leads to different DM contents of single fruit, an independent DM and water accumulation must be implicated. Evidences for such independence were also described by EHRET and

HO (1986) and HO *et al*. (1987) for tomatoes. GUICHARD *et al*. 2005 showed that a complicated system of phloem, xylem and transpiration fluxes exist in tomatoes.

On the other hand, in the present results no connection between the fruit rank and the DM content was present in the cultivars ‘Senga Sengana’ and ‘Ciflorette’. Even the separate consideration according the infructescences of the plant No. 5 of ‘Ciflorette’ revealed no connection between rank and DM or fruit weight and DM (see also figure 16). The reason for that stays unclear. One possibility could be a distorted competition situation caused by the pickings. HANSEN (1989) showed that the DM assimilation of subordinated fruit increases minimally if a fruit of higher rank order is removed. However, the fruit of all three cultivars were picked when fully ripe and the picking intervals were similar. An important difference could therefore be that the fruit of ‘Ciflorette’ were few in number. GUICHARD *et al*. (2005) showed for tomato fruit that the competition for assimilates is likely being reduced under low fruit load. Further, the fruit of ‘Senga Sengana’ were in average smaller and more uniform than the fruit of ‘Elsanta’. The competition for assimilates could have therefore been stronger between fruit of ‘Elsanta’ differing greater in size. The distorted competition caused by removing of fruit could also be an explanation for the presented contradictory results of higher DM in late pickings (D 1.3) and lower DM in fruit of lower order (D 1.2). As mentioned above, the fruit of the late pickings are in most instances few, smaller and of lower rank. However, the small number and missing fruit of higher order in combination with limited capacity of fruit enlargement, caused by the fixed lower number of achenes, could lead to the higher DM in the fruit of late pickings.

The DM differences of the fruit ranks in ‘Elsanta’ were only present if the plants and the picking dates were considered. The plotting of the DM versus the fruit weight of all fruit of ‘Elsanta’ revealed no correlation between the variables (figure 15). Within the different fruit ranks also no correlation was present between these two traits. It is assumed that the variation between the single plants as well as between the picking dates covered the differences between the ranks. Also no correlation was also present for ‘Ciflorette’ and ‘Senga Sengana’.

Therefore, it can be assumed that the accuracy of the DM measurement is not influenced by a possibly existing connection between the fruit rank and the DM content if the berries of several plants of a genotype are investigated as a pooled sample. Thus, the selection can be conducted on all ranks the same good.

Research into other crops showed that the partitioning in the fruit can be relevant in regard to DM. FISHER (1975) showed that the total plant dry weight in reproductive tissues of tomatoes is significantly negatively correlated with the dry weight in leaves and in roots. Genotypes of different growing and fruiting habits of tomato (*Lycopersicon esculentum* Mill.) or cucumber (*Cucumis sativus* L.) accumulate similar amounts of total DM per plant (HO 1996, MARCELIS 1991). On the other hand, the partitioning of this total DM in different fruit organs is affected by their habits (HO 1996). For instance, the fruit of the tomato cherry-type habit were small but numerous. Plants of this type accumulated the smallest proportion of DM in the fruit in comparison to other common tomato fruit habits. Most DM of the cherry-type habit plants was accumulated into the leaves, stems and trusses. The author assumes that the small size of the fruit may have caused the low partitioning to fruit. On the other hand, the cherry-type fruit had with 8.0% DM a noteworthy higher content than the other investigated habits beefsteak and round (5.5% and 6.0% DM). POPENOE (1994) mentioned that the partitioning of DM to the plant organs is hypothesized to differ between different growth habits in red raspberries (*Rubus idaeus* L.) but contradictory result were found. The partitioning varied between the cultivars but was not related to the growth habits. POPENOE (1994) concluded that the differences in DM partitioning are caused by the different status of the *Rubus* breeding effort at release date of a cultivar. For example, the older cultivar ‘Boyne’, released in 1960, accumulated more DM to the vegetative organs and less to the fruit in comparison to other more modern cultivars. Additionally, MARCELIS *et al*. (1998) emphasize that there seems to be great diversity in the way a crop partitions it’s assimilates.

Figure 49: Plants of a selection of a backcross of *F. ×ananassa* with *F. virginiana*.

In strawberries, for field or glasshouse grown plants of standard cultivars, the proportion of the DM partitioning in the fruit is normally around 40% in comparison to other plant organs (FORNEY and BREEN 1985a, OLSEN *et al*. 1985). If an influence of the fruit habit on the DM partitioning exists also in strawberries is not clear. In the present work, selections of backcrosses of *F.* ×*ananassa* with *F. virginiana* showed uniform, small and numerous berries (figure 49). This kind of fruit habit deviates significantly from the other standard strawberry cultivars and is comparable with the cherry-type of tomatoes. The partitioning proportion to the fruit was not investigated but no obvious deviating DM levels in the fruit were detectable. An influence of the fruit number of strawberries on the vegetative growth was reported by LENZ and BÜNEMANN (1969). An increased number of fruit per plant coincided with decreased vegetative growth. Since vegetative growth is needed for photosynthesis and further assimilation, the total dry matter of the plant was lowered as a consequence. Plants like tomatoes or cucumbers can counterbalance such a tendency by flower or fruit abortion at too high fruit or flower load (MARCELIS 1994, BERTIN 1995). However, no flower or fruit abortion was observed for strawberries. These results show how complicated the relation between vegetative and generative growth could get in strawberries, if the fruit or growing habit or the fruit DM are altered.

E 3 Influence of Location

The ANOVA table of the GLM analysis (annex G 8) indicates only significant effects of the cultivar and the interaction of cultivar by picking date by location for the response DM content. The significance of the interaction effect implies that the DM content depends upon the combination of cultivar, picking and location. This interaction is certainly difficult to interpret. Further, the high significant effect of the cultivar has to be regarded with caution. All three cultivars were chosen because of their different DM levels in preceding investigations (annex G 12). ‘Mieze Schindler’ as an old German cultivar for the house garden had a high DM content, ‘Roxana’ as a North-Italian contemporary cultivar for the fresh market and ‘Senga Sengana’ as the European standard for processing a low DM level. However, a commonality is that all three are cultivars and market-proven. This requires a constancy of traits over the years and at different locations. The influence of the environment and the genotype by environment interaction effect on the main traits can therefore be

considered as low. This was confirmed by the results of the GLM analysis and the interaction plots for the trait DM. Only at the location Geisenheim, the cultivar ‘Roxana’ showed a higher DM than ‘Senga Sengana’ (figure 19). Certainly, the trait DM was not a breeding goal for any of the investigated cultivars. Nevertheless, the trait DM is connected with important quality traits, predominantly the soluble solids (see chapter below). This could have led to an indirect selection for a constant DM content. While the DM level of the cultivars is constant, selections of lower selection stages could have much lower constancies. This is shown in D 2.1.2, D 2.2.1 and D 3.3.2 and will be discussed in E 9. Consequently, the main conclusion of this GLM analysis is not only that the trait DM is in common constant at different locations with different cultivation methods, but that a high constancy of DM can be reached by breeding work, even at different levels of the trait.

The results of the response Brix are analogous to the ones of DM. This is not surprising, in view of the fact that a correlation between the two traits exits, which will be discussed below. In accordance with the own finding, the trait total soluble solids, which is measured as Brix, was also characterized by STURM *et al*. (2003) as quite constant in different strawberry cultivars. In contrast, SHAW (1990) reported that the expression of this trait was not constant across test location and cultural treatments.

The trait citric acid showed deviating results. Only the interaction cultivar by picking by location showed a significant effect. As already mentioned for the response DM, such an interaction is difficult to evaluate. The citric acid level of different cultivars was dissimilar at different picking dates and at different locations. One aspect of this complicated connection is also illustrated in the interaction plot of figure 19 by its inconsistent ranking of cultivars across the locations. Only the locations Dresden and Geisenheim showed similar sequences of rankings. The non significant effect of the cultivar is surprising, because acid levels in ratio to the sugar level has been related to flavor quality (SWEENEY *et al.* 1970) and citric acid is the dominant acid in strawberries (SISTRUNK and CASH 1973). Consequently, the citric acid level is an important breeding goal, which should also implicate a constancy of the trait in commercial cultivars. One explanation could be that the variation of the trait citric acid has a high environmental and/or genotype by environment interaction effect. This would result in limited breeding success. A high variability of acids over years was described by SISTRUNK and CASH (1973) and SWEENEY *et al*. (1970). The influence of the genotype by environment interaction was therefore assumed to be a

main source of variation. KALT and McDONALD (1997) reported also of substantial differences in citric acid levels of cultivars between seasons due to stress. SHAW *et al*. (1987) and SHAW (1988) reported contradictory findings on seedling surveys and subsequent clonal tests. The authors reported no significant genotype by harvest effects for citric acid in a set of selections selected for high, intermediate and low expression of titratable acids. This indicated that the relative ranking of genotypes is constant across harvest dates. In continuing investigations the trait also turned out to be constant across test locations and cultural treatment, while the trait soluble solids was not constant for this factor (SHAW 1990). The heritability of titratable acids was calculated as large and a good selection response was detected. These results are contrary to the findings of the present work. One reason could be that the investigations of SHAW *et al*. (1987) and SHAW (1988, 1990) were carried out on seedlings and clones from 40 different populations. The range of a trait is normally larger in populations than in commercial cultivars. Selections for high, intermediate and low titratable acid levels should therefore result in genotypes of higher deviating acid levels. This can lead to a higher importance of the genotype as a source of variation. Evidence could be that the interaction genotype by harvest was significant for citric acid in a set of selections selected for high, intermediate and low expression of soluble solids (SHAW 1988).

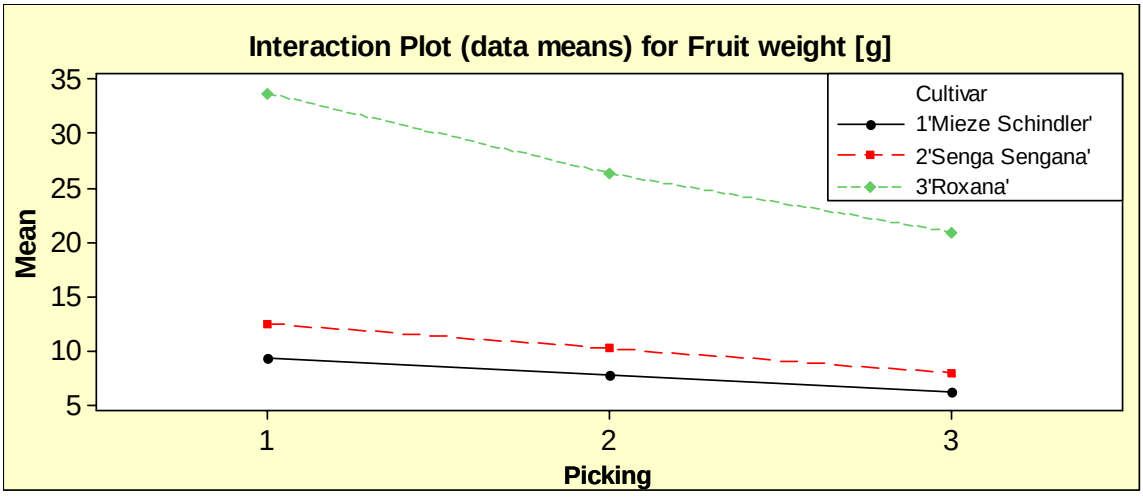


Figure 50: Interaction plot for average fruit weight [g].

The trait average fruit weight showed a significant effect of the cultivar, pickings, cultivar by pickings and cultivar by location. Similar results were reported by LOPEZ-MEDINA *et al*. 2001. The effects of the cultivar and the pickings are not astonishing, since cultivars of different markets were chosen and the average fruit weight declines with the pickings (shown also in figure 13). The significant interaction cultivar by picking can be interpreted as an effect of the pickings upon the average fruit weight depending upon the cultivar. This context is illustrated in the interaction plot of figure 50.

The cultivar 'Roxana' had in principle a higher fruit weight, but the decline over the picking is greater than in the smaller-fruited cultivars. MOORE *et al.* (1970) obtained similar results for the fruit size. The interaction cultivar by location is more difficult to analyze. However, the figure 50 shows by parallel lines that the effect of the cultivar upon average fruit weight does not depend upon the location. Consequently, this interaction has not to be considered so strongly.

Present correlations of DM with other traits were already reviewed in the introduction part. The correlation of DM with the Brix value is evident in several crops and is not astonishing, since the soluble component of the DM is the Brix value. The importance of this correlation for the present work results from the fact that it is more convenient in applied breeding programs to sometimes select for a correlated response than for the desired trait itself. The factor sample throughput is one of the most crucial factors for selection work. Due to the much less time consuming determination of the Brix value in comparison to the determination of DM by gravimetrical methods, the trait Brix is much more effective to select. The same high correlation coefficients at all locations underline that a selection for DM by using the Brix value could be conducted at all four locations.

While the Brix value represents the soluble part of the DM, the citric acid level, even as the dominant acid in strawberries, accounts for just a smaller proportion (see also D 1.7). As result, no correlation between the DM and the citric acid occurs for the location Skiernievice and Vienna and a low positive correlation for the location Geisenheim and Dresden.

The plots of DM *vs*. the average fruit weight revealed a low negative correlation. DARROW and WALDO (1932) also reported that small berries have a higher DM than large ones. Unfortunately, the author gives no more information about this connection. However, a correlation does not imply causation between two traits. It

can be based on causation or it can be coincidental that, in the present experiment, the cultivar ‘Mieze Schindler’ with the highest DM had also a lower fruit weight and the cultivar ‘Roxana’ with the highest fruit weight had a lower DM. The clusters of the three picking dates within a cultivar provide an argument for a coincidental distribution. In the case of the cultivar ‘Roxana’ at the location Skiernievice even a positive correlation occurred. This, together with the results of other DM *vs*. average fruit weight plots (shown in D 1.5), indicates that the correlation in this plot is an inhomogeneity correlation. It is based on the assortment of the investigated cultivars. Nevertheless, causation between the two traits could exist indirectly. The cultivar ‘Roxana’ is a newer cultivar than ‘Mieze Schindler’ or ‘Senga Sengana’. These cultivars reflect, by their fruit weights, the progress of strawberry breeding for higher fruit weight from the 1920s to the 1990s. Another important trait in strawberry breeding was and is always the yield. Due to a possible negative connection between yield and DM, which is discussed in E 8, the selection for high fruit weight in combination with high yield could also result in lower DM.

E 4 DM Variability within Fruit

The variability of a certain component within a fruit is of importance, in particular, if nondestructive determination techniques are used like NIR, NMR, sonic and ultrasonic or if just parts of fruit are analyzed due to the size (for example avocado, WOOLF *et al*. 2003). Nondestructive techniques are gaining in importance. They are based on calibration models derived from a correlation between a physical measurement and the trait of interest. Thereby, morphological fruit parameters and the variability influence the prediction capability of the calibration model, especially if small volumes of samples are scanned (GUTHRIE *et al*. 2005, CLARK *et al.* 2003, PEIRIS *et al*. 1999). This is the case for applied breeding programs. A comprehensive overview of the variability of soluble solids or DM within fruit, bulbs or tubers of different fruits is given by PEIRIS *et al*. (1999). No strawberries were investigated. The traits were measured by NIR and on the fruit along the proximal to distal, circumferential midway between the proximal and distal ends, and radial from the center of the interior to the outer surface. Differences in the variability were present between the fruits in all measured directions.

The results shown in D 1.6 were achieved by NMR. This technique is one of the leading candidates for non-invasive determining of internal fruit quality. In strawberries, it was already applied to indicate the variability within fruit (MAAS *et al*. 1992). The technique was also used to study the drying progress of strawberry slices during osmotic and air drying (EVANS *et al*. 2002).

In figures 21 and 22, the heterogenous dispersion of the free water is clearly visible. The main reason for that are the vascular bundles which have a higher amount of free water. Such a high contrast between fruit tissues does not have to be the standard. Cucumbers, which are berries in botanical terms, have a very low contrast in comparison (BLÜMLER P. pers. comm. 2005). The remaining tissue showed no considerable contrast. This finding differs from previously reported results of MAAS *et al*. (1992). The authors illustrated by a pass-through scan that higher water content exists in the pith. A similar result could also be drawn from the three-dimensional projection of the present work (figure 23 a. and b.), which suggests that the most free water was present in the centre of the fruit and decreased towards the skin. However, these display formats can be deceptive. The effect results from the overlay of several scans and not from a concentration of free water in the centre of the fruit body. The figures x and x demonstrate clearly that the free water was distributed homogenously in all areas. Only the vascular bundles which are enclosing the pith and diverging from it through the pulp tissue to the achenes had higher free water content. Therefore, it is also most likely that the overlay of the vascular bundles of the pith led to the conclusion of MAAS *et al*. (1992). Support for this explanation is provided by MAAS *et al*. (1992) themselves. The measured T2 times, the transverse spin relaxation times which indicate the freeness or boundness of water of the pith were similar to those of the surrounding pulp. The achenes did not image, which is in accordance with the findings of MAAS *et al.* (1992) and WILLIAMSON *et al*. (1992), who investigated red raspberries by NMR. Two causes were discussed by MAAS *et al*. (1992). First, the free water in the achenes is too tightly bound and the sensitiveness of the instruments is insufficient. Secondly, the hard coat of the achenes deflects the radio waves. Taking into consideration, that the pilot plant of BLÜMLER is able to be displayed with sharper contrast and higher resolution but the achenes were not imaging, the presented result support the second explanation of MAAS *et al*. (1992). However, further research is needed.

Overall, the NMR technique was proven to be a powerful tool to study the water respectively DM partitioning within strawberry fruit. The illustrated high contrast between the vascular bundles and the surrounding tissues underline this statement. On the other hand, this variability within the fruit could lead to imprecise measurements for several techniques which do not scan the whole fruit, for example NIR which has a limited depth of penetration. In conclusion, the whole fruit has to be scanned or, if this is not possible due to the technique, the variability has to be assessed for the development of the measurement method. The achenes could be another interference factor. It is conceivable that they deflect the beam of, for example, infrared light. The economical aspect of these methods has to be considered for an applied breeding program. The equipment as well as the development of the measurement method are expensive and time consuming. The industry estimates that the development and adjustment of a calibration model for a new crop or a new trait can be obtained by a Dr.-student within 3 years (PATZWALD M. pers. comm. 2004). Additionally, field capable NIR equipment from the market leader JENA ZEISS costs at least 20.000 € and NMR pilot field equipment around 100.000 € (PATZWALD M. pers. comm. 2004, BLÜMLER P. pers. comm. 2005).

E 5 DM Composition

Besides the partitioning of the DM within the fruit, the composition of the DM is of great interest, especially in the connection with the DM level or the change of the DM over the pickings, as shown in table 12. The values obtained for the cultivars and the selection 97/369 are comparable with those of the literature (SOUCI *et al*. 1994, PERKINS-VEAZIE and COLLINS 1995 and 1997, KALLIO *et al*. 2000, HERRMANN 2001). The amount and the ratio of sugars and acids are, due to their influence on the taste, of special interest for the fresh market (KADER 1991, SIMS *et al*. 1997). Because the taste is not a major breeding goal for freeze-dried strawberries, the relevance is not given at this stage of the presented breeding program. However, it could gain in importance in the future. KADER (1991) showed for fresh-market strawberries that a higher sugar amount and ratio resulted in a higher sensory sweetness, which was positively correlated with strawberry flavor intensity, which was negatively correlated with off-flavor. Sourness was positively correlated with off-flavor. An off-flavor is also a negative aspect for processed cultivars, but another

factor has to be additionally considered. Freeze-dried strawberries are not the end-product but ingredients of highly processed products. Thus, the sensory quality of the freeze-dried strawberries has to be always regarded in combination. For example, a very sweet freeze-dried strawberry coated with sweet chocolate would create a low flavorful contrast. This could lead to a sensory debasement. Therefore, it could be valuable to select for different flavor types of freeze-dry cultivars, depending on their dissimilar applications. However, the negative correlations between the DM and the citric acid proportion of the cultivars and seedlings (figure 24 and 26) show that a selection for high DM and high acid proportion and therewith sourness will be limited. Nevertheless, a high DM genotype does not necessarily have to be very sweet. In this context, the cultivar ‘Ciflorette’ can not be seen as an example, because it was specially selected for sweetness (ROUDEILLAC P. pers. comm. 2005). The success of the breeder is also confirmed by the results of table 12: the sugar amount per fresh weight and the sugar to acid ratio was increased. This also implicated a higher DM. For that reason, the positive correlation in figure 24 has also to be considered with caution. The plot of the seedlings revealed no significant correlation between the DM and the sugar proportion (figure 25). This leads to the conclusion that, *vice versa*, a high DM genotype does not necessarily imply a higher sugar content and therewith high sweetness.

HERRMANN (2001) reported slightly higher content of fructose than glucose. This was confirmed in every measurement of the cultivars and in the selection 97/369. Additionally, the ratio of the sugars fructose, glucose and sucrose was 2:2:1, as described in (SOUCI *et al*. 1994, MOING *et al*. 2001). Deviating results were obtained for the seedlings. Most striking were two seedlings (12/87 and 18/49) with significantly higher and one seedling (19/109) with significantly lower sucrose levels. High sucrose levels were also reported from PERKINS-VEAZIE and COLLINS (1997) for ‘Seascape’ and the *F. moschata* cultivar ‘Capron’. These cultivars had 53.3%, respectively 70.5% sucrose in relation to total sugars. The lowest sucrose proportion, reported in that publication, was 18.1% in the cultivar ‘Klondike’. The seedlings 12/87, 18/49 and 19/109 of the present work had 39.2%, 42.1% and 8.5% sucrose in relation to total sugars. Especially the very low sucrose proportion provides support for the assessment of PERKINS-VEAZIE and COLLINS (1997) that a high variability of the sugar ratios is present in the gene pool. The relevance of these results is not directly obvious but still given. A side effect of a breeding program for freeze-dry

suitability could be the intension of PERKINS-VEAZIE and COLLINS (1997). They tried to identify high sucrose genotypes in order to lower the crop losses from frugivorous birds. These birds have aversions to fruit with high sucrose content because they are lacking the intestinal enzyme sucrase (BRUGGER and NELMS 1991). The crop losses caused by birds are significant. In the US, the value of the crop losses caused by birds is estimated to be around $400/ha (PERKINS-VEAZIE and COLLINS 1997). Also, in Poland, the major processing strawberry producer in Europe, birds cause crop losses with an upward trend over the last years (MASNY A. pers. comm. 2006). However, it is not clear if and how these birds would respond to high sucrose strawberries. Another relevance could be due to the fact that fructose is, because to its stereomeric structure, the sweetest naturally occurring sugar. Fructose is estimated to be twice as sweet as sucrose. Consequently, the alteration of the fructose/glucose to sucrose ratio and their total amount could also be a possibility to influence the sensory appearance as already discussed above. In this regard, it has also to be considered that the freeze-dry process itself can alter the ratio of the sugars by sucrose hydrolysis and further glucose and fructose breakdown (FLINK 1983). This breakdown of the reducing sugars coincided with nonenzymatic browning, which is not desired. Also, influences on the technological freeze-dry suitability could follow from the ratio and concentration of the sugars (VASUNDHARA *et al*. 1992). The result of the seedlings 12/87 and 18/49, with contrary DM contents of “very high” and “low” but at similar sucrose proportion, show that a connection can not be expected. Therefore, the breeding of high DM cultivars with different sucrose amounts and ratios seems to be possible.

