Pseudogamic production of dihaploids and monoploids in *Solanum tuberosum* and some related species

E.W.M. van Breukelen

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Abstract

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Attempts were made to maximize frequencies of dihaploids from *Solanum tuberosum*, obtained through pseudogamy after pollination with *S. phureja*. Factors influencing dihaploid frequencies were studied: genetics of the pollinator effect, genetics of the seed parent effect and interaction between the two effects on dihaploid frequencies. Temperature influences were determined in a growth chamber experiment. The mechanism of dihaploid formation was studied with the aid of cytological techniques. The pollinator effect was confirmed. Five or more loci were involved and the within-locus interaction was intermediate. High numbers of hybrids had a negative but small effect on numbers of dihaploids. The seed parent effect was also confirmed. The frequency of dihaploids was determined by the sporophyte rather than by the gametophyte of the seed parent. No interaction was found between the pollinator and seed parent effect on the dihaploid frequency. Low temperature had a positive effect on the dihaploid frequency via the pollinator, but no effect was found via the seed parent. Not the 2n-pollen but the n-pollen proved to be instrumental in dihaploid induction. Monoploids were produced from diploid *S. tuberosum* and *S. verrucosum* using several *S. phureja* genotypes as pollinator. The n-pollen induced the haploids in this case as well. Doubled monoploids were obtained with good female fertility.

Free descriptors: *Solanum tuberosum*, *S. phureja*, *S. verrucosum*, haploid, diploid, monoploid, pseudogamy, parthenogenesis, genetics, cytogenetics, pollen tube mitosis, certation, 2n-gametes, growth chamber, breeding, chromosome-doubling.

This thesis will also be published as Agricultural Research Reports 908.

Stellingen

1. In tegenstelling tot de opvatting van Irikura en Sakaguchi is het mogelijk om het aantal chromosomen van de dihaploide aardappel te halveren door pseudogamie.

Y. Irikura & S. Sakaguchi, 1972. Potato Research 15: 170-173.
Dit proefschrift.

2. Het verdient aanbeveling om te onderzoeken of U.V.-bestraling tijdens de pollenbuisgroei een positieve invloed heeft op het aantal monoploiden dat gevormd wordt uit kruisingen tussen twee diploiden.

3. Diploide regeneranten uit microsporen die homozygoot zijn voor een kenmerk waarvoor de ouder heterozygoot is, zijn niet noodzakelijk uit haploide microsporen ontstaan.

E. Jacobsen & S.K. Sopory, 1978. Theoretical and Applied Genetics 52: 119-123. G. Wenzel, 1980. In: D.R. Davies & D.A. Hopwood (eds.), The Plant Genome, p. 185-196.

4. De door Hermsen geformuleerde maat voor efficiëntie van dihaploiden-inductie kan worden verbeterd door het aantal dihaploiden per 100 zaden minder zwaar mee te laten tellen dan het aantal dihaploiden per bes.

J.G.Th. Hermsen & J. Verdenius, 1973. Euphytica 22: 244-259.

5. Boeren in ontwikkelingslanden zijn eerder geholpen met voorzieningen voor gezond zaaizaad dan met verbeterde rassen.

6. Bezuinigingen op het Nederlandse veredelingsonderzoek zullen op den duur hun weerslag hebben op de uitvoer van zaaizaad en pootgoed.

7. Als pleiotropie het faillissement is van de genetica van het eiwitgehalte van tarwe, is de oogst-index het faillissement van de gewasfysiologie.

Th. Kramer, 1980. Landbouwkundig Tijdschrift 92: 279-284.

8. De neoscholastieke leer van het hylemorfisme kan een dynamische werkelijkheid niet beschrijven.

9. De produktie van kunstvoeding voor zuigelingen zou in ontwikkelingslanden niet als bijdrage tot het nationaal produkt moeten worden gerekend.

10. In een land waar geen grote zoogdieren meer in het wild voorkomen, is het gemakkelijk om voorstander te zijn van de bescherming van deze dieren elders.

11. Bij de voortgezette verkleining van elektronische apparatuur wordt het steeds moeilijker zich achter een computer te verschuilen.

Proefschrift van E.W.M. van Breukelen
Pseudogamic production of dihaploids and monoploids in *Solanum tuberosum* and some related species
Wageningen, 22 april 1981

Woord vooraf

De werkzaamheden aan dit proefschrift vielen uiteen in twee periodes: het verzamelen en verwerken van gegevens in Wageningen en het meeste schrijfwerk in Nairobi. Om de gegevens te verkrijgen zijn 50 000 kruisingen gemaakt en meer dan 600 000 zaden één voor één bekeken. Een klein deel van de zaden is uitgezaaid om de zaailingen te beoordelen, maar dat waren er toch nog vele duizenden. In beide periodes heb ik van verschillende mensen veel hulp gehad en ik wil ze graag bedanken.

Professor dr. ir. J.G.Th. Hermsen confronteerde mij tijdens mijn ingenieursstudie met de raadsels van de aardappel-dihaploïden. Dit leidde - via een onderzoeksvoorstel ingediend bij de Faculteitsraad - tot het hier gepresenteerde werkstuk. Ik dank hem als promotor voor zijn grote enthousiasme en de kritische zin, waarmee hij allerlei theorieën met me doorsprak.

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Dr. M.S. Ramanna dank ik voor de geduldige manier waarop hij me in de praktische cytologie inleidde en de vele gesprekken die we hadden over de interpretatie van cytologische aspecten van dihaploïden en monoploïden.

De kennis van ing. J. Verdenius van het *Solanum*-materiaal is van groot nut geweest. De uitstekende opkweek en verzorging van de planten door E. van de Scheur, J. Rijksen en Chr. Loo waardeer ik zeer. Een deel van het grote aantal kruisingen is gemaakt door J. Dregmans, J. Wilmer en P. Korte als stagiaire of vakantiehulp. Bij het saaie, bijna eindeloze uitwassen van zaden werd ik geholpen door E. van Rijckevorsel, J. Eerbeek en D. Kelholt. E. van Rijckevorsel heeft ook veel chromosoomtellingen gedaan, waarbij onder meer de eerste monoploïden als zodanig herkend werden. Ing. Z. Sawor en J. de Hamer hebben ook een nuttige bijdrage geleverd aan het cytologische werk.

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Voor het werk in klimaatcellen werd gebruik gemaakt van het fytotron in de afdeling Landbouwplantenteelt en Graslandcultuur. De hulp van ing. K. Scholte en van J.H. Möhring bij het ter beschikking stellen van de klimaatcellen en de afstelling ervan is door mij zeer gewaardeerd.

De meeste foto's zijn verzorgd door H. Sengers.

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Curriculum vitae

De auteur werd op 15 oktober 1943 geboren in Den Haag en slaagde in 1961 voor het eindexamen Gymnasium-β aan het Aloysius College aldaar. In 1966 behaalde hij het licentiaat in de filosofie aan de Filosofische Faculteit Berchmanianum te Nijmegen, waarna hij zijn studie aan de Landbouwhogeschool te Wageningen begon. De praktijktijd werd doorgebracht op het Plant Breeding Station te Njoro, Kenya. In april 1973 studeerde hij met lof af in de studierichting plantenveredeling met als keuzevakken erfelijkheidsleer en planteziektenkunde. Van juni 1973 tot januari 1977 was hij als promotie-assistent verbonden aan het Instituut voor Plantenveredeling (IvP) van de LH. Sinds april 1977 is hij als lecturer verbonden aan de MSc-opleiding in plantenveredeling aan de landbouwfaculteit van de Universiteit van Nairobi, Kenya. Tevens doet hij daar onderzoek aan resistentie van *Phaseolus vulgaris* tegen roest.

Contents

1 Introduction and terminology

1.1 INTRODUCTION

Haploidy plays an important role in the life of plants. The alternation of haplophase and diplophase makes recombination of genetic material possible. In lower plants the haplophase is the longest phase in the life cycle. It was reduced during evolution in size and duration, so that the haplophase in higher plants consists of a few cells only with a short life-span. The success of the diplophase in higher plants indicates, that there are advantages for plants in having chromosomes in pairs.

With the systematic production of haploids in higher plants by biologists and breeders, the haplophase is extended again and can be as important as the diplophase. Artificial life cycles can be made with haplophase and diplophase of equal length and importance.

Haploids can be used in basic research. In plant breeding the advantages of both the n and 2n condition can be combined. The sterile haploids from heterozygous diploids and allopolyploids can be used to produce homozygous plants. More applications can be expected from autopolyploids as the polyhaploid is usually a well growing plant which can be fertile. For successful breeding it is necessary that plant material is available with sufficient diversity. Systematic work with haploids can only be carried out if many haploids can be produced from many different 2n genotypes.

Much research has been done already on the pseudogamic production of dihaploids in *Solanum*. Dihaploids of autotetraploid *Solanum tuberosum* (2n=48) can be produced in large numbers by pollination with diploid *S. phureja* (2n=24) (Hougas et al., 1958). The genotype of both seed parent and pollinator proved to be important for the dihaploid frequencies obtained (Gabert, 1963). Cytological studies have shown the importance of vital endosperm for the development of dihaploid embryos (Von Wangenheim et al., 1960). Over the years the frequencies of dihaploids were increased from 80 dihaploids per 100 berries (Gabert, 1963) to 400 dihaploids per 100 berries (Hermsen & Verdenius, 1973). This led to the question whether the maximum rate of dihaploid formation had been reached, and if not, how it could further be improved.

The availability of a large number of *S. phureja* genotypes with a diversity in dihaploid inducing ability at the Institute of Plant Breeding (IvP), Wageningen, together with good potato crossing facilities provided an excellent opportunity for research on haploid production in *S. tuberosum*

and other *Solanum* species.

The objective of this study was to answer the question whether with the available *S. phureja* genotypes the limit of inducing dihaploids in *S. tuberosum* was reached. This was to be done by investigating the genetic and cytogenetic background of the differences in dihaploid inducing ability in *S. phureja* genotypes and the differences in dihaploid producing ability of cultivars of *S. tuberosum*. In addition the influence of the environment on dihaploid frequencies had to be taken into account.

Accordingly experiments were set up to determine the influence of different seed parent and pollinator genotypes on dihaploid formation during four crossing seasons. Cytological experiments were included to obtain more insight in the mechanism of dihaploid formation. In the course of the study it was discovered that pseudogamy could produce *S. tuberosum* monoploids as well. After initial success this part of the study was expanded because of its own importance and since it was hoped that monoploids would throw light on the origin of dihaploids as well.

Literature on haploids is presented in Chapter 2. A general part is followed by literature relevant for the experimental chapters. Experiments to gain information on the genetics of the influence of pollinator and seed parent are reported in Chapter 3 and 4 respectively. The interaction between the two parental influences is dealt with in Chapter 5. In Chapter 6 experiments are described which were carried out in growth chambers under controlled conditions. These experiments aimed at determining the effect of the temperature on dihaploid frequencies. Cytology and the mechanism of dihaploid formation are covered in Chapter 7. Attempts to induce *S. tuberosum* monoploids by pseudogamy are reported in Chapter 8 along with a discussion about the origin of monoploids. In the last chapter questions about the maximum possible level of dihaploid production and the efficiency of techniques for dihaploid induction are discussed.

1.2 TERMINOLOGY

The terminology used in this study follows the recommendations of De Fossard (1974) as much as possible. The symbol x is used for the basic chromosome number and for multiples of it, e.g. x = monoploid, 2x = diploid, 3x = triploid. The symbol n is used for the gametic (gametophytic) number of chromosomes and 2n for the somatic (sporophytic) number.

A major source of confusion in ploidy terminology is the double meaning of the word 'diploid'. It can mean both 2x (in the series monoploid, diploid, triploid) and 2n (as opposed to haploid (n)). Here especially the recommendation of De Fossard (1974) is followed 'to restrict the use of the word 'diploid' to the 2x condition, to use the word 'haploid' for the n condition and to leave the 2n condition undescribed by a word'.

Haploid plants are sporophytes carrying the gametophytic chromosome num-

ber (Riley, 1974), or the gametic chromosome number of a species (De Fossard, 1974). As the haploid is a sporophyte its chromosome number should be indicated as 2n. On the other hand haploids have the gametic chromosome number of the parent and therefore they could be indicated by n. The chromosome number of a haploid plant is denoted by 2n in this study to emphasize that it concerns a sporophyte and to distinguish a haploid from the parental gametes.

'Haploid' is a relative concept. A haploid has half the number of chromosomes of the parent, irrespective of the ploidy level of the parent. Haploids of different ploidy levels are distinguished by prefixes indicating their ploidy level, e.g. dihaploids and trihaploids are haploids carrying two and three genomes respectively. The word 'haploid' is used for haploid sporophytes in general, irrespective of their ploidy level.

The words 'diploid' and 'dihaploid' can be used for the same plant, the first indicating the 2x condition, the second also indicating that the parent was a tetraploid. In this study the word 'dihaploid' is only used for 2x plants derived directly from 4x plants; in all other cases 2x plants are called 'diploids'.

The same distinction as between 'diploid' and 'dihaploid' could also be made for 'monoploid' and 'monohaploid'. As there is no reason for confusion, only the word 'monoploid' is used.

Haploids in *Solanum* are often formed through pseudogamy, after pollination. Plants used as pollen source with the aim to induce dihaploids are called 'pollinators'. A good pollinator is a plant, which induces dihaploids in a relatively high frequency. The potential of pollinators to induce dihaploids is called dihaploid inducing ability (d.i.a.) and the potential of seed parents to produce dihaploids is called dihaploid producing ability (d.p.a.).

2 Literature review

The first part of the literature review (Sections 2.1 and 2.2) gives general information about sources of haploids and their uses with emphasis on *Solanum*. The other parts deal with literature related to the experimental chapters about parental influence and external influence on dihaploid frequencies and about the mechanism of pseudogamy.

2.1 INTRODUCTION

2.1.1 Sources of haploids

The first report on a haploid plant was by Blakeslee et al. (1922). They described a *Datura stramonium* plant. It had 12 chromosomes and was obtained after cold treatment. The oldest known haploid probably is the 'gracilis' type of *Thuja plicata*, an ornamental tree, which was first described in 1896 by Beissner and later recognized as a haploid by Pohlheim (1968).

After a period in which several single haploids were found and isolated work on them was done, e.g. by Jørgenson (1928) with *Solanum nigrum*, the search for haploids as well as their production started to be done more systematically. Chase (1949, 1969) worked extensively with maize. He clearly saw the applications for plant breeding (Chase, 1963b, 1964a). Hougas & Peloquin (1957) were pioneers in research on dihaploids in *S. tuberosum*. The expansion of the haploid work can be seen in the reviews of Kimber & Riley (1963), Magoon & Khanna (1963), Chase (1969) and in the symposium 'Haploids in higher plants' held at Guelph, Canada (Kasha, 1974). Haploids are now available in many cultivated species, e.g.: *Gossypium barbadense* (Harland, 1936), *Lycopersicon esculentum* (Cooper & Brink, 1945), *Capsicum frutescens* (Morgan & Rappleye, 1954), *Prunus persica* (Hesse, 1971), *Populus alba* (Kopecky, 1960), *Medicago sativa* (Bingham, 1969), *Beta vulgaris* (Kruse, 1961), *Theobroma cacao* (Dublin, 1974) and *Secale cereale* (Müntzing, 1937).

After the more conventional methods of haploid production like distant crosses, interploidy crosses and experimentation with temperatures, less conventional schemes were developed. Since Guha & Maheshwari (1964, 1966) produced embryos and haploid plants by culturing anthers of *Datura innoxia*, anther culture and even pollen culture opened up a rich field for the large-scale production of haploids, especially in tobacco (Bourgin & Nitsch, 1967; Nitsch & Nitsch, 1969). Review articles were written by Sun-

derland (1974) and Nitsch (1974).

The discovery of a gene in maize for indeterminate growth in the female gametophyte, which leads to high frequencies of androgenesis, increased the possibilities of pseudogametic androgenesis (Kermicle, 1969, 1974).

Kasha & Kao (1970) placed the poorly growing embryos from the cross *Hordeum vulgare* × *H. bulbosum* on a medium. The chromosomes of *H. bulbosum* were selectively eliminated during the early development of the embryo (Subrahmanyam & Kasha, 1973) and haploid *H. vulgare* plants resulted. This process is under genetic control (Ho & Kasha, 1975).

Semigamy appears to be a very efficient system of haploid production. In cotton it can lead to two types of haploids from the same cross, as the nuclei do not really fuse at fertilization. The result is a chimeric embryo, growing into a plant with maternal and paternal haploid sectors, which often double spontaneously (Turcotte & Feaster, 1967, 1969, 1974).

Tsunewaki et al. (1974) used alien cytoplasm to stimulate haploid formation. Lines of a wheat variety, each with a different *Aegilops* cytoplasm, were pollinated with normal wheat. This way many trihaploids were obtained. Kihara & Tsunewaki (1962) had done similar work with alien cytoplasm before.

The influence of chemicals on haploid frequencies is dealt with in Section 2.4.

2.1.2 Solanum haploids

The first reports on haploids in tuber bearing *Solanum* concerned individual plants from interspecific crosses. Lamm (1938) reported a 2x-4x twin from *S.* × *chauca* × *S. tuberosum*, Ivanovskaja (1939) a haploid from the cross *S. tuberosum* × *S. phureja* with maternal characters. Interspecific crosses yielded haploids in *S. demissum* (Bains & Howard, 1950; Dodds, 1950) and *S. polytrichon* (Marks, 1955) as well.

A report on a single *S. tuberosum* dihaploid from a cross with *S. phureja* in 1957 (Hougas & Peloquin, 1957), followed by 28 dihaploids in the next year (Hougas et al., 1958), was the start for using *S. phureja* as a pollinator systematically. This species was also a successful pollinator with other *Solanum* species: *S. chacoense* (Hermsen, 1969), *S. acaule* (Hermsen, 1971) and *S. andigena* (De la Puente, 1973; Hermsen, unpubl.). Other diploid species did also induce dihaploids. Jakubiec (1964) used *S. vernei* and *S. megistacrolobum* with some success. *S. stenotomum* was as successful as *S. phureja* in experiments of Buketova (1970) and Buketova & Yashina (1971). Budin & Broksh (1972) obtained haploids with *S. goniocalyx* and *S. canasense* as pollinators. Dihaploids of *S. tuberosum* were also able to induce other dihaploids (Hermsen et al., 1974a). Dihaploids from a cross between cultivars of tetraploid *S. tuberosum* were reported (Cooper & Rieman, 1958; Rieman et al., 1959), but never followed up.

Within *S. phureja* certain genotypes proved to have a higher dihaploid inducing ability (d.i.a.) than others. Gabert (1963; Hougas et al., 1964) tested a *S. phureja* population for d.i.a. and found three superior pollinators. His material has been used by several workers (Jakubiec, 1964; Van Suchtelen, 1966; Frandsen, 1967) and was improved by Hermsen & Verdenius (1973), who incorporated an embryo marker and selected further for high d.i.a.

The first attempts to apply anther culture to *Solanum* were only partly successful. Irikura & Sakaguchi (1972) announced a monoploid from diploid *S. verrucosum*, but from tetraploid *S. tuberosum* Dunwell & Sunderland (1973) could report only dihaploid embryo formation. Irikura (1975a,b) later produced mono-, di- and trihaploid plants from anthers of 17 tuber bearing and 2 non tuber bearing *Solanum* species. He obtained, however, only one dihaploid from tetraploid *S. tuberosum*. He was not successful with anthers from pure *S. tuberosum* dihaploids, but the closely related *S. phureja* and *S. stenotomum* yielded monoploids.

The first pseudogamic monoploids from pure *S. tuberosum* have been reported by Van Breukelen et al. (1975), apart maybe from a single *Solanum* monoploid mentioned by Frandsen (1968), where androgenesis could not be excluded. Van Breukelen et al. (1975, 1977) reported 82 monoploids from *S. tuberosum* and 3 from *S. verrucosum*, all from crosses with *S. phureja*. Jacobsen (1978) and Frandsen & Wenzel (mentioned in Wenzel, 1979) also used pseudogamy to produce monoploids. They produced two and five monoploids respectively.

Two *S. tuberosum* monoploids from anther culture were reported by Foroughi-Wehr et al. (1977), two others by Jacobsen & Sopory (1978). Wenzel et al. (1979) did not have success with pure *S. tuberosum* diploids, but obtained monoploids from anthers of *S. phureja* and *S. phureja*-dihaploid *S. tuberosum* hybrids. Recently (Wenzel, 1980) they reported a large number of monoploids from six clones. Also diploids were produced which were spontaneously doubled according to them. The highest frequency of regenerated plants was one per four plated anthers.

2.1.3 Screening for haploids

Haploid detection evolved with the techniques of haploid induction. The first haploids were detected because of aberrant seed size (Gaines & Aase, 1926), small plant size, or the fact that the plant differed from the hybrid progeny and resembled one of the parents only. The final proof of haploidy was a chromosome count.

For large-scale hybrid production markers were developed to distinguish haploids from hybrids more easily. A dominant character in the pollinator was used to find maternal haploids and in the seed parent to detect androgenetic haploids. Seedling markers as hypocotyl colouration have been used

(Peloquin & Hougas, 1959; Bingham, 1969), but embryo or seed markers were introduced where possible for even earlier detection. Different seed markers have been used in maize (Chase, 1949, 1969; Coe & Sarkar, 1964), an embryo marker in maize (Nanda & Chase, 1966) and an embryo marker in potato (Hermsen & Verdenius, 1973). Screening for polyembryony increased the chances of finding haploids in many species (Review: Lacadena, 1974).

Haploids can be distinguished from other plants by their growth habit. Usually they are smaller and their leaves are thinner and narrower. They have smaller cells, which results in a higher stomata density in the epidermis (Capot et al., 1968) and smaller pollen grains (Kostoff, 1942). Numbers of chloroplasts in the guard cells of the stomata are lower with lower ploidy levels. This has been used successfully for prescreening by Butterfass (1958), Rothacker et al. (1966), Frandsen (1967, 1968), Najcevska & Speckmann (1968), Broksh (1969), Chaudhari & Barrow (1975) and Van Breukelen et al. (1975, 1977).

The final proof for haploidy is a chromosome count. Often the counts are done on root tips of seedlings in an early stage of development of the plant. When a plant is suspected to be chimerical because of spontaneous chromosome doubling, however, diameters of pollen grains are a fair indication, but only counts from meiotic cells are decisive. Root cells originate from another tissue layer in the plant than the layer which eventually forms the gametes (Klopfer, 1965).

2.2 USE OF HAPLOIDS

2.2.1 Haploids in research

Haploids offer special opportunities for genetic studies. The direct use of monoploids is limited, especially because of their sterility. Pairing of chromosomes at meiosis can give information on homology within the basic chromosome set of a species. Monoploids are good material for mutation research. Spontaneous and induced mutations can be detected directly, as recessive alleles are not obscured by dominant alleles (Melchers, 1960; Devreux & De Nettancourt, 1974). These mutants can contribute to our knowledge of plant physiology (Zenk, 1974), not only as plants, but also in haploid cell cultures (Binding, 1974). Haploids can be used to construct a more complete polyploid series for study of gene dosage effects. Seven ploidy levels are now available in *Medicago sativa* (Bingham & Saunders, 1974). Study of the non-homologous pairing at meiosis in monoploid pollen mother cells can give information on the basic chromosome number and on relationships between genomes in allopolyploids (Sadasivaiah, 1974).

Inheritance studies are much easier to perform with dihaploids than with tetraploids, because of the smaller populations needed to detect recessive alleles.

2.2.2 *Haploids in breeding*

Some haploids can be used as they are, mainly in ornamentals. A *Pelargonium* cultivar, which was attractive because of its small size, proved to be a haploid (Daker, 1966), as was the case with the 'gracilis' type in *Thuja plicata* (Pohlheim, 1968). Most haploids, however, are used as a tool in breeding. The great potential of haploids is the fast production of homozygous lines. These can be produced faster than by inbreeding and save one to three years in maize (Chase, 1952b) or many more years in forest trees (Winton & Stettler, 1974). More important than time saving is the fact that haploids are the way to homozygosity for self-incompatible species, when inbreeding is impossible. Even completely homozygous tetraploids can thus be made. An increase in homozygosity in autotetraploids can be accomplished via a tetraploid-diploid-tetraploid cycle, which is comparable to 3.8 generations of selfing (Obajimi & Bingham, 1973). Androgenesis saves many backcrosses in a program to transfer cytoplasm (Chase, 1963a).

The production of haploids on a larger scale gave rise to high expectations for the use of haploids in practical breeding. Chase proposed breeding schemes for allopolyploid cotton and wheat (Chase, 1964a) and the autotetraploid potato (Chase, 1973). Later he reviewed the utilization of haploids in breeding diploid species (Chase, 1974). Collins & Legg (1974) discussed the potential of haploids for breeding allopolyploids. They produced doubled haploids of tobacco which compared well with the parent varieties. By this method they eliminated many undesirable alleles. Nakamura et al. (1974a,b) produced three promising tobacco lines in their breeding program via haploids. In asparagus entirely male F1 progenies have been made with a great homogeneity (Thévenin, 1974). In oil seed rape the cultivar Maris Haplon was derived from a haploid (Thompson, 1972).

Reinbergs et al. (1975) saw their doubled haploids as fixed gametes. They used them to estimate the yield potential of barley crosses in early generations (Reinbergs et al., 1976). Their lines had the same yield potential as randomly chosen F4 lines from the same cross (Song et al., 1978). So, with a three year gain in time, they did not loose yield potential. This confirmed a computer study of Walsh (1974), who also pointed out that the pedigree method is better if linkage is involved.

In autogamous crops the good homozygous lines may become cultivars, but in other crops heterozygosity is necessary. Dunbier & Bingham (1975) made two populations from haploid derived autotetraploids with the same gene frequencies, but different levels of heterozygosity. The more heterozygous double cross had the highest yield, presumably because of the higher number of tri- and tetra-allelic loci. Doubled haploids from these crops can only be used for a hybrid program.

2.2.3 *Solanum haploids in research*

Both monoploids and dihaploids in *Solanum* have their own applications in research just as other haploids. Non-homologous chromosome association at meiosis has been observed in monoploids (Van Breukelen et al., 1975). The most important application of monoploids is the fast production of homozygous diploid plants.

S. tuberosum dihaploids are smaller than tetraploids, but they may be vigorous and give a reasonable tuber yield. Leaves are smaller and narrower (Hougas & Peloquin, 1957). The flowering behaviour may be comparable with that of tetraploids, but the net female fertility is lower (Carroll & Low, 1975). Male fertility is greatly reduced (Van Suchtelen, 1966; Carroll & Low, 1976; Hermsen, unpubl.). The percentage of plants producing functional pollen can be as low as 3% (Peloquin et al., 1966).

Simple segregation ratios in dihaploids (Hougas & Peloquin, 1958) have led to several articles on genetic markers (Kessel & Rowe, 1974; Hermsen et al., 1978a), localization on a particular chromosome (Hermsen et al., 1973), genetics of self-compatibility (Hermsen, 1978a,b; Hermsen et al., 1978b) and gene-centromere mapping (Mendiburu & Peloquin, 1969; Ross & Langton, 1974; Mok et al., 1976; Mendiburu & Peloquin, 1979).

Dihaploid plants are more suitable for mutation studies than tetraploids (Van Harten, 1978). They also can produce aneuploids after crosses with triploids as seed parents (Vogt & Rowe, 1968; Wagenvoort & Lange, 1975). Aneuploid diploids can also be found amongst the normal dihaploids from a 4x × 2x cross (Hermsen et al., 1970).

2.2.4 *Solanum haploids in breeding*

The two main advantages of the use of dihaploids in potato breeding are (a) their disomic inheritance, which facilitates the production of homozygosity for selected characters and (b) the increased possibilities to make crosses with wild or primitive diploid *Solanum* species (Hougas & Peloquin, 1958, 1960). With these crosses the diversity in the potato germ plasm can be greatly increased, for instance with respect to resistance to diseases and pests.

The most serious problem for the use of dihaploids is their male sterility. This is added to the normal problems of diploids: self-incompatibility and male sterility in some interspecific hybrids (Ross et al., 1964; Abdalla & Hermsen, 1972; Perez-Ugalde et al., 1964) or unilateral incongruity (Hermsen et al., 1974b). According to Carroll (1975) this male sterility can occur in various degrees in different genotypes.

Dihaploids from one tetraploid plant form a 'gametic sample'. They have the genotypes of the gametes and show which characters are hidden in the tetraploid genotype. They can also provide an estimate of the degree of he-

terozygosity for certain characters. This gives dihaploids an application in conventional breeding. The gametic sample is an aid in choosing the right parents in 4x × 4x crosses.

The dihaploids can also be used themselves in breeding. Rothacker & Schäfer (1961) envisaged to make tetraploids with a high degree of homozygosity as parents for a breeding program, exploiting heterosis. Dihaploids would be crossed with wild *Solanum* species, made homozygous for selected characters and then doubled. The homozygous tetraploids would constitute good breeding material as the frequencies of genes for desired characters in the progeny will be high.

Chase (1963b) introduced the name 'analytic breeding' for the procedure of reducing a polyploid to its diploid components, crossing and selecting at the diploid level, resynthesizing the polyploid form and testing it.

The exploitation of 'unreduced gametes' or rather '2n gametes' has been the subject of many studies of Peloquin c.s.. They investigated the cytology of 2n gamete formation and distinguished between two meiotic restitutions: first division restitution (FDR) and second division restitution (SDR). FDR gametes transmit the genotype of the parent largely intact, including the non-additive gene actions. Their use provides a powerful breeding technique (Wang et al., 1971). SDR increases homozygosity. The cytology and genetics of 2n pollen formation has been studied further by Mok & Peloquin (1975). According to them three mechanisms of 2n pollen formation can be distinguished and they are simply inherited. FDR maintains existing heterozygosity, which is important in autotetraploids. Mendoza & Haynes (1974) showed that heterozygosity was more important than dominance for yield in potato. Dunbier & Bingham (1975) demonstrated in alfalfa that tri- and tetra-allelic loci increased yields. Doubling of the chromosome number either spontaneously or with colchicine increases the degree of homozygosity and might influence yield in negative direction.