The breeding for a certain sugar or sugar to acid ratio could be important secondary breeding goals for the future. Nevertheless, in order to maintain the variability for those traits, it is yet already advisable to monitor changes of the DM composition caused by a breeding program for high DM.

It is obvious and trivial that berries with a higher/lower DM content have a lower/higher water accumulation. Nevertheless, the question is tricky, if the water accumulation is the reason or the consequence of a certain DM level. The investigations of the DM composition in different pickings revealed interesting conclusions. The genotypes reacted to precipitations similar to the genotypes in which DM contents were investigated during the harvest seasons (D 1.3). The second and third picking of ‘Korona’ and the second picking of 97/369 were carried

out during or after the two major rainfalls of the harvest season 2006. The DM contents of these pickings were, in accordance with D 1.3, lower than the previous pickings. On the other hand, the decrease of DM content at June 14th of the cultivars 'Roxana' and 'Ciflorette' is not allegeable with the climate data. It could still be possible that the field was irrigated, due to the preceding dry period and beginning harvest. However, if only a higher availability of water caused a higher accumulation of water into the fruit, the proportion of the DM components would still be the same. Table 12 shows, that this is not the case. The proportion of the main DM components is changing in all four investigated genotypes. Within a cultivar, the DM content changed in the same direction as the sugar proportion, while the proportions of citric acid and residuals were changing in the different direction. This is also illustrated in table 12. Therefore, it can be concluded that not only an altered accumulation of water into the fruit is the reason for a certain DM.

According to the literature, the component residues should be mainly composed of proteins, minerals, fat and fibres like cellulose, pectin and soluble and insoluble polysaccharides, as well as minor acids and carbohydrates (HERRMANN 2001, SCHERZ and SENSER 1994). A major amount of fat and proteins should go into the achenes. Since the achenes have a DM content of around 95% (AABY *et al.* 2005), the effect on the proportion of the component residues and the total DM is evident. This is also shown in the percentage of achenes in DM (table 13). The achenes contribute significantly to the total DM, for example 28.3% in *F. nilgerrensis* or 32.7% and 36.1% in the *F. vesca* cultivars 'Rügen' and 'Mignonette' respectively. In comparison, the DM of a typical *F.* ×*ananassa* cultivar like 'Senga Sengana' comprised out of 10.5% achenes. For that reason, the high DM in the diploid species *F. nilgerrensis*, *F. viridis* and *F. vesca* could be the result of a shift in the ratio between the achenes and the fruit pulp. Even though *F.* ×*ananassa* accessions normally have a higher thousand-seed weight and a higher number of achenes per fruit, *F. nilgerrensis*, *F. viridis* and *F. vesca* have much smaller fruit (table 13). This caused, in comparison to *F.* ×*ananassa*, a higher proportion of the seed weight to the total fresh weight as well as DM. The consequence is demonstrated in figure 51 which illustrates the DM and Brix values of table 13. *F.* ×*ananassa* accessions are marked black, the species *F. nilgerrensis*, *F. viridis* and *F. vesca* are marked red. The green values are the Brix values of the diploid species plotted against the Brix values summated with the seed proportion per fresh weight of 'Senga Sengana'. Therewith,

these calculated values simulate the DM level which would be reached with an average seed proportion of a *F.* ×*ananassa* accessions. The water content of the achenes is neglected. The location of these values in the cluster of the *F.* ×*ananassa* accessions show that the significantly higher DM content of the whole fruit is caused by the higher weight proportion of achenes in the mentioned diploid species.

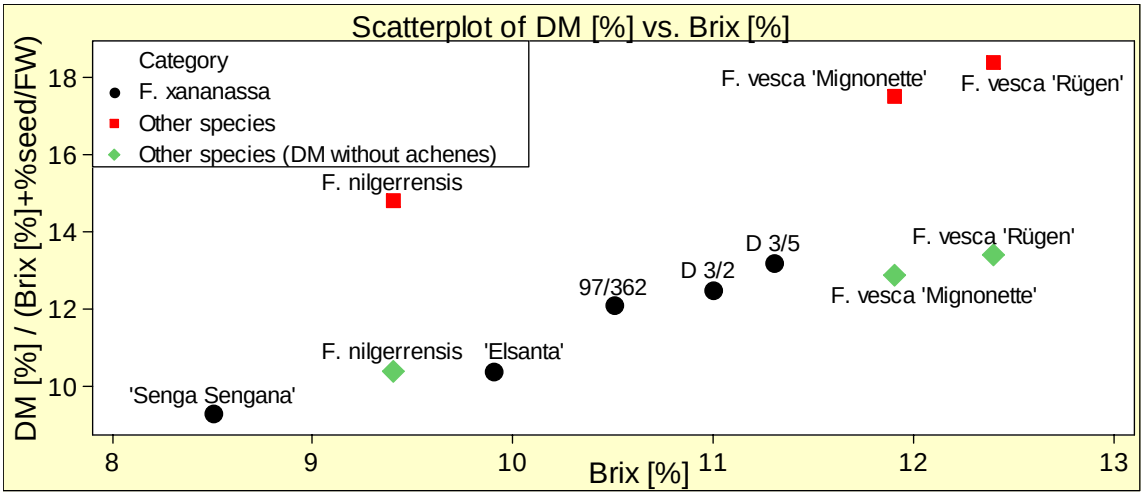


Figure 51: Scatterplot of DM [%], respectively (Brix [%]+%seed/FW) of 'Senga Sengana', *vs*. Brix [%]. Explanation is in the text.

On the other hand, the cultivar 'Ciflorette' in figure 25 exemplifies that a higher DM in *F.* ×*ananassa* genotypes does not have to be based on a higher achenes proportion. The higher amount and proportion of sugars in this cultivar was already proven above. Further, the single fruit analysis showed that a connection can also be excluded within *F.* ×*ananassa* genotypes. The smaller fruit of the cultivar 'Elsanta' had even a lower DM content (D 1.2). Another factor which limits the possibilities of an alteration of the achenes to fruit pulp proportion in *F.* ×*ananassa* will be that the fruit size is determined by the total number of achenes and the number of achenes per surface (WEBB *et al*. 1974). Anyway, due to their applications, the size of the processing strawberries is fixed to a certain range and to this a higher proportion of achenes could not be accepted by the consumer. Hence, the important statement for the breeding program is that it does not make sense to inbreed the high DM accessions of *F. nilgerrensis*, *F. viridis* or *F. vesca*. Further, the achenes proportion factor should be especially considered in evaluation of *Fragaria* species or cultivars with small fruit.

E 6 Genepool Screening

The screening of the gene pool over three years provided a good impression of the available DM variability. The highest values in all three years were reached by accessions of *F. nilgerrensis*, *F. viridis*, *F. virginiana* or *F. vesca*. These high DM values are similar to the by SALO and SUOMI (1972) as wild strawberry referred genotype with 15.5% DM. However, due to the high achenes proportion as the reason for the high DM (D 1.7.1), these accessions are not of interest, themselves or as a crossing partner, for the objectives of the present breeding program. Anyhow, due to their lower chromosome level than *F.* ×*ananassa*, the incrossing in the cultivated strawberry is difficult (ULRICH *et al.* 2007). Consequently, they are excluded in the further consideration and it is focused on *F.* ×*ananassa* genotypes and its initial species.

The DM values which were observed for *F.* ×*ananassa* accessions principally agree with those reported in the literature. For example, the European processing standard cultivar ‘Senga Sengana’ varied at the test field in Dresden, Germany from 9.5% DM in 2004, 10.3% DM in 2005 to 9.2% DM in 2006. PLOCHARSKY (1989) reported for this cultivar in Poland variability between 8.6% and 12.3% DM over the period from 1963 till 1986. The average DM during these 24 years was 10.0%. Similar values were found for ‘Senga Sengana’ independent on the location. In Norway SKREDE (1980) reported a DM value of 10.3%, SALO and SUOMI (1972) in Finland a value from 10.2% to 11.3%, IVANOV and STAMBOLIEV (1973) in Bulgaria an average DM value of 9.9% over a period of four years and SELVARAJ *et al*. (1976) in Bangalore also a DM value of 10.0%. Deviating DM values were published by SKUPIEN and OSZMIANSKI (2004). The ‘Senga Sengana’ fruit in this publication had a DM of 14.7% in one year and also the values of ‘Elsanta’ and ‘Kent’ were with 12.3% and 13.4% DM higher than those presented here (10.7%, 10.3% and 9.6% respectively -, 9.6% and 9.1% DM). A higher DM value in *F.* ×*ananassa* was also found by HEMPHILL *et al*. (1992) in the U.S., 258 selections values varied from 6.9% to 17.6% DM. However, these were selections of the only breeding program for high DM, mentioned in literature.

The variability of the trait DM content ranged over the three years from 7.5% to 14.9% DM. Remarkable are the comparatively low ranks of the European processing cultivar ‘Senga Sengana’ and the slightly better ranks of the U.S.-American

processing cultivars ‘Hood’ and ‘Totem’ in 2005 and 2006 with 10.8%, 10.8% DM and 11.2%, 11.4% DM, respectively. HEMPHILL *et al*. (1992) reported for ‘Totem’ a comparable average DM of 10.6%. Therefore, a negative effect on the DM by our location can be excluded for the cultivar ‘Totem’. The moderate DM values of the analyzed processing cultivars are explainable with the breeding history and especially by the date of release. ‘Senga Sengana’ was brought to the market in 1954. At this time other parameters were important, like the pulp color or the suitability for freezing, while the DM percentage was neglected (HONDELMANN W. pers. comm. 2005, BAUMUNK and HONDELMANN, 1969). The North American cultivars were launched in the 60th and 70th of the last century. Therefore, it is also likely that the breeding goals at that time did also not include a high DM. In this context it should also be taken into account that the breeding of a new strawberry cultivar takes around ten years.

The ranking of the main processing cultivars clarifies the dilemma of the freeze-dry strawberry processors: The available cultivars are old (in the case of ‘Senga Sengana’ more than 60 years) and thereby differ with the demands of today. ‘Tillamook’, the new release of FINN, is also not expected to be more appropriate for freeze-drying. It was released as a dual (fresh/processing) purpose strawberry with no special focus on drying suitability (FINN C.E. pers. comm. 2007).

However, the screening showed that several selections and cultivars of *F.* x *ananassa* had higher DM values than the present processing cultivars. Due to interest or availability, not all genotypes were evaluated in each year. The genotypes which were investigated for more than one year showed variation in the trait over the years (figure 27). Provided that soluble solids and its constancy are important fruit quality parameters and that soluble solids have a high correlation with DM, the genotypes should show a low variation of the trait DM. Nevertheless, high variations were observed for the cultivar ‘Weiße Ananas” and D- and 97er-numbers. This is not remarkable. The ‘Weiße Ananas’ is, because of its white fruit color, no more than a cultivar for strawberry enthusiasts. Thus, low importance was set by the breeder into the constancy of traits like soluble solids. The D- and 97-numbers are selections of a special backcross approach with *F. chiloensis*. This means that they were not evaluated over several years and therefore for the constancy of traits. The variation of the selections underlines the need for DM evaluation over several years in order to detect and to eliminate selections with inconstant traits. On the other hand, figure 27

shows genotypes which have constant DM levels in the same years of evaluation. For example, the DM of the French cultivar ‘Ciflorette’ was quite constant and significantly higher in all three years than those of the known processing cultivars. However, ‘Ciflorette’ was particularly selected for the fresh market and an intensive cultivation under plastic/glass; thus not at all convenient for processing purposes (ROUDEILLAC P. pers. comm. 2006). Additionally, and maybe as a result of the inappropriate cultivation method and deviating climate, the yield was comparatively low. This can be seen from the data of the single fruit analysis (D 1.2). Also, the other identified high DM genotypes did not meet the demand of further relevant processing traits, especially in regard to fruit color or the uniformity and size of fruit. Due to their small growth habitus or their low fruit set, the evaluated old cultivars ‘Mieze Schindler’, ‘Sieger’, ‘Markee’, ‘Korbinskaya Rannyaya’, ‘L’Oz du Rhin’, ‘Asiropa’ or ‘Dresden’ are expected to have an inappropriate yield. Even though, the genotypes with high or very high DM level imposingly demonstrate the potential of the gene pool for breeding programs for the trait high DM.

As stated above, the DM as the main breeding goal for freeze-dry strawberries can not be considered by itself. Consequently, possible correlations with other traits are also of high importance. They could restrict or loosen the breeding progress for the breeding goal high DM and further quality traits. It has to be remembered that the presented plots of figure 28 to 32 are based on values of cultivars and selections. Therefore, possible correlations can also be the result of certain breeding efforts and do not have to be based on physiological correlations. The figure 28 illustrates a plot between DM and the average fruit weight. The missing significant correlation indicates that a high DM content does not depend on a certain fruit weight or size. This is crucial, because the fruit size and weight of a freeze-dry cultivar have to be in certain ranges, smaller than those of the fresh market cultivars. This result also provides support for the hypothesized inhomogeneity correlation of figure 20 c. Such an inhomogeneity correlation was also demonstrated in the plot DM *vs*. firmness (figure 29) and also the similarity between the clusters of DM *vs*. firmness and DM *vs*. average fruit weight is based on a inhomogeneity correlation of firmness *vs*. average fruit weight (figure 30). This implies that also no significant correlation is present between the DM and the firmness. This result is concordant with those of SELVARAJ *et al*. (1976), who used a subjective rating system for firmness. A positive correlation of r= 0.619 between DM and firmness, measured with an instrument using a similar

method as those in the presented work, was reported by HALLER *et al*. (1933). However, the values of two years were combined and a separate consideration showed a significant correlation just in one year. To this, the number of investigated genotypes was with only nine respectively ten genotypes respectively low.

In the presented work, the firmness was not listed in particular as a breeding goal in the paragraph breeding parameters B 3. Nevertheless, a sufficient firmness is necessary for the breeding goal “good harvest performance”. Even if the berries are directly frozen at the field, the picking and the storage until freezing demand an adequate firmness. The European standard processing cultivar ‘Senga Sengana’ showed in comparison to other cultivars a low firmness of 211.3 g/mm. If this firmness can be defined as sufficient, at least it was sufficient until now in practice, it should be not too difficult to select for a higher DM and a higher firmness. This can also be seen in the several cultivars and selections exceeding ‘Senga Sengana’ in both traits (figure 29). In this regard it is interesting to note that the fresh market cultivar ‘Elsanta’, which dominates the North-European market, was also measured with a firmness of only 211.2 g/mm. This is remarkable, since the firmness has an influence on the shelf-life and this trait is crucial for a fresh-market cultivar. LEFEVER *et al*. (2004) were using the dynamometer DUROFEL of the company AGRO-TECHNOLOGIE, France, a similar instrument to measure the firmness, but they obtained deviating results. The cultivar ‘Senga Sengana’ showed a low firmness of 46.6% while ‘Elsanta’ differed significantly with 68.0% firmness. These contradictory findings and the disagreement of the low firmness of a successful fresh-market cultivar in the own results can be explained by two connected aspects. First, two main factors are assumed to contribute to the shelf-life of the berries, the firmness of the pulp and the toughness of the skin (JAMIESON *et al*. 2002). The cylindrical flat-ended probe of the in the present work used instrument had a surface of 4.9 cm^2 and deformed the whole fruit. Therewith, the instrument measured mainly the pulp firmness and to a lower extent just the toughness of the skin. Consequently, it is assumed that ‘Elsanta’ has soft pulp firmness but still could exhibit a sufficient shelf-life by high skin toughness. MOMMA and KAMIMURA (1978) even concluded that the skin toughness is the most important characteristic for shelf-life. A different characteristic in skin toughness and pulp firmness can be possible. OURECKY (1972) reported that a correlation was not always present between these two traits in seedling populations and MOMMA and KAMIMURA (1985) found that the correlation

was not high. These results vary from those obtained by MORI (2000) for seedlings or BUTTNER *et al*. (1987) for cultivars and selections. They found correlations of r= 0.93 to 0.98 and r= 0.67, respectively.

The second aspect explains the contradictory results of LEFEVER *et al*. (2004) to the presented result. The authors used a similar instrument but with a flat-ended cylindrical probe of only 0.25 m^2 surface. It is assumed that this smaller probe did not deform the whole fruit, but punctured just a part of the surface. In doing so, it measured more the toughness of the skin and less the firmness of the pulp. Therewith, the results of LEFEVER *et al*. (2004) would be consistent with the own findings. The problematic of different used methods and instruments as well as the often occurring low correlation between the results is reviewed by DOVING *et al*. (2005). The authors recommend for reliable result the use of two different methods in order to evaluate the pulp firmness and the skin toughness. Due to the discussed problems with the own findings, this recommendation is assented.

In the year 2006 a low positive correlation was found between DM and citric acid in the cultivars ‘Mieze Schindler’, ‘Roxana’ and ‘Senga Sengana’ at the location Dresden (figure 20 b.). Such a correlation was not present in the plot of several accessions in the year 2004. Moreover, the values of the cultivars differed between these two years. ‘Senga Sengana’ had less than 1000 mg/ml citric acid in 2006 and more in 2004. ‘Mieze Schindler’ varied vice versa. ‘Roxana’ had in both years less than 1000 mg/ml citric acid. These results support the in E 3 discussed assumption that the trait citric acid has a high environmental and/or genotype by environment interaction effect influence. Interesting is that the comparative newer cultivar ‘Roxana’ varied less between the locations (D 1.5) and between the years (figure 27). This could indicate a breeding success in regard to citric acid constancy.

The high correlation between DM and Brix is consistent with the result of E 3, where already the cause and the consequences were discussed.

E 7 F_1 Clone Populations

The Gaussian distribution of figure 33 characterizes the DM as a quantitative trait, which was assumed. This was also reported for the fruit DM in a kiwi fruit population by CHENG *et al*. (2004). Interesting is that the ranges of these distributions are similar to the range of the gene pool screening. In conclusion, the potential variability

of the *F.* ×*ananassa* gene pool can be reached by the segregation of a F_1 population of a single cross combination. Further, the mean of the population is located between the mean of the parents, which excludes a heterosis effect for this specific cross combination. Similar results were reported for the soluble solid content of strawberries by MOMMA and TAKADA (1991), DUEWER and ZYCH (1966). However, the variability in the populations does not only have to be caused by genetic factors. It can be assumed that the environment has a significant influence on the DM content. An indication is given by the differences of the population means. The mean of the year 2006 was 1.2% DM lower than the year 2005 in an absolute scale. However, the single selections did not respond similar in regard to direction and extent to this year effect. This can be seen in figure 34, which shows a plot between the corresponding DM values of 78 genotypes of 2005 and 2006. This indicates a specific genotype by environment interaction. The dotted line indicates the same values in both years relativized by the year effect of the total population and the deviating variances in the populations of the two years. According to the agronomic or also entitled dynamic concept of stability, genotypes which are located on this line can be called stable (BECKER 1981, THOMAS 2006). Their DM content complies with the respective potential of the two environments (years). Genotypes, which drift from this line, have a higher DM by environment interaction. This concept has to be differentiated from the until now applied term constancy, which is consistent with the biological or static concept. The low correlation effect of r= 0.517 between these two years indicates that the stability of the total population was only small to average. Nevertheless, for single genotypes no conclusion can be derived even if the selection for a stable or constant DM content seems to be very difficult.

This is also demonstrated by the green and red marked genotypes with the selection limits of $\leq$ 9.7% and $\geq$ 12.0% DM in the year 2005 (figure 34 a.). In order to evaluate the success of these selection examples it is necessary to state more precisely the selection goal. Two reasonable goals are conceivable.

First, the processing industry demands a DM content above a defined value. In the case of the example with a selection limit of 12.0% DM, the extent of the selection success would be devastating: only, one single genotype with a DM of 13.3% in 2005 and 14.5% DM in 2006 are up to the limits. No other genotype reached the selection limit in 2006. This is obviously a consequence of the lower population mean in 2006 caused by the year effect. To this, figure 34 a. demonstrates by the means and the

distribution that the genotypes selected for high DM in the year 2005 decreased more than the year effect of 1.2% DM of the total population. The mean of these genotypes must have been, of course, bigger than 12.0% in 2005 but the mean in 2006 was only 10.4%. Nevertheless, even with only one genotype which would have been selected, it has to be remembered that a single genotype can be enough if it fits all the other desired traits. *Vice versa*, it would have been much easier to select for a DM $\leq$ 9.7%; 15 genotypes were fitting the limits.

Second possible selection goal; the processing industry demands a stable DM which is above a defined value. Again, stable means in this case that the year effect is taken into account. For the selection limit 12.0% DM in the year 2005, the corresponding limit in 2006 would be 11.1% DM. These selection limits are illustrated by the green rectangles in figure 34 a. Five genotypes would have been selected in this case for high DM. However, in order to calculate the selection limit adjusted by the year effect it is necessary to measure the whole population. This will not be practical in applied breeding programs, in which genotypes are permanently eliminated that do not fit defined traits. Therefore, it could be more effective to define the selection limit on the basis of a comparison to a standard cultivar, for example 'Senga Sengana'. As a reference value the mean of several pickings or the single value of the same picking day could act. Problematic is that 'Senga Sengana' is the only standard processing cultivar in Europe and its first picking date is often not syncing with those of the genotypes to evaluate. Consequently, the DM or Brix value of a genotype has often to be measured without a reference value. An alternative would be the definition of several cultivars which cover the possible harvest season and to use those as reference cultivars. Due to logistical reasons, the recording of the data of the to evaluate genotypes and the subsequent comparison with the reference value could be only practicable for selections or cultivars. For numerous seedlings the effort will be too big. Anyhow, for seedling populations it has to be regarded that the performance of genotypes in the seedling stage deviates from those of the clonal propagated plant (HANKE 1989).