To use the potential of 2n gametes with FDR Mendiburu et al. (1974) proposed two ways of sexual polyploidization in potato breeding: unilateral (4x × 2x) and bilateral (2x × 2x). In a 4x × 2x cross an adapted variety would be crossed with a selected diploid, preferably with FDR, to obtain a high heterotic response (Mendiburu & Peloquin, 1971). In reciprocal crosses between 4x and 2x plants Kidane-Mariam & Peloquin (1974) associated the higher yields in 4x × 2x crosses with FDR in the diploid. It seems, however, that negative interaction between *S. tuberosum* genes and *S. phureja* cytoplasm could not be ruled out completely.

The proposed 2x × 2x cross (Mendiburu et al., 1974) is a more interesting type. It can be applied in many ways. For instance, a combination of dihaploid *S. tuberosum*, *S. phureja*, dihaploid *S. andigena* and *S. chacoense* was suggested. *S. chacoense* was incorporated to avoid cytoplasmic male sterility. Breeding was to be done at the diploid level, followed by two crosses between two parents to produce two intermediate diploid hybrids, which were

again to be crossed to give a tetraploid that could be multiplied vegetatively. FDR in 2n pollen and 2n egg formation in the last cross would accomplish that the diploid genomes were incorporated largely intact in the tetraploid. Cytological work or test crosses would be needed during the process to screen for plants with FDR (Mendiburu et al., l.c.). This is a promising breeding scheme, as it can combine genes from four sources. The cytogenetic explanation of FDR and SDR has been questioned by Ramanna (1979), who suggested that several cytological observations, e. g. parallel spindle, could not be interpreted as genetical processes. His own investigations with diverse material also showed that various processes can lead to restitution in meiosis, even in one anther. Unravelling the genetics of FDR would be more complicated than was suggested. Even if the genetic background of FDR were not as simple as Mok & Peloquin (1975) stated, the heterosis reported in the experiments is worth exploiting.

Hermsen (1974, 1977) proposed a modification of this scheme: instead of maintaining the tetraploid vegetatively, it can be reconstituted in the form of hybrid seeds from the vegetatively propagated diploid hybrids. Potatoes from seeds might carry a promise for the future in tropical regions where virus is a year round problem and where enough labour is available to transplant seedlings from a nursery to the field.

Screening for resistances in vitro is a recently developed method in which protoplast techniques are used. Dihaploid potato cells have been regenerated into plants (Behnke, 1975). Behnke (1979) selected irradiated and non-irradiated dihaploid calli for resistance to *Phytophtora infestans* in vitro. Plants from surviving calli proved to be more resistant than controls. With refinement of in vitro techniques selection might in the future take place in laboratories rather than in fields or greenhouses.

Wenzel et al. (1979) incorporated such laboratory techniques in their proposed potato breeding scheme. Dihaploids were to be produced from tetraploids through pseudogamy. Monoploids would be made via anther culture and doubled to obtain homozygous diploids. Selected diploids should then be crossed in pairs. Protoplasts from their progenies were to be isolated and fused in pairs. After regeneration highly heterozygous tetraploids would be obtained, provided that the diversity of the original tetraploids was big enough.

2.3 *INFLUENCE OF THE PARENTS ON FREQUENCIES OF HAPLOIDS FROM PSEUDOGAMY*

Pseudogamy is the development of seeds after pollination, but without fertilization of the egg cell. Pseudogamy is sometimes called parthenogenesis, but this indication is less precise, as it also includes cases where no pollination is involved. Pseudogamic haploidy often occurs after interspecific or intraspecific crosses where different ploidy levels are involved (interploidal crosses) (Rowe, 1974).

The roles of the two parents in haploid production are very different. Accordingly it is less important to know the genetics of the seed parent effect than the genetics of the pollinator effect, in order to improve the efficiency of haploid production. If haploids are wanted from a certain plant then the yield of haploids can only be changed by choosing other pollinators. Pollinator improvement is possible without influencing the type of haploids produced, as the haploid does not receive chromosomes from the sperms.

Several measurements of dihaploid frequencies have been used for comparisons between parents or between methods. Frandsen (1967) gave three different ratios in his tables: dihaploids per 100 pollinated flowers, per 100 seedlings and per 100 berries. He used only the last one in his summary. Irikura (1975b) gave the same three ratios in his tables, but used dihaploids per 100 pollinated flowers in his text. This ratio is a measure for the effort needed to obtain dihaploids. However, adverse weather conditions, which cause pollinated flowers or young fruits to drop, influence this ratio very much. There is variation in fruit set between *S. tuberosum* cultivars as well. If no seed marker is available the ratio per 100 seedlings is to be preferred (Bender, 1963) as raising and screening of seedlings is the most laborious part of the production of dihaploids in that situation. As the number of seeds per berry is a highly variable character, dihaploids per 100 seedlings is not appropriate for comparisons between parents.

Dihaploids per 100 berries (d/100b) is the most widely used measure (Gabert, 1963; Frandsen, 1967; Hermsen & Verdenius, 1973). It is also used in this study. It is not influenced very much by environmental conditions or the seed parent and reflects the number of crosses needed to produce one dihaploid.

2.3.1 Influence of the pollinator

Plants producing pollen that gives rise to haploids through pseudogamy are called pollinators, as they pollinate the seed parent without fertilization of the egg cell.

The closer the parents were related, the more research has been done on the influence of the pollinator genotype on haploid frequencies. Maize haploids have been derived from intraspecific 2x × 2x crosses. When its haploid production was studied systematically, Chase (1949, 1952b) found the choice of the pollen parent to be important. Coe & Sarkar (1964) came to the same conclusion and they transferred genes for high haploid inducing ability from one stock into another (Sarkar & Coe, 1966). Later Sarkar (1974) reported doubling of the haploid inducing ability of a pollinator population by two cycles of selection for that character. Later he obtained a further doubling after the third cycle (Aman & Sarkar, 1978). The charac-

ter was highly heritable and controlled by a large number of genes with additive effect.

In the cross *Populus tremula* × *P. alba* the pollen can be inactivated by toluidine blue and still induce haploids (Illies, 1974a,b). The genotype of the pollen parent is probably unimportant. Bingham (1971) found pollinators to have influence in the interploidal intraspecific crosses that yielded dihaploids in *Medicago sativa*. In other interploidal crosses, *Fragaria ananassa* × *Potentilla anserina* and *P. fructicosa*, which produced *Fragaria* haploids, Janick & Hughes (1974) did not mention different genotypes within a species.

In *Solanum* species pseudogamic haploids are usually induced using selected plants of *S. phureja* as pollinators. Such pollinators fertilize the central nucleus and thus contribute to the indispensable vital endosperm (Von Wangenheim et al., 1960). Dihaploids of *S. tuberosum* originate from a 4x × 2x interploidal cross. As *S. phureja* is closely related to *S. tuberosum* and even *S. tuberosum* diploids can be used as pollinator (Hermsen et al., 1974a), the interploidal character of the cross may be more important than the interspecific character.

Influence of the genotype of the pollinator on haploid frequencies in *S. tuberosum* has been established by several authors (Gabert, 1963; Hougas et al., 1964; Jakubiec, 1964; Frandsen, 1967; Hermsen & Verdenius, 1973; Irikura, 1975b). From the work of Hermsen & Verdenius (1973) it can be seen that selection for high d.i.a. is possible in *S. phureja*. Gabert (1963) was the first to screen a large number of *S. phureja* clones and he found good and bad pollinators. He observed a discontinuity between the two groups, few being good pollinators. He crossed pollinators and obtained good pollinators only from the combination good × good. His conclusion was that the character was heritable, high d.i.a. being recessive with only one or a few genes involved. Frandsen (1967) reported a clear difference between two of these pollinators both in d.i.a. and in hybrid production. Hermsen & Verdenius (1973) produced a sib-F2 population of *S. phureja* and studied 29 plants of this population extensively for their d.i.a.. The average d.i.a. of these plants was higher than that of their parents and much higher than in Gabert's (1963) experiments. Although they found a large variation in d.i.a., no clear-cut discontinuity was detected. Irikura (1975b) studied the selfprogeny of a *S. phureja* clone. He found both positive and negative transgression for d.i.a. and concluded that low d.i.a. was the dominant character and inherited quantitatively. This conclusion did not explain the negative transgression in his inbred population.

The genes for high d.i.a. were recessive according to both Gabert (1963) and Irikura (1975b). Irikura analysed one population and Gabert (unpubl.) used rather small populations for his analyses. None of the authors mentioned arguments for dominance of high d.i.a.. Intermediate inheritance was never considered, as far as the author is aware.

Berries from crosses between *S. tuberosum* and *S. phureja* usually contain hybrid seeds apart from seeds with dihaploid embryos. Hermsen & Verdenius (1973) distinguished pollinators in those with high seed set and low seed set. They suggested a relationship between seed set and dihaploid numbers per berry, especially when using low seed set pollinators.

A good pollinator needs a marker to be useful. The first marker applied in screening for dihaploids in *S. tuberosum* was purple hypocotyl, used by Peloquin & Hougas (1959). They suggested to use embryo spot as a seed marker for even earlier screening. This spot is an accumulation of anthocyanin at the base of the leaves and is caused by two genes: B and P (or R). Dominant P provides the anthocyanin (also in the hypocotyl), dominant B causes the accumulation, provided P is present (Dodds & Long, 1955, 1956). The character is pleiotropic: the spots are visible on all parts of the plant homologous to a leaf base. The dominant alleles of the non-linked genes B and P are not present in European cultivars of *S. tuberosum*. Attempts have been made to produce *S. phureja* plants homozygous for embryo spot, which initially failed (Frandsen, 1967). Hermsen & Verdenius (1973), however, succeeded in breeding *S. phureja* plants which combined homozygosity for embryo spot with high d.i.a.. This increased the efficiency of dihaploid induction considerably and also made selection for monoploids possible, where thousands of seeds have to be screened to obtain one monoploid (Van Breukelen et al., 1975).

2.3.2 Influence of the seed parent

An influence of the seed parent on the haploid frequency is expected rather than an effect of the pollinator (Hougas et al., 1964). The seed parent influence could work through nuclear genes, through the cytoplasm or both. Lethal genes can reduce the number of viable haploids. The number of ovules per berry might influence the number of haploids directly. Differences in numbers of ovules in ovaries of several potato cultivars have been found (Arnason, 1943; Carroll & Low, 1975). The influence of lethal genes is difficult to determine, unless lethality occurs during the germination of the seeds or after emergence of the seedlings. Montelongo-Escobedo (1969) found lethal genes not to be a major factor in the potato clones he used. Hermsen et al. (1978a) found four lethal genes in Gineke, which is still a good dihaploid producer. Lethal genes, which cause seed abortion of dihaploids, usually escape detection.

Genes for pseudogamy can work at either of two levels: sporophytic or gametophytic. In other words, the mechanism to produce haploids is controlled by the genotype of either the plant or the egg cell. Bender (1963) ascribed what he called an 'apomictic tendency' to the egg cell, but this was more a name than an explanation. He proposed an experiment to prove gametophytic determination. If the egg cell has a genetic constitution which

promotes pseudogamy, then doubling the chromosome number of a haploid will result in plants in which genes for pseudogamy have accumulated. Such plants are expected to produce on the average more haploids than the original plant. Chase (1952a) found doubled monoploid lines to produce more monoploids than unselected inbred lines.

Differences in haploid producing ability between maize stocks have been reported (Chase, 1949, 1952b; Sarkar & Coe, 1966; Sarkar, 1974). Coe & Sarkar (1964) were able to incorporate the high haploid producing ability of their best seed parent into another stock by backcrosses. Homozygosity increased the haploid frequency in maize (Sarkar, 1974). This might be due either to homozygosity for genes for haploid production or to reduction of the number of lethal genes. Bingham (1971) found differences in haploid producing ability between *Medicago sativa* genotypes, Stringham & Downey (1973) in *Brassica napus*. Differences between *Nicotiana tabacum* lines in haploid frequencies from crosses with *N. africana* were found by Burk et al. (1979).

Potato dihaploids can be extracted from almost any seed parent (Gabert, 1963). Frandsen (1967) investigated 117 clones. Although 13 did not actually produce dihaploids, he himself was not convinced that they were unable to produce them. Irikura (1975b) extracted dihaploids from 66 out of 83 seed parents. The general occurrence of dihaploid producing ability (d.p.a.) in *S. tuberosum* means that a broad genetic base may be made available for the application of dihaploids. However, dihaploid frequencies vary.

The influence of the seed parent on the frequency of dihaploids has been recognized from the beginning of systematic work on potato dihaploids (Gabert, 1963; Jakubiec, 1964; Rothacker et al., 1966; Frandsen, 1967; Irikura, 1975b). Genetic factors that influence dihaploid production have not been studied extensively, autotetraploidy being a hindrance. Montelongo-Escobedo (1968) made crosses between dihaploids of a good and a bad seed parent to determine the inheritance of the seed parent effect. The meiotically doubled tetraploid progeny was again used as seed parent. From the dihaploid frequencies obtained he concluded that high d.p.a. was dominant, relatively few genes being involved. However, positive genes are overrepresented in dihaploids from a bad seed parent, if Bender's (1963) theory is correct. The tetraploid-diploid-tetraploid cycle concentrates good genes, but to a larger extent in bad seed parents than in good seed parents.

Apart from chromosomal influence there may be a cytoplasmic influence. Frandsen (1967) found the cytoplasmic differences between *Solanum* species to be important. His own data did not support this very strongly. The uniformity within each cytoplasm group was not very high and ranges were overlapping: e.g. the group with *S. demissum* cytoplasm yielded 15-233 d/100b (mean: 65d/100b) and with *S. tuberosum* cytoplasm 15-433 d/100b (mean: 150 d/100b). Frandsen did not come to a conclusion about cytoplasmic differences within *S. tuberosum*.

2.3.3 *Interaction between pollinator and seed parent influence*

Pollinators can only be evaluated using seed parents, seed parents only by using pollinators. The idea of interaction between pollinator and seed parent effect on dihaploid frequencies did not receive much attention in earlier studies. Gabert (1963), who studied both effects, gave only a slight suggestion that good pollinators were good irrespective of the seed parent. Hougas et al. (1964) stated this more clearly. This same tendency could be observed in the data of others who worked with several potato varieties and a few pollinators (Jakubiec, 1964; Frandsen, 1967; Hermsen, unpubl.). On the other hand, Hermsen & Verdenius (1973) found the correlation between dihaploid production of two varieties with a range of pollinators not to be highly significant. They explained this as a differential reaction of the two seed parents to the same series of pollinators. This could be interpreted as interaction. The different frequencies of hybrids found by Frandsen (1967) between *S. tuberosum* and *S. andigena* with two *S. phureja* clones could also be seen as an interaction between seed parent and pollinator.

Chase (1949) noticed that certain seed parents in maize produced more monoploids than others with each of the pollinators used. The same can be concluded from data of Coe & Sarkar (1964). In their data a multiplicative effect can be found but no interaction in a strict sense between the effects of seed parent and pollinator.

2.4 EXTERNAL INFLUENCE ON HAPLOID FREQUENCIES

It is apparent from Section 2.3 that haploid production is genetically controlled. In this section literature on the influence of external factors on the frequency of haploids is presented. These factors include temperature, light, radiation and chemicals.

Extreme temperatures have induced haploids in some species. Blakeslee et al. (1922) obtained their first haploids in *Datura stramonium* by using low temperatures. Müntzing (1937) reported a haploid plant in *Secale cereale* after cold treatment and Nordenskiöld (1939) after heat treatment. In *Solanum tuberosum* many authors found dihaploid rates changing from year to year (Gabert, 1963; Frandsen, 1967; Hermsen, unpubl.).

Gabert (1963) investigated the influence of temperature on dihaploid frequencies under natural light conditions in an air-conditioned greenhouse. Though he stressed the big contribution of the pollen parent to dihaploid frequencies, he controlled temperature influences on the female parent only. He used small compartments with temperatures ranging from 15-30 °C. He pollinated cut stem inflorescences (Peloquin & Hougas, 1959) and placed them for four days in a compartment with either fixed or alternating temperatures. None of the treatments enhanced dihaploid frequency,

when compared with the frequency found at gradually changing temperatures of 21-24 °C during the day and 13-16 °C at night. Gabert (l.c.) concluded that temperature probably affected fruit set and fruit development.

Wöhrmann (1964) also carried out systematic experiments to determine the influence of temperature on the production of dihaploids in potato, especially during pollen tube growth. He used the cut stem technique. Flowers of four cultivars were placed at a temperature of 20, 25 or 30 °C for 10 or 20 h immediately after pollination. For the remainder of the first eight days after pollination all flowers were kept at 20 °C. He found that higher temperatures lowered the numbers of berries per flower, seeds per berry and dihaploids per berry considerably. The number of dihaploids per seed was lowered by higher temperatures to a smaller extent. Duration of treatment seemed to have more influence than temperature.

His treatments were rather short if one considers that pollen tube growth takes 36-48 h until the moment of fertilization. He also did not exclude influences on the pollen parent and on the seed parent before anthesis. The two treatments of 10 and 20 h at 20 °C, each followed by 20 °C for eight days, were not considered as replications. There was a considerable difference between these almost identical treatments for each cultivar with respect to numbers of seeds per berry and dihaploids per berry. Apparently there were factors that were not controlled in his experiments.

Frandsen (1967) was the first to note that seasonal influences before the period of fertilization might affect dihaploid rates, but did not mention any influence of environmental factors on the d.i.a. of pollinators.

Meiosis takes place in the anther before meiosis in the ovule in *S. tuberosum* (Rees Leonard, 1935; Clarke, 1940) and in *S. phureja* (Maherchandani & Pushkarnath, 1960). This means that environmental influences on both meiotic processes can be different.

An important aspect of environmental influence is formed by the growing conditions of the parental plants as far as they influence flowering and fruit set and indirectly the dihaploid frequency (Gorea, 1968, 1970). The decapitation technique, described by Peloquin & Hougas (1959), increased fruit set five to ten times. Grafting of potato onto tomato rootstock resulted in profuse flowering over a long period and improved berry set, thus increasing the efficiency of greenhouse space and labour (Hermsen, 1979). Gorea (1968, 1970, 1973) concluded from his potato dihaploid inductions that the total yield of dihaploids was promoted by suitable cultural conditions during fruit development. Fruit set can be reduced by high temperatures during flowering (Bienz, 1958) or low night temperatures (Bodlaender, 1960). Most *Solanum* species flower only at long day length, but a high light intensity might compensate for too short day lengths (Driver & Hawkes, 1943; Krug, 1957). This has consequences for the duration of the crossing season and for the choice of artificial conditions in growth chambers.

Apart from manipulations with natural factors as temperature and light, technical methods have been tried to raise haploid frequencies (Review: Lacadena, 1974). Most methods reduced the vitality of the pollen, others aimed at inducing restitution of the pollen tube mitosis.

Kopecky (1960) reduced pollen vitality of *Populus alba* by a fermentation process. He obtained more haploids after pollinations with this pollen. Heat-treated poplar pollen produced haploid-diploid mixaploids and maternal diploids, probably doubled haploids (Winton & Einspahr, 1968). Illies (1974a,b) obtained significantly higher frequencies of haploids after treatment of pollen or the pollinated style with toluidine blue. The pollen tube mitosis was blocked and the subvital pollen was only a stimulus. The endosperm was apparently not important for seed development in *Populus*. Toluidine blue did not give an increase of haploid frequency in tomato and maize (Al Yasiri & Rogers, 1971). Dumas de Vaulx & Pochard (1974) treated pollinated flowers of *Capsicum annuum* with N_2O. This treatment increased the frequency of haploid-diploid twins in one cultivar. Treatment of the flowers before pollination resulted in the production of single haploids. Ecochard et al. (1974) inactivated the male sperm specifically with thermal neutrons just before fertilization and obtained two tomato haploids with this technique.

Several workers applied X-rays to *Solanum* pollen. Bender (1963) obtained increased dihaploid frequencies, whereas Wöhrmann (1963) reported similar or lower frequencies of dihaploids per berry. Bukai (1973) obtained a fourfold increase of the dihaploid frequency. Tarasenko (1974) reported a sixfold increase in dihaploids per seed. Berry set and seed set were found to be lower after X-ray treatment by Bender (1963) and Wöhrmann (1963). It seems that dihaploids are less sensitive to X-rays than hybrid seeds, probably because irradiated pollen can still be functional in endosperm, when it is not functional any more in a zygote. Montezuma-de-Carvalho (1967) and Montelongo-Escobedo & Rowe (1969) treated *S. phureja* pollen with N_2O and colchicine respectively in order to increase dihaploid production by stimulating restitution of the pollen tube mitosis. Increased restitution was observed in both studies and in the latter also increased dihaploid frequencies were found (see also Section 2.5.2.3).

It may be concluded that optimalization of the growing conditions of seed parents and pollinators was the only manipulation of external factors that considerably increased dihaploid frequencies of a seed parent pollinator combination.

2.5 MECHANISMS OF PSEUDOGAMIC HAPLOID PRODUCTION

When haploids are formed through pseudogamy the reproductive mechanism is switched to an abnormal development. Many authors have studied haploid producing mechanisms applying embryological and cytological techniques.

Literature about these mechanisms is given here for 2x × 2x and 4x × 2x crosses with emphasis on the latter, especially in *Solanum*. The origin of hybrids is also taken into account.

2.5.1 2x × 2x crosses

The mechanism of pseudogamy is extensively studied in maize. Maize haploids may occur in low frequencies in progenies of 2x × 2x crosses. Most cytological data come from experiments by Chase (1964b) and Sarkar & Coe (1966). Chase used a dominant endosperm marker, which regulated the intensity of yellow colour according to the number of alleles present. With the marker in one of the parents he could distinguish triploid from tetraploid endosperm. He found monoploids to have triploid endosperm. Sarkar & Coe (1966) confirmed this using another endosperm marker. Apparently one sperm fertilized the central nucleus and the other was lost. This is called the 'maize mechanism' (Hermsen, 1971). Chase (1969) proposed a hypothesis of early division of the egg cell or synergid before fertilization to explain this. The tendency of precocious division would depend on the maternal genotype. This may lead to the higher monoploid rate observed after delayed pollination. Smith (1946) found a higher haploid frequency after delayed pollination in *Triticum monococcum*.

2.5.2 4x × 2x crosses

Pseudogamic dihaploids from *S. tuberosum* originate almost exclusively from 4x × 2x crosses. This is an interesting type of cross as the outcome is not triploid hybrids, which is mathematically the expectation. Triploids occur in very low frequencies per berry. The majority of the progeny consists of tetraploid hybrids. Dihaploids are produced as well in certain combinations of parents, sometimes in frequencies of up to five per berry. The total number of seeds produced is usually very low compared with that in 2x × 2x and 4x × 4x crosses.

2.5.2.1 Triploid hybrids

The lack of triploids in a 4x × 2x cross has been reported frequently and was described as 'triploid block' by Marks (1966a). He called it an enigma, as triploid plants grow well. Koopmans & Van der Burg (1952), Marks (1966b), Van Suchtelen (1966, 1973) and Hanneman & Peloquin (1967) mentioned low triploid frequencies in their crosses. Rothacker & Schäfer (1961) found 40 triploids per 100 berries. Frandsen (1967) obtained much lower frequencies: around 1 per 100 berries. Hermsen & Verdenius (1973) reported relatively high triploid frequencies from crosses involving low seed set pollinators. Calculations on their data show that these pollinators

produced 15-30 triploids per 100 berries. Triploid frequencies from high seed set pollinators could be of the same magnitude. Jackson et al. (1978) found similar frequencies in Andean potato cultivars. Hanneman & Peloquin (1968) and Ross & Jacobsen (1976) observed lower frequencies of triploids in bigger progenies, which is not unexpected as they expressed the triploid frequency as a fraction of total seed set. Triploid frequencies can vary from year to year (Sudheer, 1977) and be influenced by the parental genotypes (Gorea, 1970; Van Suchtelen, 1976).

Beamish (1955) crossed hexaploid *S. demissum* with diploid *S. phureja*. She obtained less than one seed per berry, predominantly tetraploids, comparable to the triploids in a 4x × 2x cross. In other genera low triploid frequencies in 4x × 2x crosses were found, e.g. *Dactylis* (Carroll & Borrill, 1965), *Brassica* (Nishiyama & Inomata, 1966), *Primula* (Skiebe, 1967) and *Medicago* (Bingham, 1971).

A barrier to a better understanding of the triploid block was the opinion, that fixed ratios between the ploidy levels of maternal tissue, endosperm and embryo were necessary for the development of a seed. This ratio should be 2:3:2 or 4:6:4 (Müntzing, 1930). Cooper & Brink (1945) found irregularaties in *Lycopersicon* endosperm after crosses between 4x and 2x plants, followed by poor embryo development leading to shrivelled seed. They stressed the importance of the ratio between maternal tissue and endosperm. Study of endosperm development by Von Wangenheim (1961) showed that the absolute ploidy level of the endosperm was important. He found 3x, 6x and 9x endosperm to behave normally during seed growth, irrespective of the ploidy level of the embryo, sometimes even without an embryo. In 4x endosperm he observed small cells without vacuoles and with large intercellulars. Pentaploid endosperm had its own characteristic type of degradation as it did not form cell walls. Similar defective endosperm development has been found in 4x × 2x crosses in *Brassica* by Nishiyama & Inomata (1966). It is the lack of vitality of 5x endosperm that makes the seed set low in 4x × 2x crosses. The majority of ovules, containing triploid embryos, does not develop beyond initial stages. Skiebe (1973) supported the conclusions of Von Wangenheim (1961). Exceptions of this theory have been explained by Valentine & Woodell (1963) by introducing a 'genetic value', a factor that makes endosperm chromosome numbers a multiple of 3x. The values they found look unnatural. Den Nijs (1977) and Den Nijs & Peloquin (1977) introduced a hypothesis about endosperm balance factors (EBF) to explain the different crossing behaviour of Mexican tetraploids and *S. acaule* compared to other tetraploid *Solanum* species. Endosperm would only develop normally if there were a 2:1 ratio between maternal and paternal genomes. A tetraploid would normally have 4 EBF, but *S. acaule* would have only 2 EBF and therefore easier produce triploids in crosses with diploids than tetraploids in crosses with tetraploids. Johnston et al. (1980) introduced the name 'endosperm balance number' for the number of EBF of Den Nijs (l.c.).

2.5.2.2 Tetraploid hybrids

Most hybrids from tetraploid *S. tuberosum* × diploid *S. phureja* crosses are tetraploid (Frandsen, 1967; Höglund, 1970; Hermsen & Verdenius, 1973). Höglund (l.c.) wanted to explain the high number of tetraploids from 4x × 2x crosses. She studied microsporogenesis and pollen mitosis in vitro of one *S. phureja* clone. The spindle formation in metaphase II was irregular, resulting in about 50% 2n gametes. After second pollen mitosis she found, apart from tubes with two reduced sperms, also tubes with two 'unreduced' sperms, the 2n sperms contributing to tetraploid hybrids. She suggested a cytoplasmic influence from the tetraploid pistil or diploid egg cell leading to preferential fertilization by 2n pollen grains. She mentioned the possibility of differences in growth rate between 2n and n pollen. This should explain why the frequency of tetraploid progeny was higher than the frequency of 2n pollen. She did not take into account the lethality of the triploids. Preferential fertilization is a very rare phenomenon according to Haustein (1967). Differences in pollen growth rate, called 'certation' by Heribert-Nilsson (1920), are more common. The term was originally used for types of pollen differing in genotype, but can also be used for pollen differing in ploidy level. Skiebe (1966) did not find differences in growth rate in vivo for 2x and x pollen of diploid *Primula malacoides*, neither did Esen et al. (1978) in *Citrus*. In sugar beet x pollen grows faster than 2x pollen (Matsumura, 1958).

Ramanna (1974) found aberrant cytokinesis to be the major mechanism in the occurrence of 2n pollen. Mok & Peloquin (1975) distinguished three mechanisms of 2n pollen formation in diploid potatoes on cytological grounds. Regarding the genetic consequences there are two mechanisms. All these mechanisms lead to tetraploid hybrids from tetraploid-diploid crosses. In a re-examination of the mechanisms of 2n pollen formation Ramanna (1979) doubted whether cytological observations could be interpreted genetically in the way Mok & Peloquin (l.c.) did.

2.5.2.3 Dihaploids

Dihaploids may originate from 4x × 2x crosses as well, but by which mechanism? If the maize mechanism (Section 2.5.2.1) were involved, the resulting seeds would have dihaploid embryos in lethal pentaploid endosperm and dihaploids would occur in even lower frequencies than triploids. Von Wangenheim et al. (1960) investigated the chromosome numbers in 112 ovules of *S. tuberosum*, pollinated by a *S. phureja* clone with high d.i.a.. They counted 35 ovules with hexaploid endosperm, of which 30 lacked an embryo and 5 contained a dihaploid embryo. Bender (1963) did similar counts in 614 ovules. The chromosome numbers of both endosperm and embryo could be

counted in 114 ovules. Four dihaploid embryos were found in hexaploid endosperm. Three other dihaploid embryos were found in an endosperm of which chromosomes could not be counted. Again a high number of ovules with hexaploid endosperms without embryo was found. These nine dihaploids in hexaploid endosperm are the only ones in which the ploidy level of embryo and endosperm has been determined. Never was a dihaploid mentioned in other endosperm. It can be assumed that dihaploids generally occur in hexaploid endosperm. A dihaploid in pentaploid endosperm has only a small chance to survive, but with the survival rate of triploids of about 1 in 1000 (Von Wangenheim, 1956) it is possible that an occasional dihaploid in pentaploid endosperm occurs, the frequency of which being too low for detection by chromosome counts in ovules. A hexaploid endosperm will have received 2x chromosomes from the pollen. If the egg cell is not fertilized, there are basically two possibilities for the central nucleus to be fertilized by 2x chromosomes: either by 2n pollen or by reduced pollen. In case of 2n pollen either one sperm fertilizes the central nucleus and the other degenerates, or - as Bender (1963) suggested - only one single 2n sperm is available to fertilize the central nucleus due to failing pollen tube mitosis. With n pollen two sperms fertilize the central nucleus either individually or connected by a chromatid bridge or fused after restitution.