As mentioned in the introduction part, the planting in August and a two year crop growing is common for extensive strawberry cultivation, especially of processing cultivars. Figure 34 b. shows the plot between the one year old and a two year old planting of the above discussed population. An effect of the planting is not given for the total population, which is also already shown by the minor differences of the

population means. However, some genotypes differed significantly between the two plantings. Because the second picking date of both corresponding clones could not be carried out at the same date, the difference between the plantings can be due to environmental and planting effects. Nevertheless, the correlation coefficient of r= 0.564 shows again that it will be very difficult to find a genotype with a stable DM value.

All conclusions are based on only two year studies and are for that reason somewhat vague. For more precise conclusions, investigations have to be carried out over a longer period of time and best on the basis of different populations. According to the presented results, the selection for high DM is not promising. Positive prospects are given due to the results of the literature. In kiwifruit the heritability was high for soluble solids and DM (CHENG *et al*. 2004) and moderate for soluble solid content of strawberries (SHAW 1990).

E 8 Bi-Parental Cross

No information about the influence of the cross combination itself can be gained from the populations of the cross combination ‘Mieze Schindler’ x ‘Elsanta’. In order to gain such information, the bi-parental crosses (C 2.1.2.5) were carried out.

The location of the population mean of the populations with ‘Ciflorette’ as one parent between the means of the parents is in accordance to the already mentioned clonal populations. Deviating are the means of the populations of the other combinations with 97/369 as one parent. The means of these populations were higher than the mean of any of their parents. This would point to a heterosis effect, which has to be excluded due to the potential of 97/369 in regard to DM content. In 2004, the year of the assortment of the crossing partners, the average DM mean of 97/369 was 12.4% DM. Therefore, the results of the figure 36 and table 14 have to be handled with caution in regard to the mean of the parent 97/369. Further, the selection of 97/369 shows that no conclusion for the progenies can be drawn from the DM level of the parents. Nevertheless, the phenotypic variance and the occurrence of seedling which exceeded the parent with the highest DM level, in the case of 97/369 also the DM level of 2004, promises a wide scope for selection (table 14).

The means of the eight populations varied among 11.1% to 13.2% DM content. This is an important result, since it indicates that the cross combination was an influencing

value. Otherwise, the means of the population would have been more or less the same, due to similar acting environmental effects. No conclusion can be derived about the influence of these environmental effects, since only one year could be investigated. Surprising was that the DM levels of respectively two populations were grouped together (figure 37). An influence of the field, due to the planting system of the populations, can be excluded. The change of the DM mean from population to population was not blurred. To this, the figure in annex G 21 illustrates, by means of the allocation of the DM content of the seedlings of population 15, that no systematic increase or decrease of DM was detectable between the planting positions. The indication for a missing field effect is given also by the seedlings with significantly lower DM content in immediate vicinity of seedlings with high DM content.

The most striking commonness of the grouping populations are the maternal parents. This is also imposingly illustrated by the confidential intervals of figure 37. A paternal effect can be excluded. Since the reciprocal cross combinations as well differed significantly in their DM levels, it has to be assumed that influence can be ascribed to a maternal effect. Consequently, the above expressed conclusion that the cross combination had an influence on the DM level of the progenies has to be limited to the choice of the maternal parent and not the combination of two partners.

It is amazing that the populations with ‘Korona’ as maternal parent reached the highest DM means, while the populations with 97/369 or ‘Ciflorette’ as maternal parents with the highest potential for high DM content resulted just in populations of low to average DM levels. This shows again, that no conclusion can be drawn from the DM level of the parents. The ability of a genotype for a cross combination for high DM levels is best done by an evaluation as mother. This is also demonstrated by the overview in table 15.

Contradictory results were reported by MOMMA and TAKADA (1991) for Japanese cultivars. They found higher soluble solid content in populations from crosses between parents both having high soluble solids. No maternal effect was detected but can not be ruled out, due to the lack of reciprocal crosses and only two different maternal parents used in two different years. OHTSUKA *et al*. (2004) found additative genetic effects for sugar content, the main component of the DM/soluble solids, and similarly concluded that the optimal breeding strategy for high sugar cultivars is the combination of parents with high total sugar contents. Once more, their described design of the crosses did not allow the discovery of a maternal effect for this trait. To

this, the produced populations comprised only 18 to 26 seedlings. These findings differ from DUEWER and ZYCH (1966) where the soluble solid contents of populations were not necessary higher in cross combinations of parents with high content. In these populations also no possible maternal effects were detected due to the lack of reciprocal crosses. The design of the cross combinations was also the basis why possible maternal effects had no chance to be found for other traits like color, fruit size, ascorbic acid content or acids (BAKER 1952, LUNDERGAN and MOORE 1975, MACLACHLAN 1974, OVERCASH *et al*. 1943, SHERMAN *et al*. 1956, DUEWER and ZYCH 1966). There are only a few publications which mention a maternal effect. HARLAND and KING (1957) found evidences in strawberries for maternal effects on powdery mildew manifestation in progenies of several cross combinations. The susceptibility differed in reciprocal crosses and the effects persisted in F_2 populations and in back cross generations. A maternal effect for the transmission of susceptibility or resistance to mildew was also reported by MACLACHLAN (1978). BARITT (1982) reported of non-reciprocal maternal effects for the inheritance of early flowering. Non-reciprocal maternal effects were also reported for other horticultural crops. LAYRISEE *et al*. (1980) and DWIVEDI *et al*. (1989) found such effects in peanuts (*Arachis hypogaea* L.) for yield parameters like fruit number or fruit weight per plant, fruit length and fruit weight. A maternal effect for fruit weight was also reported by SUBHADRABANDHU and NONTASWATSRI (1997) for papaya (*Carica papaya* L.).

The source for the maternal effect is another important question. It is well known and approved in several studies that the cytoplasm of plants contains genetic information that is transmitted maternally (MICHAELIS 1958). The maternal effect in the presented work could be therefore a cytoplasmatic effect. The mode of action on the DM content could be due to the photosynthetic and metabolism performance, which is determined by the cytoplasmatic inheritated cell organelles chloroplasts and mitochondria. Nevertheless, the assimilate capacity is not shown in the DM content but the DM content multiplied with the yield, which shows the actual synthesized and incorporated dry weight in gram. Figure 38 illustrates that the means of the yield of the populations do not show a maternal effect. Because the yield is, in the above mentioned multiplication, a much higher factor on the assimilated dry weight as DM, the maternal effect is consequently present only for the DM content and thus for the assimilate incorporation. The incorporation of assimilates could be controlled by plant

hormones like abscisic acid or cytokines. Abscisic acid was shown to stimulate the accumulation of sugars in fruit pulp of strawberries (JOHN and YAMAKI 1994, ARCHBOLD 1988). Similar results were reported for sugar beet (*Beta vulgaris* var. *attissima* Doell) (SAFTNER and WYSE 1984). In tomato, MARTINEAU *et al*. (1995) were able to raise the soluble solid content by altering the cytokine level. Nevertheless, plant hormones were not measured in the present work and no conclusion can be drawn.

Besides a cytoplasmatic effect, other possibilities are assumable (MICHAELIS 1958). The maternal plant could continue to have an effect on its embryos and their development, for example by the incorporation of nutrients or active components into the embryo or the egg cell. Nevertheless, these aroused differences would have been adnated very fast. An unnoticed selfing instead of a cross combination can also be excluded due to the hybrid habitus character in the progenies of all populations.

Whether a real cytoplasmatic effect or another unknown effect caused these grouping of DM levels in the progenies, the importance is very high and demands further research. It is necessary to exclude all possible effects which could appear as a maternal effect. This could be done by a repetition of this experiment and further testing to determine if the maternal effect persists in F_2 populations. If a cytoplasmatic effect really exists for the DM content of strawberry fruit, the consequences on strawberry breeding programs would be dramatic. Not only the choice of the parents has to be reconsidered but also whole breeding programs have to be realigned. DALE and SJULIN (1990) followed the pedigrees of 134 North American strawberry cultivars back to only 17 original maternal parents and consequently cytoplasms. This would imply a narrow cytoplasmatic germplasm base. If the influence of the cytoplasm is really as significant as seen in the presented results, the extension of the cytoplasmatic gene pool would be an essential part of future breeding programs.

In applied breeding programs no breeding goal can be regarded independently from other important traits. One of the most basic and consequently crucial factors is the yield. As discussed above, the yield of the first two pickings of the populations did not show a maternal effect. Nevertheless, a negative correlation with the DM content is still present, which can be seen by the plot of figure 39. A negative correlation of r= -0.33 to -0.62 between the soluble solid content and the yield was previously

reported for F_1 strawberry progenies (MOMMA and TAKADA 1991). In kiwifruit, a similar negative correlation was found by CHENG *et al*. 2004 and in tomatoes this negative correlation is considered to be responsible for the restricted progress in tomato breeding for high soluble solid processing cultivars (STEVENS 1986). The question if the present negative correlation between DM content and yield could also hinder the selection work can be best discussed by means of the figure 40 with the single values of all populations. The delta-allocation in all populations is remarkable and has consequences for the selection. Genotypes with high levels of both traits are desired but not present, most likely due to a physiological limitation of assimilation and assimilate incorporation capacity. Genotypes with low levels of both traits are present but not desired. Consequently, the interesting seedlings are those which exhaust the physiological potential most and which are found at the upper edge of the cluster. Due to the almost linear running diagonal cluster edge, these desired seedlings always represent a compromise between DM content and yield: it is possible to select seedlings with a high DM and low yield, low DM and high yield or average DM and average yield. The selection of the seedlings will arise from the already discussed selection goals and to define selection limits. Unfortunately, no comparison with a processing cultivar is possible, because of the already mentioned deviating performance of seedlings and cultivars.

A negative correlation between DM and yield occurred also during sugar beet breeding for high sugar content (OLTMANN *et al.* 1984). The answer to this problem was the implementation of indices for different directions of selection. Z-types were bred for high sugar levels, E-types for high total yield and N-types for genotypes which combine both characteristics on an intermediate level. This solution could be also necessary in high DM strawberry breeding. Furthermore, for onions a negative correlation between the bulb weight and the DM content was reported by McCOLLUM (1968). NIEUWHOF (1969) estimated that with an increase in the DM content of 1% in an absolute scale, the productivity decreases by 10% in an absolute scale in onions. If this estimation is transferred to strawberry breeding, a decrease of 10% yield and a supposed price increase of the IQF strawberries by 10% could be acceptable. The reason for this is that the 1% more DM bestows 10% less production costs and these are much higher than the cost for the IQF berries. As well, it has to be considered that for the beginning a new freeze-dry cultivar has only to surpass

‘Senga Sengana’ which is low in yield and low in DM content. Nevertheless, attention has to be paid to the yield level in order to prevent a decrease.

The yield of the populations is also linked to the vigor of the plants, which is reflected in the rates of mortality and plants without fruit. Interesting is that the populations 12 and 13 which had the lowest yield and highest DM content had also the highest rate of these undesired characteristics. In general, these populations and so their cross combinations have to be considered as not favorable. Maybe their low vigor caused a low yield which resulted in a higher DM incorporation in the fruit? Population 15 was the best population in regard to analyzable plants. This high vigor most likely caused the highest yield of all investigated populations. However, the DM mean of this population was not the lowest but average. This is a promising result for a breeding program. The absence of populations with high vigor, yield and DM level demonstrate that compromises have not only be done at the stage of selection of a seedling within a population but also at the stage of the choice of the cross combination.

Another trait which demonstrate the excellence of a cross combination is the rate of genetical defects like dwarfism or chlorophyll defects in the populations. Dwarfism occurred in all population and no special mode of inheritance could be noticed. Further, no correlation to the rate of analyzable plants was present. Therefore, it can not be concluded from the rate of dwarfism on the vigor of the population.

Chlorophyll defects which are also called June Yellows occurred only in the populations 14, 15, 17 and 19. This defect is not uncommon in breeding population (HUGHES 1989). Remarkable is that the defect was present in four of eight populations and all of those four populations had ‘Roxana’ as maternal or paternal parent.

McWHIRTER (1955), SCOTT and LAWRENCE (1975) and ROSE (1992) assumed that June Yellows is controlled by cytoplasmatic genes and WILLIAMS (1955) and WILLS (1962) reported concordant that the inheritance do not fit a Mendelian pattern. However, a non-reciprocal cytoplasmatic inheritance is not in agreement with the own result, to which a nucleoplasm inheritance is more assumable. Nevertheless, it has to be regarded that the defects do no have to occur in every year and sometimes even arise long after the release of a cultivar (REID 1954, JAMIESON and SANFORD 1996). Therefore, the own result can not be seen as finally completed. The rate of the mildew susceptibility was following a similar pattern as the chlorophyll defect rates. The lowest susceptibility rates were found in the populations 14, 15, 17

and 19. Again, the literature describes evidences for a cytoplasmatic inheritance of mildew susceptibility (HARLAND and KING 1957, MACLACHLAN 1978), which are again in contrast to the own findings. A nucleoplasm inheritance of ‘Roxana’ for moderate resistance or a nucleoplasm inheritance of ‘Korona’ for susceptibility is more supposable.

No data is presented for the internal and external color of the strawberries. Nevertheless, the figures 42 and 43 illustrate impressively the manifestation of these traits in the populations. No direct relationship between the external and the internal color of the berries was present, but the internal color of the berries was never darker than the external color. An appropriate variation for skin and pulp color was given in all populations. Similar results were reported by LUNDERGAN and MOORE (1975) who observed also a high heritability for the fruit color. Contrary results were reported from MACLACHLAN (1974) who found a low heritability and recommended that no special breeding procedure has to be adopted except the choice of well colored parents. The crosses should involve at least one well colored parent. This statement can be agreed only with limits. The internal color of the populations with 97/369 as maternal or paternal parent seemed to be brighter than the other populations. The internal and external color of the progenies was also described by MURAWSKI (1968) to be similar to the parents. It is assumed that this brighter color was transmitted by the parent 97/369, which derived from a cross with a bright pulp colored *F. chiloensis* accession as maternal parent. BLAKE (1954) reported similar that the cross combination of *F.* ×*ananassa* with an accession of *F. virginiana* showed dominant inheritance of the bright pulp color of the wild species. Therefore, it is recommended that both parents should be well colored, if this trait is demanded in the progenies. Further, according to the figure 42, 97/369 is also assumed to transmit the negative tendency of cavities. As it can be seen in figure 43, the cavity is strongly pronounced in the parent.

Similar as for the taste, it has to be considered that also the color of the strawberries can be changed due to the freeze-dry process. HAMMAMI and RENE (1997) observed that the color of freeze-dried strawberries depend deeply on the process temperature. SHISHEGARHA *et al*. (2002) measured a decrease in hue angle, which is according to ABERS and WROLSTAD (1979) the most significant correlation with visual scores, by 22.5% for the skin and by 42.4% for the pulp. As a consequence, the freeze-drying process pronounced the red color of strawberries.

Figure 52: Appearance of a strawberry of ‘Senga Sengana’ before (left) and after (right) freeze-drying.

Figure 52 illustrates the effect of the process on the appearance of a strawberry cultivar ‘Senga Sengana’. The literature and figure 52 show that color evaluation after the freeze-drying process is appropriate for optimal performance. The color of several cultivars after freeze-drying was already evaluated by THUESEN (1985), but in this evaluation no cultivar was better than ‘Senga Sengana’.

The actual impact of the discussed characteristics on an applied selection process is shown in the selection rates of table 16. Of course, these selection rates reflect also a subjective rating of the breeder. Nevertheless, interesting parallels arise from the discussed data and the selection rates. No plants were selected from the populations 13, 14 and 19. Since plant breeding always comprises the comparison to standards, fictive parameters or present plants, this result certainly interacts with the other present populations. In this case, the plants of the named population could not stand the comparison to the plants of the other cross combinations. The populations 12 and 13 were definitely the worst populations in regard to plant vigor and yield. The selection rates of these populations are therefore very low. The population 14 had the lowest rate of mortality and susceptibility to mildew. This should indicate a very good combination and indeed the plants were very vigorous, but not a single plant was selected from 160 seedlings after the second selection stage. It is assumed that this cross combination resulted in plants with a too high vigorousity and therefore maybe too less fruit set. The average rate of plants without fruit and the third lowest mean yield can be seen as an indication. Additionally, the population 14 had, due to the parent 97/369, a high rate of the negative characteristics bright pulp color and cavities (see also figure 43). This was also the reason for the elimination of the three

selected seedlings of the pre-selection; one seedling had cavities and two had bright internal color. It is interesting that in comparison the other cross combination with ‘Roxana’ as mother resulted in the very potential population 15. This population had the highest rate of analyzable plants and the highest end-selection rate. Together with the already mentioned highest yield at an average DM level, this population was the most promising one. The reciprocal cross of population 15 was population 19. However, not a single seedling was pre- or end-selected from this population. Further, the cross combination of population 18, ‘Ciflorette’ x ‘Korona’ with the same maternal parent as 19, resulted in selected seedlings. Also the populations 16 and 17 with 97/369 as maternal parent resulted in selections. This is remarkable, since the population 14 with 97/369 as paternal parent was characterized as too vigorous with a too high rate of bright colored fruit and cavities transmitted by 97/369 as paternal parent.

These results show that the choice of the maternal and paternal parent as well as the direction of the cross had a crucial impact on the performance of the progenies and therefore on the success of selection.

E 9 Practical Realization / Selections

The populations mentioned above were primarily established for research purposes. Nevertheless, during the presented work, selection work was carried out on additional populations in order to develop promising selections for a future cultivar and to evaluate the selection progress on the basis of an applied program.

According to the paragraph “Breeding parameters”, the selection was generally focused on the DM content. Consequently, the development of an adequate DM selection method was needed. Due to the high correlation between DM content and Brix value and the comparatively laborious DM determination, the Brix value was used as a correlated response for the desired DM content. For each breeding program, the selection method is the central key of success. The sample throughput preordains highly the success of a selection method. The classical example of a high throughput selection method is the selection of sweet lupines (*Lupinus luteus* L.) (SENGBUSCH 1942). Based on the cognition of DARWIN and according the homologic law of VAVILOV, BAUR and SENGBUSCH concluded that alkaloid-free genotypes must also exist in the lupine gene pool. BAUR and SENGBUSCH

screened 1.5 Million single lupines and selected 5 alkaloid-free plants (BECKER 1993). The determination of the Brix value did not offer the possibility to screen such a high number of genotypes and it also might not be the most elegant or sophisticated method. However, it offered the possibility to measure the parameter sufficiently on the field, at an appropriate sample throughput and by low costs. The increase of one Brix unit corresponded roughly with an increase of 1% DM in an absolute scale. HEMPHILL *et al*. (1992) also observed a high correlation between the total DM contents and the total Brix values of strawberry selections. However, a lack of consistenency was found in all harvests of these selections. The authors stated that the Brix measurement can not be relied upon to identify high-DM selections and DM should be measured directly. These conclusions are in direct contrast to the own findings. The mean values and the single harvests values of the own selections resulted in high correlation coefficients (figure 46 and 53). In the case of the single harvest values the correlation was still very high (r= 0.924, p-value < 0.001), which is also illustrated in figure 53.

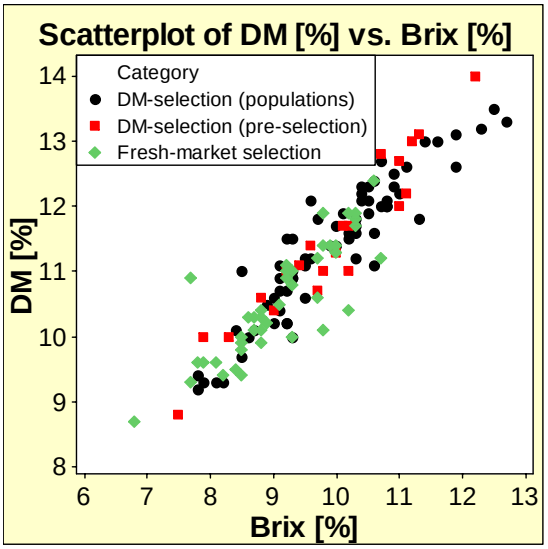


Figure 53: Scatterplot of DM *vs*. Brix of all measurements of the B-selections analyzed 2006. Grouped according to the selection approach.

The reason for these deviating results and conclusions is not clear. The range of DM content, in the results of HEMPHILL et al. (1992), varied comparatively from 8.4% to 17.6% DM within three years of investigation. Moreover, the sample size was with 126 evaluations sufficient for an informative conclusion. Most likely, the practical realization in regard to sampling and measurement differed and caused the deviating results.

The Brix value was used previously as a correlated response in sugar beet and onion breeding programs to higher the main components (MANN and HOYLE 1945, SENGBUSCH 1939). Today, the Brix measurement is replaced in these crops by

newer techniques like NIR or NMR. The main reason for this is the noninvasiveness, which is very important for the measurement on a vegetative part which is still needed for propagation, the high sample throughput by an easier operation and the more precise measurement of certain components. Nevertheless, the use of such a technique was out of question for the implementation of the own objectives. The reasons were already discussed in E 3. Also, the determination of the density, which was successfully applied in potato and kiwifruit breeding (SENGBUSCH 1939, JORDAN *et al*. 2000) for the measurement of DM content, is not appropriate due to the cavities in strawberries.

The selection was carried out by two different approaches in 2004 as described in D 3.1.1. Unfortunately, in 2005 only four A-selections were present and consequently not informative. The reasons for this were, similar to the above mentioned and in figure 42 and 43 shown populations with 97/369, the high rate of bright internal and external colored progenies. It is most likely that this characteristic was transmitted by 97/362, a selection with the same *F. chiloensis* accession as maternal parent as 97/369. Figure 44 illustrates that significant differences were present in the DM median between the A-selections of the selections for fresh-market and for DM. This result demonstrates a successful selection. The B-selections in 2005 again showed significant differences between the DM levels of the selections for fresh market and DM selection (figure 45).

The lack of a high significant correlation between the average fruit weight and the DM of the selections in figure 46 b. is in accordance to the findings of the gene pool screening and therefore not surprising. Nevertheless, figure 47 demonstrates that significant differences were present between the selections approach “population” and the other two approaches. The reason for this is not a hidden direct correlation between the two traits but the carried out selection and pre-selection for a certain fruit weight by the fresh-market strawberry breeder OLBRICHT. Consequently, the means of figure 46 b. reflect the selection success of the fresh-market and DM program for these two traits. The fresh-market selections had the highest mean average fruit weight after the second selection stage and the lowest mean DM content. The pre-selection of the year 2004 resulted in selections of a middling mean average fruit weight and high DM content and the selection for just DM content had the lowest mean average fruit weight but also a high mean DM content. The pre-selection had no direct influence on the DM level of the selections. Nevertheless, the removal of

pre-selected genotypes for the fresh-market can be disadvantageous. The breeding goals of the different approaches deviate mainly in the parameters of fruit weight and color. As shown for the fruit weight, it can be assumed that the pre-selection for the fresh-market results in seedlings with too high and too bright internal and external color. An indication of this is that a lot of the B-clones of the pre-selection were eliminated because of their skin and pulp color. As a conclusion, a strict separation of the selection processes is mandatory.