Fertilization of the central nucleus only and degeneration of the second sperm has been observed cytologically. Håkansson (1943) reported a premature division of the egg cell in *Poa alpina*, followed by the fusion of one sperm with the central nucleus, while the other sperm degenerated. Bannikova & Khvedynich (1974) compared the fertilization process of intraspecific crosses with that of wide crosses in *Nicotiana*. In the wide cross *N. rustica* × *N. paniculata* one sperm fused with the egg cell or the central nucleus, the other degenerated often in or near a synergid.

The general occurrence of 2n pollen in *S. phureja* pollinators could be an argument in favour of the role of this pollen in dihaploid induction, but then it has to be explained why the second sperm does not function. Premature division of the egg cell is not as likely here as it is in maize. Guignard (1902) reported that the development of the embryo in *Solanum* started after several endosperm divisions. This was confirmed by Clarke (1940), who observed the first endosperm divisions two days after pollination and the first division of the zygote after five days in *S. tuberosum*. A similar time interval was recorded by Williams (1955). Delayed pollination to exploit the tendency of premature division of the egg cell did not increase dihaploid frequencies in Gabert's (1963) work.

X-ray treatment (Bender, 1963; Wöhrmann, 1963; Bukai, 1973) and colchicine (Montelongo-Escobedo & Rowe, 1969) increased dihaploid frequencies in *S. tuberosum*. It is not impossible that one sperm was made subfunctional in the process. This way 2n pollen might induce dihaploids.

Double fertilization of the central nucleus has been reported in some species, often when apomixis was involved. Gaines & Aase (1926) discovered a *Triticum* haploid originating from a large kernel. They assumed that the giant endosperm was caused by a fusion of both sperms with the central nucleus. Rutishauser (1956) found double fertilization of the central nucleus in pseudogamous *Ranunculus auricomus*. Fused sperms and sperms connected by a bridge have been observed in *S. phureja*. Bender (1963) found occasionally bridge formation between two sperm nuclei and restitution nuclei in vivo. The frequency was higher after irradiation. Montezuma-de-Carvalho (1967) observed the metaphase plate to be absent in pollen tube mitosis of *S. phureja* in vivo. He found the chromosomes to lie in rows, an arrangement easily leading to abnormalities. Montelongo-Escobedo & Rowe (1969) studied pollen growth in vitro. They found one single restitution nucleus in 35% of the tubes in two good pollinators, in bad pollinators only in 3%. These percentages need not be a reflection of what happens in vivo, as the authors found abnormalities in their in vitro pollen germination, e.g. a second pollen mitosis inside a pollen grain.

Complete or partial restitution during pollen tube mitosis occurs in *S. phureja*. Several attempts have been made to increase the restitution frequency. Montezuma-de-Carvalho (1967) applied N_2O to pollinated flowers at the time of pollen tube mitosis. After treatment he found up to 70% restitution nuclei or dumb-bell shaped nuclei with a bridge between sperms due to lagging chromosomes. Montelongo-Escobedo & Rowe (1969) gave different colchicine treatments to pollen before using it in pollinations. Fruit set was reduced, seed set varied and the dihaploid frequency was slightly increased. Treated pollen germinated in vitro showed 100% restitution nuclei. Treated pollen from bad pollinators did not yield as many dihaploids as untreated pollen from good pollinators. Treatment doubled the performance of good pollen. The origin of the hybrids obtained was not explained in their experiment. Some pollen must have escaped the treatment. Bender (1963) increased dihaploid frequencies with X-ray treatment of pollen. In accompanying cytological studies he found an increase in restitution nuclei and chromatid bridges from 4 in 1000 tubes studied to 10 in 800 tubes at the optimum radiation dose.

These are some experiments that support the theory that fertilization of the central nucleus by two (fused) n sperms is the mechanism leading to dihaploid formation. This was called the '*Solanum* mechanism' by Hermsen (1971).

Bender (1963) stated on theoretical grounds that n pollen must be important as otherwise more dihaploids would have been found from 4x × 4x crosses in potato, apart from the dihaploids claimed by Cooper & Rieman (1958) and Rieman et al. (1959). This is not necessarily true, as dihaploids would be difficult to detect in the large progenies of 4x × 4x crosses. Apart from that, nobody ever selected for good tetraploid pollinators as

was done for diploid pollinators.

The role of 2n pollen could be determined by the correlation between frequencies of 2n pollen and dihaploid induction for several pollinators. This can be done directly or indirectly when hybrid production is taken as a measure for the frequency of 2n pollen. The triploid frequency per berry can be neglected. Rothacker et al. (1966) did not find correlations between dihaploids and total seed set. Hermsen & Verdenius (1973) presented results of dihaploid induction with 29 *S. phureja* clones. They did not calculate between-pollinator correlations, but only within-pollinator correlations between berries. Buketova & Yashina (1973) reported absence of correlation between the percentage of dyads in nine pollinators and the induced dihaploid frequencies. Also these results point to the importance of reduced gametes.

3 Pollinator influence on dihaploid and hybrid frequencies

3.1 INTRODUCTION

S. tuberosum dihaploids are usually produced by pseudogamy using selected plants of *S. phureja* as pollinator. Such pollinators do not contribute to the genotype of the dihaploid, but fertilize the central nucleus and thus contribute to the genotype of the endosperm. The nature of the stimulus to the egg cell causing cell division without fertilization is not known.

As described in Section 2.3.1 the influence of the genotype of the pollinator on dihaploid frequencies was well established in several studies. Frequencies of hybrid seeds were also influenced by the pollinator. Recessive inheritance of dihaploid inducing ability (d.i.a.) in pollinators was reported by Gabert (1963) and Irikura (1975b). The former mentioned that only one or a few loci were involved. Dominance and intermediate inheritance were never reported. As d.i.a. might be based on a gametophytic process in which interaction between alleles is absent because of the haploidy, intermediate inheritance must be considered.

The positive trangression in d/100b found by Irikura (1975b) and the selection response obtained by Hermsen & Verdenius (1973) raise the question whether d.i.a. can be further improved by breeding and to what extent.

Hybrid seeds are a by-product of dihaploid induction. As dihaploids and hybrids are formed from the same limited number of ovules in a berry, there must be a relationship between the number of dihaploids and hybrids produced by a pollinator. This relationship is of interest since the frequencies of hybrids might influence the frequencies of dihaploids. Hermsen & Verdenius (1973) found a 3:1 ratio for low vs. high hybrid frequencies. They suggested a monogenic dominant inheritance of low hybrid frequencies, but did not exclude the possibility of more genes being involved.

The objectives of the experiments described in this chapter were:
- to confirm the influence of the pollinator on dihaploid and hybrid frequencies,
- to study the process of inheritance of d.i.a. and hybrid frequencies in the pollinator,
- to find out whether with the existing *S. phureja* genotypes the maximum d.i.a. had been reached,
- to study the quantitative relationship between dihaploid and hybrid frequencies.

For these purposes pollinators with known performance were used, crosses between pollinators were made and their progenies tested. The d.i.a. of the pollinators was assessed by making pollinations on a *S. tuberosum* tester during four seasons. The number of dihaploids per 100 berries in the progeny was the measure for comparison of the d.i.a. of different pollinators.

3.2 MATERIAL AND METHODS

Most *S. phureja* plants were derived from material developed by Hermsen & Verdenius (1973), who were first to combine high d.i.a. with homozygosity for embryo spot (Section 2.3.1). The donor for high d.i.a. was PI 225682.22 (Gabert, 1963) and the source of embryo spot was PI 225702.2. Full-sib F2 plants from this cross were indicated as IVP-clones. All were homozygous for the hypocotyl marker P, but some proved to be heterozygous for the gene for embryo spot B. The performances for d.i.a. and seed set were known from work of Hermsen & Verdenius (1973) and confirmed in a preliminary experiment. Genotypes for B and lists of pollinators with high d.i.a. and high seed set are given in Table 1. Full-sib F3 and self progenies were made from IVP-clones. They are listed in Table 2. Reciprocal crosses between IVP48 and *S. tuberosum* dihaploid G609 (from cv. Gineke) yielded two sib families, IVP48 × G609 and G609 × IVP48, which were heterozygous for both B and P. Plant 57 of G609 × IVP48 was backcrossed with IVP48 as mother, whereas plant 3 was backcrossed with IVP48 as father. In this way one population had *S. phureja* cytoplasm and the other *S. tuberosum* cytoplasm, coded $IVP48^2$ × G609 and G609 × $IVP48^2$ respectively.

As seed parent in tests for d.i.a. of pollinators cultivar Gineke of autotetraploid *S. tuberosum* was used, which had no genes from other *Solanum* species. In earlier studies this cultivar had shown the ability to produce

Table 1. Genotypes for embryo spot and lists of pollinators with high d.i.a. and high seed set, respectively.

Genotype		High d.i.a.	High seed set
BBPP	BbPP		
IVP6	IVP1	IVP1	IVP10
IVP10	IVP24	IVP10	IVP32
IVP26	IVP32	IVP26	IVP35
IVP35		IVP35	
IVP48		IVP48	
IVP66			
IVP71			

Table 2. Full-sib F3 and self progenies derived from the IVP series of pollinators (Hermsen & Verdenius, 1973). Populations indicated with S are segregating for embryo spot.

Population	n		Population	n
IVP1 selfed	12	S	IVP(48 × 10)	10
IVP(1 ⊗ 10)	5	S	IVP(48 × 35)	24
IVP(24 × 10)	18	S	IVP48 selfed	5
IVP(32 × 48)	47	S	IVP(66 × 6)	8
IVP(48 × 1)	5	S		

n. size of the population

many dihaploids. In addition, it flowered profusely for a long period when grafted onto tomato. It was easy to emasculate and produced many berries. The high correlation (r=0.65) between d.i.a. of 22 pollinators tested with *cv. Gineke* and *cv. Radosa* in one year (Hermsen & Verdenius, 1973) justified the use of one tester cultivar to determine the relative d.i.a. of pollinators. This was corroborated by other tests by Hermsen and by own preliminary trials (Van Breukelen, 1972).

All pollinators and seed parents were grafted onto tomato rootstock to enhance flowering. They were planted in a greenhouse with high relative humidity and temperature set at 20 °C. Temperatures did not drop below 20 °C, but were occasionally as high as 35 °C for 2-4 h on very hot days. Flowering usually lasted from late June to mid September.

For pollination mostly fresh pollen was used. Sometimes the pollen was collected from flowers that had been dried overnight. All Gineke flowers were emasculated and labelled individually. Pollinations were made in cycles. Ten emasculated flowers of Gineke were pollinated per pollinator until all pollinators had been used. This took several days. The cycle was repeated until 15-30 berries per combination were obtained. The number of pollinations per pollinator ranged from 15 to 150.

The seeds were carefully extracted from every berry separately and screened for embryo spot with the aid of a dissecting microscope (magn. 20-40×). Spotted seeds, spotless seeds and doubtful cases were counted and kept apart. The fraction of spotless seeds in the progeny of pollinators homozygous for embryo spot can be regarded as the fraction of dihaploids produced. However, the spotless seeds from pollinators heterozygous for gene B contained also hybrids, which showed a purple hypocotyl as a seedling. All the spotless seeds from heterozygous pollinators were therefore planted in the next spring, after the dormancy was over. From the emerging seedlings data were taken to enter them in the group of dihaploids or hy-

brids. In the first two years all the other spotless seeds were planted as well, together with the doubtful cases. This way mistakes in screening could be rectified. The percentage of emergence was calculated for all groups of seeds. The germination rate was very high, except for the doubtful cases, where the absence of an embryo had been suspected. Only emerging seedlings without purple hypocotyl or leaf base were finally recorded as dihaploids. This group included lethal types that did not survive the first two weeks. Green tetraploid plants constituted less than 1% of the plants from spotless seeds. They were detected by their wide leaves and confirmed as tetraploids by counting the number of chloroplasts in the guard cells of the stomata. This method reliably distinguishes 2x from 4x plants (Frandsen, 1968).

From the seeds of 1975 and 1976 only a sample of the spotless seeds from Gineke × 'homozygous' pollinators was planted, to determine the percentage of emerging dihaploids. The total number of spotless seeds from a cross was then multiplied by this percentage to give an estimate of the number of emerging dihaploids. The percentages did not vary much from year to year.

As a routine seeds with embryo spot were not planted. They were counted as hybrids, together with seedlings with a purple hypocotyl or leaf base.

Absolute numbers of dihaploids and hybrids from a cross were converted to numbers per 100 berries: d/100b and hy/100b.

Dihaploid numbers in berries have a distribution that is close to a Poisson distribution (Van Breukelen, 1972). Means of numbers of dihaploids per berry per day are probably closer to the normal distribution. Notwithstanding a certain deviation from normality, analysis of variance (anova) was carried out with pollinator and year or crossing date as main effects.

Pollen quality was determined up to three times per season for every pollinator by staining pollen with lactophenol acid fuchsin and counting the percentage of regularly shaped deeply stained pollen grains. This is a fast method which gives a fair indication as to whether pollen is good or poor (Janssen & Hermsen, 1976). Samples with more than 95% stained pollen grains can be considered as very good; lower than 60% indicates poor pollen and with less than 10% the chances of seed set are very low.

3.3 RESULTS

Results of crosses varied with the years. Prolonged hot periods, such as occurred in 1975 and 1976, lowered berry set and the number of seeds per berry considerably. As for berry set, 1974 was the best year and 1971 and 1973 were good. Since most crosses were repeated throughout a season, part of the temperature influence was evened out.

Table 3. Dihaploids per 100 berries (d/100b) and hybrid seeds per 100 berries (hy/100b). Berries formed on cv. Gineke, after pollination with seven related pollinators. Results are averages of around 30 berries for each of three years (upper part) or averages of 5-10 berries for each of four different days in 1975 (lower part).

		Pollinator (IVP nr.)							
		6	10	26	35	48	66	71	mean
d/100b	1968[a]	37	53	46	236	111	9	0	70
	1975	103	235	304	297	406	46	41	205
	1976	192	156	402	237	207	27	28	178
	mean	111	148	251	257	241	27	23	151
hy/100b	1968[a]	7	7838	42	1453	17	117	115	1370
	1975	40	465	31	150	39	125	60	130
	1976	72	1950	97	1323	25	196	157	546
	mean	40	3418	57	975	27	146	111	682
		Pollinator (IVP nr.)							
		6	10	26	35	48	(48 × 35)		mean
d/100b	18/8	68	84	348	265	290	395		242
	21/8	62	115	255	225	376	541		262
	25/8	119	330	312	479	568	646		409
	28/8	56	367	238	256	401	974		388
	mean	76	224	288	306	409	639		324
hy/100b	18/8	0	200	25	73	20	13		55
	21/8	0	420	38	38	67	46		102
	25/8	50	375	60	211	33	70		133
	28/8	20	850	0	378	25	50		221
	mean	18	461	31	175	36	45		128

a. data calculated from Hermsen & Verdenius (1973)

3.3.1 *Influence of the pollinator on dihaploid and hybrid frequencies*

Cv. Gineke was pollinated with the same seven pollinators in three seasons and d/100b and hy/100b were calculated (Table 3, upper part). The same data were collected from six pollinators on four different days in one month in 1975 (Table 3, lower part). Analyses of variance from both groups of data are given in Table 4. The influence of the pollinator on d/100b was significant in both cases. It was less marked on hy/100b, where it was significant in the four date experiment, but only marginal in the three year experiment. Variations between years and days were high and even significant for d/100b.

Notwithstanding environmental influences, the ranking of the pollinators was almost identical from year to year: IVP48 > IVP35 > IVP10 = IVP1 =

Table 4. Analysis of variance from the data of Table 3.

	Source	Sum of squares	d.f.	Probability
Three year experiment				
d/100b	pollinators	187716	6	0.01
	years	70959	2	0.02
	error	74718	12	
	total	333394	20	
hy/100b	pollinators	28248879	6	0.12
	years	5574976	2	0.31
	error	25880246	12	
	total	59704101	20	
Four date experiment				
d/100b	pollinators	717522	5	0.00
	dates	127022	3	0.06
	error	209097	15	
	total	1053641	23	
hy/100b	pollinators	601120	5	0.00
	dates	87535	3	0.16
	error	219245	15	
	total	907900	23	

d.f.. degrees of freedom

IVP26 > IVP6 > IVP24 > IVP71 > IVP32. Thus seven levels of d/100b were found.

3.3.2 Relationship between dihaploid and hybrid frequencies

The different cytological processes leading to formation of dihaploids and hybrids will be discussed in Chapter 7. Here only the quantitative relationship between frequencies of pseudogamic dihaploids and hybrid seeds will be dealt with.

Among some well tested pollinators the levels of d/100b and hy/100b differed as follows:

low d/100b	high d/100b	low hy/100b	high hy/100b
IVP24, 32	IVP10, 35, 48	IVP24, 48	IVP10, 32, 35

High d/100b did not always coincide with high hy/100b, nor with low hy/100b. The derived populations IVP(24 × 10), IVP(32 × 48) and IVP(48 × 35) had a wide range for both d/100b and hy/100b and there was no clear positive or negative trend, as can be seen in Fig. 1. Correlation between the two characters and regression of d/100b on hy/100b were calculated for every population and for parts of the populations (Table 5). The pattern in the three populations was similar. From 0-100 hy/100b, d/100b was rising

Table 5. Analysis of the relationship between d/100b and hy/100b from the untransformed data of Fig. 1. n = (sub)population size; r = correlation coefficient; slope = the slope of the regression of d/100b on hy/100b.

Population	Range of hy/100b	n	r	Slope
IVP(24 × 10)	0- 1000	7	0.36	0.08
	1000-20000	11	-0.63	-0.01
	total	18	-0.66	-0.01
IVP(32 × 48)	0- 100	9	0.44	1.58
	100- 1000	13	-0.35	-0.11
	1000-20000	25	-0.59	-0.01
	total	47	-0.67	-0.01
IVP(48 × 35)	0- 100	9	0.66	3.69
	100- 1000	8	-0.19	-0.12
	1000-20000	7	-0.87	-0.03
	total	24	-0.39	-0.02

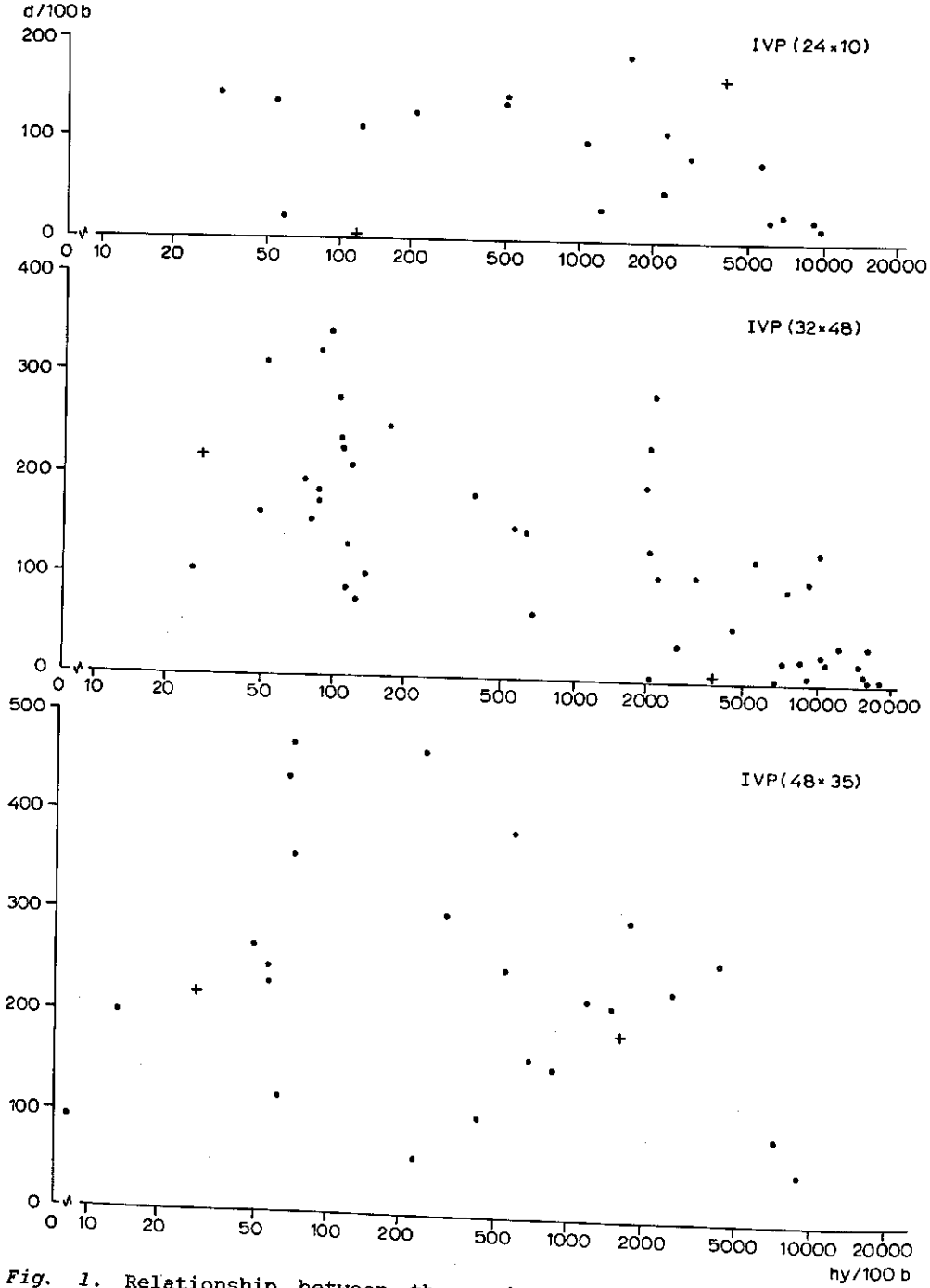

Fig. 1. Relationship between the number of dihaploids per 100 berries (d/100b) and hybrids per 100 berries (hy/100b) of three full-sib F3 populations of *S. phureja*: IVP(24 × 10), IVP(32 × 48) and IVP(48 × 35). Data are averages of at least five berries from crosses with cv. Gineke in 1973. +. parental value.

(not visible in IVP(24 × 10) with only few plants in this range). In the range of 100-1000 hy/100b correlation was very low and the level of d/100b decreased slowly. Beyond 1000 hy/100b the average number of dihaploids decreased even slower. High values of d/100b occurred even when hy/100b was very high. The area in Fig. 1 with less than 50 d/100b and less than 50 hy/100b was empty. It would have contained pollinators which produced less than one seed per berry. Berries with zero, one or very few seeds usually drop before maturity. Especially in IVP(48 × 35) the positive correlation was reasonably high in the range of 0-100 hy/100b.

The slope of the regression of d/100b on hy/100b varied from -0.01 to -0.02 between populations.

3.3.3 Inheritance of dihaploid inducing ability

Apart from the pollinator populations in Fig. 1, other full-sib *S. phureja* populations were made and tested for d.i.a. with cv. Gineke (Table 6). In addition to these, also the progenies of dihaploid *S. tuberosum* × *S. phureja* crosses were tested (Table 7). Crosses between good and bad *S. phureja* pollinators yielded plants with a wide range in d/100b and showed both positive and negative transgression (Table 6). Besides giving good pollinators, selfing of IVP1 yielded also very bad ones, whereas the small self progeny of IVP48 did not give low values. In the population IVP(66 × 6), the progeny of two poor pollinators, one high value occurred.

The plants from IVP48 $\overset{\varnothing}{\times}$ G609 were rather uniform in d/100b (Table 7). Of the two backcross populations, G609 × IVP48^2 and IVP48^2 × G609, the former had a higher average d/100b than the latter (222 vs. 124 d/100b), reflecting the difference between the two plants that were backcrossed: 157 vs. 86 d/100b in 1974. The parent G609 induced only 2 d/100b in 1973.

The cross between the best pollinators available, IVP48 and IVP35, was made to see whether pollinators with even higher d.i.a. could be produced. Positive transgression for d/100b was not only found from this cross, but also from other crosses, even where a bad pollinator as IVP32 was used as one of the parents (Fig. 1, Table 6). IVP(32 × 48) yielded many good pollinators, IVP1 selfed, IVP(48 × 1) and IVP(1 × 10) several. Even IVP(66 × 6) produced a good pollinator.

In order to investigate whether this transgression could be reproduced over the years, test crosses were made in the following years (Table 8). Not all new plants produced tubers, so that some could not be repeated. Clones 1 and 5 from the population IVP(48 × 35) repeatedly showed transgression; clones 2, 3 and 4 did not perform better than IVP48 in the long run. The transgression of IVP48 selfed,1 was consistent; IVP(48 × 10)1 and IVP(66 × 6)1 also showed repeatedly positive transgression.

Table 6. Values of d/100b and hy/100b induced by *S. phureja* genotypes of F3 full-sib populations. Means of at least five berries from crosses made during one season with cv. Gineke as seed parent. Data of parents (P1 and P2) are also included.

1973		1974							
IVP48 selfed		IVP1 selfed		IVP(1 ♀ × 10)		IVP(48 × 1)		IVP(66 × 6)	
d/100b	hy/100b	d/100b	hy/100b	d/100b	hy/100b	d/100b	hy/100b	d/100b	hy/100b
394	353	520	39	444	56	666	73	356	144
287	50	350	90	389	67	514	136	188	2413
273	80	340	128	223	8	475	244	40	940
228	21	310	433	208	133	307	57	40	447
211	1567	270	111	175	100	103	38	22	337
		220	85					14	593
		180	33					6	2623
		160	59					0	60
		140	60						
		120	1857						
		94	44						
		50	93						
P1 217	28	251	75	251	75	445	81	18[a]	290[a]
P2				209	8569	251	75	163	78

a. mean of 1975 and 1976

3.3.4 Inheritance of hybrid-producing ability

The *S. phureja* progenies from Section 3.3.3 were also studied with respect to the inheritance of hy/100b. The number of hybrids produced by these plants in crosses with *S. tuberosum* showed a remarkable positive transgression in (G609 × IVP48^2)5 and IVP48 selfed,5 (Tables 6 and 7). The two *S. phureja*-dihaploid *S. tuberosum* backcross populations differed in hy/100b (534 vs. 61) in the same direction as the backcrossed plants: 347 vs. 74. The range of hy/100b was greater than that of d/100b. Values up to 18000 hy/100b were reached. Especially in the higher part of the range, data were very much spread out.

In order to test the repeatability of the levels of hy/100b observed for a pollinator, four plants of IVP(24 × 10), producing 32, 54, 209 and 9252

Table 7. Values of d/100b and hy/100b induced by *S. phureja* × dihaploid *S. tuberosum* populations. Means of at least five berries from crosses with cv. Gineke as seed parent. Data of parents (P1 and P2) are also included.

	1974		1975			
	IVP48 ♀ × G609		G609 × IVP48[2]		IVP48[2] × G609	
	d/100b	hy/100b	d/100b	hy/100b	d/100b	hy/100b
	160	67	550	914	217	67
	157	347	282	86	171	59
	140	235	270	53	150	75
	130	27	242	79	128	56
	130	24	216	3310	111	144
	104	381	206	86	111	16
	96	16	157	50	100	17
	86	14	120	96	89	93
	79	526	100	133	86	45
	14	100	75	81	78	39
P1	445	81	157[b]	347[b]	322	74
P2	2[a]	25[a]	322	74	86[b]	14[b]

a. measured in 1973
b. measured in 1974

hy/100b respectively, were used again as pollinators in 1974. In that year they produced 92, 88, 280 and 20794 hy/100b respectively. The level was higher in 1974, but the order was about the same. The plant with an intermediate level was still intermediate. Similarly IVP66 produced usually between 100 and 200 hy/100b, more than IVP48 (<85 hy/100b) and less than IVP32 and 35 (>500 hy/100b).

3.4 DISCUSSION

3.4.1 Influence of the pollinator on dihaploid and hybrid frequencies

This study confirmed that the pollinator influenced the frequency of dihaploids formed in *S. tuberosum* significantly. Whereas most authors worked with only a few pollinators (Jakubiec, 1964; Frandsen, 1967) or with a

Table 8. Positive transgression for d.i.a.. Values of d/100b of several clones in different years, compared with the value of the parents recorded in the same year.

Pollinator	1973			1974			1975			1976		
	F1	P1	P2	F1	P1	P2	F1	P1	P2	F1	P1	P2
IVP(48 × 35)1	470	217	184	800	445	342	556	322	246	309	278	272
2	462			487			372					
3	437			394						322		
4	383			488			147			313		
5	360						713					
IVP48 selfed,1	394	217	217							418	278	278
IVP(48 × 10)1							547	322	229	390	278	152
IVP(66 × 6)1				356		163	450	26	68			

larger group, containing only a few good pollinators (Gabert, 1963; Irikura, 1975b), now a group of pollinators with a wide range of d.i.a. was tested over several years. The overall level of dihaploid production varied between years and days. This, however, did not affect in a major way the relative differences in d.i.a. between pollinators and their ranking.

The gap between good and bad pollinators, described by Gabert (1963), was not found in this material.

Hybrid production was also influenced by the pollinator. The high yearly fluctuations did not upset the ranking of the pollinators, except for the ones with very low hy/100b. The great variation between years and days was probably due to environmental influences during pollen formation (Chapter 6).

3.4.2 Relationship between dihaploid and hybrid frequencies

The quantitative relationship between the dihaploid and hybrid progeny from Gineke and a certain pollinator was weak. Correlations between the two were negative and not very high in three larger populations as a whole (Table 5). With regard to the positive correlation in the range of 0-100 hy/100b it might be suggested that low pollen quality could have been the reason for the combination of low d/100b and hy/100b, but correlation between pollen stainability and either d/100b or hy/100b proved to be very low. In the range from 100-1000 hy/100b, dihaploid and hybrid numbers seemed to vary independently. The higher (negative) correlations in plants with more than 1000 hy/100b were accentuated by some very high values of

hy/100b. Factors for high d/100b and high hy/100b seem to have been distributed independently in the populations, but in their expression they might have influenced each other. High numbers such as 100-180 hybrid seeds per berry will limit the number of ovules available for production of dihaploids. The negative slope of the regression of d/100b on hy/100b can thus be interpreted as a reduction in dihaploids of an average pollinator, caused by extra hybrids in a berry. This reduction was 1 to 2 dihaploids per 100 hybrids. It was higher in IVP(48 × 35) with a higher mean for d/100b than in IVP(32 × 48) and IVP(24 × 10). This is not a major influence on average dihaploid production. In the 10% of the pollinators producing more than 10000 hy/100b, the reduction in d/100b will have been 100-200. A high seed set pollinator as e.g. IVP32 will probably be underestimated for its genetic potential for inducing dihaploids. Apart from reducing the number of dihaploids produced, the production of very high numbers of hybrids is an unfavourable character in a pollinator, because it involves more work in seed extraction and screening. However, it is possible for an individual pollinator to produce high numbers of both dihaploids and hybrids per berry, as can be seen in IVP10.

Hermsen & Verdenius (1973) studied the correlation of total numbers of seeds and dihaploids per berry. They did not calculate between-pollinator correlations, but the within-pollinator correlations between berries. The positive correlations in their study were the result of using the total number of seeds instead of number of hybrids, especially for low seed set pollinators. In low seed set pollinators, like IVP48, dihaploids form the largest fraction of the seeds and a correlation between the total and its largest fraction must be positive and high.

3.4.3 Inheritance of dihaploid inducing ability

Inheritance of d/100b was not controlled by the cytoplasm, as the progeny of G609 × IVP48 was not lower than its reciprocal cross, which had a superior pollinator as source of cytoplasm.

The range in d/100b was almost continuous in the populations studied. This meant that many genes were involved and/or that environment had a great influence on the expression of the genes. Amongst the IVP pollinators a constant ranking was found and seven levels of d/100b could be distinguished (Section 3.3.1), but some could have been caused by high numbers of hy/100b. Several genes must have been involved in producing these differences. On the basis of the data available it was attempted to estimate the type of allelic interaction and the number of genes involved in d.i.a.. It was assumed that all genes for d.i.a. had the same influence and that the allelic interaction was of the same type. Alleles increasing d.i.a. of a pollinator are called positive alleles (+alleles).

3.4.3.1 Mode of inheritance

In the dominant type of allelic interaction, positive alleles are not hidden in a heterozygous combination. Therefore, d.i.a. levels higher than that of the higher parent cannot be expected after selfing. As there was positive transgression in the self progenies of IVP1 and 48, as well as in that of *S. phureja* 253 (Irikura, 1975b), dominance was not a likely way of inheritance.

Recessivity has been suggested by Gabert (1963) and Irikura (1975b). Data from this study did not support their conclusion. In a recessive model d.i.a. levels lower than that of a parent are not expected after selfing. Plants were found in self progenies with a much lower level of d/100b than the parent (Table 6). Inbreeding depression and bad pollen might have been a cause for the lower d.i.a., but some of the plants with low d/100b produced many hybrids, e.g. IVP1 selfed, plant 10. Data from Irikura (1975b) also showed negative transgression for d/100b, often combined with a high male fertility of the pollinator.

If complete dominance and recessivity are both excluded, intermediate inheritance remains. There are arguments in favour of intermediate inheritance. Gineke dihaploid G609 had a very low d.i.a. (2 d/100b), which level could be regarded as spontaneous or basic. G609 was unrelated to *S. phureja* IVP48, a superior pollinator. It is unlikely that both clones had genes in common which contributed to pollen irregularities (Chapter 7) and would reduce generative vitality. The reciprocal F1 populations from G609 and IVP48 had a good level of d.i.a. and were remarkably uniform (Table 7). An exception was one plant with 14 d/100b, which had low pollen stainability and produced only 100 hy/100b. This uniformity indicated a high level of homozygosity for +alleles in both parents. This is not unlikely in IVP48 because of inbreeding and selection for high d.i.a.. For G609 this would mean a complete lack of +alleles.

With intermediate inheritance all +alleles would be expressed. If none were present in G609 and many in IVP48, the number of +alleles in the F1 must have been half of that in IVP48. The level of d/100b of the F1 can thus be expected to be about halfway between G609 and IVP48. This was not the case on a linear scale, but the F1 was halfway on a square root scale. Such a scale means that the effect of every additional +allele is higher than the preceding one. The increased effect is brought about in a gametophytic system by (a) a lower fraction of microspores without +alleles and by (b) more +alleles per microspore. Not only the frequency of microspores capable of inducing a dihaploid is increased, but also the efficiency of the individual microspores in inducing dihaploids. A logarithmic scale shows a similar increasing effect, but there problems are encountered with low values.

3.4.3.2 Number of loci

Assuming a square root scale a hypothesis can be formulated on the number of loci for d.i.a. in IVP48 and related pollinators. The uniformity of the F1 of IVP48 and G609 pointed to high homozygosity of the parents for d.i.a.. The small differences amongst F1 plants were caused by heterozygous loci in IVP48 and/or environmental influences. If environmental influences are not considered, the difference between the two groups in the F1 plants - 4 plants: 79-104 d/100b and 5 plants: 130-160 d/100b - will reflect the number of heterozygous loci in IVP48. In that case the lower group will have the same number of +alleles as IVP48 has homozygous +loci. With only one heterozygous locus in IVP48, the effect of one +allele would bring the dihaploid frequency from 90 d/100b to 145 d/100b. On a square root scale the distances 0-90 d/100b and 145-444 d/100b are each 3.8 times the distance 90-145 d/100b. With one heterozygous locus in IVP48 the level of IVP48 (445 d/100b) would have been caused by 9 +alleles on 5 loci. On a square root scale the values for 1-10 +alleles would be an expansion of the series $5.5x^2$: 6, 22, 50, 88, 137, 200, 270, 350, 445 and 550 d/100b (level of 1974). If IVP48 were heterozygous for 2 or 3 dihaploid inducing loci, the range in the F1 would be caused by 2 or 3 +alleles and the number of +alleles in IVP48 would be 12 and 17 respectively on 7 and 10 loci. Another indication for the number of loci involved was found in the differences in d.i.a. between IVP pollinators and their constant ranking. Seven levels were distinguished, but the lower pollinators were probably underrepresented, as better pollinators got more emphasis in studies. A number of five or seven loci would agree better with the seven levels than ten loci.

The transgression found in IVP48 selfed agreed well with a total number of five loci, four being homozygous for +alleles and one heterozygous.

3.4.3.3 Test for intermediate inheritance

The hypothesis of intermediate inheritance can be tested in the three pollinator populations from 1973 (Fig. 1), using the estimate of the number of alleles involved in determining the d.i.a., intermediate inheritance meaning that the progeny mean must be close to the mid parent value. The general level of d/100b was lower in 1973 than in 1974. If IVP48 is assumed to have 9 +alleles, an adapted series for 1-10 +alleles for 1973 would be: 3, 13, 29, 52, 81, 117, 160, 208, 263 and 325 d/100b. With these values the number of +alleles was determined for every plant of the three populations and the parents. The observed numbers of d/100b were increased with 1% of hy/100b in IVP(32 × 48) and IVP(24 × 10) and 2% of hy/100b in IVP(48 × 35) because of the negative influence of hy/100b on d/100b (Section 3.4.2). This correction was not needed for IVP48 $\overset{\times}{\times}$ G609, as hy/100b was low. Population IVP(48 × 35) had a mean value of 8.8 +alleles and a range of 4-12

+alleles. The mid parent value was 8.5 +alleles. For IVP(24 × 10) the progeny mean was 5.9 +alleles, the mid parent value was 5.5, assuming that IVP10 carried 10 +alleles, which is reasonable with a number of 6500 hy/100b and a correction factor of 2%. Progenies of IVP10 were usually high in d/100b. The progeny mean of IVP(32 × 48) was 6.9 +alleles, whereas the mid parent value was 6.5, if 4 +alleles would be present in IVP32 (in 1973 4090 hy/100b). The three population means tended to be higher than the mid parent values, but not much. These results point to intermediate inheritance. This conclusion does not depend on the assumption about the number of loci involved. With seven or ten loci for d.i.a. the progeny mean would likewise be close to the mid parent value.

3.4.3.4 Transgression

With the big increase in levels of d.i.a. as a result of the selection work of Hermsen & Verdenius (1973), the limits of dihaploid induction had not yet been reached. Further increases proved to be possible with the same material (Table 8). Repeatable positive transgression was found in several full-sib F3 progenies, to levels higher than the best pollinators. Within the available material the upper limit of d.i.a. may have been reached, but with other sources of genes for dihaploid induction further improvement may be possible.

3.4.4 Inheritance of hybrid producing ability

Frequencies of hy/100b were very variable between seasons. Three levels were found: 0-100 hy/100b, 100-300 hy/100b and >300 hy/100b. It is quite possible that more levels were present in the material, but with the high variation they could not be distinguished. Monogenic dominance of low numbers of hybrids, as suggested by Hermsen & Verdenius (1973), would result in two levels only. With the three levels found, either inheritance must be intermediate, or more genes must have been involved.

4 Seed parent influence on dihaploid and hybrid production

4.1 INTRODUCTION

Influence of the *S. tuberosum* seed parent on the frequency of dihaploids it produces after pollination with *S. phureja* has been reported by several authors (Section 2.3.2). Such influence may work through genes for pseudogamy, cytoplasmic factors, lethal genes or the number of ovules per berry. The last factor can also influence hybrid frequencies.

The dihaploid producing ability (d.p.a.) of a seed parent can either be controlled by the genotype of the sporophyte or by that of the gametophyte. Little is known about the mechanism inducing an unfertilized egg cell to divide. Therefore this question cannot be solved cytologically; it must be solved genetically. To do this Bender's (1963) suggestion was followed to check whether a haploidization-diploidization cycle would accumulate genes for pseudogamy.

Cytoplasm played an important role in the level of d.p.a. according to Frandsen (1967). Montelongo-Escobedo (1968) concluded about the chromosomal inheritance that high d.p.a. was dominant and based on few genes. Van der Knaap (unpubl.) found F1 values for d.p.a. between the lower parent and the mid parent value in a study of the inheritance of d.p.a.

In this study it was assumed that the seed parent had an influence on the production of dihaploids. Data were obtained to confirm this with more pollinators and over several years. At the same time seed parents from different origins were pollinated during several years to determine the genetics of d.p.a., the influence of cytoplasmic inheritance and the stage of functioning: gametophytic or sporophytic.

4.2 MATERIAL AND METHODS

Seed parents from different origins were tested for their d.p.a.. All plants were grafted onto tomato rootstock, to enhance flowering and fruit set. For crosses with several pollinators the following *S. tuberosum* cultivars were used: Gineke, Radosa, Ultimus, Sirtema, Record, Libertas, Multa, Merrimack and Katahdin. In addition a *S. demissum* (2n=6x) genotype was included, a seedling from accession WAC 3095. A clone of allotetraploid *S. polytrichon* was tested with several pollinators as well.

To test for cytoplasmic influence, reciprocal crosses had been made between Gineke and Libertas, which differed in d.p.a. and had no parents in

common in the last four generations. From each of the two resulting F1 populations fifteen unselected seedlings were grafted and used as seed parents. For the same purpose A18, a Neotuberosum clone selected at Wageningen from material derived by Dr. N.W. Simmonds from pure *S. andigena*, was crossed with *S. tuberosum* cv. Sirtema. Both parents and three F1 plants were studied as seed parents.

In order to distinguish between gametophytic and sporophytic determination of d.p.a., several Gineke derivatives were used, as described in Table 9. Two different colchicine doubling methods were applied to seeds and to plants. Seeds were placed on filter paper in petridishes, soaked with 0.25% solution of colchicine and left to germinate. Germinating seeds were placed in seed boxes. Vigorous plants of tetraploid appearance and having a high chloroplast number were potted and later grafted. The tetraploidy was later verified by counting the number of chromosomes in the root tips and finally in pollen mother cells. The colchicine-doubled plants of G609 were obtained by the Dionne method (Ross et al., 1967; Langton, 1974). Buds in leaf axils were removed and cotton wool soaked with 0.25% colchicine placed in the axil. Chloroplasts were counted of new shoots that looked doubled. Shoots with a high number of chloroplasts were grafted onto tomato rootstock. At flowering time the chromosome number was determined in pollen mother cells.

As pollinators the *S. phureja* clones IVP1, 10, 35 and 48 were used. The origin of the pollinators and the methods of crossing and screening for dihaploids are described in Section 3.2.

Table 9. Derivatives of cv. Gineke used as seed parent.

Name	Number of genotypes	Origin
Gineke pseudogamic tetraploids	3	pseudogamic tetraploids from Gineke, preselected on leaf shape; the tetraploid character was determined from the chloroplast number
(G × G254)4x	2	tetraploid progeny from Gineke and its highly fertile dihaploid G254
Gineke colchicine-doubled dihaploids	2	plants from dihaploid seeds from Gineke treated with colchicine (seed treatment)
G609 colchicine-doubled	3	colchicine-doubled plants from Gineke dihaploid G609 (plant treatment)
Gineke selfed	4	plants from seeds obtained after selfing of Gineke

Numbers of ovules per berry were determined according to the method of Carroll & Low (1975).

4.3 RESULTS

4.3.1 Influence of the seed parent on dihaploid and hybrid frequencies

Six *S. tuberosum* cultivars and a *S. demissum* genotype were pollinated with four *S. phureja* pollinators in 1974. Values for d/100b and hy/100b are given in Table 10, the analyses of variance in Table 11. The seed parent effect on d/100b was highly significant, as was the pollinator effect. Considering one pollinator at a time, ranking of the seed parents was similar. Six cultivars were pollinated with two good pollinators in 1975 and 1976. Data of this two year comparison are given in Table 12 and the analyses of variance in Table 13. Again the seed parent effect was highly significant. The ranks of the seed parents were similar for both pollinators. In both experiments Gineke and Radosa ranked highest and Record low. Libertas was very low. More data on the seed parent effect can be found in Chapters 3 and 5 (Tables 3, 17 and 19). All, including the three and four year comparisons of Table 17, indicated a significant seed parent effect.

From more than 250 flowers of *S. polytrichon*, pollinated with several pollinators, a few berries were obtained; but no dihaploids were found.

The seed parent effect on hy/100b was not significant in the two experi-

Table 10. Numbers of d/100b and hy/100b produced by *S. tuberosum* cultivars and a *S. demissum* (2n=6x) genotype after being crossed with *S. phureja* pollinators. Crosses were made in 1974. Average number of berries was 38.

Seed parent	d/100b pollinator (IVP nr.)					hy/100b pollinator (IVP nr.)				
	1	10	35	48	mean	1	10	35	48	mean
Gineke	251	209	342	445	312	75	8569	2065	81	2698
Radosa	361	154	250	700	366	128	8802	2195	86	2803
Ultimus	253	291	193	283	255	87	1082	924	50	536
Sirtema	154	117	116	315	176	178	12900	2163	138	3845
Record	131	59	155	248	148	77	4334	624	33	1267
Libertas	114	81	41	113	87	100	3038	3076	75	1572
S. demissum[a]	125	100	109	119	113	0	1811	293	34	535
mean	198	144	172	318	208	92	5791	1620	71	1894

a. *S. demissum*, a hexaploid, produced trihaploids

Table 11. Analysis of variance of the data from Table 10.

	Source	Sum of squares	d.f.	Probability
d/100b	seed parents	264801	6	0.00
	pollinators	121900	3	0.01
	error	135993	18	
	total	522694	27	
hy/100b	seed parents	37866392	6	0.28
	pollinators	152814121	3	0.00
	error	83378472	18	
	total	274058985	27	

d.f.. degrees of freedom

Table 12. Numbers of d/100b and hy/100b produced by *S. tuberosum* cultivars after pollination with two *S. phureja* pollinators in two years. Average number of berries was 21.

Seed parent		d/100b pollinator (IVP nr.)			hy/100b pollinator (IVP nr.)		
		35	48	mean	35	48	mean
Gineke	1975	297	406	352	150	39	95
	1976	237	207	222	1323	25	674
Radosa	1975	301	448	375	254	25	140
	1976	134	160	147	2714	27	1371
Multa	1975	279	349	314	89	33	61
	1976	239	180	210	252	14	133
Record	1975	188	126	157	100	0	50
	1976	89	93	91	3880	27	1954
Merrimack	1975	0	130	65	0	50	25
	1976	148	248	198	455	67	261
Katahdin	1975	31	21	26	67	14	41
	1976	49	91	70	4285	38	2162
mean		166	205	185	1131	30	580

Table 13. Analysis of variance of the data from Table 12.

	Source	Sum of squares	d.f.	Probability
d/100b	seed parents	189331	5	0.01
	pollinators	9087	1	0.10
	years	20475	1	0.03
	s × p interaction	14308	5	0.38
	s × y interaction	82953	5	0.02
	p × y interaction	3775	1	0.24
	error	10866	5	
	total	330794	23	
hy/100b	seed parents	3770058	5	0.48
	pollinators	7271004	1	0.03
	years	6289408	1	0.03
	s × p interaction	3930302	5	0.47
	s × y interaction	3744606	5	0.49
	p × y interaction	6213872	1	0.03
	error	3623714	5	
	total	34842965	23	

d.f.. degrees of freedom

ments. However, it was almost significant (p=0.08) in the 1974 experiment, after logarithmic transformation to remove multiplicative effects (Chapter 5). Variation was very high for hy/100b. The coefficients of variation for d/100b in Tables 10 and 12 were 42% and 25%; from the same experiments the values for hy/100b were 147% and 114%.

Correlation between numbers of ovules per berry of nine cultivars and d/100b was low; correlation with hy/100b was high only with two pollinators in 1974 (r=0.90). Numbers of ovules per berry ranges from 600-950.

4.3.2 Cytoplasmic inheritance

The possibility of cytoplasmic inheritance of d.p.a. was studied within *S. tuberosum* and in a *S. tuberosum* × *S. andigena* cross. F1 plants from reciprocal crosses between the good seed parent Gineke and the unrelated bad seed parent Libertas and the parents themselves were pollinated with IVP35. The resulting numbers of dihaploids are given in Table 14. Notwithstanding the grafting onto tomato rootstock, several F1 plants did not flower. This resulted in unequal numbers of F1 plants from the reciprocal crosses being used as seed parents. The overall performance of the F1 plants was low. Two

Table 14. Numbers of d/100b produced by *S. tuberosum* cultivars Gineke and Libertas and their two reciprocal F1 progenies after pollination with *S. phureja* clone IVP35 in 1974. Also given are the means per F1 and the mid parent value. F1 plants yielded 17 berries on the average.

	d/100b						Mean
Libertas × Gineke	17	40	64	75			49
Gineke × Libertas	6	25	36	42	54	67	64
	67	83	86	108	128		
Gineke	342						192
Libertas	41						

out of fifteen plants produced less dihaploids than the lower parent, Libertas. None of the others reached the mid parent value. The population with Gineke cytoplasm had a slightly higher mean (64 d/100b) than the population with Libertas cytoplasm (49 d/100b), but the difference was not significant (Wilcoxon rank test, p=0.14).

Three plants with *S. andigena* cytoplasm, the progeny of a cross between Neotuberosum clone A18 and Sirtema, a good *S. tuberosum* cultivar with high d.p.a., were pollinated with IVP48. Results are given in Table 15. The mean level of dihaploid production of the progeny (186 d/100b) was slightly lower than the mid parent value (201 d/100b).

4.3.3 Sporophytic vs. gametophytic determination

Different types of progenies from Gineke all produced dihaploids in lower frequencies than Gineke itself when pollinated with IVP35 or 48 (Table 16). There was variation within and between groups. Especially the tetraploids derived from Gineke × G254 yielded very few dihaploids. The three colchicine-doubled G609 plants should be genetically identical. Their differences were possibly due to after-effects of colchicine. They formed another low producing group. The other two colchicine-doubled dihaploids from Gineke, in fact doubled egg cells, were the highest producing group (33% of Gineke), but not much higher than the self progeny (31%) or the pseudogamic tetraploids (25%). It was not clear whether such tetraploids originated from 2n egg cells or whether they were spontaneously doubled during pseudogamy. In the latter case they would be genetically comparable with doubled dihaploids.

Table 15. Numbers of d/100b produced by a *S. andigena* genotype (A18), *S. tuberosum* cv. Sirtema and their progeny after pollination with *S. phureja* clone IVP48 in 1974. Also given are means of the F1 and the mid parent value. F1 plants yielded 10 berries on the average.

	d/100b	Mean
A18 × Sirtema, pl. 1	190	
pl. 2	267	186
pl. 3	100	
A18	86	201
Sirtema	315	

4.4 DISCUSSION

4.4.1 Influence of the seed parent on dihaploid and hybrid frequencies

The effect of the seed parent on the production of dihaploids was confirmed. The ranking of seed parents with a series of pollinators was rather constant over several years. The effect on hybrid production was not significant, probably due to the high variation of this character and multiplicative effects (see Chapter 5). A big seed parent effect could not be expected, as most hybrids were tetraploids, whose frequency was determined by 2n pollen frequencies in the pollinators (Chapter 7). Female fertility of the seed parent could have influenced hy/100b, but the correlation of ovule numbers with hy/100b was high in a few cases only.

Trihaploids from *S. demissum* have been reported earlier (Bains & Howard, 1950; Irikura, 1975a,b). The overall frequency of one trihaploid per berry confirmed that the cytologically unbalanced nature of a triploid is apparently no obstacle to vegetative growth (Marks, 1966a). Allotetraploid *S. polytrichon* has proved to be able to produce dihaploids after a cross with *S. stoloniferum* (Marks, 1955) or via anther culture (Irikura, 1975a,b). A reason for failure in this study might be, that *S. phureja* is not the right pollinator. *S. polytrichon* might have a number of endosperm balance factors (Section 2.5.2.1) different from *S. tuberosum*. The cross *S. polytrichon* × *S. phureja* can yield many triploids (Ramanna & Abdalla, 1970).

Table 16. Numbers of d/100b and hy/100b from cv. Gineke and tetraploid progeny of Gineke. Results from pollinations with IVP35 and IVP48. Dihaploid production is also expressed as percentage of the production of Gineke in the same year with the same pollinator. Mean number of berries from progeny plants was 14.

Pollinator		Seed parent		Dihaploid production			hy/100b
				d/100b	as % of Gineke	mean %	
IVP35	1974	Gineke		342	100		2065
		Gineke pt	pl. 1	30	9		140
			pl. 2	70	20	23	260
			pl. 3	136	40		1791
		(G × G254)4x	pl. 1	20	6	3	104
			pl. 2	0	0		109
IVP48		Gineke		445	100		81
		Gineke pt	pl. 3	130	29		140
IVP48	1975	Gineke		406	100		39
		Gineke selfed	pl. 1	100	25		80
			pl. 2	0	0	31	50
			pl. 3	118	29		92
			pl. 4	278	68		75
		(G × G254)4x	pl. 1	32	8		16
IVP48	1976	Gineke		207	100		25
		Gineke cdd	pl. 1	67	32	33	133
			pl. 2	68	33		140
		G609 cdd	pl. 1	10	5		90
			pl. 2	44	21	12	122
			pl. 3	22	11		67

G. Gineke
pt. pseudogamic tetraploid
cdd. colchicine-doubled dihaploid

4.4.2 Cytoplasmic inheritance

Concerning cytoplasmic differences within *S. tuberosum*, the results from Gineke ♀ ♂ Libertas progenies did not indicate cytoplasmic inheritance of d.p.a. (Table 14). The big difference in d.p.a. between the two parents was not reflected in the two reciprocal progenies. The results from the few plants from the cross between A18 and Sirtema - all with *S. andigena* cytoplasm - did not support the theory of Frandsen (1967) about interspecific cytoplasm differences (Table 15). Even his own data did not support this cytoplasmic influence very strongly. Apparently the cytoplasm of the seed parent was not a major factor in determining d.p.a..

4.4.3 Sporophytic vs. gametophytic determination

When studying the genetics of dihaploid production, it is important to know whether it is the sporophytic or the gametophytic genotype that influences pseudogamy. This question was investigated on the basis of the hypothesis of Bender (1963) who stated that doubled dihaploids should be effective dihaploid producers, if the 'apomictic tendency' was determined by the gametophytic genotype. The tetraploid progeny of seed parent Gineke produced via dihaploidization should have accumulated alleles for pseudogamy and should accordingly produce more dihaploids than Gineke itself or its progeny obtained through selfing, unless Gineke was homozygous for all genes involved. The results did not support this (Table 16). The doubled dihaploids were similar to the self progeny of Gineke, their d.p.a. being 33% and 31% respectively of the d.p.a. of Gineke. The doubled G609 plants produced even fewer dihaploids. The pseudogamic tetraploids also did not seem to be the result of a successful selection for alleles for d.p.a., nor did the tetraploid F1 plants of Gineke with two of its dihaploids produce dihaploids in high numbers. The bad performance of double G609 plants and doubled dihaploids of Gineke could not be explained by reduction of the fertility due to inbreeding, as the production of hybrid seeds per berry was even higher than that of Gineke itself.

The populations used were very small, but on the average they should have had a d.p.a. at least equal to Gineke, if the gametophytic phase were determinative. It can be concluded that the genetic determination of pseudogamy was not on the dihaploid, gametophytic level.

Dihaploidization works as a sieve for lethal genes. One might assume that d.p.a. was not genetically determined, but that the differences between the seed parents in d.p.a. were only caused by a different occurrence of lethal genes. In that case as well, the progeny of dihaploids of Gineke should have performed at least as well as Gineke itself. Since this was not the case, apparently lethal genes were not determinative either. The pro-

duction of dihaploids is most probably determined in the sporophytic phase, modified by the lethal genes, which work in the gametophytic phase.

4.4.4 Mode of inheritance of dihaploid producing ability

Assuming that cytoplasmic effects on d.p.a. were minor and that the sporophytic genotype determined pseudogamy, the results obtained can be analysed as being governed by tetraploid chromosomal inheritance. It is difficult to reach definite conclusions as the sizes of the populations used were small and tetraploid inheritance is complicated in itself. Chances of recovering a good genotype in the progeny are relatively high, when a character is dominant, but are much lower for a recessive character, especially in tetraploids. With intermediate inheritance a mean progeny value is expected close to the mid parent value.

The two reciprocal progenies of Libertas and Gineke can be regarded as one F1 population (Table 14). The low level of dihaploid production in this progeny did not point to dominance with one or a few loci, like Montelongo-Escobedo (1968) concluded. If many loci were involved, dominance would be possible. Four or more additive loci present in Gineke as simplex or duplex could also result in a dispersal of the positive genotype of Gineke to such an extent that no plant in this small progeny would receive all the positive alleles. The *S. andigena* × *S. tuberosum* progeny was close to the mid parent value, pointing to intermediate inheritance (Table 15). All different Gineke derivatives had a lower d.p.a. than Gineke itself. Amongst the data of Tables 14, 15 and 16 no case of positive transgression occurred. Only two plants came close to the better parent: Gineke selfed, plant 4 (68%) and A18 × Sirtema, plant 2 (85%). Recessivity could fit with some of the data, but if pseudogamy were recessive, the differences in d.p.a. between the *S. tuberosum* cultivars would have had to be caused by as many additive loci. Pseudogamy is common in *Solanum* and most *S. tuberosum* clones produce dihaploids. It is not very likely that so many (tetraploid) genotypes are homozygous recessive for several loci for a character which has no obvious advantage to the plant. From that point of view intermediate inheritance is more likely. This was also supported by findings of Van der Knaap (unpubl.), who found progeny means close to the mid parent value in two reciprocal progenies of the cultivars Radosa and Libertas.

It is likely that the inheritance of dihaploid producing ability was intermediate, but dominance can not be excluded, provided that many loci were involved.

5 Interaction between pollinator and seed parent influence

5.1 INTRODUCTION

Only a few genotypes were used as testers for pollinator and seed parent influence in Chapters 3 and 4. When using only few testers one assumes that, apart from multiplicative effects, there is no interaction between the effects of pollinator and seed parent on dihaploid production. This implies that one main effect can be studied irrespective of the level of the other. One can distinguish a multiplicative and a non-multiplicative effect in interaction. The multiplicative effect will not change the ranking of the pollinators or seed parents and this effect can be eliminated from an analysis of variance (anova) by a logarithmic transformation. The interaction which then remains is the interaction in a strict sense.

The approach of not considering interaction was based on preliminary experiments (Van Breukelen, 1972). At that time it was found that the ranking of pollinators was similar with each of the two cultivars they were crossed to. In the course of this research (late 1974) the need was felt to verify the assumption of no interaction, especially since a high variation was observed in dihaploid and hybrid frequencies of IVP35 with several cultivars during a season. The 'no interaction' hypothesis could at that moment be tested with available data using years as replications. However, analysis of the results of the two years without pooling the results from different crossing dates, raised the suspicion that 'crossing date' was a major source of variation in these experiments. When temperature influence was confirmed in a growth chamber experiment (Chapter 6), a plan was devised in which complete sets of crosses were to be carried out in a single day with an interval of three to four days. This way the influence of the date would be the same for all combinations. Crossing date combines two influences: the age of the plants and the environment on that day, as temperature and humidity. The experiment of 1975 was planned with a limited number of cultivars and pollinators. After failure of many of the crosses owing to high temperatures the experiment was repeated in 1976 on a smaller scale. Both experiments were planned especially to determine whether among the best pollinators some would induce more dihaploids with certain cultivars than others, or whether the dihaploid production of a specific pollinator-seed parent combination could be predicted from the performance of the individual parents. Collection of data on hybrid production was included as hybrid production might influence dihaploid frequencies.
Other two-factor interactions were studied as well.

5.2 MATERIAL AND METHODS

The procedure for crossing and growing plants has been described in Section 3.2. For the interaction experiment the cultivars Gineke, Record, Multa, Radosa and Katahdin were used as seed parents and IVP6, 10, 35 and 48 as pollinators (Section 3.2). The seed parents were arranged in the greenhouse in a way that environmental differences between them were minimized. Crossing took place according to the following schedule: every three to four days ten pollinations were made for every combination of the selected pollinators and seed parents. Seeds were screened for embryo spot as described in Section 3.2.