Nevertheless, a pre-selection of the seedling could be beneficial. This pre-selection should be based on relevant processing selection limits like dark colored internal and external fruit, easy detachability of the calyx, no cavity and processing appropriate fruit size and uniformity. The DM or Brix determination could also be carried out on the A-clone or B-clone stages. This would also have the advantage that the number of measurements is reduced and the complexity of problems, in regard to the assumably low correlation between seedling and clonal stage as well as the low precision of DM determination on single plants (E 2), is skipped. Clonal plants would also provide the opportunity to evaluate on several locations, which could be helpful to detect and evaluate the next discussed feature.

In the box-plots of figure 45, the lower whiskers of all three approaches are similarly low. This was not the case for the A-selections of the year 2005 where the lower whiskers of the approaches for high DM reached only up to the interquartile range of the fresh-market approach. The reason for that is shown in the plot between the DM values of the years (figure 48). The mean of the selections was 1.0% in 2006 in an absolute scale lower than 2005. This is a comparable year effect as already shown for the clonal population of the cross combination ‘Mieze Schindler’ x ‘Elsanta’. Nevertheless, not all selections reacted to the year effect similar. From the gradient of the equation of the main axis of correlation and from the plot it can be seen that the genotypes with a high DM value in 2005 reacted stronger with a decrease in DM in 2006 than the genotypes with a low DM. These genotypes were highly unstable for the trait DM. In this regard, in 1917 the famous German breeding researcher RÖMER was already asking in the title of an article about several crops: “Sind die ertragsreichen Sorten ertragssicherer? (Are the high yield cultivars more stable in their trait?)”. Transferred to the present work, the question has to be: are the high DM cultivars more stable in this trait and the answer has to be: no, it looks like they are more unstable.

Also, the two different selection approaches for high DM reacted divergently. Table 48 a shows that the selections of the approach “population” greatly decreased in their DM. One explanation could be based on the different cross combination backgrounds of the selections. Figure 48 b marks the main cross combinations of the selections and revealed different responses to the different environments. The selections of the cross combination ‘Fraroma’ x ‘Honeoye’ comprised higher and lower DM values than the year effect. In contrast, the selections of ‘Honeoye’ x ‘Fraroma’ showed higher DM content and the selections of ‘Fraroma’ x ‘Senga Sengana’ showed lower DM content than the year effect in 2006. This deviating pattern according to the cross combination could be the hint for an inheritance of a certain genotype by environment interaction mode. Consequently, the different reactions of the high DM selection approaches could be the result of certain cross combinations in the respective approach. However, the results are quite vague and more research is needed to verify this consideration. A possibility to reduce the characteristic of the genotype by environment interaction effects by experimentally ascertained cross combinations would be a powerful tool. Certainly, it will be necessary to evaluate in several environments, in order to identify and to select genotypes with a stable or constant DM content.

E 10 Breeding Strategies

Because the mean of the DM content of the F_1 populations of chapter D 2.2.2 was shown to be partly genetically influenced by the choice of the cross combination, a classical *modus operandi* of combining ability tests and subsequent realization of the most favorable crosses in a higher seedling number would be promising. A careful selection and combination of parents would assure populations with the maximum frequency of progenies high in DM and other demanded traits. The results of the bi-parental diallel (D 2.2.2) demonstrated that it is unachievable to combine several extreme traits in one genotype. A compromise will be most likely necessary and has to be defined by lower selection limits for all important traits. Due to the only few in number and old processing cultivars, it is presumable that several cross combinations and selection steps will be necessary. Some of the obtained selections for high DM might be the basis for the establishment of a breeding program with lower variation in certain demanded traits.

Deviating breeding strategies from this standard pedigree method are already reviewed in B 2. The reasons for the introgression of wild species or polyploidizations mainly were the transmission of particular characteristics into *F.* ×*ananassa*. A backcross with wild species is not interesting for the main breeding goal high DM, since the gene pool of *F.* ×*ananassa* has already a sufficient variability of DM. The variability can also be reached by a cross. Further, the high DM of the investigated wild species is based on the achenes to pulp ratio. Nevertheless, such an approach could gain in importance by the already discussed tricky connection between DM and total yield (E 8). Breeding for high DM and high yield will lead to an approximation to the physiological barrier shown in figure 40. An expansion of this restriction can only be realized by an alteration of the assimilate translocation into the fruit and/or by a higher rate of net photosynthesis. RETAMALES J. B. (pers. comm. 2006) showed that hybridizations between *F.* ×*ananassa* and an accession of *F. chiloensis* with higher net photosynthesis resulted in hybrids with a rate in between. This could be a potentiality for a shift of the physiological limit in *F.* ×*ananassa*. Of course, such a program could only be long-termed orientated and the chances of success are difficult to estimate. (HANCOCK 1990).

Another interesting approach is the breeding of decaploid cultivars suitable for mechanical harvest, as reported in B 2. The incrossing of *F. vesca* leaded to populations which made it easier for the breeder to select a genotype with up ride infructescences and easy detachable calyx. The idea of mechanical harvest was not realized in practice due to the missing simultaneous ripening of the cultivars. This problem could not exist no longer by a discriminate picking harvester: a harvest robot. Several approaches are on its way (ARIMA *et al*. 2004, KONDO *et al*. 2005). Such a robot would also reshuffle the whole strawberry production, due to the reduction of the labor cost. However, it is questionable if a high fruit quality level can be assured. The in the present work analyzed decaploid genotype ‘Spadeka’ had only an average DM content of 9.9%, but DM was not a selection parameter of ‘Spadeka’. Unfortunately, to less decaploid genotypes are available to estimate the variation of this trait on that chromosomal level. In this regard it is a legitimate question if the octoploid level is necessarily the best ploidy level for cultivated strawberries. A lower or higher ploidy level, especially the hexaploid or decaploid level could be advantageously. The limited success of the decaploid cultivars ‘Spadeka’ or ‘Florika’ are no proof for supremacy of the octoploid level. It has to be

considered that with the cultivar ‘Spadeka’ a stage in decaploid breeding was reached which is comparable with the year 1750 for the octoploid stage (SCHIMMELPFENG H. pers. comm. 2007). The situation is similar with the development of the WANKEL rotary engine in 1954 (YAMAGUCHI 2003). The concept of a rotating rotor which orbitally revolves chambers makes more sense than the OTTO stroke engine. However, the stroke concept was invented in 1876 and consequently has had 78 years of technical improvement and investments. Therefore, this technology is preferred. Nevertheless, other than the ban of rotary-engine cars from racing, there should be no ban of strawberry ploidy breeding affords. No fast result can be expected but especially the easier calyx detachability and the better suitability for mechanical harvest are tempting features.

However, altered surrounding conditions of applied breeding programs are the main reason why alternative breeding strategies should be considered. The standard approach of cross combination tests and subsequent pedigree crosses is possible. However, it depends on two factors: the quantity of the cross combinations and seedlings as well as the quality of the selection. For example, ‘Senga Sengana’ was selected out of a population of 40,000 seedlings and 10,000 selections (25% of the seedlings!) were analyzed for freezing and thawing ability in the A-selection stage (DARROW 1966). Today, these high numbers of seedlings and selections can not be reached anymore by a governmental breeding program. For comparison, the current German strawberry breeding program for the fresh-market is now instructed to limit the seedlings to less than 10,000 per annum. It has to be emphasized that this are less seedlings than SENGBUSCH had A-selections. Also, the other governmental European strawberry breeding programs do not reach much higher seedling numbers. BARTUAL *et al*. (1990) reported of 18,000 seedlings in only one year for a special program in the Spanish region of Valencia.

SENGBUSCH and his colleagues mentioned this capacity problem already in 1982 (MELLENTHIN *et al*. 1982). They declared that due to the heterozygoty of strawberries the combination of demanded traits in one genotype will only be reachable by high numbers of seedlings but that exactly this will not be possible anymore in the future. They concluded that an alternative breeding strategy will be necessary and proposed an inbreeding of genotypes, not for heterosis breeding but for the creation of parents. These parents should be “homozygotized” in special

breeding traits and should be available for crossings for cultivar development. Unfortunately, no further report about the success of this approach is reported.

Another alternative breeding strategy could be the use of pollen mixtures. These pollen mixtures are successfully used in applied ornamental breeding programs. Nevertheless, there is no information present about the influence of these pollen mixtures on for example the proportions of the several paternal parents in the progeny populations. Table 17 lists these proportions for a conducted pollen mixture approach. It has to be noticed that the achieved results are only to refer to this specific experiment. It can be seen that the potential paternal parents did not participate in a balanced ratio. Too many various causes are possible for this imbalance, like a different number of pollen in the mixture, differences in the speed of the pollen tube growing or fertility grades. As a consequence no scientific speculation can be conducted. The main question is anyway, are the pollen mixtures an advantageous or not. A comparison with the selection rates of the defined cross combination shows that the higher proportion of the paternal parent ‘Korona’ in the pollen mixture population was advantageously. The defined cross combination ‘Korona’ x ‘Fraroma’ had one of the highest selection rates of all four combinations. On the other hand, the other superior cross combination ‘Fraroma’ x ‘Honeoye’, with 2.4% selected genotypes, was underrepresented in the pollen mixture population and consequently not selected. This was a disadvantageous. Therefore, no clear conclusions can be drawn about the benefit of this kind of breeding approach. The only clear result is that certain cross combinations can be underrepresented and other combinations overrepresented. The direct comparison of the total selection rates of the two approaches is not permissible, since this rate can be based on the second important breeding factor: the breeder. Every seedling is evaluated by a human and not strictly according to certain defined parameters. Therefore, the selection rates of table 18 could be the result of a subjective decision of the breeder (conducted consciously or unintentionally) or by an advantage of the pollen mixture approach in this special case. An interaction between the breeder’s decision and the breeding approach is also assumable, in this way that the pollen mixture approach did not result in a population with a higher proportion of superior genotypes but in populations which made it easier for the breeder to select superior genotypes; maybe due a higher divergence between the plants. The aspects which influence the

decision-making of the breeder are largely ignored but these are interesting and significant factors (TIMMERMANN 2006). Further research is strongly needed.

Molecular biological approaches are also assumable and several genetic transformations, integrations and transgenic gene expression were reported for strawberries (HANCOCK 1999, HOKANSON and MAAS 2001). However, these experiments are cost intensive and were mostly done for research. So far not a single transgenic strawberry cultivar is worldwide on the market. Anyway, the in the present work described phenotypic variances of the strawberry gene pool in regard to the quantitative traits DM content, color or yield are wide enough to promise faster and cheaper results by applied breeding approaches.

The use of molecular marker is also assumable for strawberries but again the capacity efforts for the development and implementation are in no ratio to an applied governmental breeding program.

E 11 Summary

In the presented work, the new strawberry breeding goal DM was characterized in regard to influence factors, composition, location within fruit as well as to the inheritance and interaction with other important traits. A high phenotypic variance for DM content was demonstrated in several F_1 populations as well as in the gene pool. This variance is the basis for an improvement of the trait by selection. Due to a high correlation between the DM content and the Brix value, the selection for high DM content can also be carried out by the easier to measure correlated response. The high DM of species other than *F. ×ananassa* was shown to be based on an altered ratio of achenes to pulp. Therefore, the incrossing of these species for high DM populations is not recommended. Additionally, problems are expected due to different chromosome numbers. Further, the mean of the DM content of F_1 populations was shown to be also genetically influenced by the choice of the cross combination. This implies also a genetic inheritance of the DM content. The detected potential maternal effect has to be evaluated in further research. No negative correlations were present between DM content and important quality traits like fruit size, soluble solids, sugar composition or color. Therefore, the combination of DM content and these important traits in one genotype seems to be reachable. Correlation with other important traits like the detachability of the calyx have still to be

evaluated. A negative correlation between DM content and yield was observed. Nevertheless, due to the low DM content and low yield of the old freeze-dry standard cultivar ‘Senga Sengana’ a genotype with superior traits seems to be possible. Further, the DM content of established cultivars was characterized as constant across different environments. On the other hand, a high genotype by environment interaction was found in seedling and clonal populations. An inheritance of the reaction of genotype by environment influence is possible, but needs further research. Together with the described problems of a precise measurement on single plants, the DM selection in higher selection stages on several locations seems to be most beneficial. Additionally, the freeze-dry performance and the appearance after the process should be evaluated in these stages. The breeding strategy of preceding combining ability tests and the subsequent realization of the most promising crosses in high seedling numbers seem rewarding. Nevertheless, the success of this approach depends highly on the capacity in regard to number of crosses, seedlings and selections. Therefore, the discussed alternative approaches of breeding methods should be taken into consideration.

E 11 Zusammenfassung

Die vorliegende Dissertation hatte die wissenschaftliche Erarbeitung und die Etablierung eines Zuchtprogrammes für eine Kulturerdbeere (*F.* ×*ananassa*) mit Gefriertrocknungseigenschaften zum Inhalt.

Es wurde der für die Kulturerdbeere neue Qualitätsparameter prozentuale Fruchtrockenmasse (TM) im Hinblick auf Einflussfaktoren, Zusammensetzung, Lokalisation innerhalb der Frucht sowie dessen Vererbung und Interaktion mit anderen bedeutenden Merkmalen charakterisiert. Eine große phänotypische Varianz des Parameters wurde für verschiedene F_1 Populationen und den Genpool nachgewiesen. Diese Varianz ist die Basis für eine züchterische Verbesserung durch Auslese. Durch eine hohe Korrelation zwischen der TM und der löslichen Trockensubstanz (Brix) kann die Selektion auch an diesem korrelierenden und einfacher zu bestimmenden Parameter durchgeführt werden. Die hohe TM von verschiedenen anderen Erdbeerarten (Wildarten) basierte auf dem veränderten Masseverhältnis von Samen zu Fruchtfleisch. Eine Nutzung oder Einkreuzung dieser Arten ist daher nicht zu empfehlen. Des weiteren wurde nachgewiesen, dass der TM-

Mittelwert von unterschiedlichen Populationen durch die Wahl der Kreuzungspartner bestimmt ist. Untersuchungen zur Vererbung bezüglich des Parameters wurden durchgeführt. Der aufgefundene maternale Effekt könnte von großer Bedeutung in der weiteren Züchtung sein, bedarf aber weiterer Prüfungen. Es wurde keine negative Korrelation zwischen der TM und anderen wichtigen Qualitätsmerkmalen wie Fruchtgröße, Brix, Zuckerzusammensetzung oder interne und externe Färbung nachgewiesen. Dadurch ist eine Kombination zwischen der TM und den genannten Merkmalen in einem Genotyp möglich. Dies ist insofern entscheidend, als dass neben der Trockenmasse Strukturausprägungen, Färbungen und Fruchtgröße für die Gefriertrocknungseignung bedeutsam sind. Mögliche Interaktionen mit anderen wichtigen Merkmalen wie der Entkelchbarkeit bedürfen noch weiterer Untersuchungen. Es wurde eine negative Korrelation zwischen der TM und dem Frischmasseertrag nachgewiesen. Dennoch erscheint aufgrund des niedrigen Niveaus von ’Senga Sengana’ in diesen Merkmalen ein verbesserter Kultivar möglich.

Die TM wurde in etablierten Kultivaren als konstant in verschiedenen Umwelten charakterisiert. Andererseits wurde eine hohe Genotyp-Umwelt-Interaktion in Sämlingen und einer Klon-Population dokumentiert. Eine Vererbung der Ausprägung dieser Interaktion ist möglich und sollte weiter in Untersuchungen Beachtung finden. Zusammen mit der aufgeführten Problematik der präzisen Bestimmung des Parameters an Einzelpflanzen scheint die TM Selektion in höheren Selektionsstufen und an verschiedenen Orten Erfolg zu versprechen. Die Gefriertrocknungseignung und die Qualität nach Trocknung sollte auch in diesen höheren Selektionsstufen vorgenommen werden.

Die Züchtungsstrategie einer Kombinationseignungsprüfung mit nachfolgender Realisierung der aussichtsreichsten Kreuzungen in hoher Sämlingszahl ist möglich. Leider beruht der Erfolg dieses Ansatzes ganz erheblich auf der Kapazität im Hinblick auf Kreuzungs-, Sämlings- und Zuchtklon-Zahlen und ist zudem zeitlich ineffizient. Alternative Züchtungsstrategien sollten daher in Betracht gezogen werden.

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G Annex

G 1: RAPD primers used in presented work.

Primer name	Sequence	Tm. [°C]	Source reference
RAPD-FR1	5'-CCG CAT CTA C-3'	32	Hancock *et al.* (1994)
RAPD-FR2	5'-TGG ACC GGT G-3'	34	Hancock *et al.* (1994)
RAPD-FR3	5'-GGA CCC AAC C-3'	34	Hancock *et al.* (1994)
RAPD-FR4	5'-GGT AGC AGT C-3'	32	Graham *et al.* (1996)
RAPD-FR5	5'-CCA CCG CCA G-3'	36	Graham *et al.* (1996)
RAPD-FR6	5'-CAG TTC TGG C-3'	32	Graham *et al.* (1996)
RAPD-FR7	5'-AGC CAG CGA A-3'	32	Graham *et al.* (1996)
RAPD-FR8	5'-AAT CGG GCT G-3'	32	Graham *et al.* (1996)
RAPD-FR9	5'-TCC GCT CTG G-3'	34	Degani *et al.* (1998)
RAPD-FR10	5'-GAA GCC AGC C-3'	34	Degani *et al.* (1998)

G 2: SSR primers used in presented work.

Marker		Sequence	Tm. [°C]	Size [bp]	Source reference	Multi-plex
ARSFL2 (681)	F	5'-GCG AAG CGA AGC GGT GAT G-3'	59	237	Lewers *et al.* (2005)	MP1
	R	5'-GCG AAC GTC GAG GAG CAT TCT CAT-3'	62		Lewers *et al.* (2005)	
ARSFL3 (681)	F	5'-GCG GGT GCT TAG GTT TTC ACA ACT-3'	59	291	Lewers *et al.* (2005)	
	R	5'-GCG CAA GTG GTA TTT AAG GGT TAG-3'	55		Lewers *et al.* (2005)	
ARSFL7 (781)	F	5'-GCG CGC ATA AGG CAA CAA AG-3'	58	256	Lewers *et al.* (2005)	
	R	5'-GCG AAT GGC AAT GAC ATC TTC TCT-3'	58		Lewers *et al.* (2005)	
ARSFL22 (781)	F	5'-GCG AAC CCC ATT AAC AGC TTC A-3'	58	158	Lewers *et al.* (2005)	
	R	5'-GCG ATC AAA TTC CCC TCT AAC AAT-3'	57		Lewers *et al.* (2005)	
ARSFL24 (681)	F	5'-GCG TGG ATC TAT GAT CAG TTT GCC-3'	59	195	Lewers *et al.* (2005)	
	R	5'-GCG GGG TTC TTC TTC TGG GAA ATG-3'	63		Lewers *et al.* (2005)	
ARSFL4 (681)	F	5'-GCG GTC GCA TTG AGT TGG AGG ATA-3'	63	166	Lewers *et al.* (2005)	MP2
	R	5'-GCG TAG CCA AAC ACC GAT CTA CC-3'	59		Lewers *et al.* (2005)	
ARSFL10 (781)	F	5'-GCG TCA GCC GTA GTG ATG TAG CAG-3'	60	252	Lewers *et al.* (2005)	
	R	5'-GCG CCA GCC CCT CAA ATA TC-3'	58		Lewers *et al.* (2005)	
ARSFL11 (681)	F	5'-GCG AAG CAT AAC TGG CAG TAT CTG-3'	57	279	Lewers *et al.* (2005)	
	R	5'-GCG GGC CTA GGT GAT CTT GGA-3'	60		Lewers *et al.* (2005)	
ARSFL14 (681)	F	5'-GCG TTA AAC GGA AAC TTA GAG AGA-3'	53	233	Lewers *et al.* (2005)	
	R	5'-GCG GAA CGG CTC AAA CAT C-3'	55		Lewers *et al.* (2005)	
ARSFL17 (781)	F	5'-GCG CAT CAC AAT CGC CAT AGA AAC-3'	61	218	Lewers *et al.* (2005)	
	R	5'-GCG AAC ACG CCT TCA ACA ACC AC-3'	62		Lewers *et al.* (2005)	
01H05 (781)	F	5'-GGG AGC TTG CTA GCT AGA TTT G-3'	55	246	Bassil *et al.* (2006)	MM1
	R	5'-AGA TCC AAG TGT GGA AGA TGC T-3'	56		Bassil *et al.* (2006)	
02G01 (781)	F	5'-ACG AGG TGG GTT TTG TGT TGT-3'	57	159	Bassil *et al.* (2006)	
	R	5'-CCC AGA TGA AGA AAC CGA TCT A-3'	54		Bassil *et al.* (2006)	
04G04 (681)	F	5'-ACG AGG CCT TGT CTT CTT TGT A-3'	56	187	Bassil *et al.* (2006)	
	R	5'-GCT CCA GCT TTA TTG TCT TGC T-3'	55		Bassil *et al.* (2006)	
08H09 (681)	F	5'-CTT CAC CTA ATC ACT TGC CTG A-3'	55	188	Bassil *et al.* (2006)	
	R	5'-GGT CTG TTC CTT TCC TTG TTT G-3'	54		Bassil *et al.* (2006)	

G 3: Means, SD and ANOVA of the tested different DM determination methods.

	DM [%]			
	Filter paper (70°C)	Sea sand (60°C)	Sea sand (70°C)	Laboratory Freeze-dryer
Repetition 1	10.85	10.17	10.94	11.81
Repetition 2	10.86	10.65	12.01	10.82
Mean	10.86	10.41	11.48	11.32
SD	0.01	0.34	0.76	0.70

One-way ANOVA: Filter paper (70°C), Sea sand (60°C), Sea Sand (70°C), Laboratory Freeze-dryer

```
Source  DF     SS     MS     F      P
Factor   3  1.386  0.462  1.57  0.329
Error    4  1.178  0.294
Total    7  2.564

S = 0.5426   R-Sq = 54.07%   R-Sq(adj) = 19.62%
```

G 4: DM content of different ripening stages and cultivars.

		DM [%]					
		Repetition					
Cultivar	Ripening stage	1	2	3	Mean	SD	CV
‘Avalon classic’	unripe	10.5	10.3	10.4	10.4	0.1	1.1
‘Avalon classic’	half-ripe	11.0	10.9	10.8	10.9	0.1	1.0
‘Avalon classic’	ripe	10.8	10.5	10.2	10.5	0.3	2.9
‘Avalon classic’	overripe	15.7	13.9	14.5	14.7	0.9	6.1
‘Dover’	unripe	9.7	10.1	9.9	9.9	0.2	2.0
‘Dover’	half-ripe	10.1	9.8	9.6	9.9	0.2	2.5
‘Dover’	ripe	10.0	10.8	10.2	10.3	0.4	3.8
‘Dover’	overripe	13.4	14.0	14.7	14.0	0.6	4.5
‘Elsanta’	unripe	13.6	14.4	14.1	14.0	0.4	2.8
‘Elsanta’	half-ripe	10.5	10.3	10.4	10.4	0.1	1.1
‘Elsanta’	ripe	10.7	10.6	10.5	10.6	0.1	0.9
‘Elsanta’	overripe	15.5	14.1	15.2	14.9	0.7	4.9
‘Lambada’	unripe	10.7	10.6	10.9	10.7	0.2	1.6
‘Lambada’	half-ripe	11.1	11.2	10.7	11.0	0.3	2.3
‘Lambada’	Ripe	11.6	11.5	12.2	11.7	0.4	3.1

One-way ANOVA: DM [%] vs. ripening stage for ‘Avalon Classic’

```
Source          DF      SS      MS      F      P
Ripening Stage   3  38.243  12.748  53.67  0.000
Error            8   1.900   0.238
Total           11  40.143

S = 0.4873   R-Sq = 95.27%   R-Sq(adj) = 93.49%

                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level      N    Mean  StDev  -----+---------+---------+---------+----
half-ripe  3  10.900  0.100     (----*---)
```

```
overripe   3  14.700  0.917                                (---*---)
ripe       3  10.500  0.300   (---*---)
unripe     3  10.400  0.100  (---*----)
                             -----+---------+---------+---------+----
                               10.5      12.0      13.5      15.0
Pooled StDev = 0.487

Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of Ripening Stage

Simultaneous confidence level = 82.43%

Ripening Stage = half-ripe subtracted from:

Ripening
Stage       Lower   Center   Upper  -------+---------+---------+---------+--
overripe   2.8824   3.8000  4.7176                              (--*--)
ripe      -1.3176  -0.4000  0.5176               (--*--)
unripe    -1.4176  -0.5000  0.4176              (--*--)
                                   -------+---------+---------+---------+--
                                       -3.0       0.0       3.0       6.0

Ripening Stage = overripe subtracted from:

Ripening
Stage       Lower   Center    Upper  -------+---------+---------+---------+--
ripe      -5.1176  -4.2000  -3.2824  (--*--)
unripe    -5.2176  -4.3000  -3.3824  (--*--)
                                    -------+---------+---------+---------+--
                                        -3.0       0.0       3.0       6.0

Ripening Stage = ripe subtracted from:

Ripening
Stage       Lower   Center   Upper  -------+---------+---------+---------+--
unripe    -1.0176  -0.1000  0.8176               (--*--)
                                   -------+---------+---------+---------+--
                                       -3.0       0.0       3.0       6.0
```

One-way ANOVA: DM [%] vs. ripening stage for ‘Dover’

```
Source          DF      SS      MS      F      P
Ripening stage   3  36.643  12.214  69.80  0.000
Error            8   1.400   0.175
Total           11  38.043

S = 0.4183   R-Sq = 96.32%   R-Sq(adj) = 94.94%

                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level      N    Mean  StDev  --------+---------+---------+---------+-
half-ripe  3   9.833  0.252  (---*--)
overripe   3  14.033  0.651                              (---*--)
ripe       3  10.333  0.416     (---*---)
unripe     3   9.900  0.200  (---*---)
                             --------+---------+---------+---------+-
                                  10.5      12.0      13.5      15.0
Pooled StDev = 0.418

Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of Ripening stage
```

```
Simultaneous confidence level = 82.43%

Ripening stage = half-ripe subtracted from:

Ripening
stage       Lower  Center   Upper     +---------+---------+---------+---------
overripe   3.4123  4.2000  4.9877                                        (--*--)
ripe      -0.2877  0.5000  1.2877                          (--*--)
unripe    -0.7210  0.0667  0.8543                       (--*--)
                                    +---------+---------+---------+---------
                                  -5.0      -2.5       0.0       2.5

Ripening stage = overripe subtracted from:

Ripening
stage       Lower   Center    Upper
ripe      -4.4877  -3.7000  -2.9123
unripe    -4.9210  -4.1333  -3.3457

Ripening
stage        +---------+---------+---------+---------
ripe           (--*--)
unripe       (--*---)
             +---------+---------+---------+---------
          -5.0      -2.5       0.0       2.5

Ripening stage = ripe subtracted from:

Ripening
stage       Lower   Center   Upper     +---------+---------+---------+---------
unripe    -1.2210  -0.4333  0.3543                    (--*--)
                                    +---------+---------+---------+---------
                                  -5.0      -2.5       0.0       2.5
```

One-way ANOVA: DM [%] vs. ripening stage for 'Elsanta'

```
Source          DF      SS      MS      F      P
ripening stage   3  48.876  16.292  89.68  0.000
Error            8   1.453   0.182
Total           11  50.329

S = 0.4262   R-Sq = 97.11%   R-Sq(adj) = 96.03%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level      N    Mean  StDev  ----+---------+---------+---------+-----
half-ripe  3  10.400  0.100  (--*---)
overripe   3  14.933  0.737                                (---*--)
ripe       3  10.600  0.100   (---*--)
unripe     3  14.033  0.404                          (---*--)
                            ----+---------+---------+---------+-----
                             10.5      12.0      13.5      15.0
Pooled StDev = 0.426

Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of ripening stage

Simultaneous confidence level = 82.43%

ripening stage = half-ripe subtracted from:
```

```
ripening
stage       Lower  Center   Upper  -------+---------+---------+---------+--
overripe   3.7308  4.5333  5.3358                            (--*--)
ripe      -0.6025  0.2000  1.0025                  (--*-)
unripe     2.8308  3.6333  4.4358                        (--*--)
                                   -------+---------+---------+---------+--
                                       -3.0       0.0       3.0       6.0

ripening stage = overripe subtracted from:

ripening
stage       Lower   Center    Upper  -------+---------+---------+---------+--
ripe      -5.1358  -4.3333  -3.5308  (--*-)
unripe    -1.7025  -0.9000  -0.0975             (--*--)
                                   -------+---------+---------+---------+--
                                       -3.0       0.0       3.0       6.0

ripening stage = ripe subtracted from:

ripening
stage      Lower  Center   Upper  -------+---------+---------+---------+--
unripe    2.6308  3.4333  4.2358                          (-*--)
                                   -------+---------+---------+---------+--
                                       -3.0       0.0       3.0       6.0
```

One-way ANOVA: DM [%] vs. ripening stage for 'Lambada'

```
Source          DF      SS      MS      F      P
ripening stage   2  1.7267  0.8633  10.94  0.010
Error            6  0.4733  0.0789
Total            8  2.2000

S = 0.2809   R-Sq = 78.48%   R-Sq(adj) = 71.31%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level      N    Mean  StDev  ---+---------+---------+---------+------
half-ripe  3  11.000  0.265       (-------*-------)
ripe       3  11.767  0.379                 (-------*-------)
unripe     3  10.733  0.153  (-------*-------)
                            ---+---------+---------+---------+------
                            10.50     11.00     11.50     12.00

Pooled StDev = 0.281

Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of ripening stage

Simultaneous confidence level = 89.08%

ripening stage = half-ripe subtracted from:

ripening
stage       Lower   Center   Upper     +---------+---------+---------+---------
ripe       0.2055   0.7667  1.3278                          (------*------)
unripe    -0.8278  -0.2667  0.2945               (------*------)
                              +---------+---------+---------+---------
                           -1.60     -0.80      0.00      0.80

ripening stage = ripe subtracted from:
```

```
ripening
stage       Lower   Center    Upper
unripe    -1.5945  -1.0333  -0.4722

ripening
stage       +---------+---------+---------+---------
unripe      (------*------)
            +---------+---------+---------+---------
          -1.60     -0.80      0.00      0.80
```

G 5: Data of C 2.1.2.2.

Ciflorette'

Plant ID	Rank	Truss	Date	Fruit weight [g]	DM [%]
1	A		8.6	31.7	11.3
1	A		16.6	25.0	13.6
1	A		16.6	26.6	12.2
1	B		8.6	18.1	10.9
1	B		16.6	15.3	12.7
1	B		21.6	11.9	15.7
1	B		16.6	16.7	11.1
1	B		21.6	14.3	11.6
1	C		16.6	3.6	8.0
1	C		16.6	6.6	8.5
2	A		13.6	20.7	12.1
2	A		8.6	30.5	12.9
2	A		16.6	28.2	13.1
2	A		21.6	16.0	13.3
2	A		21.6	30.0	13.5
2	B		21.6	14.0	13.9
2	B		16.6	8.5	10.4
2	B		16.6	13.3	11.7
2	B		13.6	9.3	11.8
2	B		21.6	15.2	12.1
2	B		16.6	12.3	12.3
2	B		21.6	9.5	12.5
2	B		21.6	11.6	12.7
2	B		13.6	10.0	13.0
2	B		13.6	15.5	13.8
3	A		10.6	18.8	11.4
3	A		8.6	14.9	11.9
3	A		8.6	14.3	12.3
3	A		21.6	19.9	12.8
3	B		16.6	15.3	11.5
3	B		16.6	16.3	11.8
3	B		16.6	13.2	12.0
3	B		16.6	16.4	12.2
3	B		16.6	16.5	12.4
3	B		16.6	16.2	12.6
3	B		16.6	10.3	12.6
3	B		16.6	16.8	12.8
3	B		16.6	14.6	13.6
3	C		16.6	9.6	11.5
3	C		16.6	8.8	12.5

Ciflorette'

Plant ID	Rank	Truss	Date	Fruit weight [g]	DM [%]
4	A		16.6	19.6	12.1
4	A		8.6	23.9	12.6
4	A		8.6	26.4	12.2
4	A		13.6	22.0	14.4
4	B		16.6	11.1	11.0
4	B		16.6	11.8	12.8
4	B		16.6	12.3	11.4
4	B		13.6	16.7	13.0
4	B		13.6	15.5	14.9
4	C		21.6	3.0	13.3
5	A	1	10.6	18.7	8.2
5	A	2	10.6	19.8	11.5
5	A	3	13.6	20.3	11.3
5	A	4	10.6	19.6	11.8
5	A	5	10.6	29.2	11.6
5	B	1	13.6	10.0	7.1
5	B	1	13.6	8.7	7.5
5	B	4	13.6	14.1	11.1
5	C	4	16.6	9.6	11.2
5	B	4	16.6	13.0	11.5
5	B	4	16.6	11.0	12.3
5	B	3	13.6	7.9	12.5
5	B	2	10.6	10.3	12.9
5	B	5	13.6	12.0	13.3
5	B	5	13.6	12.4	13.4
5	C	5	13.6	9.5	13.5
5	C	1	16.6	5.1	7.1
5	C	1	16.6	7.0	8.3
5	C	4	13.6	10.4	10.9
5	C	3	16.6	4.7	11.8
5	C	5	16.6	7.8	11.9
5	C	5	16.6	10.2	12.5
5	C	2	16.6	8.6	12.9
6	A		8.6	21.6	13.4
6	A		8.6	25.9	12.8
6	A		13.6	21.6	14.4
6	C		21.6	7.6	12.6
6	C		16.6	7.8	13.4
6	B		16.6	12.0	14.0
6	B		13.6	11.2	13.7
6	B		16.6	9.2	14.2
6	B		13.6	10.4	15.1
6	C		21.6	6.3	16.6

Elsanta'

Plant ID	Rank	Date	Fruit weight [g]	DM [%]
1	A	16.6	26.3	9.9
1	A	16.6	21.3	12.3
1	A	18.6	23.8	10.0
1	B	16.6	22.6	8.9
1	B	16.6	21.9	9.2
1	B	16.6	23.3	9.2
1	B	18.6	13.3	7.9
1	B	18.6	12.4	8.3
1	B	18.6	10.1	8.4
1	B	18.6	11.3	8.5
1	B	18.6	20.2	8.8
1	B	18.6	13.9	9.0
1	B	18.6	13.8	9.5
1	B	18.6	16.2	10.0

Elsanta'

Plant ID	Rank	Date	Fruit weight [g]	DM [%]
4	A	16.6	39.8	9.8
4	A	16.6	28.3	10.6
4	A	21.6	21.5	9.9
4	A	21.6	19.2	12.4
4	B	16.6	13.0	9.0
4	B	16.6	13.6	11.3
4	B	18.6	20.0	9.3
4	B	21.6	12.2	10.0
4	B	21.6	11.4	10.3
4	B	21.6	10.1	10.5
4	B	18.6	12.8	9.7
4	B	18.6	13.5	9.6
4	B	18.6	11.9	9.9
4	B	18.6	17.6	10.0

1	B	18.6	11.7	10.1
1	B	18.6	13.3	10.1
1	B	18.6	14.4	10.3
1	B	21.6	14.2	10.6
1	B	21.6	13.5	10.7
1	B	16.6	18.9	9.6
1	B	18.6	13.9	9.2
1	B	18.6	16.6	10.7
1	C	21.6	6.3	9.9
1	C	21.6	8.2	12.6
1	C	21.6	7.7	13.4
1	C	21.6	7.3	10.1
1	C	21.6	8.5	8.6
1	C	21.6	6.1	11.2
1	C	21.6	8.4	8.6
2	A	16.6	14.5	9.7
2	A	18.6	17.5	8.7
2	A	18.6	22.7	8.9
2	A	21.6	17.8	9.7
2	A	21.6	27.6	11.2
2	A	18.6	16.8	9.4
2	B	16.6	14.9	8.6
2	B	16.6	19.7	8.8
2	B	16.6	14.9	9.0
2	B	16.6	21.0	9.3
2	B	16.6	12.3	10.9
2	B	18.6	9.5	8.3
2	B	18.6	17.2	8.4
2	B	18.6	10.5	8.4
2	B	18.6	14.6	8.6
2	B	18.6	14.8	8.6
2	B	18.6	16.5	8.8
2	B	18.6	15.8	8.8
2	B	18.6	13.9	8.9
2	B	18.6	14.0	9.0
2	B	18.6	9.9	9.2
2	B	18.6	14.4	10.1
2	B	18.6	11.5	10.9
2	B	21.6	13.2	10.0
2	B	21.6	19.7	10.2
2	B	16.6	13.5	9.3
2	C	18.6	5.8	8.3
2	C	18.6	8.3	8.3
2	C	21.6	6.1	8.7
2	C	21.6	11.2	9.0
2	C	21.6	7.8	9.1
2	C	21.6	4.1	10.0
2	C	21.6	11.4	10.1
2	C	21.6	7.8	10.2
2	C	21.6	10.3	10.7
3	A	16.6	34.5	10.1
3	A	16.6	28.0	11.4
3	A	16.6	26.9	9.7
3	A	16.6	24.1	11.3
3	B	16.6	19.6	8.8
3	B	16.6	16.9	9.2
3	B	16.6	22.5	9.3
3	B	16.6	21.6	9.6
3	B	16.6	18.4	9.7
3	B	16.6	20.7	9.8
3	B	16.6	22.9	11.4
3	B	18.6	15.7	8.8
3	B	18.6	12.8	8.9
3	B	18.6	15.5	9.0
3	B	18.6	15.7	9.2
3	B	18.6	14.1	9.4
3	B	18.6	17.4	9.5
3	B	18.6	18.1	9.6
3	B	18.6	12.3	9.8
3	B	18.6	20.0	11.2
3	B	18.6	16.1	11.4
3	B	21.6	11.7	8.3
3	B	21.6	14.0	11.8
3	B	21.6	11.6	12.7
3	C	21.6	8.7	10.4
3	C	21.6	9.3	10.9
3	C	21.6	9.4	11.2
3	C	21.6	6.3	13.0
3	C	21.6	10.1	13.8
3	C	21.6	8.5	14.8
4	B	21.6	14.3	10.4
4	C	18.6	7.1	6.6
4	C	18.6	10.5	8.4
4	C	18.6	7.8	8.5
4	C	18.6	9.1	9.3
4	C	18.6	7.6	9.3
4	C	21.6	8.6	8.3
4	C	21.6	8.5	9.1
4	C	21.6	7.8	8.6
5	A	16.6	14.9	8.7
5	A	21.6	33.7	12.8
5	A	16.6	24.5	9.4
5	B	16.6	11.0	8.9
5	B	16.6	13.3	9.3
5	B	16.6	18.9	9.7
5	B	16.6	13.0	9.9
5	B	16.6	18.8	10.2
5	B	16.6	15.0	10.3
5	B	16.6	10.7	10.4
5	B	18.6	16.2	8.6
5	B	18.6	16.5	9.5
5	B	21.6	8.3	8.4
5	B	21.6	10.9	8.6
5	B	21.6	11.4	8.8
5	B	21.6	19.3	9.7
5	B	21.6	12.5	9.7
5	B	21.6	13.8	10.3
5	B	21.6	12.2	11.2
5	B	18.6	13.5	9.0
5	B	18.6	12.3	9.3
5	B	18.6	15.1	9.9
5	C	21.6	6.4	7.8
5	C	21.6	7.9	10.5
5	C	21.6	8.5	9.5
5	C	21.6	8.1	10.1
5	C	21.6	9.8	9.2
6	A	16.6	25.2	9.3
6	A	16.6	25.7	9.9
6	A	16.6	23.6	11.5
6	A	21.6	17.9	8.8
6	A	21.6	16.8	9.6
6	A	21.6	18.8	9.9
6	A	21.6	18.2	10.3
6	A	21.6	22.8	10.5
6	A	21.6	20.6	11.7
6	B	16.6	21.5	9.0
6	B	16.6	18.6	9.0
6	B	16.6	25.3	9.2
6	B	16.6	24.8	9.6
6	B	16.6	20.9	9.6
6	B	16.6	21.2	10.0
6	B	16.6	21.2	10.1
6	B	18.6	16.0	9.1
6	B	18.6	13.2	9.1
6	B	21.6	8.4	8.8
6	B	21.6	7.9	9.3
6	B	21.6	12.4	9.4
6	B	21.6	10.2	9.7
6	B	21.6	7.8	9.7
6	B	21.6	10.0	10.2
6	B	21.6	9.9	10.9
6	C	16.6	11.6	8.1
6	C	16.6	11.5	8.2
6	C	16.6	10.8	8.4
6	C	16.6	15.2	8.5
6	C	16.6	13.7	8.8
6	C	16.6	10.7	9.2
6	C	16.6	13.5	9.2
6	C	16.6	18.1	9.3
6	C	16.6	14.8	9.4
6	C	16.6	12.4	9.8
6	C	21.6	6.6	8.6
6	C	21.6	6.3	9.2
6	C	21.6	6.8	9.3

Senga Sengana'

Plant ID	Rank	Date	Fruit weight [g]	DM [%]
1	A	16.6	10.13	9.28
1	A	16.6	19.23	9.31
1	A	16.6	19.07	10.12
1	A	16.6	10.78	10.48
1	A	18.6	11.24	9.25
1	A	21.6	14.42	11.44
1	A	21.6	13.73	11.58
1	B	16.6	9.59	10.22
1	B	18.6	9.83	9.16
1	B	18.6	8.89	9.45
1	B	18.6	8.99	9.68
1	B	18.6	8.13	10.82
1	B	21.6	9.6	10.31
1	B	21.6	9.45	10.58
1	B	18.6	8.96	9.23
1	B	18.6	8.99	9.78
1	B	18.6	9.56	9.91
1	B	18.6	9.34	10.34
1	B	18.6	9.56	10.75
1	C	21.6	5.63	8.35
1	C	21.6	6.62	9.37
1	C	21.6	3.96	10.61
1	C	21.6	5.5	11.09
1	C	21.6	6.06	9.06
1	C	21.6	5.55	10.45
1	C	21.6	4.67	10.88
1	D	21.6	3.755	10.65
1	D	21.6	2.14	14.02
1	D	21.6	1.487	14.12
2	A	16.6	14.53	9.57
2	A	16.6	10.65	10.05
2	A	16.6	11.26	10.21
2	A	16.6	24.89	10.37
2	A	16.6	13.13	10.43
2	A	16.6	10.12	10.77
2	B	16.6	8.94	10.18
2	B	16.6	9.43	10.18
2	B	18.6	6.69	8.82
2	B	18.6	7.05	9.65
2	B	18.6	9.34	9.74
2	B	18.6	8.4	9.76
2	B	18.6	8.11	10.11
2	B	21.6	8.93	9.74
2	B	21.6	5.69	10.19
2	B	21.6	8.45	10.65
2	B	21.6	7.45	11.41
2	B	18.6	8.67	9.58
2	B	18.6	9.27	10.16
2	B	18.6	9.22	10.57
2	B	18.6	8.04	11.03
2	C	21.6	5.21	11.13
2	C	21.6	3.74	12.03
2	C	21.6	5.08	9.67
2	C	21.6	4.69	10.23
2	C	21.6	4.23	10.61
3	A	16.6	19.09	11.05
3	A	16.6	23.44	11.65
3	A	16.6	17.29	12.55
3	A	16.6	18.41	12.98
3	A	18.6	14.82	10.53
3	A	18.6	12.73	10.53
3	A	21.6	11.46	11.52
3	B	16.6	12.39	11.70
3	B	16.6	11.23	12.20
3	B	18.6	8.78	11.62
3	B	18.6	8.23	11.91
3	B	21.6	10.57	10.03
3	B	21.6	8.81	11.58
3	B	18.6	9.13	10.56
3	B	18.6	8.69	10.97
3	B	21.6	10.21	9.89
3	B	21.6	10.04	10.78
3	C	21.6	7.27	10.59
3	C	21.6	7.33	11.60
3	C	21.6	4.99	12.02
3	C	21.6	6.1	15.74
3	C	21.6	6.92	10.66

Senga Sengana'

Plant ID	Rank	Date	Fruit weight [g]	DM [%]
4	A	16.6	19.19	9.33
4	A	16.6	13.38	10.01
4	A	16.6	17.6	10.17
4	A	18.6	16.27	9.47
4	A	18.6	13.83	9.98
4	A	18.6	16.05	10.47
4	B	16.6	8.65	9.71
4	B	16.6	8.28	10.51
4	B	18.6	9.54	8.18
4	B	18.6	8.49	8.72
4	B	18.6	8.93	8.85
4	B	18.6	8.5	9.29
4	B	18.6	12.69	9.93
4	B	18.6	7.17	10.32
4	B	21.6	10.92	8.52
4	B	18.6	9.45	9.86
4	B	21.6	9.53	8.73
4	B	21.6	8.32	8.98
4	B	21.6	10.23	9.90
4	B	21.6	9.71	10.33
4	C	21.6	4.9	9.18
4	C	21.6	4.07	9.58
4	C	21.6	4.75	9.68
4	C	21.6	4.3	9.77
4	C	21.6	4.98	9.44
4	C	21.6	5.68	9.68
5	A	16.6	14.39	10.01
5	A	16.6	15.91	10.12
5	A	16.6	15.16	10.29
5	A	16.6	14.57	10.57
5	A	16.6	18.31	10.65
5	A	16.6	13.58	11.12
5	A	18.6	14.51	9.58
5	A	21.6	16.13	11.59
5	B	16.6	7.57	9.11
5	B	16.6	6.8	9.56
5	B	16.6	7.37	9.77
5	B	16.6	6.67	9.90
5	B	16.6	6.72	9.97
5	B	16.6	7.58	10.16
5	B	16.6	7.27	11.14
5	B	18.6	8.93	8.96
5	B	18.6	9.54	9.12
5	B	18.6	8.15	9.20
5	B	18.6	7.96	9.30
5	B	18.6	8.98	9.47
5	B	18.6	10.55	9.95
5	B	21.6	8.21	10.35
5	B	21.6	8.2	11.59
5	B	18.6	9.88	9.83
5	B	21.6	8.55	10.21
5	C	21.6	7.43	9.02
5	C	21.6	7.31	9.30
5	C	21.6	6.11	10.31
5	C	21.6	7.04	9.43
5	C	21.6	6.73	9.87
5	C	21.6	7.14	10.18
5	D	21.6	3.73	8.58
5	D	21.6	4.83	8.70
6	A	16.6	9.96	6.83
6	A	16.6	11.57	10.20
6	A	16.6	12.13	10.22
6	A	18.6	14.68	8.92
6	A	18.6	12.74	9.26
6	A	18.6	13.47	9.43
6	A	18.6	20.2	11.04
6	A	18.6	15.79	11.72
6	B	16.6	5.88	7.31
6	B	16.6	7.5	10.13
6	B	16.6	10.22	10.37
6	B	16.6	7.8	10.38
6	B	16.6	7.46	10.46
6	B	16.6	8.28	10.87
6	B	16.6	6.16	12.82
6	B	18.6	7.64	8.25
6	B	18.6	10.06	8.65
6	B	18.6	9.7	8.66
6	B	18.6	8.73	8.93

6	B	18.6	9.93	9.26
6	B	18.6	8.75	9.83
6	B	21.6	7.18	8.50
6	B	18.6	9.12	8.98
6	B	18.6	9.34	9.97
6	C	21.6	5.51	8.71
6	C	21.6	4.87	8.83
6	C	21.6	5.02	9.16
6	C	21.6	5.34	9.74
6	C	21.6	4.58	9.83
6	C	21.6	6.53	9.95
6	C	21.6	5.76	9.79

G 6: Data of C 2.1.2.3.