A sample was planted of the spotless seeds and of the seeds without a visible embryo of each cultivar. The number of emerging dihaploids was determined for each group. With the emergence ratio the number of emerging dihaploids per 100 berries (d/100b) was calculated for every crossing date and for every combination. The spotted (hybrid) seeds were not planted. Their numbers were converted to hybrids per 100 berries (hy/100b).

The preliminary experiment (Van Breukelen, 1972) had shown that numbers of dihaploids of single berries had a distribution close to a Poisson distribution. Therefore a square root transformation could have been used to bring the data closer to a normal distribution. However, a logarithmic (ln) transformation was preferred, as it eliminates the multiplicative effect from the interaction. The ln transformation was carried out on d/100b and hy/100b with the formula $y=100\ln(x+1)$, with x=original value and y=transformed value. This formula was preferred to $y=100\ln x$, as the value x=0 occurred.

Anovas were carried out on original and transformed values regarding three main effects: seed parent, pollinator and year (or date) and regarding three two-factor interactions. Three-factor interaction was assumed to be small and considered as error. It could not be separated from the error as there were no replications. Values for the critical level p (one-tailed) of the variance ratio statistic (F) were calculated.

It would have been possible to use the data of individual berries, so that berries were replicates. Such an analysis would have given an estimate of the three-factor interaction. This analysis is hard to carry out owing to the variation in the number of berries. Another difficulty with this analysis is the square root transformation used for the normalization of the variance, which gives problems in interpretation. Amongst possible ways of analysis a choice was made for a log-linear model with main effects and three two-factor interactions. This permitted a test of the three-factor interaction. The analysis resulted in a χ^2 value, which would be high if the three-factor interaction were high compared to the significance level. Not numbers of d/100b and hy/100b were used, but dihaploids and hybrids per

berry, multiplied with the mean number of berries in that experiment. The outcome of the test has a limited value as the number of berries varied between combinations. It was still adhered to as the most practical way to find the order of magnitude of the three-factor interaction. High χ^2 values, indicating the presence of a three-factor interaction, mean that in an anova the main effects and two-factor interactions are tested against too high a mean square of error. This can obscure small effects.

For between-year comparisons, data from experiments described in Chapters 3 and 4 were used.

5.3 RESULTS

The interaction experiments were very much affected by spells of hot weather during the peak of flowering, both in 1975 and 1976. Therefore the data were not as complete as planned. An exact analysis of all (incomplete) data would have given problems in interpretation of contrasts which are difficult to estimate. The problem of analysis with missing data was circumvented by making separate analyses of complete parts of the data. In every part the effects were orthogonal. This way some of the data served several times and others were omitted. The number of berries obtained on one day from one seed parent-pollinator combination varied as the berry set was not constant.

χ^2 values for the estimates of the three-factor interaction were high in the between-year comparisons indicating that a three-factor interaction was present. Consequently, F-ratios in anovas were underestimated and critical levels overestimated. χ^2 values were low and not significant in the interaction experiment in all anovas for d/100b and in one anova for hy/100b. Therefore the combined three-factor interaction was not higher than what error was expected to be. Here the found F-ratios and critical levels were probably of the correct order of magnitude.

5.3.1 Dihaploid production

The first results presented are from experiments in which pollination days were not necessarily the same for all combinations. In the first years mostly good pollinators were used, so that no between-year comparison could be made with orthogonal effects and including bad pollinators. Data are given in Table 17 and anovas of three orthogonal parts of the data in Table 18, both for the original and for the transformed data. Main effects were significant with and without transformation. In part I the seed parent × pollinator (s × p) interaction was not significant. In parts II and III the significance of this interaction was low in the original data ($p=0.18$ and 0.15 resp.), but even this significance disappeared after transformation

Table 17. Numbers of d/100b and hy/100b, original values. Data are means from crosses made on several days in a year. Also given are means of main effects of parts of the data with orthogonal effects.

Seed parent		d/100b pollinator (IVP nr.)				hy/100b pollinator (IVP nr.)		
		1	10	35	48	10	35	48
Gineke	1973	268	160	184	217			
	1974	251	209	342	445	8569	2065	81
	1975		235	297	406	465	150	39
	1976		156	237	207	1950	1323	25
Record	1973	110	86	69	123			
	1974	131	59	155	248	4334	624	33
	1975		122	188	126	86	100	0
	1976		151	89	93	1653	3880	27
Radosa	1974		154	250	700	8802	2195	86
	1975		322	301	448	614	254	25
	1976		223	134	160	4586	2714	27
Ultimus	1973	291	91	126	261			
	1974	253	291	193	283			
Libertas	1973	100	34	41	57			
	1974	114	81	41	113			

Means of main effects		d/100b part			hy/100b part
		I	II	III	II
seed parent	Gineke	260	282	258	1630
	Record	123	137	126	1193
	Radosa		299		2145
	Ultimus	224			
	Libertas	73			
pollinator	IVP1	190			
	IVP10	126	181	147	3451
	IVP35	144	221	195	1478
	IVP48	218	315	233	38
year	1973	139		140	
	1974	201	285	243	2977
	1975		272	229	192
	1976		161	156	1798
mean number of berries		43	39	47	39

Table 18. Analyses of variance of orthogonal parts of the data from Table 17 and of their transformed values (y=100ln(x+1)).

	Source	d.f.	Original values		Transformed values	
			sum of squares	proba-bility	sum of squares	proba-bility
d/100b						
I	seed parents	3	180892	0.00	86541	0.00
	pollinators	3	42522	0.03	20679	0.02
	years	1	30690	0.01	11325	0.01
	s × p interaction	9	16985	0.76	6564	0.75
	s × y interaction	3	5990	0.60	88	0.99
	p × y interaction	3	13715	0.28	3416	0.44
	error	9	27498		10363	
	total	31	318292		138976	
II	seed parents	2	142863	0.00	32438	0.00
	pollinators	2	84498	0.01	9211	0.02
	years	2	82973	0.01	12254	0.01
	s × p interaction	4	38482	0.18	2493	0.50
	s × y interaction	4	23130	0.38	2026	0.59
	p × y interaction	4	94976	0.03	15662	0.02
	error	8	38168		5459	
	total	26	505089		79543	
III	seed parents	1	104808	0.00	32708	0.00
	pollinators	2	29628	0.01	6528	0.09
	years	3	48141	0.01	10272	0.07
	s × p interaction	2	7526	0.15	294	0.85
	s × y interaction	3	9948	0.18	577	0.88
	p × y interaction	6	26745	0.10	8811	0.27
	error	6	8613		5222	
	total	23	235409		64411	
hy/100b						
II	seed parents	2	4085695	0.10	57672	0.01
	pollinators	2	52840393	0.00	898659	0.00
	years	2	35152158	0.00	291334	0.00
	s × p interaction	4	7047428	0.11	14015	0.39
	s × y interaction	4	6498731	0.13	43459	0.05
	p × y interaction	4	46767826	0.00	39612	0.07
	error	8	5193141		23477	
	total	26	157585372		1368229	

(p=0.50 and p=0.85). Some more combinations of pollinators and seed parents in several years were analysed, apart from the ones presented, and no significant s × p interaction was found.

The complete interaction experiment of 1975 consisted of a combination of five seed parents and six pollinators crossed on seven days in a three week period. In 1976 four seed parents were used and three pollinators on

Table 19. Numbers of d/100b and hy/100b, original values. Data are means of crosses made on one day. Also given are means of main effects of parts of the data with orthogonal effects.

Seed parent		d/100b pollinator (IVP nr.)				hy/100b pollinator (IVP nr.)		
		6	10	35	48	10	35	48
Gineke	75-08-18	68	84	265	290	200	73	20
	75-08-21	62	115	225	376	420	38	67
	75-08-25		330	479	568			
	75-08-28	56	367	256	401			
	76-06-09		43	56	123	5814	3167	33
	76-07-23		471	463	276	1292	583	21
Radosa	75-08-18	93	193	349	387	467	250	20
	75-08-21	0	124	133	235	567	30	0
	75-08-25		373	440	998			
	75-08-28	83	457	312	406			
	76-06-09		74	264	80	7447	3833	33
	76-07-23		571	312	322	1800	475	20
Multa	75-08-18	33	197	374	246	233	50	75
	75-08-21	0	60	176	183	133	0	13
	75-08-25		337	332	543			
	75-08-28	38	328	263	184			
Record	75-08-18		107	172	44	86	133	0
	75-08-21		0	104	88	0	33	0
	76-06-09		107	78	43	3850	7700	33
	76-07-23		191	39	87	371	0	20
Katahdin	75-08-18		13	19	12	67	75	50
	75-08-21		7	49	47	100	33	33

four days. Four complete (orthogonal) sets of data were extracted which are given in Table 19. Anovas of original and transformed data are given in Table 20. In a combination of three seed parents and three pollinators on four days all main effects were significant, the s × p interaction was not (part I). Part II included five seed parents, three pollinators and two crossing days, and part III three seed parents, four pollinators and three days. Both seed parent and pollinator effect were highly significant. In both cases the s × p interaction was significant in the original data (p=0.03 and 0.00 resp.). The ln transformation lowered the contribution of this interaction to the total variance to such an extent, that it was not significant any more. Where the scarce data from 1976 were combined with those from 1975 (three seed parents, three pollinators, part IV) the pollinator effect was not significant, nor was the s × p interaction.

Table 19. Continued.

Means of main effects

		d/100b part				hy/100b part	
		I	II	III	IV	II	IV
seed parent	Gineke	313	226	214	232	136	977
	Radosa	360	223	224	247	222	1245
	Multa	269	206	174		84	
	Record		86		88	42	1019
	Katahdin		25			60	
pollinator	IVP6			48			
	IVP10	249	92	216	175	227	1860
	IVP35	300	187	261	205	72	1360
	IVP48	393	181	290	188	28	22
date	75-08-18	254	177	207	199	120	139
	75-08-21	183	129	142	158	98	128
	75-08-25	489					
	75-08-28	330		263			
	76-06-09				96		3546
	76-07-23				304		509
mean number of berries		7	6	7	8	6	8

Table 20. Analyses of variance of orthogonal parts of the data from Table 19 and of their transformed values (y=100ln(x+1)).

	Source	d.f.	Original values		Transformed values	
			sum of squares	proba-bility	sum of squares	proba-bility
d/100b						
I	seed parents	2	50618	0.05	4188	0.05
	pollinators	2	128644	0.00	15307	0.00
	dates	3	465202	0.00	51605	0.00
	s × p interaction	4	37174	0.29	3912	0.18
	s × d interaction	6	63386	0.23	7169	0.10
	p × d interaction	6	186184	0.01	16884	0.01
	error	12	78709		6264	
	total	35	1009918		105328	
II	seed parents	4	204364	0.00	274155	0.00
	pollinators	2	56472	0.00	76680	0.04
	dates	1	16898	0.01	12526	0.23
	s × p interaction	8	39499	0.03	31628	0.81
	s × d interaction	4	30891	0.01	37987	0.35
	p × d interaction	2	14848	0.02	33885	0.16
	error	8	9377		59425	
	total	29	372350		526285	
III	seed parents	2	17183	0.00	18564	0.14
	pollinators	3	315902	0.00	358154	0.00
	dates	2	87020	0.00	92760	0.00
	s × p interaction	6	32807	0.00	36495	0.26
	s × d interaction	4	31243	0.00	37642	0.11
	p × d interaction	6	90685	0.00	87534	0.03
	error	12	10548		48229	
	total	35	585388		679378	
IV	seed parents	2	184188	0.00	114761	0.01
	pollinators	2	5504	0.61	19718	0.35
	dates	3	205004	0.00	68480	0.10
	s × p interaction	4	17795	0.52	6590	0.94
	s × d interaction	6	90334	0.06	49052	0.50
	p × d interaction	6	115044	0.03	74766	0.28
	error	12	63012		104182	
	total	35	680880		437549	

Table 20. Continued.

hy/100b

II	seed parents	4	126835	0.01	191563	0.06
	pollinators	2	219945	0.00	259937	0.01
	dates	1	3674	0.34	90640	0.03
	s × p interaction	8	186581	0.01	302065	0.08
	s × d interaction	4	21933	0.29	74491	0.31
	p × d interaction	2	19366	0.13	16798	0.55
	error	8	28785		105419	
	total	29	607119		1040913	
IV	seed parents	2	498735	0.78	224769	0.05
	pollinators	2	21655459	0.00	895682	0.00
	dates	3	73768016	0.00	704883	0.00
	s × p interaction	4	6270671	0.23	45128	0.80
	s × d interaction	6	1617241	0.94	138483	0.57
	p × d interaction	6	37140551	0.00	146895	0.54
	error	12	11593584		332539	
	total	35	152544257		2488377	

The seed parent × year (date) interaction was never significant after ln transformation. Pollinator × year (date) interaction, however, was several times significant, even after transformation.

It can be concluded that the most important interaction for dihaploid production, namely the s × p interaction, was only present, but not always, where both main effects were significant in the original data. After ln transformation no s × p interaction was found.

5.3.2 *Hybrid production*

Variation was higher for hybrid production than for dihaploid production in the same experiments. The coefficient of variation for d/100b in e.g. Table 17 part II was 29%, whereas it was 49% for hy/100b from the same crosses. In a compilation of results from three years (Table 17 and 18) s × p interaction was hardly significant (p=0.11) and even less after ln transformation (p=0.39). The seed parent main effect was not very strong in the original data, as could be expected from the results of Chapter 4.

Combined results from the interaction experiments of 1975 and 1976 for hy/100b are given in Table 19. As the hybrid production is less important, less data are given. Two anovas of parts of the data with orthogonal effects are given in Table 20. In part II, including five seed parents, the

s × p interaction was significant (p=0.01); it had even some significance after transformation (p=0.08). Part IV (three seed parents, three pollinators, four dates from two years), where the seed parent effect was significant after transformation only, showed no significant s × p interaction.

Of the other two-factor interactions the pollinator × year (date) interaction was sometimes present, but after transformation it was reduced; only in the between-year comparison (Table 17) the critical level was still low (p=0.07).

About s × p interaction it can be concluded that it was found once for hy/100b. Logarithmic transformation reduced this, but did not remove it completely.

5.4 DISCUSSION

5.4.1 Dihaploid production

The analysis of data from Table 17 showed no interaction between seed parent and pollinator effect on the production of dihaploids. In the interaction experiment (Table 19), where the influence of pollination date was the same for all combinations, the s × p interaction was found only in those parts where data of a bad pollinator or low producing seed parent could be included (Table 20, II and III). It was probably due to the more careful collection of data that this interaction was significant here and not in the between-year comparison. It was also here that χ^2 values for the three-factor interaction were low. The fact that the ln transformation reduced the interaction component in the anova to a not significant level, means that only the multiplicative part of the s × p interaction had been present. Where only pollinators and seed parents with high d.i.a. and d.p.a. were involved, the multiplicative effect could not appear, hence the absence of interaction there, even in the original data. The multiplicative effect means that the combination of a pollinator with high d.i.a. with a seed parent with high d.p.a. gives even higher dihaploid production than could be expected from their individual performances. The absence of interaction in a strict sense implies that a good pollinator is good irrespective of the seed parent used. For practical dihaploid production this means that the best pollinator for a certain seed parent is also the best for others. For dihaploid research the implication is that testing for d.p.a. can be done with one pollinator and testing for d.i.a. with one seed parent. With the limited data and the multiple use of part of the data such a conclusion should be drawn with some caution, especially as the genetic variation in the group of pollinators was limited. The parentage of the cultivars of *S. tuberosum* seems to have been sufficiently diverse to assume general applicability of the conclusion at the side of the seed parent.

These observations did not provide an argument for introducing the ter-

minology of general and specific dihaploid producing or inducing ability in *S. tuberosum* as Eenink (1974) proposed for *Brassica oleracea*. In *Solanum* the dihaploid production of specific parental combinations can apparently be deduced from the general performance of the parents, provided that the multiplicative effect is taken into account.

Experimental error proved to be considerable in interaction experiments, even when crossing was carried out according to a balanced plan. This error might have been the reason why the correlation for dihaploid production found by Hermsen & Verdenius (1973) between two cultivars was not as high as they expected. Their theory of a differential reaction of the two cultivars to the same series of pollinators is not necessary as explanation. The reasonably high correlation they obtained (r=0.65; n=22) can support the no interaction conclusion.

The pollinator × year (date) interaction found fitted with the temperature sensitivity of the pollinator for d/100b in the growth chamber experiment (Chapter 6). Apparently the different pollinators are not sensitive to the environment to the same extent, hence the interaction.

5.4.2 Hybrid production

The high variation in hybrid production might have been the reason why an existing interaction was not observed. In the more accurate interaction experiment a s × p interaction was found once, but it was not very strong. There is probably no s × p interaction for hy/100b, but the existence of this interaction could not be excluded with certainty. Even if it would exist, it would not be an important factor in determining the level of hybrid formation. As the removal of hybrids that are produced along with dihaploids only plays a minor role in the amount of work involved in making dihaploids, this possible interaction is not of practical importance.

The pollinator × year (date) interaction found was expected from the observation that IVP10 and 35 were very variable in hy/100b during a season, in contrast with IVP48.

6 Influence of temperature on frequencies of dihaploids and hybrids

6.1 INTRODUCTION

It follows from Chapters 3 and 4 that dihaploid and hybrid production are determined genetically. In this chapter the influence of one external factor, temperature, on the frequency of dihaploids and hybrids is studied.

Gabert (1963) did not find temperature influence on d/100b, but Wöhrmann (1964) found a negative influence of high temperature on d/100b and berry set (see also Section 2.4). Both authors experimented with temperature treatments after pollination only.

Information available on variability is not very detailed. Data on dihaploid frequencies are usually pooled per year, so that the fluctuations during the year are lost. Several authors have found year to year fluctuations in d/100b and hy/100b (Gabert, 1963; Frandsen, 1967; Hermsen, unpubl.). A preliminary study with unpooled data from crosses on different days in 1971 indicated that fluctuations occurred within the season in a conditioned greenhouse (Van Breukelen, 1972). There was more variation in hy/100b than in d/100b, especially in high seed set pollinators like IVP35 and 32. When a similar pattern was found in 1973, an experiment was set up to determine the influence of the temperature on d/100b.

The purpose of the experiment was to find the best temperature conditions for dihaploid production and also to obtain more insight in the mechanism of dihaploid production. As this cannot be separated completely from the mechanism of hybrid production, the influence of temperature on hybrid production was also looked into. The experiment was carried out in growth chambers, in order to be able to keep the temperature constant. To separate the temperature influences on the seed parent and on the pollinator, which are confounded in the greenhouse, two chambers with different temperature regimes were used. This made it also possible to transfer plants between growth chambers and to give them a controlled change in temperature, in order to see at which moment the change in temperature becomes effective in changing dihaploid or hybrid frequencies. This would give additional information about the mechanisms involved.

6.2 MATERIAL AND METHODS

Cultivar Gineke was used as seed parent in crosses and *S. phureja* clones IVP35 and 48 as pollinators (see Section 3.2). All plants were grafted onto

tomato rootstock, potted in well drained plastic buckets of 8-10 l and placed in growth chambers about 14 days before the first flowers opened. Gineke and *S. phureja* plants were distributed at random over two growth chambers. Codes indicating plants and treatments are given in Table 21.

The growth chambers used were part of the phytotron at the Department of Field Crops and Grassland Husbandry of Wageningen Agricultural University. Each chamber measured 4.5 m × 3.2 m × 2.2 m. Plants were placed on tables so that the flowers were about 0.5 m below the lamps. Fluorescent tubes (Philips TL33/40 W) and some additional incandescent bulbs provided a radiation intensity of 175 $J \cdot m^{-2} \cdot s^{-2}$ at plant height. CO_2-concentration was kept at 300 $mg \cdot kg^{-1}$. Temperature, moisture and daylength were set as follows. Lights were on for 16 h per day, during which period the relative humidity was 70%. During the 8 h dark period the relative humidity was 90%. In the chamber with low temperature (L) the temperature was kept at 18 °C throughout. In the other chamber (H) the temperatures for the first 12 h of light were 22 °C in 1974 and 23 °C in 1975. During the remaining 4 h of light and 8 h of darkness the temperature was 18 °C (in 1975 after 6/8: 15 °C in darkness). Good air circulation made the temperature equal in all parts of a growth chamber.

White fly (*Trialeurodes vaporariorum*) teemed during the crossing season of 1974 and was controlled with dichlorephos. This caused abortion of pol-

Table 21. Codes indicating plants and examples of treatments in two growth chambers with different temperature regimes and a greenhouse. All transfer dates were in August 1975.

G	= *S. tuberosum* cv. Gineke
35	= *S. phureja* IVP35
48	= *S. phureja* IVP48
L	= plant in chamber with low temperature
H	= plant in chamber with high temperature
Gr	= plant in greenhouse
LH17	= plant transferred from chamber L to chamber H on 17/8
HL5	= plant transferred from chamber H to chamber L on 5/8

Examples of treatments:

GL × 48H	= cross between cv. Gineke in chamber L with IVP48 in chamber H
GH × 35LH16	= cross between cv. Gineke in chamber H with IVP35, transferred from chamber L to chamber H on 16/8
GHL5 × 35L	= cross between cv. Gineke, transferred from chamber H to chamber L on 5/8, with IVP35 in chamber L

linated flowers in crosses made from one day before until 4-5 days after spraying. The negative effect of dichlorephos on berry set was determined only by the end of the crossing period owing to misleading results of a former spraying experiment. In 1975 *Encarsia formosa*, a parasitic wasp, was successfully introduced into the chambers to keep the pest at low levels.

Fresh pollen was applied to emasculated flowers of Gineke. Seeds were extracted from each berry separately and screened for embryo spot (see Section 3.2). Three groups were distinguished: seeds with embryo spot, seeds without embryo spot and seeds without a visible embryo. The third group was discarded. All seeds without embyro spot were classified as dihaploids. Previous experiments actually displayed less than 1% pseudogamic tetraploids and less than 1% hybrids. If some spotless seeds would not have been dihaploids, the resulting error would have been very small.

Gineke plants in specified conditions crossed with *S. phureja* plants in specified conditions on several successive days are called a treatment. In 1974, four to eight flowers were pollinated per treatment per day. This was increased to ten flowers in 1975. Data from single berries were pooled per day within one treatment. Nearly 7000 flowers in all were pollinated.

Most plants were left in the same growth chamber throughout the crossing period. By using pollen from both chambers to pollinate seed parents in one chamber, four treatments could be made per pollinator, e.g. GL × 35L, GL × 35H, GH × 35L and GH × 35H. These are called 'fixed treatments'. The results of these treatments were compared with those of the conditioned greenhouse, described in Chapter 3. In other treatments plants were exchanged between the two chambers. This was done in both years, but only in 1975 data were complete enough to be reported.

Out of the Gineke plants in each chamber a group of plants was chosen and pollinated every day with pollen from 35L. After eight days, on 5/8, the plants were transferred from chamber L to H (GLH5) and from H to L (GHL5). Pollinations with 35L were continued for 21 more days. Two other groups were selected, also pollinated with 35L and after 12 days, on 17/8, transferred (GLH17 and GHL17), after which pollinations with 35L were continued for 16 days.

One IVP35 plant from each chamber was transferred to the other chamber on 16/8 (35LH16 and 35HL16). Pollinations were made in the period 15/8-31/8 on seed parents in chamber L. Two other plants were transferred on 27/8 (35LH27 and 35HL27) and used from 27/8-31/8 on seed parents in chamber H.

Analysis of variance (anova) was carried out with days as replications and temperature regimes of the seed parent and pollinator as factors.

6.3 RESULTS

From the experiment in 1974 it was learned that the temperatures used were suitable for flowering and berry set. Berry set was almost nil in the

period in which insecticides were used. The best berry set was obtained in chamber L and with pollinator IVP35. Therefore these were used as much as possible in 1975 in those treatments, in which plants were transferred. The milder pest control in 1975 led to higher berry set. Since the last two weeks showed the best berry set, most data used are from that period. For 1974, only data from the first ten days were used and only of the fixed treatments. Data from transfers in that year were very incomplete. They served, however, to improve the transfer procedure for the next year.

6.3.1 Treatments with fixed temperatures

Coefficients of variation for d/100b and hy/100b between crossing days within treatments in 1975 were calculated both for the whole period (40 days) and the last 15 days with high berry set. They were compared with the variation in a conditioned greenhouse during the crossing seasons of 1974 and 1975 (Table 22). Variation in d/100b was about equal in treatments with IVP35 and 48 in the growth chambers, both in the whole crossing period and in the last 15 days. Variation was higher in hy/100b, especially for IVP35. Variation in the greenhouse in 1974, a good crossing year, was similar to

Table 22. Range, mean and coefficient of variation (c.v.=s/$\bar{x}$) for dihaploids per 100 berries (d/100b) and hybrid seeds per 100 berries (hy/100b). Data from crosses in growth chambers (1975) and a greenhouse (1974 and 1975). For codes see Table 21.

Treatment	Number of days		d/100b			hy/100b		
	total	crosses[a]	range	mean	c.v.	range	mean	c.v.
GL × 35L	40	20	50- 650	231	0.64	71-2943	572	1.29
GL × 35L[b]	15	8	100- 400	199	0.49	71- 290	179	0.61
GGr × 35GR[c]	84	12	100- 540	318	0.55	100-6963	2059	0.88
GGr × 35Gr[d]	73	17	33- 389	171	0.60	0-3000	817	1.30
GL × 48L	40	21	38- 525	244	0.58	25- 150	62	0.61
GL × 48L[b]	15	9	38- 525	271	0.59	25- 88	38	0.69
GGr × 48Gr[c]	77	11	200-1230	505	0.53	0- 100	60	0.57
GGr × 48Gr[d]	47	7	100- 513	278	0.58	0- 80	27	1.21

a. number of days, on which crosses were made
b. the shorter crossing period was part of the total crossing period in the growth chamber
c. in 1974
d. in 1975

that of the shorter period in the growth chambers, whereas the greenhouse variation of 1975 was usually higher and closer to the whole growth chamber period. Means of d/100b and hy/100b in the growth chambers were closer to those of 1975 than to those of 1974 in the greenhouse.

The influence of the temperature on the seed parent and the pollinator separately was studied with data of the relatively stable first period in 1974 and last period in 1975. Numbers of d/100b and hy/100b are given in Table 23. Data are means of four or five crossing days with one day interval. Conclusions about positive or negative influence of high or low temperatures are given in Table 24. On the four treatments with the same pollinator an anova was carried out with the crossing days as replications. Since for IVP48 in 1974 not enough complete data were available for an anova, a sign test (two tailed) was applied to data from the whole crossing period. Resulting significance levels of differences between the effects of the two chambers on seed parents or pollinators are also presented in Table 24. Differences due to temperature were more consistent in 1975 than in 1974. Results of both years and both pollinators were similar. About the direction of the differences it can be concluded that, whereas the influence of the temperature on the seed parent in d/100b was variable or absent, low temperature had a small but consistent positive effect on the pollinator in d/100b. For hy/100b low temperature had a negative influence on the seed parent when IVP35 was used, but for IVP48 data were inconsistent. Low temperature had a positive influence on both pollinators, especially on IVP35. From all differences those in hy/100b for IVP35 were most marked.

Table 23. Means of d/100b and hy/100b from crosses between seed parent Gineke and two pollinators in growth chambers with low (L) and high (H) temperatures.

	Seed parent	Pollinator	1974		1975	
			IVP35	IVP48	IVP35	IVP48
d/100b	L	L	240	218	251	266
		H	121	132	199	263
	H	L	325	156	246	224
		H	145	150	181	201
hy/100b	L	L	850	39	236	31
		H	428	25	133	19
	H	L	1400	21	430	43
		H	541	7	174	37

Table 24. Influence of low (L) and high (H) temperatures on d/100b and hy/100b via seed parent and pollinator. Positive (>) and negative (<) influence of low temperatures is concluded from data in Table 23. Critical levels (p) are for differences between L and H and calculated with an anova on an orthogonal part of the data, for IVP48 in 1974 with a sign test.

	Influence via	1974		1975	
		IVP35	IVP48	IVP35	IVP48
		p	p	p	p
d/100b	seed parent	L<H 0.21	L=H 0.45	L>H 0.67	L>H 0.61
	pollinator	L>H 0.00	L>H 0.02	L>H 0.36	L>H 0.16
hy/100b	seed parent	L<H 0.12	L>H 0.12	L<H 0.08	L<H 0.11
	pollinator	L>H 0.01	L>H 0.77	L>H 0.03	L>H 0.80

6.3.2 Treatments in which the seed parent was transferred

It is of interest to know whether seed parents react immediately to transfer to another temperature regime or only after a lapse of time. As the clearest influence on the seed parent was with IVP35 in 1974, only IVP35 pollen was used on transferred seed parents in 1975. Mean dihaploid and hybrid frequencies are given in Table 25 for periods of five to seven consecutive days of crossing before and after transfer of two groups of seed parents. As could be expected from the fixed treatments, no big difference in d/100b was observed between the treatments. For hy/100b clear differences between the treatments were observed. Before and after the first transfer more hybrid seeds were formed on seed parents originally in chamber L than on seed parents from chamber H, but often the difference was small. Of the seed parents of the second transfer the plants from chamber L produced consistently less hy/100b, both before and after transfer.

There was no change at the moment of transfer for d/100b and hy/100b. The direction of the differences in hy/100b was inconsistent: it was different for the two transfers.

6.3.3 Treatments in which the pollinator was transferred

From the clear effect of temperature on the pollinators in fixed treatments, especially on IVP35, changes could be expected after transfer of the

Table 25. Means of d/100b and hy/100b from crossing periods of 5-7 days. Data from seed parents transferred from one growth chamber to another, compared with data from non-transferred seed parents. All pollinations with 35L. Dates in August 1975. For plant codes see Table 21.

Date	d/100b				hy/100b			
	GLH5	GHL5	GL	GH	GLH5	GHL5	GL	GH
1- 5	236	263[a]	-	-	1166	513[a]	-	-
6-12	191	189	209	210	448	188	283	370
15-20	218	221	198	202	333	302	197	232
21-26	365	332	254	275	613	541	221	562
	GLH17	GHL17			GLH17	GHL17		
12-17	143	160			181	390		
19-24	233	238			337	530		
25-30	223	269			190	500		

a. only one observation
- no data

pollinators from one temperature regime to the other. Mean frequencies of dihaploids and hybrids for successive periods after transfer are given in Table 26.