Values of the location Dresden, Germany (left table) and Geisenheim, Germany (right table).

Code: M: ‘Mieze Schindler’, R: ‘Roxana’, S: ‘Senga Sengana’, numbers: blocks

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
M1	18/6	10.8	12.1	1136.9	9.3
M2	18/6	11.5	12.7	1128.5	9.6
M3	18/6		11.8		7.4
M1	20.6	10.2	11.7	1025.7	9.1
M2	20.6		11.8		8.9
M3	20.6	9.6	11.2	975.0	8.8
M1	25.6	9.8	11.3	899.3	6.4
M2	25.6	10.0	11.6	897.7	6.3
M3	25.6	10.0	11.3	1020.7	5.7

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
R1	12.6	8.8	9.5	805.6	42.0
R2	12.6		10.6		40.1
R3	12.6	8.6	9.7	887.3	36.0
R1	14.6	7.9	9.1	884.6	30.1
R2	14.6	8.3	9.4	866.9	27.2
R3	14.6	8.7	10.0	950.2	25.3
R1	16.6	8.4	9.2	899.9	23.8
R2	16.6	9.9	10.7	987.0	22.1
R3	16.6	9.0	9.7	871.6	22.0

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
S1	14.6	10.1	11.8	852.3	13.4
S2	14.6	10.2	12.1	881.0	15.4
S3	14.6	10.1	11.3	932.1	17.0
S1	16.6	8.3	10.0	872.9	10.8
S2	16.6	9.5	9.8	933.1	11.7
S3	16.6	9.7	10.3	1032.8	12.4
S1	18.6	8.3	10.0	963.6	11.1
S2	18.6	8.8	10.6	917.8	11.1
S3	18.6	8.7	10.6	995.1	12.0

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
M1	19/6	12.0	13.1	1079.7	7.3
M2	19/6	11.9	12.9	1093.4	6.6
M3	19/6	11.4	12.2	1167.2	7.1
M1	22/6	11.4	12.9	1084.6	5.9
M2	22/6	11.5	12.9	1157.5	6.2
M3	22/6	11.3	12.7	1138.2	7.3
M1	26/6	12.1	12.4	990.1	6.0
M2	26/6	11.6	12.0	1072.2	6.0
M3	26/6	10.9	11.8	879.7	6.8

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
R1	6/6	9.8	10.2	985.9	41.4
R2	6/6	9.5	10.6	939.0	35.3
R3	6/6	10.0	10.8	1002.5	22.7
R1	12/6	8.9	10.5	888.3	33.6
R2	12/6	10.3	11.2	996.3	21.5
R3	12/6	10.4	11.1	909.6	25.0
R1	14/6	9.0	9.9	904.5	22.5
R2	14/6	11.0	12.2	927.6	27.0
R3	14/6	10.0	10.8	969.5	20.8

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
S1	14/6	9.2	10.5	954.5	10.2
S2	14/6	9.2	10.7	1032.3	9.3
S3	14/6	9.7	11.2	1028.6	10.0
S1	16/6	8.8	9.9	973.4	9.0
S2	16/6	8.7	9.6	1004.7	8.9
S3	16/6	9.2	10.4	1055.3	10.0
S1	19/6	9.4	11.0	907.2	10.4
S2	19/6	9.7	11.2	984.1	6.8
S3	19/6	8.8	10.6	916.6	7.5

Values of the location Skiernievice, Poland (left table) and Vienna, Austria (right table).

Code: M: ‘Mieze Schindler’, R: ‘Roxana’, S: ‘Senga Sengana’, numbers: blocks

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
M1	21/6	10.8	12.1	1055.2	11.0
M2	21/6	11.4	13.2	868.8	10.0
M3	21/6	10.9	12.5	1140.9	9.0
M1	23/6	11.8	12.0	1308.2	9.1
M2	23/6		12.3		6.3
M3	23/6	9.8	11.5	924.9	9.6
M1	26/6	10.9	13.0	1117.6	5.6
M2	26/6	11.1	13.2	1191.8	6.0
M3	26/6	10.1	12.2	1129.5	6.9

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
R1	16/6	9.1	10.0	1078.7	26.9
R2	16/6	9.7	11.0	1022.6	33.9
R3	16/6	10.0	10.9	978.8	33.7
R1	19/6	8.6	9.9	1094.5	35.9
R2	19/6	8.8	9.4	1056.0	16.3
R3	19/6	8.6	9.5	1065.3	26.5
R1	21/6	7.8	8.8	981.4	20.7

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
M1					
M2	14/6	11.1	11.7	906.1	12.8
M3	14/6	11.3	12.4	904.9	12.1
M1	20/6	10.7	11.4	1023.1	8.7
M2	20/6	11.4	11.2	1177.0	7.0
M3	20/6	10.8	12.5	967.2	7.0
M1	29/6	9.5	10.9	1130.0	5.6
M2	29/6	9.5	11.8	1008.9	8.1
M3	29/6	9.8	11.6	1157.7	5.6

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
R1					
R2					
R3	14/6	8.5	9.3	969.3	25.4
R1	20/6	9.7	10.0	863.8	24.3
R2	20/6	8.5	9.5	983.3	24.2
R3	20/6	8.6	9.2	1046.8	26.2
R1	29/6	9.5	10.3	1092.3	15.1

R2	21/6	7.2	8.3	995.8	22.8	R2	29/6	10.0	11.1	1061.3	13.2
R3	21/6	8.2	9.3	1027.0	26.9	R3	29/6	6.9	8.3	908.6	13.9

Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]	Code	Date	Brix [%]	DM [%]	Citric acid [mg/ml]	Fruit weight [g]
S1	21/6	8.2	9.8	1115.6	9.6	S1	14/6	8.9	10.5	1010.4	13.8
S2	21/6	8.5	10.1	1191.5	11.3	S2	14/6	10.4	11.9	958.8	13.6
S3	21/6	8.2	9.7	1013.0	13.0	S3	14/6	10.1	11.6	893.9	13.0
S1	23/6	7.5	9.4	1205.0	8.5	S1	20/6	9.4	10.6	856.1	10.2
S2	23/6	8.0	8.9	1236.3	9.1	S2	20/6	10.3	11.2	908.3	12.5
S3	23/6	8.0	9.2	1272.0	9.3	S3	20/6	10.6	11.4	1045.5	9.5
S1	26/6	8.6	11.2	1158.6	6.0	S1	29/6	10.2	11.7	859.4	5.7
S2	26/6	8.6	10.7	1172.3	7.4	S2	29/6	7.9	10.2	692.3	4.6
S3	26/6	9.0	10.6	1209.9	6.7	S3	29/6	9.2	10.7	830.1	5.9

G 7: Descriptive statistics of G 6

Mean, SD and COV of the samples (G 6) from Skiernievice, Poland.
Code: M: 'Mieze Schindler', R: 'Roxana', S: 'Senga Sengana', numbers: 1st, 2nd and 3rd picking.

	DM [%]			Brix [%]			Citric acid [mg/ml]			Average fruit weight [g]		
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
M1	12.6	0.5	4.2	11.0	0.3	2.9	1021.6	139.1	13.6	10.0	1.0	10.0
M2	11.9	0.4	3.2	10.8	1.4	13.1	1116.5	271.0	24.3	8.3	1.8	21.7
M3	12.8	0.5	4.2	10.7	0.5	4.9	1146.3	39.8	3.5	6.1	0.7	11.0
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
R1	10.6	0.5	5.0	9.6	0.5	4.8	1026.7	50.1	4.9	31.5	4.0	12.7
R2	9.6	0.3	2.8	8.7	0.1	1.3	1071.9	20.1	1.9	26.3	9.8	37.3
R3	8.8	0.5	6.0	7.7	0.5	6.5	1001.4	23.3	2.3	23.5	3.1	13.4
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
S1	9.9	0.2	2.1	8.3	0.2	2.1	1106.7	89.6	8.1	11.3	1.7	15.0
S2	9.2	0.2	2.7	7.8	0.3	3.7	1237.8	33.5	2.7	9.0	0.4	4.5
S3	10.9	0.3	3.1	8.7	0.2	2.6	1180.3	26.6	2.3	6.7	0.7	10.7

Mean, SD and COV of the samples (G 7) from Vienna, Austria.
Code: M: 'Mieze Schindler', R: 'Roxana', S: 'Senga Sengana', numbers: 1st, 2nd and 3rd picking.

	DM [%]			Brix [%]			Citric acid [mg/ml]			Average fruit weight [g]		
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
M1	12.0	0.5	4.3	11.2	0.1	1.3	905.5	0.8	0.1	12.4	0.5	4.2
M2	11.7	0.7	5.5	11.0	0.4	3.5	1055.8	108.6	10.3	7.6	1.0	13.1
M3	11.4	0.5	4.2	9.6	0.2	1.8	1098.9	79.2	7.2	6.4	1.5	22.7
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
R1	9.3			8.5			969.3			25.4		
R2	9.6	0.4	4.1	8.9	0.7	7.5	964.6	92.9	9.6	24.9	1.1	4.5
R3	9.9	1.5	14.8	8.8	1.7	18.9	1020.7	98.3	9.6	14.1	1.0	7.0
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
S1	11.3	0.7	6.6	9.8	0.8	8.1	954.3	58.4	6.1	13.5	0.4	3.2
S2	11.1	0.4	3.9	10.1	0.6	6.2	936.6	97.8	10.4	10.7	1.6	14.6
S3	10.9	0.8	7.2	9.1	1.2	12.7	793.9	89.2	11.2	5.4	0.7	12.7

Mean, SD and COV of the samples (Fx) from Dresden, Germany.
Code: M: 'Mieze Schindler', R: 'Roxana', S: 'Senga Sengana', numbers: 1st, 2nd and 3rd picking.

	DM [%]			Brix [%]			Citric acid [mg/ml]			Average fruit weight [g]		
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
M1	12.2	0.5	3.8	11.2	0.5	4.4	1132.7	6.0	0.5	8.8	1.2	13.5
M2	11.6	0.3	2.9	9.9	0.4	4.3	1000.4	35.9	3.6	8.9	0.1	1.7
M3	11.4	0.2	1.8	9.9	0.1	1.2	939.2	70.6	7.5	6.1	0.4	6.2
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
R1	9.9	0.5	5.4	8.7	0.1	1.6	846.4	57.8	6.8	39.4	3.0	7.7
R2	9.5	0.4	4.5	8.3	0.4	4.8	900.5	43.9	4.9	27.5	2.4	8.8
R3	9.8	0.8	7.6	9.1	0.8	8.3	919.5	60.1	6.5	22.6	1.0	4.5

Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
S1	11.7	0.4	3.4	10.1	0.1	0.6	888.4	40.4	4.6	15.2	1.8	11.8
S2	10.0	0.3	2.8	9.2	0.8	8.3	946.3	80.8	8.5	11.6	0.8	6.6
S3	10.4	0.4	3.6	8.6	0.3	3.1	958.8	38.9	4.1	11.4	0.5	4.7

Mean, SD and COV of the samples (Fx) from Geisenheim, Austria.
Code: M: 'Mieze Schindler', R: 'Roxana', S: 'Senga Sengana', numbers: 1st, 2nd and 3rd picking.

	DM [%]			Brix [%]			Citric acid [mg/ml]			Average fruit weight [g]		
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
M1	12.7	0.5	3.7	11.8	0.3	2.7	1113.5	47.1	4.2	7.0	0.3	4.8
M2	12.8	0.1	0.7	11.4	0.1	0.9	1126.8	37.8	3.4	6.5	0.7	11.4
M3	12.1	0.3	2.4	11.5	0.6	5.2	980.7	96.6	9.8	6.3	0.4	7.1
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
R1	10.6	0.3	2.9	9.8	0.3	2.6	975.8	32.9	3.4	33.1	9.6	28.9
R2	10.9	0.4	3.6	9.9	0.8	8.5	931.4	57.2	6.1	26.7	6.2	23.3
R3	11.0	1.1	10.3	10.0	1.0	10.0	933.9	32.9	3.5	23.4	3.2	13.7
Code	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV	Mean	SD	COV
S1	10.8	0.4	3.3	9.4	0.3	3.1	1005.1	43.9	4.4	9.8	0.5	4.9
S2	10.0	0.4	4.0	8.9	0.3	3.0	1011.1	41.3	4.1	9.3	0.6	6.6
S3	11.0	0.3	3.1	9.3	0.5	4.9	936.0	42.0	4.5	8.2	1.9	23.3

G 8: GLM's of C 2.1.2.3.

General Linear Model: DM [%] vs. cultivar, picking, location, block

```
Factor           Type    Levels  Values
Cultivar         fixed        3  'Mieze Schindler', 'Roxana', 'Senga Sengana'
Picking          fixed        3  1, 2, 3
Location         random       4  Dresden, Geisenheim, Skiernievice, Vienna
Block(Location)  random      12  1, 2, 3, 1, 2, 3, 1, 2, 3, 1, 2, 3
```

```
Analysis of Variance for DM [%], using Adjusted SS for Tests

Source                     DF    Seq SS   Adj SS   Adj MS      F      P
Cultivar                    2   82.5130  80.6312  40.3156  18.77  0.003 x
Picking                     2    5.3905   3.7990   1.8995   3.77  0.086 x
Location                    3    6.3575   6.5500   2.1833   1.26  0.415 x
Block(Location)             8    2.1127   1.9951   0.2494   0.84  0.574
Cultivar*Picking            4    2.3458   2.5926   0.6482   0.74  0.583 x
Cultivar*Location           6   12.6917  13.0250   2.1708   2.47  0.086 x
Picking*Location            6    2.9194   3.0440   0.5073   0.58  0.743 x
Cultivar*Picking*Location  12   10.6446  10.6446   0.8871   2.98  0.003
Error                      61   18.1833  18.1833   0.2981
Total                     104  143.1585

x Not an exact F-test.

S = 0.545973   R-Sq = 87.30%   R-Sq(adj) = 78.34%
```

General Linear Model: Brix [%] vs. cultivar, picking, location, block

```
Factor           Type    Levels  Values
Cultivar         fixed        3  'Mieze Schindler', 'Roxana', 'Senga Sengana'
Picking          fixed        3  1, 2, 3
Location         random       4  Dresden, Geisenheim, Skiernievice, Vienna
Block(Location)  random      12  1, 2, 3, 1, 2, 3, 1, 2, 3, 1, 2, 3
```

Analysis of Variance for Brix [%], using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P	
Cultivar	**2**	**68.2196**	**66.2263**	**33.1132**	**18.05**	**0.003**	**x**
Picking	2	5.9545	4.6922	2.3461	3.22	0.111	x
Location	3	13.4887	12.8247	4.2749	2.55	0.193	x
Block(Location)	8	2.9364	2.0203	0.2525	0.66	0.728	
Cultivar*Picking	4	0.6982	0.8854	0.2213	0.29	0.877	x
Cultivar*Location	6	11.0558	11.1006	1.8501	2.45	0.087	x
Picking*Location	6	4.5232	4.3972	0.7329	0.97	0.486	x
Cultivar*Picking*Location	**12**	**9.1276**	**9.1276**	**0.7606**	**1.97**	**0.044**	
Error	57	21.9647	21.9647	0.3853			
Total	100	137.9687					

x Not an exact F-test.

S = 0.620762 R-Sq = 84.08% R-Sq(adj) = 72.07%

General Linear Model: Citric acid [mg/ml] vs. cultivar, picking, location, block

Factor	Type	Levels	Values
Cultivar	fixed	3	'Mieze Schindler', 'Roxana', 'Senga Sengana'
Picking	fixed	3	1, 2, 3
Location	random	4	Dresden, Geisenheim, Skiernievice, Vienna
Block(Location)	random	12	1, 2, 3, 1, 2, 3, 1, 2, 3, 1, 2, 3

Analysis of Variance for Acid [mg/ml], using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P	
Cultivar	2	130901	128131	64066	2.46	0.166	x
Picking	2	21254	19759	9880	1.02	0.414	x
Location	3	372866	334584	111528	5.83	0.118	x
Block(Location)	8	25279	33317	4165	0.75	0.646	
Cultivar*Picking	4	14325	13787	3447	0.23	0.919	x
Cultivar*Location	6	168433	157792	26299	1.71	0.201	x
Picking*Location	6	54948	58413	9736	0.63	0.702	x
Cultivar*Picking*Location	**12**	**185739**	**185739**	**15478**	**2.79**	**0.005**	
Error	57	315920	315920	5542			
Total	100	1289666					

x Not an exact F-test.

S = 74.4477 R-Sq = 75.50% R-Sq(adj) = 57.02%

General Linear Model: Fruit weight [g] vs. cultivar, picking, location, block

Factor	Type	Levels	Values
Cultivar	fixed	3	'Mieze Schindler', 'Roxana', 'Senga Sengana'
Picking	fixed	3	1, 2, 3
Location	random	4	Dresden, Geisenheim, Skiernievice, Vienna
Block(Location)	random	12	1, 2, 3, 1, 2, 3, 1, 2, 3, 1, 2, 3

Analysis of Variance for Fruit weight [g], using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P	
Cultivar	**2**	**7238.74**	**6599.81**	**3299.91**	**93.43**	**0.000**	**x**
Picking	**2**	**764.61**	**668.44**	**334.22**	**25.00**	**0.001**	**x**
Location	3	151.57	151.10	50.37	1.28	0.362	x

Block(Location)	8	75.04	73.44	9.18	1.04	0.418
Cultivar*Picking	**4**	**303.55**	**197.04**	**49.26**	**4.99**	**0.013 x**
Cultivar*Location	**6**	**201.17**	**213.85**	**35.64**	**3.61**	**0.027 x**
Picking*Location	6	88.69	80.65	13.44	1.36	0.305 x
Cultivar*Picking*Location	12	118.75	118.75	9.90	1.12	0.362
Error	61	539.33	539.33	8.84		
Total	104	9481.46				

x Not an exact F-test.