After the first transfer (35LH16 and 35HL16) d/100b was lower for 35LH16 than for 35HL16 for ten days. In the next five days d/100b was higher for 35LH16, but the difference was small. After the second transfer (35LH27 and 35HL27) crosses could only be made for a short period. The results were similar to those of the first group: pollinators placed in chamber H induced less dihaploids. The data were analysed in an anova with the pollinator treatments and crossing days as sources of variation for the ten and fifteen day period after transfer. For the period 17/8-26/8 the critical level for pollinator influence was 0.02, whereas this was 0.16 for the period 17/8-31/8. From the original data it was also clear that the difference between 35LH16 and 35HL16 was smaller during the last days.

With respect to hy/100b pollinators transferred to chamber H had a higher production than their transferred counterparts after both transfers

Table 26. Means of d/100b and hy/100b from crossing periods of five days. Data from pollinators transferred from one growth chamber to another, compared with data from non-transferred pollinators. All pollinations on GL, except 35LH27 and 35HL27 which were on GH. Dates in August 1975. For plant codes see Table 21.

Date	d/100b				hy/100b			
	35LH16	35HL16	35L	35H	35LH16	35HL16	35L	35H
17-21	185	252	247	225	335	93	260	150
22-26	211	289	254	182	253	129	221	122
27-31	176	154	-	-	288	143	-	-
	35LH27	35HL27			35LH27	35HL27		
27-31	176	190			367	273		

- data very incomplete

and for the whole period. In an anova with pollen treatments and crossing days as sources of variation the critical level for pollinator treatments was very low (p=0.003).

6.4 DISCUSSION

In different temperature conditions the number of dihaploids per 100 berries was a rather stable character. Variation in d/100b was regular and low, compared with hy/100b, both in the greenhouse and in the growth chambers. IVP35 had higher variation than IVP48 in most conditions. Temperature had an influence on d/100b only via the pollinator. Especially in IVP35 low temperatures had a positive influence.

The higher variation in hy/100b was reflected in the differences found between the fixed treatments. Both IVP35 and 48 were temperature sensitive regarding the production of hybrid seeds. Positive influence of low temperature on the seed parent was more outspoken in treatments with IVP35 than in those with IVP48. With the higher level of hy/100b in IVP35 there was more room for influences to show. Results from 1974 and 1975 compared well.

In the experiments, in which the seed parent was transferred, no effect was expected for d/100b, considering the results of the fixed treatments.

This was confirmed by the results. For hy/100b a negative effect in chamber L was expected. This influence proved not to be consistent in this experiment. Already before transfer the plants in chamber L had higher hy/100b. This continued after the transfer. Before the second transfer plants in chamber L had lower hy/100b, as expected. These, too, were unaffected by the transfer for 12 days.

After transfer of the pollinator a positive influence of low temperatures on the pollinator was expected, considering the results of the fixed treatments, both in d/100b and in hy/100b. For d/100b this was confirmed. After both transfers 35LH induced the lower number of dihaploids. For hy/100b, however, the reverse was found: in both cases 35LH produced more hybrids. For d/100b results 'adapted' to the new temperature after transfer; for hy/100b they did not adapt, even after 15 days.

Apparently, the two temperature sensitive mechanisms in the pollinator influencing d/100b and hy/100b acted at different moments during floral development. The mechanism for the induction of dihaploids reacted fast to new conditions. Data from the first two days after transfer were not complete, but after two days the change had already taken place. For dihaploid induction events in the last phase of pollen production or in the style and ovary during pollination or immediately afterwards must be temperature sensitive.

The temperature effect on hy/100b via the pollinator must have worked on the pollinator at least ten days before pollen ripeness. The group of hybrid seeds consisted mainly of tetraploid hybrids, derived from unreduced *S. phureja* pollen. The frequency of unreduced pollen is determined for the greatest part by events during meiosis. Meiosis in *S. phureja* takes place 12-14 days prior to anthesis at 20-23 °C (Mok, pers. comm.). This could explain why during the measured period (up to 15 days after transfer) the number of hybrid seeds was still similar to that found with plants from the chamber where the pollinator had been before transfer. When the experiment ended 15 days after transfer of the pollinators, the change that could be expected was not yet observed. Not even a tendency in that direction was found during the last two days. The results from the transfer of the pollinator corresponded with the results from the fixed treatments, if one assumes a fast temperature reaction of the pollinator in d/100b and a slow reaction in hy/100b.

The absence of temperature influence on the seed parent for d/100b makes that no sensitive mechanism could be pointed at. While the influence on the seed parent for hy/100b was clear in fixed treatments, the results were inconsistent in transfer experiments. However, there was a tendency for seed parents not to be affected by transfer. This slow reaction could indicate that it was the process which determined the number of ovules in an ovary and not of seed development after pollination, which was temperature sensitive.

From the results of experiments with temperatures it can be concluded that within the range of 18-23 °C no positive effects on d/100b can be expected from a special constant or alternating temperature for the seed parent. This confirms Gabert's (1963) findings. The lowering of d/100b by high temperature found by Wöhrmann (1964) was not confirmed within the temperature range used. The fact that he and Gabert (1963) did not pay attention to conditions in which the seed parent was grown before pollination, will not have affected their results very much. The higher berry set on seed parents in chamber L could be a reason to try to maintain a temperature of 18 °C during the flowering period. Conditions for the pollinator proved to be more important. Low temperature (18 °C) had a positive influence on dihaploid induction. Dihaploid frequencies can probably not be improved by lower temperatures, as temperatures lower than 18 °C reduce flowering (Bodlaender, 1960) and are difficult to maintain in a greenhouse.

If space is limited in low temperature growing facilities, it will be advantageous to place pollinators there preferentially and to give the seed parents the second best place. Low temperature for pollinators will also result in higher hybrid frequencies, but this will not have negative consequences for practical dihaploid production.

7 The mechanism of dihaploid induction

7.1 INTRODUCTION

After the discussion of the genetics of the pollinator effect on dihaploid induction the question remained how the pollinator effect operated. Through what kind of mechanism did pollen influence the frequency of dihaploids, to which it did not contribute genetically?

Considering the processes after pollination of a 4x *S. tuberosum* flower with pollen of a 2x *S. phureja*, it can be assumed that 5x endosperm is lethal and that seeds with a dihaploid embryo have 6x endosperm (Von Wangenheim et al., 1960; Bender, 1963) (see Section 2.5.2.1). Pollen must provide 2x chromosomes for this 6x endosperm. Is this 2n or n pollen? Does dihaploid induction follow the 'maize mechanism', in which one sperm fertilizes the polar nuclei and the other sperm is lost, or does it follow the '*Solanum* mechanism' (Hermsen, 1971), where two sperms both fertilize the central nucleus, separately or fused?

This chapter deals with cytological and microscopical observations in order to try to elucidate these questions. Frequencies of 2n pollen were estimated in a series of pollinators in order to relate these to the frequencies of dihaploids and hybrids that derive from them. Confirmation was sought of the lethality of seeds with 3x embryos and an estimate was made of the lethality of seeds with 4x embryos. Numbers of dihaploids and hybrids of a range of pollinators were compared in order to find out whether dihaploids and hybrids originated from pollen with the same ploidy level. Pollen tubes were studied in vivo to screen for restitution nuclei and other abnormalities in pollen tube mitosis. To check Höglund's (1970) hypothesis of preferential fertilization or faster growth of 2n pollen, as compared with n pollen, the relative growth rate of pollen tubes of different ploidy levels was measured in styles. Finally, the effect of delayed pollination on dihaploid frequencies was determined.

7.2 MATERIAL AND METHODS

Material for experiments described in this chapter consisted of fresh pollen from *S. phureja* clones and pollinated styles and ovaries from *S. tuberosum* at different stages of development. The origin of the *S. phureja* clones is described in Section 3.2. *S. tuberosum* cultivars used were Gineke, Multa and Radosa, together with Gineke dihaploids G254 and G609, de-

scribed in Section 8.2, and dihaploid derivative GB24.

Frequencies of 2n pollen grains were estimated by the pollen diameter. Fresh pollen was dusted on a slide and stained with lactophenol acid fuchsin. The diameters of 100 to 200 pollen grains were measured in eyepiece graticule units and a frequency distribution was made. Assuming that 2n pollen grains had twice the volume of n pollen grains, their linear dimensions should differ by a factor 1.25. Grains with a diameter of about 1.25 times the diameter at the lower mode were recorded as 2n pollen grains. Data were collected in 1975 and 1976. Frequencies of pollen with four germ pores, an indication for 2n pollen, were recorded from samples of 25 pollen grains.

Seed collapse was checked in young berries of Gineke, pollinated with pollen of IVP10, 24, 35, 48, Gineke or G254. Three sets of pollinations were made at monthly intervals. Three berries of each combination were picked one and two weeks after pollination. The berries were dissected and the number of developing ovules counted. Fully developed seeds were counted after ripening of the remaining berries.

Correlation coefficients between d/100b and hy/100b were calculated from data in Fig. 1, Tables 6 and 7 and data in Van Breukelen (1972).

Sperms were studied in pollen tubes in vivo, after pollen tube mitosis was completed. The method for preparing styles was adapted from Montezuma-de-Carvalho (1967). Gineke styles, pollinated with *S. phureja* pollen, were fixed after 24 h in a 3:1 mixture of ethanol and acetic acid for one day and kept in 70% ethanol. Styles were macerated in 1 $mol \cdot l^{-1}$ HCl at 60 °C for 10 min, rinsed, placed in Feulgen stain and dissected on a slide in aceto-carmine. Only the lowest third of the style was used and dissected with needles to very fine bundles. The material was gently pressed. The number and relative size of the nuclei was recorded for all pollen tubes of which a long section near the nuclei was not broken. After initial experiments the distance between the two sperms was also recorded. Pictures were made of a number of the tubes. The negatives were enlarged to the same magnification and the nuclei, measuring 1-4 cm, were cut out very close to the edge and weighed for an estimate of the size.

Certation was measured by placing two small amounts of pollen at opposite sites on a stigma and comparing the length of the bundles of pollen tubes after 24 h. Styles were prepared for UV fluorescence according to Martin (1959). They were fixed in a mixture of acetic acid, formalin and 80% ethanol in a 1:1:8 proportion for 24 h and macerated in 8 $mol \cdot l^{-1}$ NaOH for 5 h. Staining was done in 0.1% aniline blue dissolved in 0.033 $mol \cdot l^{-1}$ (0.1 N) K_3PO_4. After staining the style was split in two halves, each containing one bundle of tubes. Before pollination the style had been marked with a razor blade to distinguish between the two halves of the style and to facilitate splitting (see Fig. 2). By this method the two halves, with different types of pollen, could be recognized even after maceration and

Fig. 2.

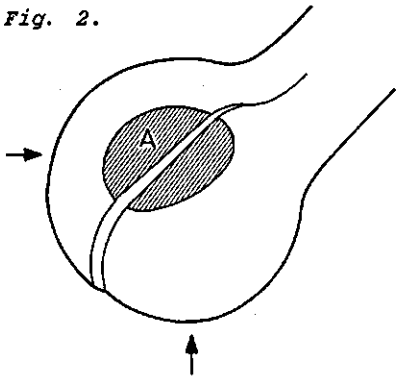

Fig. 3.

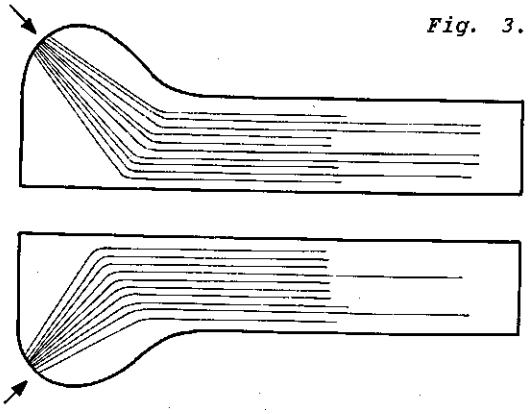

Fig. 2. Markings on the stigma for certation measurements. The stigma was split and a small part (A) removed at one side of the incision. Pollen was applied at each stigma half (arrows).

Fig. 3. Schematic picture of two half styles with thinning pollen tube bundles. The top half shows little thinning and the lower half much. Arrows: places of pollen application.

splitting. The two halves were placed alongside on one slide in glycerine and pressed gently. Both pollen tube bundles could be seen simultaneously under low magnification. For each replication of a comparison of two pollen sources five to ten styles were used. Notes were made of the relative distances covered by the pollen bundles and the thinning of the bundles in the three to eight best preparations. From these notes it was decided whether one pollen type was growing faster than the other. Some comparisons of pollen sources were replicated several times. Tetraploid styles, mostly from Multa, were used in all cases but one. Diploid styles are usually thin. Diploid GB24 was chosen because of its robust stigmas.

The effect of the age of the flower at the time of pollination on dihaploid frequencies was determined by pollinating Gineke flowers of different ages with pollen of IVP35 on one day in a growth chamber with low temperatures (see Section 6.2).

7.3 RESULTS

7.3.1 Determination of 2n pollen frequencies

Frequency distributions of pollen diameters and counts of the number of germ pores were made for several *S. phureja* clones. Frequencies of 2n pollen were calculated from the frequency distributions and are presented in Table 27. Diameters of pollen from IVP35 were measured on four other occasions as well with the following results: 23, 10, 13 and 1% 2n pollen.

Table 27. Percentages of 2n pollen of *S. phureja* pollinators determined by measuring pollen diameters. Also given are numbers of hy/100b produced by cv. Gineke with the same pollinators in two years.

Pollen source	% 2n pollen				hy/100b	
	1973[a]	1975	1976[b]	1976[b]	1974	1976
IVP1	0[c]	0	0	-	75	75
IVP10	4	6	2	6	8569	1950
IVP24	1	0	0	0	85	131
IVP26	-	0	0	-	-	97
IVP32	30	7	-	10	3283	7400
IVP35	0	0	1	5	2065	1323
IVP48	0[c]	0[c]	0	0[c]	81	25
IVP66	-	0	0	-	-	196
sample size per pollinator	200-2000	200	100	100		

- no observation
a. data kindly provided by Dr. M.S. Ramanna
b. pollen collected on two different dates in 1976
c. values between 0.0 and 0.5

IVP35 was highly variable for this character. The frequencies for the other pollinators were variable as well. High seed set pollinators (IVP10, 32 and 35) had higher frequencies than low seed set pollinators (IVP24, 26, 48 and 66). The frequencies of pollen grains with four germ pores showed the same pattern. Numbers of hy/100b for 1974 and 1976, the two years in which most of the pollinators were used, have been included in Table 27 for comparison.

7.3.2 *Ovule lethality*

Numbers of developing ovules, counted one and two weeks after fertilization, and numbers of mature seeds are given in Table 28. These numbers decreased sharply with the age of the berry. Only about 5% of the ovules, which initially started to grow, reached maturity in 4x × 2x crosses. In Gineke × Gineke (4x × 4x) the decrease was less drastic, but even there at least 40% of the developing ovules did not produce a seed. The reduction in ovule numbers was in general slower in the first set of crosses than in the

other two. Final seed set was high only with Gineke as pollen parent and reasonable with IVP10 in the third set. Variation within samples was not high.

7.3.3 Correlation between dihaploids and hybrids per berry

Correlations between d/100b and hy/100b were calculated on data from full-sib *S. phureja* populations. Correlation coefficients, including those calculated for all pollinators used in one season, are given in Table 29. All correlations but one were negative and some were high. Correlations for all pollinators from one season were not very high.

7.3.4 Pollen tube mitosis

Information about sperms after pollen tube mitosis before fertilization was obtained from observations of pollen tubes in vivo. In the majority of the tubes two or three nuclei were seen, the vegetative nucleus not always being visible. Weights of photographic prints were used as measure of sperm size. Sizes of sperms varied. Larger and smaller pairs of sperms were observed, possibly corresponding with 2n and n gametes respectively. However, the size of the sperms varied with a factor two in Gineke × Gineke crosses,

Table 28. Numbers of developing ovules or seeds per berry at different times after pollination of cv. Gineke with several pollen parents. Data are means of three or more berries. Pollinations were made on three different dates in 1976.

Date	Pollen parent	1 week	2 weeks	maturity
76-06-18	IVP10	135	42	11
	IVP35	203	129	6
	IVP48	152	78	6
76-07-20	Gineke	360	279	218
	G254	77	8	3
	IVP48	177	35	9
76-08-21	IVP10	-	64	50
	IVP24	-	6	2
	IVP48	-	9	3

- no observation

Table 29. Correlation coefficients (r) between d/100b and hy/100b of populations of pollinators crossed with cv. Radosa (1971) or cv. Gineke (1973, 1974).

	Population	n	r
1971[a]	IVP(32 × 48)	21	-0.41
	IVP(48 × 35)	13	-0.13
	total	34	-0.29
1973[b]	IVP(48 × 35)	24	-0.39
	IVP(32 × 48)	47	-0.67
	IVP(24 × 10)	18	-0.66
	total	89	-0.57
1974[c]	IVP1 selfed	13	-0.15
	IVP(1 ⊗ 10)	6	-0.58
	IVP(48 × 1)	5	0.41
	IVP(66 × 6)	8	-0.04
	IVP(48 ⊗ G609)	10	-0.00
	total	42	-0.42

n. number of genotypes in a population
a. original data in Van Breukelen (1972)
b. original data in Fig. 1
c. original data in Tables 6 and 7

where only one size of sperms was expected from pollen diameter observations. The range in IVP10 sperms was wider than in Gineke and IVP48 sperms. Thus it was not possible by this method to identify restitution nuclei with certainty.

In 38 tubes out of a group of 542 only one sperm was visible. Most of these single sperms were not big and probably not restitution nuclei. Some sperms might have been lost during preparation. In a second group of preparations only one out of 365 intact tubes showed unambiguously one sperm. In eight others only single sperms were seen, but those tubes might have been broken, or the other sperm was left in the other 2/3 part of the tube which was excluded from the preparation. Including all the doubtful cases only 5.1% of the tubes contained one sperm, the majority not having the size of a restitution nucleus.

In most of the tubes screened also the relative position of the sperms was noted. Thirty pairs (4.8%) from 615 tubes were very close together or

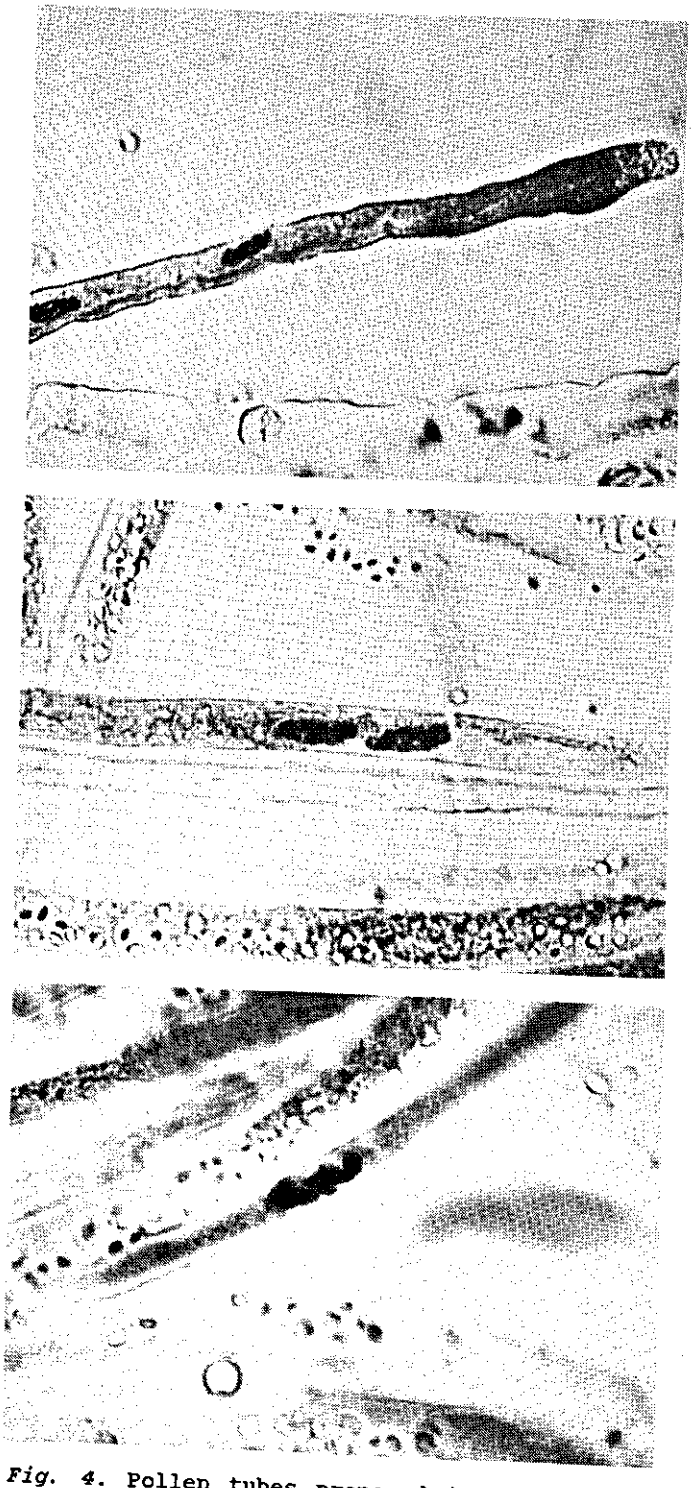

Fig. 4. Pollen tubes prepared in stylar tissue with two sperms each. The distance between the sperms is normal (top), close (middle) and very close (bottom) respectively.

touching (Fig. 4). Percentages of sperms very close together varied for the different *S. phureja* pollinators. IVP48 had the highest percentage (8.6%), followed by IVP35 (5.5%). IVP1, 10 and 24 had about 3%. Most of these sperms were small.

7.3.5 *Certation*

The relative rate of pollen tube growth was determined for pairs of pollen parents. In 4x styles two batches of Gineke pollen were compared with each other to check the accuracy of the method. No growth rate differences were found between the pollen tube bundles in three replications. Gineke pollen was growing at the same rate as pollen from Radosa and Multa, and faster than pollen from the dihaploids G254 and G609. In 2x styles Gineke proved again to be faster than the dihaploid G254 in two replications. Irrespective of the ploidy level of the style, 2x pollen was faster than x pollen in *S. tuberosum* crosses.

A more complicated pattern arose when Gineke pollen was compared with that of *S. phureja*. Pollen bundles of several *S. phureja* pollinators showed a thinning, so that some tubes were ahead of the others (Fig. 3). Pollinators producing much 2n (=2x) pollen, e.g. IVP10 and 32, had many pollen tubes growing as fast as 2x pollen from Gineke. Others like IVP48 had only few tubes reaching as far as Gineke, while the majority was shorter. In a comparison of IVP10 and 48, both bundles were thinning, but not to the same extent. Many tubes of IVP10 were as long as the few forerunners of IVP48. *S. tuberosum* dihaploid G254 was compared with IVP32, 35 and 48. G254 tubes were slower than the fastest tubes of *S. phureja* pollen in all cases.

Tubes of 2n (=2x) pollen of *S. phureja* did grow faster than those of n pollen and as fast as those of 2x pollen from *S. tuberosum*.

7.3.6 *Delayed pollination*

The age of the flower at the time of pollination influenced d/100b, hy/100b and berry set (Table 30). The stigma was not receptive before the bud started to colour. Highest dihaploid frequencies were obtained with young open flowers. Closing old flowers were lower in d/100b, hy/100b and berry set. Delayed pollination did not increase dihaploid frequencies.

7.4 DISCUSSION

7.4.1 *Relationship between 2n pollen and hybrids*

Percentages of 2n pollen in *S. phureja* clones in Table 27 (0-30%) were similar or slightly lower than those found by other authors. Bender (1963) observed 7% large sperms after pollen tube mitosis. Hougas et al. (1964) re-

Table 30. Berry set, d/100b and hy/100b of Gineke flowers of different ages, all pollinated on the same day with pollen of IVP35. Pollinations were made in a growth chamber.

Age of flower	Number of flowers	% berry set	d/100b	hy/100b
green bud	10	0	-	-
bud with colour	15	13	75	250
opening bud	20	40	200	1180
young open flower	25	96	209	954
pale older flower	25	96	172	733
closing old flower	16	35	117	786

ported 5-15% 2n pollen in their best pollinator. They considered this percentage as exceptionally high. Höglund (1970) calculated 50% 2n pollen from studies of PMC meiosis in *S. phureja*. Mendiburu (1971) found 15-25% 2n pollen in *S. phureja* × *S. tuberosum* diploids. Mok & Peloquin (1975) found up to 58% in similar material.

Frequencies of 2n pollen, as calculated from pollen size, fitted well with hy/100b (Table 27). Hybrid seeds consisted of triploid and tetraploid hybrids. As triploid frequencies would not be higher than 15 per 100 berries (Section 2.5.2.1), they could be neglected. Therefore the frequencies of tetraploid hybrids, formed by fusion of an egg cell with a 2n sperm, could be used as an indication of the level of 2n pollen production of a pollinator.

The considerable variation in frequencies of 2n pollen between the times of sampling was not unexpected, as frequencies of hybrid seeds also varied during the season (Table 3). Ramanna (1979) observed also variation during a season in frequencies of cytological processes, which led to 2n pollen formation. Jacobsen (1976) found even variation between different plants of the same clone on the same day.

7.4.2 Ovule lethality

Von Wangenheim (1957, 1961) and Bender (1963) showed that ovules with 3x embryos and 5x endosperm were lethal. Triploids degenerated between 5 and 15 days after fertilization. The reduction of ovules in this study occurred in the same period. Only a very low percentage of the ovules developed to maturity in 4x × 2x crosses. The mature seeds were mostly tetraploids, with some dihaploids. Only tetraploids were expected in the Gineke × Gineke cross, hence the high seed set. IVP10 yielded many tetraploids as well, which could be expected from the high numbers of 2n gametes in this clone.

Where high numbers of tetraploids were formed, relatively high numbers of ovules were found in two week old berries.

These results implied that the low numbers of seeds in several 4x × 2x crosses were not the result of failure of fertilization, but of high lethality of especially the ovules fertilized with n pollen. They were the majority of the ovules in most crosses.

7.4.3 The role of 2n pollen in dihaploid induction

Hougas et al. (1964) suggested a positive influence of 2n gametes on dihaploid induction. The best dihaploid inducer in their material produced the highest number of seeds (8 per berry). Overall seed set was low. The high number of seeds without embryo gave them reasons not to exclude a role for the n pollen. Frandsen (1967) worked with two pollinators. Again the highest dihaploid inducer had the highest seed set. Buketova & Yashina (1973) did not find a correlation in nine clones.

Larger numbers of pollinators, some with high seed set, were studied by Hermsen & Verdenius (1973). Calculations from their data on 28 pollinators gave a correlation coefficient between d/100b and hy/100b of -0.21. This low and negative correlation together with the negative correlations found in this study (Table 29) throw some light on the origin of dihaploids. If dihaploids originated from 2n pollen, a positive correlation would be expected: high frequencies of 2n pollen giving rise to high numbers of tetraploid hybrids and a certain fraction inducing dihaploids, if the second 2n sperm would not fertilize the egg cell. High numbers of hybrid seeds did not go with higher dihaploid numbers generally. Therefore dihaploids were probably not induced by 2n pollen.

Rowe (1974) also decided that 2n gametes were not responsible for dihaploid induction. His argument was, however, that 2n pollen could only induce pseudogamy when one sperm was lost. According to him the loss of a sperm would not be under genetic control, whereas the effect of the pollinator on dihaploid frequency was.

There are other arguments against a role of 2n pollen in dihaploid induction. If dihaploids were induced by 2n pollen there should be a reason why one sperm would not function. In maize this was explained by assuming premature division of the egg cell (Chase, 1969), after which the egg cell could not be fertilized anymore. This way delayed pollination increased the haploid frequency. It was confirmed (Table 30) that in *S. tuberosum* delayed pollination did not increase the dihaploid frequency, as Gabert (1963) already found. Several studies have shown that premature egg cell division is unlikely to occur in *Solanum*. Embryological studies of Clarke (1940) in *S. tuberosum* revealed that the first division of the zygote took place between four and five days after pollination, two days after the first endosperm division. Dnyansagar & Cooper (1960) found a similar sequence in

S. phureja. Functioning of only one sperm is thus not as likely in *Solanum* as in maize. This strengthens the likelihood that 2n sperms do not induce dihaploids.

The negative slope (-0.01--0.02) of the regression of d/100b on hy/100b (Table 29) gave an indication of the order of magnitude of the number of ovules (50-100) that needs to be fertilized by n pollen in order to produce one dihaploid, if an average pollinator is involved.

If n pollen induced dihaploids, it has to be explained, how both n sperms fertilized the central nucleus to provide 6x endosperm. Study of pollen tube mitosis could give the information needed.

7.4.4 Pollen tube mitosis

If n pollen contributes 2x chromosomes to the 6x endosperm of a dihaploid, these can come from two separate sperms or from two fused sperms from pollen tube mitosis restitution. Chances that double fertilization of the central nucleus takes place by two completely separated sperms are very low.

Single sperms from pollen tube mitosis restitution in n pollen might induce dihaploids, but most single sperms found in pollen tubes were possibly from broken tubes and they were often not big. Their frequency was too low to explain the dihaploid numbers found. The most reliable count of single sperms in pollen tubes gave 1% single nuclei in 200 tubes of IVP48, a good pollinator. A 1% frequency means eight potential dihaploids in a berry with 800 ovules. Assuming 50% general lethality (cf. lethality in 4x ovules, Table 28), 50% failure of the unfertilized egg cell to divide and no lethal genes in the embryo, which were all generous assumptions, only two dihaploids per berry would result. This frequency is lower than the observed frequency, as IVP48 induced more than four dihaploids per berry in 1974.