S = 2.97346 R-Sq = 94.31% R-Sq(adj) = 90.30%

G 9: ANOVAS for C 2.1.2.3

One-way ANOVA: For location Skiernievice

Source	DF	SS	MS	F	P
Code	2	13.687	6.843	11.62	0.009
Error	6	3.533	0.589		
Total	8	17.220			

S = 0.7674 R-Sq = 79.48% R-Sq(adj) = 72.64%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
Mieze Schindler	3	12.433	0.473	(------*------)
Roxana	3	9.667	0.902	(------*-------)
Senga Sengana	3	10.000	0.854	(-------*------)

9.0 10.5 12.0 13.5

Pooled StDev = 0.767

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Code

Individual confidence level = 97.80%

Code = Mieze Schindler subtracted from:

Code	Lower	Center	Upper
Roxana	-4.6895	-2.7667	-0.8438
Senga Sengana	-4.3562	-2.4333	-0.5105

Code
Roxana (--------*---------)
Senga Sengana (---------*--------)
-4.0 -2.0 0.0 2.0

Code = Roxana subtracted from:

Code	Lower	Center	Upper
Senga Sengana	-1.5895	0.3333	2.2562

Code
Senga Sengana (---------*--------)
-4.0 -2.0 0.0 2.0

One-way ANOVA: For location Vienna

```
Source  DF      SS      MS      F      P
Code     2  7.0200  3.5100  47.86  0.000
Error    6  0.4400  0.0733
Total    8  7.4600

S = 0.2708   R-Sq = 94.10%   R-Sq(adj) = 92.14%

                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level            N    Mean  StDev  -----+---------+---------+---------+----
Mieze Schindler  3  11.700  0.300                           (----*----)
Roxana           3   9.600  0.300  (----*----)
Senga Sengana    3  11.100  0.200                    (----*----)
                                    -----+---------+---------+---------+----
                                       9.60     10.40     11.20     12.00
Pooled StDev = 0.271

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Code

Individual confidence level = 97.80%

Code = Mieze Schindler subtracted from:

Code             Lower   Center    Upper
Roxana         -2.7785  -2.1000  -1.4215
Senga Sengana  -1.2785  -0.6000   0.0785

Code           ---------+---------+---------+---------+
Roxana         (----*----)
Senga Sengana            (----*----)
               ---------+---------+---------+---------+
                     -1.5       0.0       1.5       3.0

Code = Roxana subtracted from:

Code            Lower  Center   Upper  ---------+---------+---------+---------+
Senga Sengana  0.8215  1.5000  2.1785                          (----*----)
                                       ---------+---------+---------+---------+
                                             -1.5       0.0       1.5       3.0
```

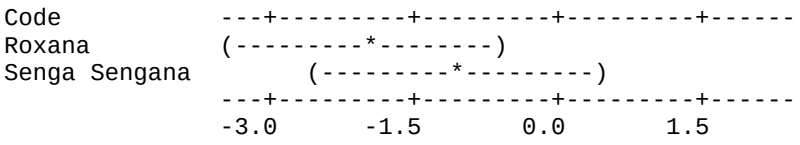
One-way ANOVA: For the location Geisenheim

```
Source  DF     SS     MS      F      P
Code     2  6.682  3.341  21.48  0.002
Error    6  0.933  0.156
Total    8  7.616

S = 0.3944   R-Sq = 87.74%   R-Sq(adj) = 83.66%

                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level            N    Mean  StDev  ----+---------+---------+---------+-----
Mieze Schindler  3  12.533  0.379                          (------*------)
Roxana           3  10.833  0.208    (------*------)
Senga Sengana    3  10.600  0.529  (-----*------)
                                    ----+---------+---------+---------+-----
                                     10.40     11.20     12.00     12.80
Pooled StDev = 0.394
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Code

Individual confidence level = 97.80%

Code = Mieze Schindler subtracted from:

Code             Lower   Center    Upper
Roxana         -2.6883  -1.7000  -0.7117
Senga Sengana  -2.9216  -1.9333  -0.9451

Code           ----+---------+---------+---------+-----
Roxana           (-------*-------)
Senga Sengana  (-------*-------)
               ----+---------+---------+---------+-----
                -2.4      -1.2       0.0       1.2

Code = Roxana subtracted from:

Code             Lower   Center   Upper
Senga Sengana  -1.2216  -0.2333  0.7549

Code           ----+---------+---------+---------+-----
Senga Sengana               (-------*-------)
               ----+---------+---------+---------+-----
                -2.4      -1.2       0.0       1.2
```

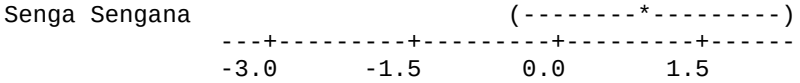
One-way ANOVA: For the location Dresden

```
Source  DF     SS     MS     F      P
Code     2  6.002  3.001  8.94  0.016
Error    6  2.013  0.336
Total    8  8.016

S = 0.5793   R-Sq = 74.88%   R-Sq(adj) = 66.51%

                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level            N    Mean  StDev   -+---------+---------+---------+--------
Mieze Schindler  3  11.733  0.416                      (-------*--------)
Roxana           3   9.733  0.208   (-------*--------)
Senga Sengana    3  10.700  0.889            (-------*-------)
                                   -+---------+---------+---------+--------
                                  9.0      10.0      11.0      12.0
Pooled StDev = 0.579

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Code

Individual confidence level = 97.80%

Code = Mieze Schindler subtracted from:

Code             Lower   Center    Upper
Roxana         -3.4515  -2.0000  -0.5485
Senga Sengana  -2.4848  -1.0333   0.4181
```



Code = Roxana subtracted from:

Code	Lower	Center	Upper
Senga Sengana	-0.4848	0.9667	2.4181



G 10: Achenes proportion

		Fruit no.				Average achene proportion [%]
		1	2	3	SD	
Korona	Fruit [g]	2.1913	2.0856	1.9987		
	Achenes [g]	0.1787	0.171	0.1619		
	Achenes [%]	8.15	8.20	8.10	0.0	8.2
Ciflorette	Fruit [g]	1.5879	1.4895	1.6431		
	Achenes [g]	0.1485	0.1422	0.1587		
	Achenes [%]	9.35	9.55	9.66	0.2	9.5
97/369	Fruit [g]	1.1976	1.3765	1.1416		
	Achenes [g]	0.1207	0.1404	0.1154		
	Achenes [%]	10.08	10.20	10.11	0.1	10.1
Roxana	Fruit [g]	1.9204	1.8976	1.4321		
	Achenes [g]	0.1949	0.1977	0.1479		
	Achenes [%]	10.15	10.42	10.33	0.1	10.3

G 11: Data of seedlings of D 1.7.

Average percentages and SD of sugars, citric acid and DM of one selected seedling with high and one with

Seedling number		Sugar [%] DM				Citric acid [%] DM	Residues [%] DM	DM [%]
		Fructose	Glucose	Sucrose	Total			
12/87	Mean	**22.2**	**21.6**	**28.2**	**72.0**	**5.6**	**22.4**	**13.5**
	SD	0.5	0.6	0.6	1.7	0.0		
12/84	Mean	**19.5**	**17.8**	**20.0**	**57.3**	**7.5**	**35.2**	**8.8**
	SD	0.5	0.5	0.7	1.8	0.1		
13/105	Mean	**27.6**	**26.9**	**16.2**	**70.7**	**7.3**	**22**	**13.2**
	SD	0.2	0.1	0.6	0.8	0.1		
13/15	Mean	**21.3**	**18.9**	**14.1**	**54.3**	**7.8**	**37.9**	**12.1**
	SD	0.1	0.2	0.6	0.7	0.1		
14/156	Mean	**24.3**	**23.5**	**13.1**	**60.9**	**5.7**	**33.4**	**12.5**
	SD	0.0	0.0	0.6	0.6	0.1		
14/105	Mean	**22.3**	**21.3**	**10.6**	**54.2**	**7.3**	**38.5**	**10.2**
	SD	0.4	0.1	0.4	0.7	0.0		
15/99	Mean	**32.7**	**29.5**	**19.4**	**81.6**	**7.5**	**10.9**	**11.2**
	SD	0.4	0.5	0.2	1.1	0.1		
15/164	Mean	**24.4**	**21.9**	**15.3**	**61.6**	**7.7**	**30.7**	**9.0**

	SD	0.5	1.1	0.4	1.6	0.1		
16/114	Mean	**21.5**	**19.4**	**15.5**	**56.4**	**6.9**	**36.7**	**11.9**
	SD	0.3	0.2	0.8	0.4	0.1		
16/3	Mean	**19.4**	**17.8**	**16.3**	**53.5**	**9.1**	**37.4**	**8.8**
	SD	0.3	0.1	0.4	0.0	0.1		
17/42	Mean	**10.3**	**9.7**	**11.8**	**31.8**	**5.6**	**62.6**	**13.9**
	SD	0.1	0.1	0.3	0.6	0.1		
17/62	Mean	**19.1**	**16.9**	**6.7**	**42.7**	**7.9**	**49.4**	**8.9**
	SD	0.4	0.4	0.2	0.9	0.1		
18/24	Mean	**21.0**	**19.5**	**14.4**	**54.9**	**7.8**	**37.3**	**14.0**
	SD	0.6	0.5	0.1	1.1	0.0		
18/49	Mean	**17.7**	**16.5**	**24.9**	**59.1**	**7.4**	**33.5**	**10.1**
	SD	0.6	0.7	0.3	1.4	0.0		
19/109	Mean	**28.0**	**27.1**	**5.1**	**60.2**	**6.2**	**33.6**	**12.9**
	SD	0.4	0.3	0.4	0.6	0.1		
19/35	Mean	**13.9**	**12.9**	**6.8**	**33.6**	**7.5**	**58.9**	**10.1**
	SD	1.8	0.2	0.1	1.9	0.0		

G 12: Data of D 2.1.1.

	DM [%]			
	Picking			
Genotype investigated 2004	1st	2nd	3rd	Mean
JH 11/2	7.5	7.4		7.5
Elsinore	7.9			7.9
Alba	9.5	9.0		9.3
Marianna	9.4	9.1	9.5	9.3
Roxana	9.7	9.6	8.8	9.4
Senga Sengana	9.3	9.3	9.8	9.5
Korona	10.2	9.8	9.2	9.7
Prelude	10.2	10.5	8.8	9.8
Cirofine	10.3	9.2	10.2	9.9
Dr. Hanke	10.7	9.8	9.3	9.9
Fraroma	10.2			10.2
Honeoye	10.6	10.2	10.0	10.3
Florence	10.5	10.3	10.2	10.3
JH 11/3	10.4	10.3		10.4
Karmen	10.6	10.2		10.4
Simida	10.6			10.6
JG 3/5		11.1	10.2	10.7
P-315	10.5	10.0	11.5	10.7
Elsanta	11.4	10.0		10.7
Yamaska	11.3	10.3	10.6	10.7
P-303	0.0	10.3	11.6	11.0
Polka	11.4	10.5		11.0
Malling Pandora	10.5	11.3	11.2	11.0
P-311		11.2	10.8	11.0
Premial	11.4	10.9	10.9	11.1
P-310		10.9	11.3	11.1
P-323		11.1	11.2	11.2
G 1/1	11.6	10.6	11.5	11.2
G 1/26	11.4	11.2		11.3
G 1/20	11.4	11.3		11.4
P-312		11.6	11.3	11.5
JG 1/3	11.6	11.8		11.7

	DM [%]			
	Picking			
Genotype investigated 2005	1st	2nd	3rd	Mean
Kent	9.7	9.5		9.6
Alba	9.7	9.5		9.6
Queen Elisa	9.9			9.9
Honeoye	9.8	10.2	10.5	10.2
Addi	9.8	10.6		10.2
Elsanta	10.1	9.9	10.8	10.3
Senga Sengana	10.5	10.2	10.3	10.3
Korona	10.8	10.1	10.4	10.4
97/362		10.4	10.5	10.5
P-323	10.6	10.4		10.5
Carmen	11.4	10.5	9.8	10.6
Polka	10.6	10.8	10.8	10.7
Prelude	11.5	10.9	9.8	10.7
Hood	10.9	10.6		10.8
NZ-8	10.8			10.8
Cijosee	10.7	11.1		10.9
Marianna	10.7	11.1		10.9
P-315	11.4	10.7		11.1
Cilady	10.8	11.3		11.1
Sachsen	11.5	10.7		11.1
Totem	11.4	11.0		11.2
Chandler	11.7	11.1		11.4
Benton	11.9	11.8		11.9
Dresden	12.2	11.5		11.9
Cigoulette	12.1	11.6		11.9
Mieze Schindler	12.0	11.8		11.9
St. Pierre	11.8	12.2		12.0
F. moschata, Bauwens	12.3			12.3
JG 1/3	13.0	12.1	11.8	12.3
Sieger	12.0	12.9		12.5
Cirano	12.7	12.3		12.5
Cifrance	12.9	12.1		12.5

P-322		11.7	11.7	11.7
St. Pierre	11.7			11.7
Mieze Schindler	11.6	11.9	12.0	11.8
E 16/6	12.8	11.5	11.6	12.0
Pill.9	12.0	12.2	11.8	12.0
JG 3/3	12.2	11.2	12.7	12.0
97/362	12.6	12.4	12.1	12.4
97/369	13.3	11.6	12.4	12.4
Weisse Ananas	12.5	12.6		12.6
F. virginiana W-9	13.2	12.5	12.7	12.8
Ciflorette	13.1	13.1	12.7	13.0
D 7/19	13.5	12.8	12.8	13.0
D 3/2	13.8		12.3	13.1
D ¾	13.6	13.3	12.8	13.2
D 4/6	13.8	13.1		13.5
D 3/5	13.7	12.9	14.0	13.5
D 5/5	14.6	12.7	13.4	13.6
F. vesca 'Mignonette'	15.7			15.7
F. vesca 'Rügen'	16.8			16.8

D 3/4	13.0	12.2		12.6
Ciflorette	13.1	12.4		12.7
Markee	13.3	12.9		13.1
L'Oz du Rhin	14.2	13.5		13.9
Korbinskaya Rannyaya	14.5	14.9		14.7
Weisse Ananas	15.2	14.4		14.8
F. nilgerrensis	14.8			14.8
Asiropa	14.7	15.0		14.8
F. vesca 'Alba'	15.1			15.1
F.vesca ssp.*vesca*, Franken	16.6			16.6
F. viridis, Usolje	17.3			17.3
F. vesca 'Alexandria'	17.7			17.7
F. vesca 'Mignonette'	17.8			17.8
F. vesca 'Rügen'	18.4			18.4

	DM [%]			
	Picking			
Genotype investigated 2006	1st	2nd	3rd	Mean
Alba	8.5	8.2		8.4
NZ-4	8.5			8.5
Kent	9.3	8.9		9.1
Senga Sengana		9.7	8.7	9.2
Carmen	9.5	9.4		9.4
Elsanta	10.2	9.9	8.7	9.6
Elkat	9.7			9.7
Heros	9.7			9.7
Luna	9.8			9.8
Roxana	10.0	9.5	9.8	9.8
Polka	10.6	9.6	9.3	9.8
Maya	10.4	9.4		9.9
Spadeka (decaploid)	10.2	9.7		9.9
Mara de Bois	10.2	9.9		10.0
Honeoye	10.2	10.0		10.1
97/362	10.9	9.3	10.2	10.1
Dukat	11.3	10.0	9.1	10.1
Korona	10.8	10.3	9.6	10.2
Salute	10.3			10.3
Tufts	10.8		9.8	10.3
97/369	10.9	9.8		10.4
NZ-6	10.4			10.4
Fara	10.4			10.4
Karmen	10.9	10.2		10.6
Marianna	11.0	10.3		10.7
Hood	11.6	10.5	10.3	10.8
Asia	11.6	10.2		10.9
P-315	11.6	10.3	11.0	10.9
D ¾	10.1	10.8	12.0	11.0
Queen Elisa	11.1			11.1
P-323	12.6	9.7		11.2
Darselect	11.5	11.1	11.2	11.3
NZ-6	11.3			11.3

	DM [%]			
	Picking			
Genotype investigated 2006	1st	2nd	3rd	Mean
Segal	11.3			11.3
D 3/2	10.7	11.9		11.3
Totem	11.7	11.1		11.4
Gemma	11.5			11.5
JG 1/3	11.5			11.5
D 5/5	11.9	11.5	11.2	11.5
P-316	12.1	12.6	10.2	11.6
Mieze Schindler	12.2	11.6	11.4	11.7
Fraroma	12.2	11.3		11.8
Cirano	11.8	11.8		11.8
D 4/6	11.9	12.1	11.5	11.8
Clery	12.6	11.7	11.4	11.9
D 3/5	12.0	11.8		11.9
P320	12.4	11.1	12.3	11.9
Earlyglow	9.8		14.1	12.0
Dresden	12.4	12.5		12.4
Benton	12.8	13.0	11.8	12.5
D 7/19	12.8			12.8
Ciflorette	14.1	13.3	12.4	13.3
Weiße Ananas	13.9	14.6		14.3
Asiropa	14.9			14.9
F. viridis, Usolje	14.9			14.9
F. virginiana W-9	15.2			15.2

class	Mean
very low	≤9.1
low	9.2 - 10.5
intermediat	10.6 - 11.9
high	12.0 - 13.2
very high	≥13.3

G 13: Brix [%], firmness [g/mm], citric acid [mg/ml], average fruit weight [g] data of D 2.1.2.1.

Brix [%]				
	Picking			
Genotype	1st	2nd	3rd	Mean
97/362			10.2	10.2
97/369	9.4		10	9.7
Alba	7.8	7.3		7.6
Ciflorette	11.3	10.9	11.2	11.1
Cirofine	8.5	9.8	8.4	8.9
D3/2				
D3/4	11.6		11	11.3
D3/5	11.4		11.3	11.4
D4/6	11.6	11		11.3
D5/5	11.4	11.8	11.1	11.4
D7/19	9.7	10.2	11.5	10.5
Dr. Hanke	9.1	8.5	8.2	8.6
E16/6	10.3	9.3	9.5	9.7
Elsanta	9.9	8.5	9	9.1
Elsinore	6.1			6.1
Florence	8.7	8	9.3	8.7
Fraroma	9.4			9.4
G 1/1	9.7	8.8		9.3
G 1/20	9.6	9.1		9.4
G 1/26	10	8.8		9.4
Honeoye	8.9	8.3	8.2	8.5
JH 11/2				
JH 11/3	9.2	8.3		8.8
JG 1/3	10.3	10.1		10.2
JG 3/3	11.4	10.4	10	10.6
JG 3/5	9.4	8.5	8.1	8.7
Karmen	9.1	8.7		8.9
Korona	8.8	8	7.4	8.1
Malling Pandora	9.1	9.2	9.7	9.3
Marianna	8.1	8.3	7.6	8.0
Mieze Schindler	10.4		10.5	10.5
P-303	10.1	9.3		9.7
P-310	9.5	8.7	8.8	9.0
P-311	9.4			9.4
P-312	9.6	10.1	9.5	9.7
P-315	8.6	7.9	7.8	8.1
P-322	9.5			9.5
P-323	8	8	8.1	8.0
Pill.9	11	10.5	10.1	10.5
Polka	9.6	9.9	9.6	9.7
Prelude	8.7	7.7	7.4	7.9
Premial	9.5	8.8	9.3	9.2
Roxana	8	8.5	7.6	8.0
Senga Sengana	8	8.2	7.2	7.8
Simida	8.8			8.8
St. Pierre	9.8			9.8
Weisse Ananas		9.9		9.9
Yamaska	9.4	9.2	8.5	9.0

Firmness [g/mm]				
	Picking			
Genotype	1st	2nd	3rd	Mean
97/362	259.1	227.6	221.4	236.0
97/369	195.8	203.7	183.3	194.2
Alba	312.7	303.2		308.0
Ciflorette	257.7	269.9		263.8
Cirofine	272.8	297.8		285.3
D3/2	122.0			122.0
D3/4	187.4	143.1	176.5	169.0
D3/5	185.7	215.9	155.1	185.6
D4/6	168.3	147.3		157.8
D5/5	163.4	185.9	167.0	172.1
D7/19	252.2	212.4	209.5	224.7
Dr. Hanke	240.7	240.2	260.7	247.2
E16/6	263.1	325.4	304.5	297.7
Elsanta	193.2	215.8	224.6	211.2
Elsinore	307.6			307.6
Florence	201.3	291.3	307.9	266.8
Fraroma	218.5			218.5
G 1/1	312.8	281.1	267.8	287.2
G 1/20	253.5	254.3		253.9
G 1/26	235.6	242.3		239.0
Honeoye	240.0	277.7	259.5	259.1
JH 11/2				
JH 11/3	213.3	237.0		225.2
JG 1/3	190.8	261.7		226.3
JG 3/3	283.4	257.6	256.3	265.8
JG 3/5	377.8	321.8	341.5	347.0
Karmen	212.6	203.4	222.3	212.8
Korona	275.3	232.3	237.6	248.4
Malling Pandora	275.6	265.8	291.1	277.5
Marianna	295.5	341.8	299.2	312.2
Mieze Schindler	146.7	147.0	160.5	151.4
P-303	146.4	132.9		139.7
P-310		121.8	112.8	117.3
P-311	151.2	156.4	160.4	156.0
P-312	128.4	125.9	126.7	127.0
P-315		128.5	142.1	135.3
P-322	148.6	125.3	162.8	145.6
P-323		119.3	104.7	112.0
Pill.9	243.4	284.3	259.5	262.4
Polka	236.3	240.1	213.3	229.9
Prelude	283.2	309.1	231.5	274.6
Premial	234.8	225.4	215.9	225.4
Roxana	281.1	289.0	280.0	283.4
Senga Sengana	204.3	202.1	227.7	211.3
Simida	256.5			256.5
St. Pierre	337.9			337.9
Weisse Ananas	109.0	106.0	107.4	107.5
Yamaska	298.8	319.5	322.1	313.5

Citric acid [mg/ml]				
	Picking			
Genotype	1st	2nd	3rd	Mean
97/362			975.5	975.5
97/369	1005.7		976.3	991.0
Alba	927.7	909.0		918.4
Ciflorette	927.1	885.4	991.9	934.8
Cirofine	930.6	928.7	862.9	907.4
D3/2	1301.1			1301.1
D3/4			938.3	938.3
D3/5			993.0	993.0
D4/6	1102.7	1361.0		1231.9
D5/5	1264.8	1256.6	1377.4	1299.6
D7/19	801.9	886.0	806.3	831.4
Dr. Hanke	1017.0	948.5	1042.4	1002.6
E16/6	911.1	787.2	936.1	878.1
Elsanta	979.9	979.7	1012.2	990.6
Elsinore	723.0			723.0
Florence	1003.4	883.9	958.4	948.6
Fraroma	1013.2			1013.2
G 1/1	987.1	1085.5		1036.3
G 1/20	730.3	844.0		787.2
G 1/26	908.2	1002.9		955.6
Honeoye	963.8	949.1	1033.2	982.0
JH 11/2				
JH 11/3	763.7	768.7		766.2
JG 1/3	874.0	992.4		933.2
JG 3/3	940.7	1056.3	1158.0	1051.7
JG 3/5	881.6	855.6	976.9	904.7
Karmen	1037.8	1033.5		1035.7
Korona	864.3	873.2	802.3	846.6
Malling	1054.8	989.6	1028.9	1024.4
Marianna	992.9	952.0	929.6	958.2
Mieze	952.0		901.4	926.7
P-303	1047.3	1220.3		1133.8
P-310	1139.7	1121.4	1108.6	1123.2
P-311	1210.1			1210.1
P-312	1250.2	1296.2	1382.2	1309.5
P-315	1522.2	1549.4	1513.7	1528.4
P-322	1150.4			1150.4
P-323	1613.2	1492.0	1472.3	1525.8
Pill.9	883.9	849.0	1004.8	912.6
Polka	1019.4	1000.1	1167.1	1062.2
Prelude	1289.9	1274.0	1055.7	1206.5
Premial	1066.8	1037.8	1057.9	1054.2
Roxana	900.3	996.7	908.3	935.1
Senga	983.0	1106.4	1023.2	1037.5
Simida	772.3			772.3
St. Pierre	1032.4			1032.4
Weisse Ananas		1177.9		1177.9
Yamaska	928.9	1105.7	964.8	999.8