More dihaploids could be induced by pollen tubes with sperms very close together or connected by a bridge as described by Bender (1963) and Montezuma-de-Carvalho (1967). The sperms found close together might function as one and both fertilize the central nucleus. They were found in a frequency of 8.6% in IVP48 and could induce 17 potential dihaploids following the same assumptions as above. As the assumptions were generous, the four dihaploids per berry actually found could be explained by this type of sperms. The frequency of close sperms found in the good pollinator IVP35 was also reasonably high, but in good IVP10 rather low as compared with bad IVP24.

For the formation of 6x endosperm for dihaploids one should not only look at complete restitution, which results in a single sperm, but also take into account the more frequently occurring incomplete or 'functional' restitution, where the two sperms are not completely separated.

Montezuma-de-Carvalho (1967) described anaphase disturbances in pollen tube mitosis in *S. phureja*. He suggested that laggards might lead to chro-

mosomal bridges. The mitosis was found to be sensitive to external influences as irradation (Bender, 1963; Bukai, 1973), N_2O (Montezuma-de-Carvalho, 1967) and colchicine (Montelongo-Escobedo & Rowe, 1969). Temperature effects on dihaploid frequencies found by Wöhrmann (1964) might also work via pollen tube mitosis. On the other hand one of the sperms might have lost its functionality by these treatments so that single 2n sperms could have induced dihaploids.

Irregularities in pollen tube mitosis do not necessarily mean that the same irregularities have to occur in other types of mitosis in the parent, as the pollen tube mitosis has special spatial conditions. In addition to mechanical factors, also genetic factors will control irregularities in mitosis. According to the conclusion from Section 3.4.3 several additive loci will be involved. Additivity of gene effects means that more genes for, or more structural changes in chromosomes leading to, chromosome bridges between the sperms will increase the chances of double fertilization of the central nucleus, and thus of dihaploid induction. Such a system will follow patterns of quantitative inheritance and function gametophytically.

7.4.5 *Certation and dihaploid frequencies*

The study of certation showed that 2x pollen grew faster than x pollen, both in 4x and in 2x styles. This is different from the suggestion of Höglund (1970) that 2x ovules (in tetraploid plants) might be preferentially fertilized by 2x pollen. If it was a matter of preference, the x egg cells should also favour x pollen (in diploids), which was not the case. Her other suggestion of differences in growth rate was confirmed. However, the tetraploid frequency she found could better be explained by triploid lethality. Potato pollen behaves differently from beet pollen where x pollen is faster than 2x pollen in a 4x style (Matsumura, 1958). Differences in growth rate are not a general phenomenon. Skiebe (1966) and Esen et al. (1978) found no indication for differences in growth rate between 2x and x pollen in *Primula malacoides* and *Citrus* respectively.

The faster growth of 2x pollen found in *Solanum* implied that the fraction of ovules fertilized by 2x pollen must be higher than the fraction of 2x pollen, if there is an overdose of pollen. With room for 3000 pollen grains on a tetraploid stigma (Janssen & Hermsen, 1976) and 800 ovules in an ovary (Section 4.3.1) there will be enough room for competition. If all 2n pollen tubes would grow faster than n pollen tubes, 2n male gametes could occupy three to four times their share of the ovules up to a maximum of 100%. However, a comparison of the percentage of 2n gametes with the number of hybrids in Table 27 shows that the hybrid frequencies were not that much higher than the 2n pollen frequencies. Apparently not all 2n pollen tubes were faster than all n pollen tubes. In 4x × 2x crosses, even with much 2n pollen, many ovules were left for n pollen to fertilize as the

seed set was generally lower than in 4x × 4x crosses.

Thus it can be concluded that the mechanism of certation reduced dihaploid frequencies of pollinators with much 2n pollen, but not to a large extent.

7.4.6 The mechanism of dihaploid induction

What happens after pollination of 4x *S. tuberosum* with 2x *S. phureja*? *S. phureja* pollen consists of a mixture of 2n and n pollen. Some pollinators are genotypically determined to produce many 2n pollen grains, but the actual frequency varies much. The 2n pollen has in general a higher growth rate than n pollen and thus occupies more than its share of the ovules, leading to 4x hybrids. The n pollen occupies the remainder of the ovules. Most of these ovules will contain 5x endosperm and be lethal. Very few triploid hybrids survive. A certain fraction of the n pollen - influenced by genotype and environment - forms a single restitution sperm or a bridge between the two sperms after the pollen tube mitosis. This can lead to double fertilization of the central nucleus and result in 6x endosperm. If the unfertilized egg cell then divides and does not carry lethal genes, a dihaploid embryo in viable endosperm will result. The frequency of the dihaploids will depend on - first the tendency of n pollen to form abnormal sperms, together with the tendency of unfertilized egg cells to divide, - secondly the number of ovules fertilized by 2n pollen. The more 2n pollen, the smaller the chances of n pollen to fertilize ovules. However, the genetic constitution of the n pollen seems to be more important than the frequency of 2n pollen, as among pollinators producing many hybrids some also induce high numbers of dihaploids.

8 Monoploids

8.1 INTRODUCTION

The first group of monoploids from *S. tuberosum* obtained by pseudogamy was induced by *S. phureja* pollinators with high d.i.a. (Van Breukelen et al., 1975). This led to the question whether it was a general phenomenon that good dihaploid inducers also induce monoploids. A positive answer will have implications for a hypothesis on the mechanism of monoploid formation in *Solanum*. *Solanum* dihaploids, originating from a 4x × 2x cross, result from the '*Solanum* mechanism', in which both reduced sperms fertilized the central nucleus. Would *S. tuberosum* monoploids from 2x × 2x crosses follow this mechanism or the 'maize mechanism', in which one sperm fertilizes the central nucleus and the other is lost (Hermsen, 1971)?

The genotypes of both the seed parent and the pollinator influence dihaploid frequencies in *S. tuberosum*. Both parents influence monoploid rates in maize (Chase, 1949; Coe & Sarkar, 1964; Sarkar & Coe, 1966). Nothing is known, however, about the relative importance of the female and male idiotype in monoploid pseudogamy in *Solanum*.

An attempt to answer these questions was made by carrying out a large number of *S. tuberosum* di(ha)ploid × *S. phureja* crosses, involving several genotypes. Most of the results were presented in Van Breukelen et al. (1977). More details and a further analysis of the data are presented in this chapter.

Irikura & Sakaguchi (1972) claimed that *S. verrucosum* monoploids could only be made via anther culture. In order to test the possibility of induction of monoploids in *S. verrucosum* through pseudogamy, some plants of this species were pollinated by *S. phureja*.

Most of the applications of monoploids require chromosome doubling. This was done and the resulting diploid *S*-allele homozygotes and their identification are reported.

8.2 MATERIAL AND METHODS

Monoploids were induced with *S. phureja* genotypes IVP6, 10, 35, 48 and (48 × 35)4, all homozygous for embryo spot (see Section 3.2).

Most seed parents were *S. tuberosum* di(ha)ploids. G609 and G254 are dihaploids from cv. Gineke. B16 is possibly also a dihaploid from cv. Gineke (Hermsen, pers. comm.), otherwise it derives from the tetraploid clone

B4495, developed by Dr. W. Black. All three have a high male and female fertility. G609 has no lethal genes and is self-incompatible (genotype *S1S2*). G254 is heterozygous for three lethal genes and is self-compatible (genotype *S1S3*). B16 contains two sub-lethal genes and is self-compatible (Hermsen et al., 1978a). The self-compatibility of G254 and B16 is caused by an *S*-allele bearing translocation, which is lethal in homozygous condition (Hermsen, 1978b). Of the F1 populations G609 × G254, G254 × G609 and G609 × B16, which segregated for self-(in)compatibility, mostly self-compatible plants were used in this study. One self-compatible clone, GB37, from G254 × B16, was also included (Olsder & Hermsen, 1976). Other dihaploids used derive from five different cultivars. Dihaploid × dihaploid derivatives and one dihaploid were kindly provided by Ir. N. van Suchtelen and Dr. B. Maris of the Foundation for Agricultural Plant Breeding, Wageningen.

Non *S. tuberosum* diploid seed parents were from the wild diploid species *S. multidissectum* and *S. verrucosum*. The *S. multidissectum* seed parent (WAC 3908) was collected in 1974 in Peru. Three populations of the self-compatible *S. verrucosum* were used: A3 selfed, WAC accessions, and Code 41. A3 selfed consisted of the self progeny of an F1 plant from CPC 2247 (3x) × PI 195172 (2x). Most plants were euploid (2n=24). The WAC population consisted of a few plants from each of the WAC accessions 3330, 3332, 3335, 3337 and 3338. Code 41 consisted of the self progeny of a parthenogenetic 2x seed (Abdalla, 1970). In addition hybrids from reciprocal crosses between *S. tuberosum* dihaploids and *S. verrucosum* were used.

All plants were grafted onto tomato rootstock and grown in a controlled greenhouse. Flowers of seed parents were emasculated and pollinated with pollen of *S. phureja* clones, homozygous for embryo spot (Section 2.3.1). Seeds were extracted from the berries and carefully screened for spots. Seeds without spots and seeds without visible embryos were kept apart. The latter group was rather big and many of these seeds were so small that they were not expected to germinate. Both groups of seeds were put on wet filter paper in petri dishes to germinate and those which germinated were transplanted into seed boxes. After one week also the seeds that had not germinated were put into seed boxes. A list was kept of all planted seeds. All plantlets showing a coloured hypocotyl or a nodal band at the base of the cotyledons were removed. Amongst them was a large number of plants with abnormal cotyledons, e.g. one peltate leaf, which showed no normal nodal band but a coloured hypocotyl. Plants without the paternal marker were left in the seed boxes until they reached a height of about 5 cm. At that stage the number of chloroplasts in the guard cells of the stomata was counted (Frandsen, 1968). Plants with a mean number of chloroplasts lower than 12.0 were kept. The chloroplast number of monoploids is around eight (Fig. 5) (Van Breukelen et al., 1975), but a large margin was used in order not to lose a single monoploid. The selected plantlets were transplanted into small pots in order to facilitate the collection of root tips for chromo-

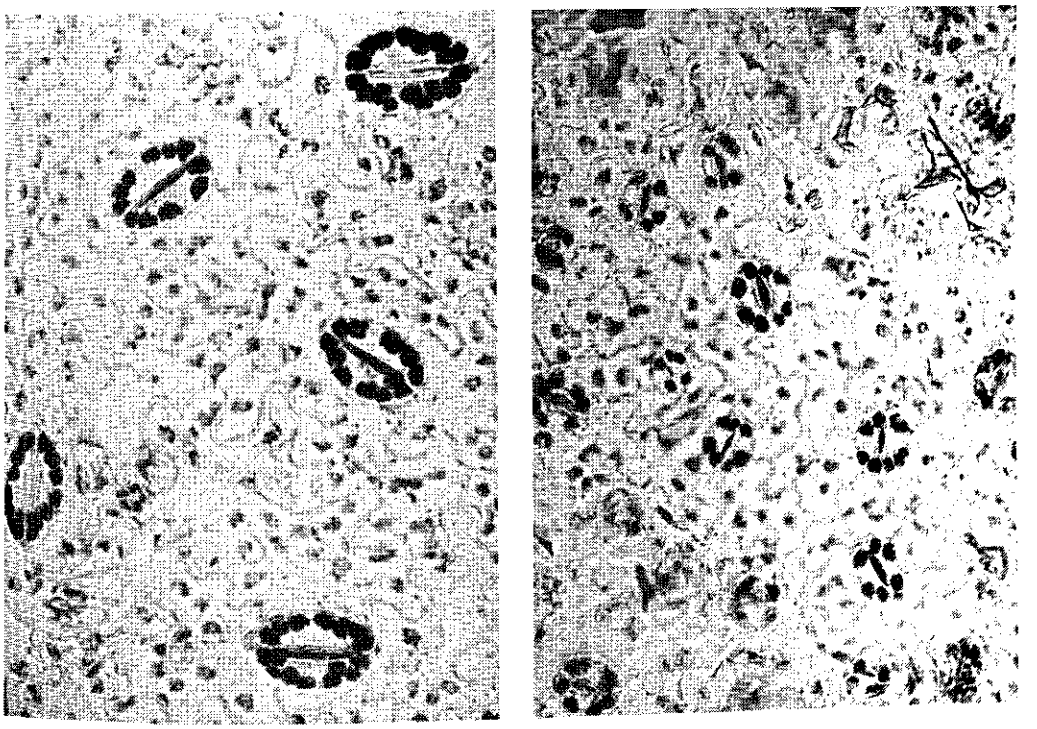

Fig. 5. Stained chloroplasts in guard cells of stomata of *S. tuberosum* plants with different chromosome numbers: diploid (left) and monoploid (right).

some counting at a later stage. Plants with 12 chromosomes in the root tips were recorded as monoploids. A few plantlets did not survive until this stage. The somatic chromosome number was also counted in plants with more than 12.0 chloroplasts. No monoploids were found in this group.

Most monoploids produced flower buds, which dropped prematurely. Meiosis was studied when possible to confirm root tip counts.

For chromosome counting root tips were pretreated with 8-hydroxyquinoline for about 24 h. Fixations were made in 3:1 ethanol acetic acid and stained in propionic acid haematoxylin according to Henderson & Lu (1968). For meiotic studies young flower buds were fixed for 48 h in a 3:1 mixture of ethanol and propionic acid saturated with iron acetate. Anthers were squashed in 1% acetocarmine (Van Breukelen et al., 1975).

For chromosome doubling Dionne's method was used (Ross et al., 1967; Langton, 1974) (see Section 4.2) with some modifications. Some monoploids had been grafted and others left on their own roots. The chloroplast number and the chromosome number were determined after passing through one tuber generation. The method was later modified. New shoots on treated plants were first checked for their chloroplast number. Shoots with more than 11.0 chloroplasts were left on the plant. The plant was then pruned to promote

the growth of the doubled shoot. Later several cuttings were made from the doubled shoot for propagation. This reduced the risk of losing a doubled plant. In addition, flowers could be obtained in a shorter time.

Frequencies of monoploids were considered as the outcome of Poisson variables and were compared using a binomial test. One frequency was taken as test statistic and the test performed conditionally on the total number of monoploids.

For observation of pollen growth, styles were cut from the ovary 24 h after pollination and prepared for UV fluorescence (Section 7.2). Individual styles were put on a slide in glycerine and gently squashed with a cover glass. Pollen tubes could then be studied under a UV microscope with a large field.

8.3 RESULTS

Most monoploids surviving the seedling stage were well growing plants with light green leaves and narrow leaflets (Fig. 6). They reached a height

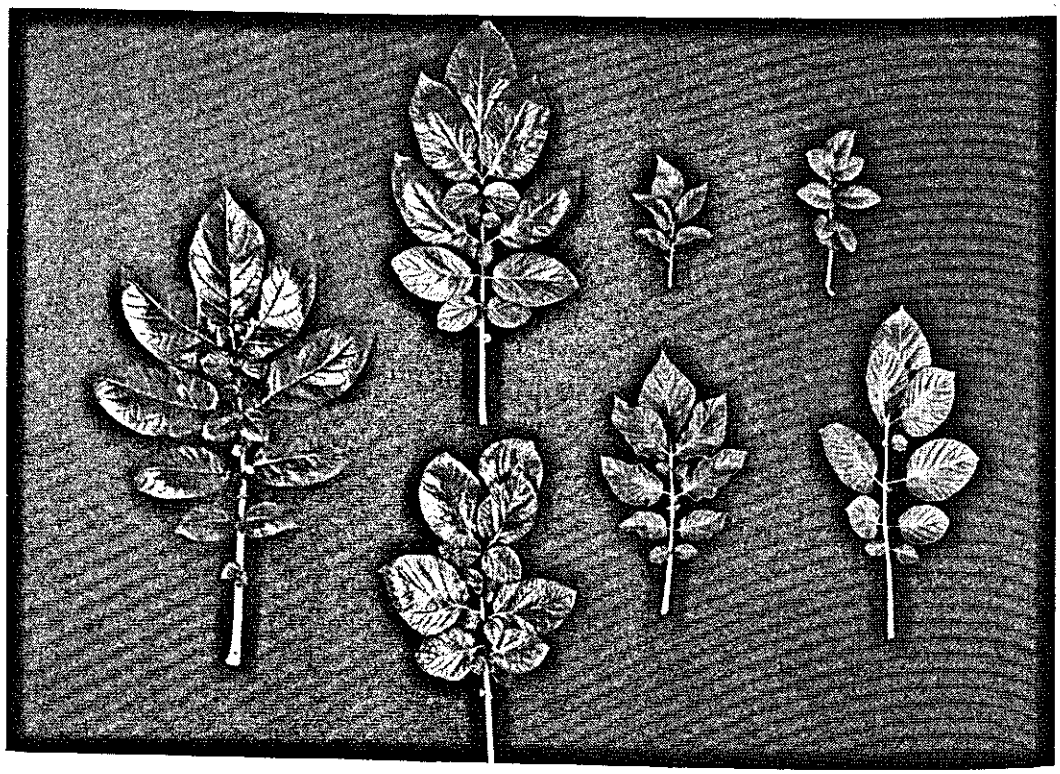

Fig. 6. Leaves of *S. tuberosum*. Top row (from left to right): Gineke (4x), G609 (2x) and monoploids M3 and M7 (x). Lower row: doubled G609 and doubled monoploids.

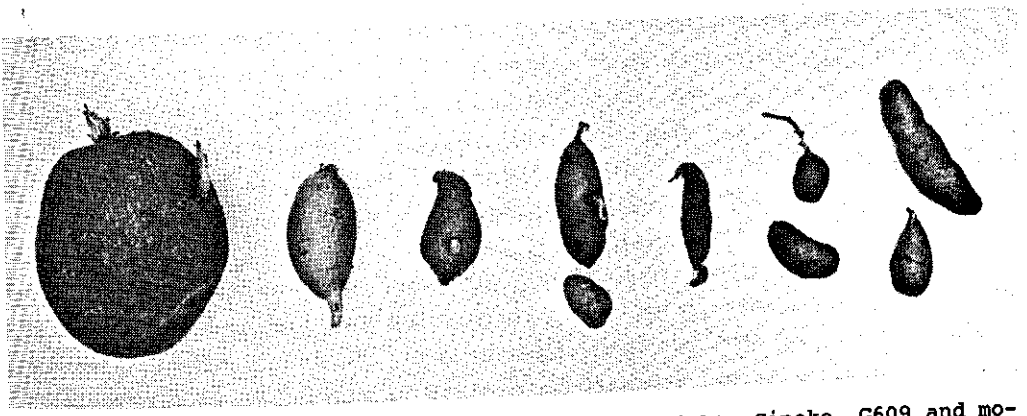

Fig. 7. Tubers from *S. tuberosum*. From left to right: Gineke, G609 and monoploids M40, M55, M109, M4 and M39.

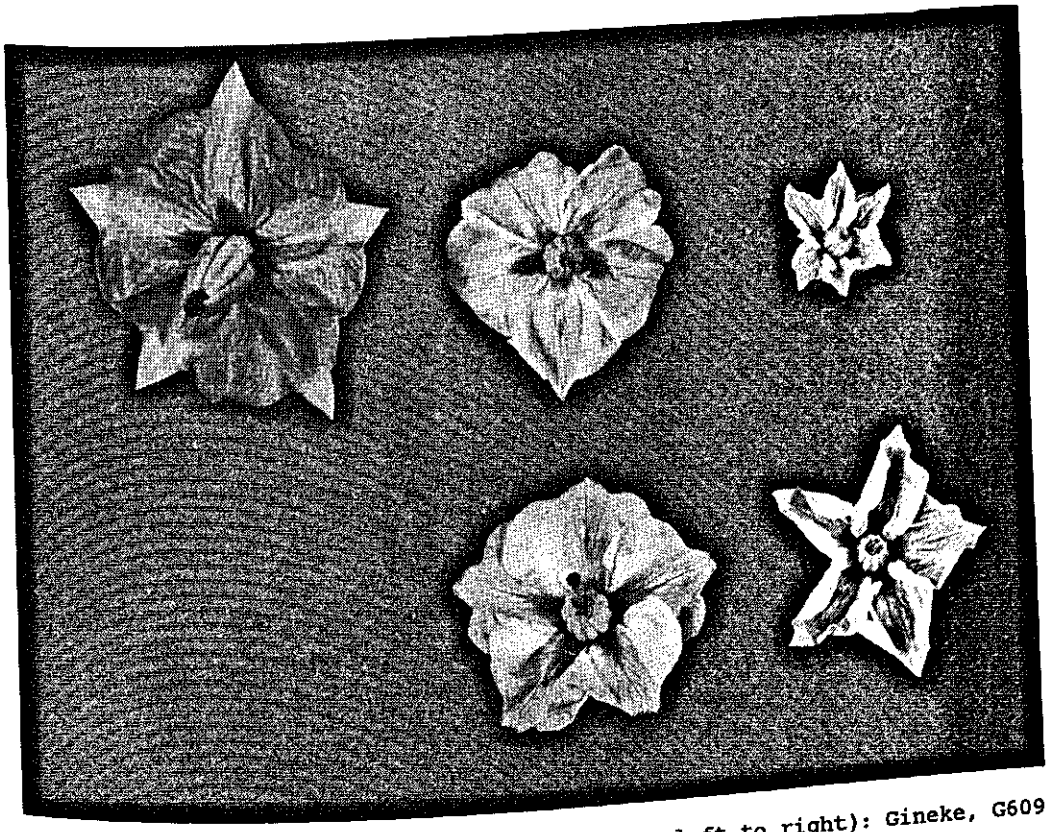

Fig. 8. Flowers of *S. tuberosum*. Top row (from left to right): Gineke, G609 and monoploid M39. Lower row: doubled G609 and doubled M39.

of about 80 cm. Most produced tubers with little or no dormancy or stolons which developed into new shoots immediately (Fig. 7). About 60% of the genotypes produced flower buds, but only two actually flowered (Fig. 8). Flowers were completely sterile.

Monoploid frequencies can be expressed as monoploids per 1000 seeds (m/1000s) or as monoploids per 100 berries (m/100b). The monoploid frequency of G609 was in general lower in 1974 than in 1975, when expressed as m/1000s (Table 31). The seed set, however, was higher in 1974. There seemed to be a negative correlation between m/1000s and seed set. The monoploid frequency of G609 was more constant over the years, when expressed as m/100b. Therefore m/100b was used to compare *S. tuberosum* seed parents.

8.3.1 Pollinator influence

The four *S. phureja* genotypes, which were used extensively for monoploid induction, had also been used as pollinators in dihaploid induction in the same years. The numbers and frequencies of haploids obtained from seed parents G609 and Gineke are given in Table 31. IVP35 and 48 were the

Table 31. Numbers and frequencies of monoploids obtained from *S. tuberosum* dihaploid G609 in 1974 and 1975, compared with dihaploid frequencies of cv. Gineke with the same pollinators in the same years.

	Pollinator	Number of		Frequency of monoploids		Number of seeds per berry	Gineke dihaploids per 100 berries
		berries	monoploids	per 100 berries	per 1000 seeds		
1974	IVP6	12	2	17	0.38	435	163
	IVP10	23	6	26	0.76	342	209
	IVP35	135	23	17	0.46	374	342
	IVP48	108	18	17	0.42	393	445
Total and means		278	49	18	0.46	382	290
1975	IVP6	21	2	10	0.42	224	103
	IVP10	12	2	17	0.90	185	235
	IVP35	34	7	21	0.80	257	297
	IVP48	26	3	12	0.52	221	406
Total and means		93	14	15	0.65	230	260

best dihaploid inducers and IVP10 was also good (cf. Section 3.3.1). The d.i.a. of IVP6 was only 41% and 24% of the mean of IVP35 and 48 in 1974 and 1975 respectively. In monoploid induction IVP6 was again the lowest pollinator but here it induced respectively 99% and 62% of the mean monoploid frequency of IVP35 and 48 expressed as m/100b in the two years. On the other hand, the first place of IVP48 in d/100b did not go together with the highest monoploid frequency. Another pollinator, IVP(48 × 35)4 (cf. Table 8), was used on a small scale. This plant with high d.i.a. yielded 1 monoploid in 15 berries, which was not an excellent performance, compared with other pollinators.

8.3.2 Seed parent influence

Three dihaploids and three F1 populations derived from them were pollinated with IVP35 in one season. Details of the berries and monoploids produced are given in Table 32. G609 was by far the best producer of monoploid plants, while G254 produced no monoploid plants at all. Neither did B16, but with only 7 berries the only conclusion that can be drawn is that B16 is not much better than G609. Ten out of 34 F1 plants produced one or more monoploids, unevenly distributed over the three populations. The two reciprocal progenies of G609 and G254 produced about the same number of berries and seeds, but the progeny which had G609 as mother produced about four times as many monoploids.

The complete results of monoploid induction in 112 seed parents, including the negative results with several seed parents from different *Solanum* species, are presented in Table 33. Apart from the positive results already

Table 32. Numbers and frequencies of monoploids obtained from three dihaploids and their F1 progenies after pollination with IVP35.

Seed parent	Number of genotypes		Number of		Frequency of monoploids	
	total	producing monoploids	berries	monoploids	per 100 berries	per 1000 seeds
G609	1	1	135	23	17.0	0.46
G254	1	0	135	0	0.0	0.00
B16	1	0	7	0	0.0	0.00
G609 × G254	13	7	247	11	4.5	0.16
G254 × G609	14	2	250	3	1.2	0.05
G609 × B16	7	1	106	1	0.9	0.03

Table 33. Summary of inductions of monoploids in several seed parents, using *S. phureja* clones as pollinators.

Seed parent	Number of				
	genotypes[a]	monoploids	berries	seeds	seeds per berry
G609	1(1)	67	411	136809	333
G254	1	0	333	65541	197
B16	1	0	7	339	48
Progenies of G609, G254 and B16	34(10)	15	603	165631	275
other dihaploids	21	0	127	11008	87
dihaploid × dihaploid	6	0	111	11274	102
S. multidissectum	1	0	244	10103	41
S. tuberosum × *S. verrucosum*	12	0	135	11041	82
S. verrucosum × *S. tuberosum*	6	0	63	5145	82
S. verrucosum A3, selfed	9(1)	1	259	7012	27
S. verrucosum WAC accessions	17(1)	2	146	1742	12
S. verrucosum code 41	1	0	89	1559	18
GB37	1	0	47	4677	100
Total	111(13)	85	2575	431881	

a. in brackets number of genotypes producing one or more monoploids

mentioned (Table 31 and 32), other monoploids derived from two populations of *S. verrucosum*. Three monoploids were obtained, which flowered profusely. Other plants and populations pollinated with *S. phureja* yielded a few seeds without embryo spot. These seeds did not yield monoploids. In all, 13 seed parent genotypes out of 112 tested produced one or more monoploids.

The ability to produce monoploids seems not to be distributed evenly over all species and genotypes.

8.3.3 Doubled monoploids

About 100 monoploid plants were treated with colchicine (10-20 meristems per plant). Twenty-one doubled individual plants were obtained from eight different genotypes. Results were better with grafted plants than with plants on their own roots. Most doubled plants flowered (Fig. 8), unlike their monoploid counterparts, and produced berries with 80-100 seeds when crossed with G254. The quality of the pollen of the doubled monoploids was

bad, stainability being not higher than 1%.

The homozygous diploids, obtained from monoploids from G609, proved to be suitable material to test *S*-allele genotypes. Crosses were made with different tester genotypes and pollen growth in the style was observed with a UV microscope. Normal functioning of the style was tested with pollen of G609 and G254. Results are given in Table 34. Both *S*-allele genotypes expected from G609 were actually found. Doubled M39 gave an incompatible reaction with pollen of tester genotype *S1S1* and must have been homozygous for *S1*. Doubled M4 and M36, compatible with *S1S1*, must have been homozygous for *S2*, the other allele from G609.

8.4 DISCUSSION

Numbers of seeds per berry were lower in 1975 (230s/b) than in 1974 (382 s/b). The number of monoploids per 1000 seeds seemed to be negatively correlated with the number of seeds per berry. IVP10 was the highest of the four pollinators in m/1000s in both years but also the lowest in seeds per berry. This can be explained by the high number of 2n pollen in IVP10, which leads to non-viable seeds with tetraploid endosperm. Apparently the number of ovules is more determinative for the monoploid number than the number of seeds formed.

Table 34. Compatible (+) and incompatible (-) reactions in styles of *S. tuberosum* plants of different ploidy levels, observed on the growth of pollen of diploid testers. The *S*-allele genotype of the monoploid M39 and the doubled monoploids (DM) are deducted from the compatibility reactions in this table.

Tested material		Tester		
		S1S2	*S1S1*	*S1S3*
Gineke	*S1S2S3S5*		-[a]	
G609	*S1S2*		-	
M39	*S1*	+	-	+
DM39	*S1S1*	+	-	+
DM4	*S2S2*		+	
DM36	*S2S2*		+	+

a. inhibition only at the base of the style

8.4.1 *Pollinator influence*

It is apparent from this study that monoploid inducers are useless without an embryo or seed marker, which differentiates pseudogamic embryos from amphimictic (and paternal) ones. Without the embryo spot marker all the 450 000 seeds which were now screened for embryo spot would have had to be planted and they would have taken 900 m^2 at a 4 cm × 5 cm spacing as seedlings. Apart from space limitations, the work involved in screening of so many seedlings would have been enormous. Consequently it was not possible to test the pollinators IVP24 and 32 with very low d.i.a. for their monoploid inducing ability, as they were heterozygous for one embryo spot locus.

The role of the pollinator is very important in dihaploid induction, but less so in monoploid induction. Data obtained from four pollinators in two seasons seemed to indicate that low dihaploid inducer IVP6 was also low in m/100b (Table 31). On the other hand the differences between frequencies of m/100b were small, whereas differences between frequencies of d/100b were as high as 300%. To test whether IVP6 was indeed lower in m/100b two hypotheses were formulated. The first was: IVP6 equals IVP35 and 48. Under this assumption the critical level (one-sided) was 0.67 in 1974 and 0.36 in 1975. From this it can be concluded that it is not impossible that IVP6 equalled IVP35 and 48. The other hypothesis was: when IVP6 is compared with IVP35 and 48, it is as much inferior in m/100b as it is in d/100b. Under this assumption the critical levels were 0.37 and 0.62 (two-sided) for 1974 and 1975 respectively. This hypothesis was also not refuted by the data. Apparently larger numbers of monoploids are needed for statistically sound statements on the significance of the differences between the effects of pollinators. It can be concluded that, although the monoploid frequencies of the pollinators looked similar, it is not impossible that IVP6 was lower in m/100b to the same extent as in d/100b compared with IVP35 and 48.