Average fruit weight [g]				
	Picking			
Genotype	1st	2nd	3rd	Mean
97/362	25.7	27.0	26.0	26.2
97/369	16.5	13.7	18.0	16.1
Alba	22.4	17.5		20.0
Ciflorette	15.4	14.9	9.0	13.1
Cirofine	16.0	13.0	12.7	13.9
D3/2	13.7			13.7
D3/4	17.4	16.9	14.6	16.3
D3/5	18.1	16.6	16.0	16.9
D4/6	19.2			19.2
D5/5	14.3	13.2	13.4	13.6
D7/19	17.1	15.2	13.2	15.2
Dr. Hanke	23.3	17.4	12.9	17.9
E16/6	20.1	18.3	14.2	17.5
Elsanta	30.6	22.6	20.6	24.6
Elsinore				
Florence	25.4	30.1	28.4	27.9
Fraroma	21.8			21.8
G 1/1	25.4	18.2	22.6	22.1
G 1/20	24.9	14.7	10.0	16.5
G 1/26	30.4	23.3	18.8	24.2
Honeoye	23.9	28.7	20.9	24.5
JH 11/2				
JH 11/3	18.2	16.5		17.4
JG 1/3	22.3	18.0	13.8	18.0
JG 3/3	24.6	19.3	17.5	20.5
JG 3/5	27.3	18.8	14.9	20.3
Karmen	27.9	19.6	15.4	20.9
Korona	35.7	24.1	18.4	26.1
Malling Pandora	24.6	20.6	19.1	21.4
Marianna	15.8	12.3		14.1
Mieze Schindler	16.8	14.1	11.7	14.2
P-303	6.8	7.7		7.3
P-310	9.2	9.2	9.2	9.2
P-311	5.8	6.2	5.6	5.9
P-312	7.0	5.5	5.3	5.9
P-315	6.1	4.5	3.8	4.8
P-322	10.9	10.0	8.3	9.7
P-323	5.4	5.2	5.1	5.2
Pill.9	26.0	22.3	14.4	20.9
Polka	27.5	20.4	20.0	22.7
Prelude	8.2	8.7	9.9	9.0
Premial	23.1	16.5	14.6	18.1
Roxana	36.4	27.5	22.1	28.7
Senga Sengana	13.3	12.5	10.1	12.0
Simida				
St. Pierre				
Weisse Ananas	10.1	7.0	6.2	7.8
Yamaska	33.5	27.1	23.3	27.9

G 14: ANOVA and FISHER's comparison test of D 2.2.1.
One-way ANOVA: Low DM selection, High DM selection, Population DM [%] 06_1

```
Source   DF      SS     MS     F      P
Factor    2   20.36  10.18  6.12  0.003
Error   114  189.58   1.66
Total   116  209.94

S = 1.290   R-Sq = 9.70%   R-Sq(adj) = 8.11%

                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level              N    Mean  StDev   -+---------+---------+---------+--------
Low DM selection  18   8.933  1.079   (--------*-------)
High DM selectio  21  10.376  1.413                      (-------*-------)
DM [%]06_1        78   9.794  1.298                 (---*---)
                                     -+---------+---------+---------+--------
                                    8.40      9.10      9.80     10.50
Pooled StDev = 1.290

Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons

Simultaneous confidence level = 87.84%

Low DM selection subtracted from:

                  Lower  Center  Upper
High DM selectio  0.622   1.443  2.263
DM [%]06_1        0.192   0.860  1.528

                   --+---------+---------+---------+-------
High DM selectio                    (-------*--------)
DM [%]06_1                      (------*-----)
                   --+---------+---------+---------+-------
                  -1.0       0.0       1.0       2.0

High DM selection subtracted from:

             Lower  Center  Upper   --+---------+---------+---------+-------
DM [%]06_1  -1.211  -0.583  0.045   (-----*-----)
                                     --+---------+---------+---------+-------
                                   -1.0       0.0       1.0       2.0
```

G 15: ANOVA and FISHER's pairwise comparison of D 2.2.

One-way ANOVA: Populations: 12 – 19 (DM [%])

```
Source   DF       SS     MS      F      P
Factor    7   364.41  52.06  22.09  0.000
Error   806  1899.62   2.36
Total   813  2264.03

S = 1.535   R-Sq = 16.10%   R-Sq(adj) = 15.37%

                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level    N    Mean  StDev  -----+---------+---------+---------+----
12      82  13.118  1.897                              (---*----)
13      55  13.147  1.473                             (-----*-----)
```

```
14     103  11.956  1.538              (---*---)
15     152  12.033  1.286               (---*--)
16     118  11.187  1.362   (---*---)
17     119  11.142  1.645  (---*---)
18      93  11.956  1.392            (----*---)
19      92  12.504  1.770                    (----*---)
                          -----+---------+---------+---------+----
                            11.20     11.90     12.60     13.30
Pooled StDev = 1.535

Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons

Simultaneous confidence level = 49.28%

12 subtracted from:

     Lower  Center   Upper  -------+---------+---------+---------+--
13  -0.496   0.029   0.554               (--*---)
14  -1.608  -1.162  -0.716        (--*--)
15  -1.498  -1.085  -0.672         (--*--)
16  -2.365  -1.931  -1.498   (--*--)
17  -2.408  -1.976  -1.543   (--*--)
18  -1.618  -1.162  -0.705        (--*--)
19  -1.071  -0.613  -0.156            (--*--)
                           -------+---------+---------+---------+--
                               -1.5       0.0       1.5       3.0
13 subtracted from:

     Lower  Center   Upper  -------+---------+---------+---------+--
14  -1.695  -1.191  -0.688        (--*--)
15  -1.588  -1.114  -0.640        (---*--)
16  -2.452  -1.960  -1.468   (--*--)
17  -2.496  -2.005  -1.513  (---*--)
18  -1.703  -1.191  -0.678        (--*--)
19  -1.156  -0.643  -0.129           (---*--)
                           -------+---------+---------+---------+--
                               -1.5       0.0       1.5       3.0
14 subtracted from:

     Lower  Center   Upper  -------+---------+---------+---------+--
15  -0.307   0.078   0.462                (--*-)
16  -1.175  -0.769  -0.363           (--*--)
17  -1.219  -0.813  -0.408           (--*-)
18  -0.430   0.001   0.432                (--*--)
19   0.117   0.549   0.981                    (--*--)
                           -------+---------+---------+---------+--
                               -1.5       0.0       1.5       3.0
15 subtracted from:

     Lower  Center   Upper  -------+---------+---------+---------+--
16  -1.216  -0.847  -0.477          (-*--)
17  -1.260  -0.891  -0.522          (-*--)
18  -0.474  -0.077   0.320               (-*--)
19   0.073   0.471   0.869                  (--*--)
                           -------+---------+---------+---------+--
                               -1.5       0.0       1.5       3.0
16 subtracted from:

     Lower  Center  Upper  -------+---------+---------+---------+--
17  -0.436  -0.044  0.347               (--*-)
18   0.352   0.770  1.187                    (--*--)
19   0.899   1.318  1.737                        (--*--)
```

```
                          -------+---------+---------+---------+--
                              -1.5       0.0       1.5       3.0
17 subtracted from:

    Lower  Center  Upper  -------+---------+---------+---------+--
18  0.397   0.814  1.231                    (-*--)
19  0.944   1.362  1.781                       (--*--)
                          -------+---------+---------+---------+--
                              -1.5       0.0       1.5       3.0
18 subtracted from:

    Lower  Center  Upper  -------+---------+---------+---------+--
19  0.105   0.548  0.991                     (--*--)
                          -------+---------+---------+---------+--
                              -1.5       0.0       1.5       3.0
```

G 16: KRUSKAL-WALLIS test of D 2.2.2.1.

Table x: KRUSKAL-WALLIS test on yield.

Kruskal-Wallis Test: yield [g] versus code

Population	N	Median	Ave Rank	Z
12	82	35.90	253.4	-6.24
13	55	32.10	246.5	-5.25
14	103	46.20	347.3	-2.76
15	152	77.55	519.3	6.54
16	118	58.00	427.4	1.02
17	118	70.05	483.4	3.82
18	93	57.90	417.4	0.45
19	92	55.10	386.4	-0.89
Overall	813		407.0	

H = 116.45 DF = 7 P = 0.000
H = 116.46 DF = 7 P = 0.000 (adjusted for ties)

G 17: 0-1-matrix of D 2.3.1.3. K: designated ‘Korona’, H: designated ‘Honeoye’, S: designated ‘Senga Sengana’, E: designated ‘Elsanta’. Green: confirmed paternal parent, Red: dissenting results. Yellow: No paternal bands.

Randomly chosen seedlings:

Multiplex:	MM1:						MP1:						MP2:						
Wavel.	800	800	800	800	700	700	700	800	800	800	800	800	700	700	700	700	700	800	800
Sample	K	H	KS	E	SE	E	E	SE	E	K	HS	KE	K	S	K	E	K	S	H
1											HS								H
3			KS		SE			SE			HS			S				S	
4			KS		SE			SE										S	
6			KS		SE			SE			HS			S					
8			KS												K				
9												KE	K		K		K		
11										K			K				K		
12			KS							K					K		K		
13	K		KS									KE	K				K		
15								SE						S					
16			KS									KE	K		K				
17	K		KS									KE	K		K		K		
18																			
19			KS					SE											
20			KS										K		K		K		

21	K												KE								
22																K					
23									SE						S					S	
24			KS						SE			HS								S	
25	K		KS																		
27		H														K	E				
28										E					S					S	
30			KS								K		KE					K			
31		H										HS									H
32											K			K				K			
33					SE							HS			S						
34																					H
36													KE					K			
37											K			K				K			
38													KE			K		K			
39																		K			
40	K										K			K		K					
41	K																				
42			KS													K		K			
43											K		KE					K			
44					SE				SE			HS									
45			KS		SE				SE			HS			S						
46			KS											K							
47			KS						SE						S					S	
48			KS								K		KE	K				K			
50															S						
51	K																	K			
52			KS													K					
53	K										K										
54			KS								K			K		K		K			
55			KS								K		KE					K			
63									SE						S						
66		H										HS									H
74	K										K		KE	K				K			
75			KS										KE					K			
79														K							
80					SE							HS									
81		H										HS									H
82	K		KS								K			K		K		K			
83									SE												
84	K		KS								K			K							
85			KS													K		K			
86																					
87			KS										KE					K			
88															S					S	
89									SE								E				
90	K		KS								K		KE			K					
91			KS								K										
92	K		KS								K							K			
93			KS															K			
94	K												KE					K			
95			KS								K					K		K			
96											K					K					
97													KE	K		K		K			
98											K							K			
99																					
100											K					K					
101											K		KE					K			
102			KS													K					
127																					
129														K		K					
130									SE												
131			KS		SE															S	
132			KS						SE				KE								
133				E	SE	E	E						KE				E				
134		H			SE									K			E				H
135	K													K		K		K			
136			KS									HS									
137			KS		SE						K		KE						S		
138			KS								K			K				K			
139											K					K					
140																K		K			
143											K		KE	K		K		K			
146											K		KE								
160					SE				SE			HS		K				K			
161			KS								K		KE	K		K		K			
162													KE	K		K		K			
163				E	SE	E			SE	E											

169	K		KS									KE	K						
174			KS										K		K		K		
176			KS		SE												K		
184								SE						S				S	
185					SE									S				S	
186														S				S	
195																			
199													K				K		
248																			
249										K			K		K		K		
250										K							K		
251	K		KS									KE	K						
252								SE			HS								
253					SE					K		KE						S	
254	K		KS							K		KE	K		K		K		
255								SE			HS			S				S	
256								SE						S					
257								SE			HS								

Selected seedlings:

Multiplex:	MM1:	MP1:	MP2:	

Wavel.	800	800	800	800	700	700	700	800	800	800	800	800	700	700	700	700	700	800	800
Sample	K	H	KS	E	SE	E	E	SE	E	K	HS	KE	K	S	K	E	K	S	H
4016			KS										K		K		K		
4017	K												K		K				
4018			KS							K									
4019											HS			S					
4020	K												K		K				
4021			KS										K		K		K		
4022	K		KS							K							K		
4023													K				K		
4024	K		KS										K						
4025			KS														K		
4026	K																K		
4027										K		KE	K				K		
4028			KS							K		KE							
4029										K			K		K		K		
4030										K		KE			K		K		
4031		H			SE						HS					E			
4032			KS							K		KE					K		
4033	K		KS									KE			K				
4034			KS							K		KE	K		K		K		
4035			KS												K		K		
4036		H			SE											E			H
4037								SE	E										
4038								K					K		K		K		
4039											HS			S				S	
4040								K									K		
4041	K																		
4042								K									K		
4043								K									K		

G 18: MOOD Median test of D 3.2.1.

Mood Median Test of DM vs. the selection methods. A-clones 2005.

```
Mood median test for dm
Chi-Square = 32.74    DF = 2    P = 0.000

                                  Individual 95.0% CIs
code  N<=  N>  Median  Q3-Q1  -------+---------+---------+---------
1       31   3   10.47   1.48  (-------*---)
2        9  12   11.60   1.40                    (--*----)
3       15  37   12.32   1.81                          (----*----)
                                  -------+---------+---------+---------
                                      10.40     11.20     12.00
```

G 19: DM [%] of B-selections selected 2005.

Selection type	Selection	12.06.06	14.06.06	16.06.06	19.06.06	21.06.06	23.06.06	Average DM [%]
Fresh-market	P-4020		10.6	10				10.3
Fresh-market	P-4021		11.9	11.7				11.8
Fresh-market	P-4022		11.2		10			10.6
Fresh-market	P-4023		10.1	9.4				9.8
Fresh-market	P-4024		10.4		9.8			10.1
Fresh-market	P-4033		11.1	9.4				10.3
Fresh-market	P-4035			9.9				9.9
Fresh-market	P-4046		10.1	10.3	10.5	9.6		10.1
Fresh-market	P-4047		12.4	11.4	10.8			11.5
Fresh-market	P-4056		11.9	11.2				11.6
Fresh-market	P-4072			11				11.0
Fresh-market	P-4078			9.9				9.9
Fresh-market	P-4099			11.3				11.3
Fresh-market	P-4107				9.3			9.3
Fresh-market	P-4177		11.4	11				11.2
Fresh-market	P-4180	9.5	10.3	10.9				10.2
Fresh-market	P-4184		11.9	10.3				11.1
Fresh-market	P-4186		10.4	9.6	8.7			9.6
Fresh-market	P-4189		11.8	10.9	9.6			10.8
Pre-selection	P-4082			11.7				11.7
Pre-selection	P-4045			10.7	10			10.4
Pre-selection	P-4049				11.4	10.4		10.9
Pre-selection	P-4064		11	11				11.0
Pre-selection	P-4079				10	8.8		9.4
Pre-selection	P-4080				12.2	11.7		12.0
Pre-selection	P-4108		12	11.1	10.6			11.2
Pre-selection	P-4127			12.7	11			11.9
Pre-selection	P-4149				12.8			12.8
Pre-selection	P-4150			13.1	11.3			12.2
Pre-selection	P-4156				14			14.0
Populations	P-4300			11.4	9.3			10.4
Populations	P-4301		12.2	12.2	10.6			11.7
Populations	P-4303				10.9		10.1	10.5
Populations	P-4304				10.1	9.2		9.7
Populations	P-4305			12.9	11.9			12.4
Populations	P-4306		11.2		10.2			10.7
Populations	P-4307		12.1	12.9	12.1			12.4
Populations	P-4309		11		9.4			10.2
Populations	P-4310						10.9	10.9
Populations	P-4311				9.3			9.3
Populations	P-4312				11.7	10.7		11.2
Populations	P-4313		12.3	13.7	12			12.7
Populations	P-4314		11.6	11.1	10			10.9
Populations	P-4315						9.3	9.3
Populations	P-4318			11.9	11.4			11.7
Populations	P-4319		12		10.2			11.1
Populations	P-4322			10.4	9.7			10.1
Populations	P-4323		11.8	11.3	10.5			11.2
Populations	P-4324		11.5	11	10			10.8
Populations	P-4325		12.3	12.4	11.9			12.2
Populations	P-4326			12.3	10.7			11.5
Populations	P-4327		12.6	12.6				12.6
Populations	P-4328		13	12.7	11.2			12.3
Populations	P-4330		11.8					11.8
Populations	P-4331		12	11.2	10.9			11.4

Populations	P-4332	12.1	13	11.7	11.8			12.2
Populations	P-4335				13.1	10	12.7	11.9
Populations	P-4337	11.8	12.1	12.2	12.5			12.2
Populations	P-4339		13.1	13.2	13.5			13.3
Populations	P-4343		12.1	11.1	11.5			11.6
Populations	P-4345	12.2	12.6	11.5	12			12.1
Populations	P-4347			11.6	11.4			11.5
Populations	P-4348	13.3	11.6	10.6				11.8
Populations	P-4350		11.1	10.2	11.2			10.8

G 20: ANOVA of D 3.3.2.

One-way ANOVA: Selection approach: DM [%]

```
Source  DF      SS     MS     F      P
Factor   2  12.027  6.014  6.97  0.002
Error   60  51.770  0.863
Total   62  63.797

S = 0.9289   R-Sq = 18.85%   R-Sq(adj) = 16.15%

                                      Individual 95% CIs For Mean Based on
                                      Pooled StDev
Level              N    Mean  StDev    -+---------+---------+---------+--------
Fresh-market sel  19  10.537  0.725    (------*------)
DM selection pre  11  11.618  1.240                  (---------*--------)
DM selection pop  33  11.430  0.917                   (-----*----)
                                      -+---------+---------+---------+--------
                                      10.20     10.80     11.40     12.00
Pooled StDev = 0.929

Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons

Simultaneous confidence level = 87.91%

Fresh-market selection subtracted from:

                   Lower  Center   Upper
DM selection pre  0.3774  1.0813  1.7853
DM selection pop  0.3584  0.8935  1.4286

                   --+---------+---------+---------+-------
DM selection pre                   (---------*----------)
DM selection pop                   (-------*------)
                   --+---------+---------+---------+-------
                  -0.70      0.00      0.70      1.40

DM selection (pre-selection) subtracted from:

                    Lower   Center   Upper
DM selection pop  -0.8348  -0.1879  0.4590

                   --+---------+---------+---------+-------
DM selection pop   (--------*---------)
                   --+---------+---------+---------+-------
                  -0.70      0.00      0.70      1.40
```

One-way ANOVA: Selection approach: Average fruit weight [g]

```
Source  DF      SS     MS      F      P
Factor   2   354.6  177.3  12.16  0.000
Error   60   875.2   14.6
Total   62  1229.9

S = 3.819   R-Sq = 28.84%   R-Sq(adj) = 26.46%


                                    Individual 95% CIs For Mean Based on
                                    Pooled StDev
Level              N    Mean  StDev  ------+---------+---------+---------+---
Fresh-market sel  19  17.705  4.687                      (------*------)
DM selection (pr  11  15.282  4.501         (--------*--------)
DM selection (po  33  12.348  2.943  (----*-----)
                                    ------+---------+---------+---------+---
                                       12.5      15.0      17.5      20.0

Pooled StDev = 3.819

Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons

Simultaneous confidence level = 87.91%

Fresh-market selection subtracted from:

                   Lower  Center   Upper
DM selection (pr  -5.318  -2.423   0.471
DM selection (po  -7.557  -5.357  -3.157

                   --+---------+---------+---------+-------
DM selection (pr          (-------*-------)
DM selection (po   (------*-----)
                   --+---------+---------+---------+-------
                  -7.0      -3.5       0.0       3.5

DM selection (pre-selection) subtracted from:

                   Lower  Center   Upper
DM selection (po  -5.593  -2.933  -0.274

                   --+---------+---------+---------+-------
DM selection (po         (-------*------)
                   --+---------+---------+---------+-------
                  -7.0      -3.5       0.0       3.5
```

G 21: Allocation of DM of the population 15 at the test field.

87	10.2	12.1	88	175	11.2	14.2	176
85	13.3	11.9	86	173		10.8	174
83	11.6	12.6	84	171		11.1	172
81	15.0	13.9	82	169	12.6		170
79	11.0	14.8	80	167	11.0	11.3	168
77	14.7	12.3	78	165		9.2	166
75	17.2	11.9	76	163	10.4	9.1	164
73	10.9	10.2	74	161	10.2	13.2	162
71	11.9	11.6	72	159	11.6	13.0	160
69	12.3	11.2	70	157	13.0	11.8	158
67	11.2	12.6	68	155		13.9	156
65		10.7	66	153	12.9	12.4	154
63	12.5	10.2	64	151	10.6		152
61	12.0	11.7	62	149	11.9	11.4	150
59	11.9	11.0	60	147	12.8	12.1	148
57	13.1	10.3	58	145	11.3	12.3	146
55	12.3	10.4	56	143	12.7	11.0	144
53	12.9	11.4	54	141	13.3		142
51	11.1	11.3	52	139	10.9		140
49	13.8	11.5	50	137		12.0	138
47		9.5	48	135	11.6	10.8	136
45	12.0	11.1	46	133		12.1	134
43	12.2	13.3	44	131	12.1	11.3	132
41	12.3	11.0	42	129	13.9	11.7	130
39	13.7	13.3	40	127	16.1	11.5	128
37	11.9	14.8	38	125	11.3	19.8	126
35	11.3	10.8	36	123		11.8	124
33	12.5	12.2	34	121	10.3	11.2	122
31	13.7	11.1	32	119	14.3	12.4	120
29		10.7	30	117	11.6	13.1	118
27	10.7	13.0	28	115	12.6		116
25	10.4	13.0	26	113			114
23	10.9	13.0	24	111	12.5	14.8	112
21	11.3	11.1	22	109	14.6	11.1	110
19		13.2	20	107	14.7	11.9	108
17	13.4	12.9	18	105	13.4	11.3	106
15	13.0		16	103	10.8	12.9	104
13	11.4	11.3	14	101		11.6	102
11	11.7	12.9	12	99	11.1	14.3	100
9		11.6	10	97	11.2	12.2	98
7	13.1	13.3	8	95	13.8	11.3	96
5	10.7	12.4	6	93	11.9	11.1	94
3	10.6		4	91	11.9	11.3	92
1	9.9	12.4	2	89	11.8	12.9	90

	DM [%]
1	<10.0
2	10.1 - 10.5
3	10.6 - 11.0
4	11.1 - 11.5
5	11.6 - 12.0
6	12.1 - 12.5
7	12.6 - 13.0
8	13.1 - 13.5
9	13.6 - 14.0
10	14.1 - 14.5
11	14.6 - 15.0
12	>15.0
	missing value

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I Lebenslauf

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