It is remarkable that every *S. phureja* pollinator tried was successful. This could imply that the genotype of pollinators is probably unimportant and that every male fertile plant might be a potential monoploid inducer.

8.4.2 *Seed parent influence*

The difference in monoploid production between G609 and G254, two dihaploids with a high female fertility, was remarkable. G254 not only produced no monoploids with IVP35 (Table 32), it neither did so with other pollinators (Table 33). It is known from work by Hermsen et al. (1978a) that G254 is heterozygous for three lethal genes and carries a translocation, which is lethal in homozygous condition. No lethal genes are known in G609. The four lethal genes of G254 would reduce the number of viable monoploids with a factor 16. The expected number of monoploids from the 333 berries of G254

- if it had the same frequency as G609 (49/278) - would have been 58.7 and this would have been reduced to 3.7 monoploids. The probability of finding 0 monoploids in 333 berries if 3.7 are expected is only 2.9%. This probability is so low that it can be assumed that the difference between G609 and G254 was not based on the known genes for lethality only. Genes for monoploid production might be present in G609 and lacking in G254.

Population (G609 × G254) produced four times as many monoploids as (G254 × G609) from the same number of berries. Under the assumption that both populations produced monoploids with the same frequency, the critical level for the actual value of (G254 × G609) was 0.03 (one-sided). One can conclude that (G254 × G609) was indeed lower than (G609 × G254). No difference could be found between (G254 × G609) and (G609 × B16). The critical level for (G609 × B16) compared with (G609 × G254) was 0.17 (two-sided), indicating that the two populations possibly differed in monoploid production.

The two reciprocal progenies of G609 and G254 produced monoploids in lower frequencies than G609 itself. F1 plants carried on average half of the three lethal genes of G254. The self-compatibility of most plants implied that most of them also carried a lethal translocation. Together this made that the plants from (G609 × G254) carried on average 2.4 lethal genes and those from (G254 × G609) 2.3. Assuming the same monoploid production per berry as that from G609 with IVP35 and a reduction by the lethal genes, the berries from the two populations were expected to produce 8.0 and 8.6 monoploids respectively, instead of the 11 and 3 monoploids actually produced. The numbers fitted reasonably well for (G609 × G254) but not for its reciprocal. The probability that the 250 berries of (G254 × G609) yielded 3 monoploids or less is 3.5% if the expected frequency is 49/278 (as G609) and 2.3 lethal genes are taken into account. There was a significant shortage of monoploids in (G254 × G609).

The two reciprocal F1 populations from G609 and G254 differed and followed the frequency of the mother in monoploid production. Such a situation usually points to a cytoplasmic influence on the character concerned. G609 cytoplasm would be superior to that of G254. The problem in this case is that G609 and G254 are dihaploids from the same cultivar. Even while G254 has many alleles in common with B16 (Hermsen et al., 1978a), it is more likely that B16 derived from Gineke than G254 from Black 4495 (Hermsen, pers. comm.). This leaves the difference between (G609 × G254) and (G254 × G609) unexplained, unless a cell organelle were involved that was not transmitted by cv. Gineke to all its dihaploids.

Only a small part of the seed parents produced monoploids. The low numbers of berries or seeds from many of the seed parents did not warrant the expectation of a single monoploid from them. M/100b was a good measure to compare pollinators used with G609, but is was not good for comparing seed parents. *S. verrucosum* produced a lower number of seeds per berry than *S. tuberosum* di(ha)ploids. In m/100b the two successful *S. verrucosum* popu-

lations were lower than the three *S. tuberosum* F1 populations (0.7 vs. 2.5 m/100b), but in m/1000s they were higher (0.3 vs. 0.1 m/1000s). Together with the low number of seeds in *S. verrucosum* this means that the number of ovules might be an important factor in the determination of m/100b in a species. The number of monoploids should be expressed per ovule to compare species. As this is inconvenient it can be expressed per seed when there is no excessive lethality.

The relatively high monoploid frequency in *S. verrucosum* might be related to the self-compatibility of this species. The consequent inbreeding reduces the number of lethal genes, compared with outbreeding species. About ten monoploids could be expected from the genotypes without monoploids on the basis of the number of seeds produced and assuming a monoploid frequency similar to G609. Lethality or other factors must have reduced this number. A monoploid from a *S. verrucosum* $\times$ *S. tuberosum* hybrid could survive, as Irikura (1975a,b) has shown. The fact that so many genotypes did not produce monoploids might mean that the ability to produce monoploids is restricted to a few idiotypes. The influence of the seed parent in monoploid production is more important than in dihaploid production, where only few clones failed.

8.4.3 Doubled monoploids

The male fertility of the doubled monoploids was unexpectedly low, considering that most derived from G609 which produced excellent pollen. Colchicine has been mentioned as a possible cause of low fertility after chromosome doubling (Skiebe, 1975). If that were the case, it should as well affect female fertility, which was not low. Spontaneous doubling through tissue culture (Murashige & Nakano, 1966) would have no adverse colchicine after-effects. Complete homozygosity has also been mentioned as a cause for sterility (Skiebe, 1975). All F1 plants from crosses between doubled monoploids and G254 had highly stainable pollen (Bonthuis & Hermsen, unpubl.), which points to a genetic explanation of the male sterility.

Both homozygous *S*-allele genotypes (*S1S1* and *S2S2*) were found, which could be expected from G609 derivatives.

8.4.4 Mechanism of monoploid formation

It was hoped that data about monoploid and dihaploid frequencies of pollinators (Table 31) would give an indication as to whether *Solanum* monoploids are formed by the same mechanism as dihaploids. Neither the presence nor the absence of a correlation could be proved and the information from the pollinators provided no starting point for a hypothesis on the mechanism.

The endosperm must play an important role in monoploid formation, but

the ploidy level of endosperm with a monoploid embryo has never been determined. It probably will never be, as the frequency of ovules with monoploid embryos is extremely low. Double fertilization of the central nucleus as in dihaploid formation is a possible mechanism. This would give rise to hexaploid endosperm with 2n pollen and tetraploid endosperm with n pollen. Hexaploid endosperm would be vital, but it would imply that monoploid frequencies of pollinators would be positively correlated with 2n pollen formation. IVP48 - very low in 2n pollen - was equal in monoploid induction to IVP10 and 35, which had a high 2n pollen frequency. Tetraploid endosperm is not very vital (Von Wangenheim, 1961), but a certain percentage of the ovules with tetraploid endosperm will grow to maturity. Monoploid frequencies would then be the product of the frequencies of two rare events: double fertilization and tetraploid endosperm vitality. If double fertilization would occur in 1 out of 100 ovules and the vitality of tetraploid endosperm were the same as that of pentaploid endosperm (20/100b, see Section 2.5.2.1), the frequency would be around 0.2/100b. This is much lower than the frequency observed in G609 and also in *S. verrucosum* which had lower ovule numbers. The mechanism of double fertilization does not seem to lead to the bulk of the monoploids. However, the possibility cannot be excluded that a small fraction of the monoploids arises through this mechanism.

A more likely mechanism, involving vital triploid endosperm, is the 'maize mechanism', where only one sperm fertilizes the central nucleus and the other sperm is lost ('single fertilization'). The question remains as to why one of the sperms would not function. Is this a genetically directed process, or is it 'spontaneous' and due to chance. If there were indeed no influence of the pollinator on the monoploid frequencies, a pollinator effect on the 'single fertilization' would not be expected. If there were a direct genetic influence through the seed parent on the loss of a sperm, monoploid frequencies would probably be higher than 1 out of 4000 ovules as found. A precocious division of the egg cell prevents one of the sperms to function in maize (Section 2.5.1), but in *Solanum* such a division would have to be advanced by more than four or five days (Section 7.4.3) to prevent fertilization of the egg cell. This would be a rather big time difference. The effect of the seed parent on the monoploid frequency - apart from that through lethal genes - probably functions differently. It might involve a stimulus on the unfertilized egg cell to divide after the first endosperm division. A seed parent with a high monoploid producing ability would not increase the frequency of 'single fertilization', but increase the chances that 'single fertilization' leads to a monoploid. Such a stimulus could be of cytoplasmic origin.

Chance plays a major role in monoploid production in this hypothesis and the cause of the loss of a sperm is not explained. Certain seed parent genotypes would exploit the chance process better and the absence of lethal

genes would reduce further losses of potential monoploids. At the pollinator side only the genetic markers to facilitate screening are very important.

9 Conclusion

The frequency of dihaploids from *S. tuberosum* is definitely influenced by the seed parent and the pollinator. The genetics of the seed parent influence is yet little known, but the influence probably functions in the sporophyte without contribution of cytoplasmic factors. High dihaploid production is most likely not recessive. Contrary to the genotype of the seed parent, the genotype of the pollinator can be manipulated to increase dihaploid frequencies. Therefore, knowledge about genetics of the pollinator effect is more important. Several loci are involved in dihaploid inducing ability (d.i.a.), possibly five or more, and the inheritance follows the intermediate pattern. Consequently the progeny of an excellent pollinator contains a reasonable number of plants with good d.i.a.. This facilitates exchange of pollinators between institutes in the form of seeds, circumventing the risks of tuber shipments. Intermediate inheritance also implies that inbreeding is necessary to attain high d.i.a.. The inbreeding will act negatively on the vigour and especially the male fertility of the pollinator.

The environment influences the frequency of dihaploids as well. Temperatures that give good growth and flowering of *Solanum* plants generally improve the total yield of dihaploids (per plant, per m^2 greenhouse or per day work). The frequency per 100 berries does not appear to be affected by a specific temperature regime as far as the seed parent is concerned. The pollinator induces more dihaploids at a constant temperature of 18 °C than at 23 °C during the day and 15-18 °C at night. It is therefore good practice to make pollinations for dihaploid induction early in the season.

One of the objectives of this study was to determine whether the maximum d.i.a. had been reached. It seems that the maximum of the IVP population has been reached in the genotype of IVP(48 × 35)1, which induces up to 800 d/100b in cv. Gineke. However, dihaploid inducing ability is not limited to the two *S. phureja* parents of the IVP population. Several other *S. phureja* populations and also other *Solanum* diploids have been reported as inducing dihaploids. Recombination between those pollinators and the best IVP pollinators would increase the dihaploid frequency beyond 800 d/100b. This would not be easy. The search for new genes, incorporation in the IVP population and sib crosses for homozygosity would take many years. Notwithstanding the fact that pollinators can be tested with one seed parent, selection of the best pollinators will be laborious. It is doubtful whether such a breeding program is worth the effort, since the present level is sufficient for

practical breeding.

In this study pseudogamy was used to produce haploids. How does this method compare with in vitro methods like anther and pollen culture for *S. tuberosum*? The two methods should be compared in costs per haploid and also in the diversity of the haploids produced.

Pseudogamy appears to have many advantages if a good pollinator is available. Investments in laboratory equipment are small and no highly trained personnel is needed. The right growing conditions have to be provided. This often means a conditioned greenhouse and good care for the plants, but this is also a - sometimes neglected - requirement for in vitro techniques. Also more *Solanum* genotypes will be suitable as seed parent than as material for pollen or anther culture, because female sterility is less frequent than male sterility. A disadvantage of in vitro techniques is that not all genotypes respond similarly to the same medium.

In practical haploid production pseudogamy is nowadays the best method for production of dihaploids. Large numbers of dihaploids have been produced by this technique from a diversity of genotypes, while only few dihaploids have so far been reported from anther culture.

For production of monoploids the picture is not that clear. Pseudogamy was only successful in part of the genotypes. Anther culture has also yielded monoploids from a few genotypes only. The two techniques might be complementary as far as diversity is concerned. No genotype has been tested by both methods.

As the influence of the pollinator on monoploid frequencies is probably small, pseudogamic frequencies may not be increased by selection of good pollinators. One person can obtain 50-200 pseudogamic monoploids in a season. Similar numbers of plants might be obtained in vitro, but most plants will be diploids. These are either from 2n gametes or from spontaneously doubled n embryos and it will be difficult to separate the two. Male sterility is also very frequent among diploid *S. tuberosum* plants. All together pseudogamy has advantages over anther culture for monoploid production as well.

Summary

Chapter 1. Haploid plants can be produced from several species. They have applications in basic research and are a valuable tool in plant breeding. Dihaploids of *Solanum tuberosum* can occur amongst the progeny of crosses with *S. phureja*. This publication describes experiments to determine the influence of genetic and environmental factors on *S. tuberosum* dihaploid frequencies and to determine whether maximum frequencies have been reached.

Chapter 2. In a literature review occurrence and use of diploids are described together with literature relevant to the experimental chapters. Haploids from diploids and allopolyploids can yield homozygous plants for hybrid breeding. Haploids from autotetraploids are used for breeding at diploid level, after which an improved tetraploid can be reconstituted; 2n gametes can play an important role in this retetraploidization.

Frequencies of *S. tuberosum* dihaploids, obtained through pseudogamy, are influenced by the genotypes of both the pollinator and the seed parent. Selection and breeding has been done for superior pollinators. Genes for high dihaploid frequencies were described as recessive. Genes in the seed parent for high dihaploid frequencies were found to be dominant.

Dihaploid frequencies can be influenced to a large extent by environmental factors like temperature, light, radiation and chemicals.

Endosperm plays a major role in development of seeds. Dihaploid embryos of *S. tuberosum* have only been observed in hexaploid endosperm. The twenty-four paternal chromosomes in the endosperm are either contributed by one 2n sperm or by two n sperms. Arguments for both have been given.

Chapter 3. Crosses were made between pollinators and the progeny tested to study the genetics of the influence of the pollinator on dihaploid frequencies. The number of loci determining dihaploid frequencies in the population studied was probably five, assuming additivity. Within-locus interaction was of the intermediate type. Several pollinators in the progeny were better than the best parent, and one pollinator yielded up to 800 dihaploids per 100 berries (d/100b). Certain pollinators produced high numbers of hybrid seeds. This had a small negative effect on dihaploid frequencies. The number of dihaploids was reduced on the average by one for every 100 extra hybrids.

Chapter 4. The existence of an influence of the seed parent on dihaploid

frequencies was confirmed. Results of an inheritance study of the seed parent effect were not conclusive. Probably several loci were involved and the inheritance was unlikely to be recessive, possibly intermediate. A major influence of the cytoplasm on dihaploid frequencies was not found. The sporophyte rather than the gametophyte determined the frequency of dihaploids.

Chapter 5. Interaction between pollinator and seed parent influence on dihaploid frequencies was studied. Apart from analysis of data over several years, a special experiment was carried out, in which 'crossing date' effects were excluded. A seed parent × pollinator interaction was found only, but not always, when both main effects were significant. This interaction disappeared after a logarithmic transformation, indicating that a multiplicative effect was responsible for most of the interaction. Interaction in a strict sense between the pollinator and the seed parent effect seemed to be absent. A pollinator × year (crossing date) interaction was found.

For hybrid production no seed parent × pollinator interaction was found, but again a pollinator × year (crossing date) interaction was found.

Chapter 6. Crosses made in a greenhouse had shown considerable variation in dihaploid frequencies during a season. An experiment in two growth chambers with high temperature (23 °C day, 15-18 °C night) and low temperature (18 °C constant) was set up to give information about the influence of the temperature. There was no clear effect of the temperature via the seed parent on d/100b. Low temperature had a positive effect on d/100b via the pollinator. Hybrid frequencies were more variable than dihaploid frequencies. High temperature had a positive influence on hybrid frequencies via the seed parent when IVP35 was the pollinator. Pollinators exposed to low temperature gave higher hybrid frequencies than those exposed to high temperature.

Chapter 7. Cytological techniques were used to elucidate the mechanism of dihaploid formation. High 2n pollen frequencies in *S. phureja* did not coincide with high dihaploid frequencies, but they did with hybrid frequencies. No correlation was found between dihaploid and hybrid frequencies. Ovule lethality proved to be high, especially when many 3x embryos were present. Irregularities were found in the pollen tube mitosis of *S. phureja* (in vivo). In 5% of the tubes only one sperm was found, but in only very few it was certain that only one sperm was present. In a further 5% of the tubes the two sperms were very close or touching. This group might represent sperms with a 'functional' restitution, leading to double fertilization of the central nucleus, a hexaploid endosperm and an unfertilized egg cell. Observations on the growth rate of pollen in styles showed that 2x pollen grew faster than x pollen in *S. tuberosum*. This was also true for *S. phureja*. Delayed pollination did not increase dihaploid frequencies.

From these elements it was concluded, that 2n *S. phureja* pollen produced tetraploid hybrid seeds after pollination of *S. tuberosum*. The n pollen, however, produced lethal triploid hybrid seeds and induced dihaploids in hexaploid endosperm. The double fertilization of the central nucleus was possible through complete or incomplete, but functional, restitution of the pollen tube mitosis.

Chapter 8. Monoploids from *S. tuberosum* were also produced through pseudogamy, using the same pollinators as for dihaploids. Differences between pollinators were not significant. Seed parents differed considerably; the highest frequency found was 17 monoploids per 100 berries, while certain seed parents did not produce any monoploid. The mechanism of monoploid formation almost certainly differed from that of dihaploid formation. Again the n pollen of *S. phureja* was involved, but one sperm did not function and the other fused with the central nucleus to form 3x endosperm.

Three monoploids from diploid *S. verrucosum* were obtained.

Chapter 9. In a concluding chapter the implications of the findings are discussed. It will be possible to breed pollinators for higher dihaploid frequencies. However, it is most likely not worth the effort, as frequencies from existing pollinators are high already.

Pseudogamy is more efficient than anther culture as a method for dihaploid production, both in costs and in genetic diversity. For monoploid production pseudogamy is more efficient as well. Both methods may be limited in the number of genotypes from which monoploids can be extracted.

Samenvatting

Geslachtscellen van planten bevatten gewoonlijk de helft van het aantal chromosomen dat in de lichaamscellen voorkomt. Een plant die zich ontwikkelt uit één enkele geslachtscel wordt een haploid genoemd. Veel haploiden zijn onvruchtbaar, omdat ze op hun beurt geen geslachtscellen kunnen vormen. De cultuuraardappel, *Solanum tuberosum*, heeft alle chromosomen in viervoud; dit wordt aangeduid als 4x, waarbij "x" één volledige set chromosomen voorstelt. Haploiden van de aardappel hebben 2x chromosomen en worden dihaploiden genoemd. Ze zijn vaak vruchtbaar. Ze kunnen in grote aantallen gevormd worden door bestuiving van bloemen met stuifmeel van *Solanum phureja*, een aardappelsoort uit Zuid-Amerika, die diploid (2x) is. De bessen die worden gevormd na deze bestuiving bevatten meestal weinig zaden. Een paar zaden kunnen dihaploid zijn en de rest normaal kruisingsprodukt (hybride).

Voor toepassingen in veredeling en onderzoek moeten dihaploiden op grote schaal gemaakt kunnen worden. Hoewel de dihaploiden geen chromosomen ontvangen van de bestuiver, beïnvloeden de bestuivers wel de frequentie van de dihaploiden. Er kan op hoge frequentie geselecteerd worden. Ook is het praktisch als dihaploiden zo vroeg mogelijk van hybriden kunnen worden onderscheiden. Dit is mogelijk door gebruik te maken van zaadstip. Bepaalde bestuivers zijn homozygoot voor zaadstip en al hun hybride nakomelingen kunnen reeds als zaad worden gescheiden van de dihaploiden.

Wilde aardappelsoorten (2x) kunnen eigenschappen - zoals resistenties tegen ziekten - hebben, die men over zou willen brengen naar de cultuuraardappel. Echter, 2x-planten zijn over het algemeen slecht kruisbaar met 4x-planten. Door uit 4x-planten dihaploiden te maken, deze te kruisen met wilde 2x-planten en van hun nakomelingen het aantal chromosomen weer te verdubbelen, kunnen de gewenste eigenschappen toch benut worden voor de veredeling van de cultuuraardappel.

Dihaploiden zijn ook nuttig voor erfelijkheidsonderzoek. Bij gebruik van 2x-planten zijn veel minder planten nodig om het voorkomen van een bepaalde eigenschap in de nakomelingen te volgen dan bij 4x-planten.

Het doel van dit onderzoek was om uit te zoeken of er bestuivers konden worden geselecteerd, die nog meer dihaploiden zouden kunnen leveren dan de beschikbare bestuivers. Daarnaast zou worden getracht om meer inzicht te krijgen in de vererving van de eigenschap om dihaploiden te leveren en in de ontstaanswijze van dihaploiden.

Verschillende bestuivers zijn met elkaar gekruist. De nieuwe bestuivers die zo ontstonden, zijn getest op hun dihaploiden-leverend vermogen. Dit

leverde nieuwe bestuivers die beter waren dan de beste ouder. De beste nieuwe bestuiver leverde gemiddeld acht dihaploiden per bes. Sommige bestuivers leverden hoge aantallen hybriden. Deze hoge aantallen hadden een negatief effect op het aantal dihaploiden: voor iedere honderd extra hybriden werd het aantal dihaploiden gemiddeld met één verlaagd.

Het testen van de nieuwe bestuivers leverde ook aanwijzingen op over de vererving van het dihaploiden-leverend vermogen. Van iedere groep bestuivers werden de verhoudingen tussen goede en slechte bestuivers vergeleken. Hieruit werd geconcludeerd, dat de erfelijkheidsfactoren voor deze eigenschap op de chromosomen liggen en niet in het celplasma. Er zijn meerdere genen die deze eigenschap bepalen; binnen de onderzochte groep bestuivers zijn er waarschijnlijk vijf. Binnen een gen is de expressie niet dominant of recessief, maar intermediair. De effecten van de afzonderlijke genen versterken elkaar.

Naast de bestuiver heeft ook de moederplant veel invloed op het aantal geproduceerde dihaploiden. Ook bij de moederplanten bleken de factoren die het aantal dihaploiden bepalen op de chromosomen te liggen. De eigenschap wordt bepaald door de erfelijke eigenschappen van de plant en niet door die van de eicellen. Er zijn waarschijnlijk meerdere genen werkzaam.

Er werd onderzocht of er interactie was tussen het effect van de moederplant en dat van de bestuiver op de dihaploiden frequentie (d.f.). Als de d.f. van een goede moederplant, bestoven door een goede bestuiver, hoger is dan op grond van ieders d.f. met een gemiddelde testplant verwacht mocht worden, is er sprake van een vermenigvuldigingseffect. Als de rangorde in d.f. van moederplanten afhangt van de bestuiver waarmee getest wordt en de rangorde van de bestuivers van de moederplant, is er sprake van interactie in strikte zin.

Er werd gebruik gemaakt van de jaargemiddelden van verschillende jaren. Ook werd er een gedetailleerder experiment uitgevoerd, waarin milieuïnvloeden zoveel mogelijk gelijk werden gehouden voor alle moederplanten en bestuivers. Groepen gegevens werden met behulp van een variantieanalyse geanalyseerd. In sommige combinaties werd inderdaad een interactie tussen moederplant- en bestuiver-effect gevonden. Deze interactie verdween echter na een logarithmische bewerking van de gegevens. Dit betekent, dat alleen een vermenigvuldigingseffect aanwezig was en geen interactie in strikte zin. Het ontbreken van deze interactie heeft als consequentie, dat het voor het zoeken naar een bestuiver met hoge d.f. voldoende is om met één moederplant te toetsen en dat een moederplant met hoge d.f. kan worden opgespoord met behulp van maar één bestuiver.

De meeste proeven zijn in een kas gedaan, waarin de temperatuur 20 °C was tenzij de buitentemperatuur te hoog opliep. In de loop van het onderzoek bleken er verschillen in d.f. te bestaan tussen kruisingen gemaakt op verschillende dagen. Vermoed werd, dat met name de temperatuur hier een rol speelde. Om deze invloed na te gaan zijn er proeven in twee klimaatkamers

gedaan: een met een constante lage temperatuur (18 °C) en een met een wisselende, gemiddeld hogere temperatuur (23 °C overdag en 15-18 °C 's nachts). Binnen de onderzochte temperatuursgrenzen bleek er geen invloed van de temperatuur via de moederplanten op de d.f. te zijn. Lage temperatuur verhoogde wel de d.f. van de bestuivers. Ook aantallen hybriden waren hoger als de bestuivers bij lage temperatuur opgroeiden.

Het is dus belangrijk om met name de bestuiverplanten op een koele plaats te zetten of om de bestuivingen vroeg in het seizoen te maken, voordat er hoge temperaturen optreden.

Om een beter inzicht te krijgen in de ontstaanswijze van dihaploiden zijn microscopische technieken gebruikt. Uit de literatuur was bekend, dat dihaploiden alleen zijn gevonden in zaden met 6x-kiemwit. Als regel bevrucht een generatieve kern van het stuifmeel de eicel en de andere de (dubbele) centrale kern van de kiemzak. Aangezien de moederplant 4x chromosomen levert voor het kiemwit, moet de resterende 2x van het stuifmeel komen, terwijl de eicel niet wordt bevrucht.

Bij sommige bestuivers bevat een deel van het stuifmeel 2x chromosomen in plaats van x. Percentages van dit 2x-stuifmeel werden bepaald voor verschillende veelgebruikte bestuivers door de doorsnede van een groot aantal stuifmeelkorrels te meten. Deze percentages liepen niet parallel met de aantallen dihaploiden, wel met de aantallen hybriden die door de verschillende bestuivers werden geproduceerd. Ook bleek er geen parallel te zijn tussen het aantal dihaploiden en hybriden. Het 2x-stuifmeel speelt dus geen directe rol bij de dihaploidenvorming, wel bij de hybridenvorming. Het was al bekend, dat de meeste hybriden 4x-planten waren. Zij worden dus gevormd uit een 2x-eicel van de moederplant en een 2x-generatieve kern van de bestuiver.

Een andere mogelijkheid voor het ontstaan van 6x-kiemwit is, dat de twee generatieve kernen van het x-stuifmeel beide de centrale kern van de kiemzak bevruchten, na mogelijk eerst samen versmolten te zijn. Om dit te onderzoeken werden de kernen van stuifmeelbuizen van de bestuivers, die 24 uur in een stijl waren gegroeid, onder een microscoop bekeken. In sommige buizen werd maar één generatieve kern gevonden, maar dit kon slechts zeer zelden met zekerheid worden vastgesteld. De frequentie was te laag om de gevonden aantallen dihaploiden te verklaren. In ongeveer 5% van de buizen werden de twee generatieve kernen vlak bij elkaar of tegen elkaar aan gevonden. Het is mogelijk dat deze kernenparen zich als een geheel gedragen en samen de centrale kern van de kiemzak bevruchten.

Hybriden met 3x chromosomen, het normale produkt van een kruising tussen een 4x- en een 2x-plant, hebben geringe levenskansen. Tellingen van de aantallen zich ontwikkelende zaadbeginsels in jonge aardappelbessen bevestigden, dat de 3x-zaden in grote meerderheid afsterven.

In het kort: het 2x-stuifmeel van *S. phureja* levert na bestuiving van een 4x-aardappelplant levenskrachtige 4x-hybriden. Het x-stuifmeel levert

grote aantallen 3x-hybriden, die in een vroeg stadium afsterven, en een paar dihaploiden in 6x-kiemwit, als beide generatieve kernen op de een of andere manier samen de centrale kern van de kiemzak bevruchten.

De groeisnelheid van 2x-stuifmeel werd vergeleken met die van x-stuifmeel door de in 24 uur afgelegde afstand in een stijl te vergelijken. Het 2x-stuifmeel bleek gemiddeld sneller te groeien dan het x-stuifmeel. Dit heeft tot gevolg, dat 2x-stuifmeel naar verhouding meer eicellen bevrucht en dat er meer 4x-hybriden optreden dan op grond van de frequenties van 2x-stuifmeel verwacht mag worden.

Bestuiving van oudere bloemen bleek geen verhoging van de d.f. op te leveren.

Het is waarschijnlijk mogelijk om bestuivers te kweken die nog grotere aantallen dihaploiden opleveren dan de tot nu toe geselecteerde bestuivers. Hiervoor zijn zeer grote aantallen planten nodig, die allemaal op hun d.f. getest moeten worden. Het is de vraag, of deze langdurige selectieprocedure de moeite waard zal zijn, aangezien de huidige bestuivers al redelijke hoeveelheden dihaploiden opleveren.

Het bleek mogelijk het chromosoomaantal van de dihaploiden van de aardappel opnieuw te halveren, weer door bestuivingen met *S. phureja*. De gevormde planten met x chromosomen worden monoploiden genoemd. De monoploiden frequentie bleek niet duidelijk afhankelijk van de bestuiver. De moederplanten hadden wel een grote invloed. Sommige produceerden in het geheel geen monoploiden, terwijl de hoogste gevonden frequentie 17 monoploiden per 100 bessen bedroeg. In totaal werden 82 *S. tuberosum* monoploiden gevonden zowel van dihaploiden als van andere 2x-aardappelplanten. Ook werden drie monoploiden van *Solanum verrucosum* (2x) verkregen.

Aangezien bestuivers met meer 2x-stuifmeel niet meer monoploiden leveren, moet bij het ontstaan van monoploiden weer het x-stuifmeel een rol spelen. Van de twee generatieve kernen bevrucht er één de centrale kern van de kiemzak, terwijl de ander verloren gaat, zodat de eicel onbevrucht blijft. Het is niet duidelijk waarom een van de generatieve kernen niet werkzaam is. Het vermogen van eicellen om onbevrucht toch tot een kiem uit te groeien is waarschijnlijk erfelijk bepaald.

Door het verdubbelen van het aantal chromosomen van enige monoploiden werden homozygote 2x-planten verkregen. Homozygote aardappelplanten zijn door inteelt niet te verkrijgen. Ze kunnen worden gebruikt in een veredelingssysteem dat op "hybride groeikracht" is gebaseerd. Een mogelijke toepassing in de verre toekomst is de teelt van hybride aardappelen uit zaad.

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