DESYNAPSIS AND FDR 2N-MEGASPORE FORMATION IN DIPLOID POTATO; POTENTIALS AND LIMITATIONS FOR BREEDING AND FOR THE INDUCTION OF DIPLOSPORIC APOMIXIS

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Proefschrift

ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
in het openbaar te verdedigen
op vrijdag 15 maart 1991
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

Aan mijn ouders

Voor Anja

Front cover:

Where normal meiosis (1) generally gives rise to the formation of a tetrad of reduced (n) megaspores (2) and desynaptic meiosis (3) causes sterility (4), pseudo-homotypic division of univalent chromosomes in desynaptic mutants (5) is followed by dyad formation and subsequent development of a single so called FDR 2n-megaspore (6).

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STELLINGEN

1. "First division restitution (FDR)" en "second division restitution (SDR)" 2n-gametenvorming als gevolg van voortijdige, equationele chromosoomdeling dienen in genetisch en cytologisch opzicht beschouwd te worden als extremen van een kontinuum.
   Dit proefschrift.

2. De door Iwanaga en Peloquin gerapporteerde, uitsluitend in de megasporogenese van diploide aardappelklonen tot expressie komende synaptische mutant *sy-1* bestaat niet.
   Iwanaga M & Peloquin SJ (1979) J. Heredity 70: 385-389.
   Dit proefschrift

3. De door Gustafsson gerapporteerde vorming van ongereduceerde megasporen in diplospore apomikten door middel van de zogenaamde pseudo-homotypische deling wordt door Nogler ten onrechte als onwaarschijnlijk afgedaan en verdient op z'n minst nader onderzoek.
   Gustafsson A (1935) Hereditas 21: 1-111.
   Nogler GA (1984) In: Johri BM (Ed.) Embryology of Angiosperms. pp. 475-518.

4. De evolutionaire rol van synaptische mutanten bij het ontstaan van polyploide complexen en diplospore apomixis wordt sterk onderschat.

5. De bewering dat genetische modifikatie van plantenrassen onvermijdelijk leidt tot een ongewenste vernauwing van het rassensortiment in de land- en tuinbouw, is onjuist.

6. Verlening van afhankelijk kwekersrecht op genetisch gemodificeerde plantenrassen dient afhankelijk te worden gesteld van de aantoonbaarheid van zowel de in deze rassen ingebouwde nieuwe genen als de geclaimde, daarmee samenhangende, nieuwe phenotypische eigenschappen.

7. De door Bonierbale *et al.* met een *Solanum*-soorthybride gekonstrueerde koppelingskaart onderschat de genetische lengte van het aardappelgenoom.
   Bonierbale MW, Plaisted RL & Tanksley SD (1988) Genetics 120: 1095-1103.

8. Genetische kaarten die zijn gebaseerd op zogenaamde "random amplified polymorphic DNA markers" of "RAPD's", zijn in de veredeling slechts beperkt bruikbaar.

9. De door "De Ziedende Bintjes" en "De Woedende Escorts" ondernomen akties tot sabotage van veldexperimenten met genetisch gemodificeerde gewassen zijn in meer dan één opzicht als milieugevaarlijk aan te merken.

10. De grootschalige ontduiking van de wettelijk voorgeschreven melkquota rechtvaardigt het gebruik van de slogan "Melk de Zwarte Motor".

Stellingen behorende bij het proefschrift "Desynapsis and FDR 2n-megaspore formation in diploid potato; potentials and limitations for breeding and for the induction of diplosporic apomixis", door Erik Jongedijk in het openbaar te verdedigen op vrijdag 15 maart 1991 in de aula van de Landbouwuniversiteit te Wageningen.

VOORWOORD

Velen hebben op direkte of indirekte wijze hun steentje bijgedragen aan de totstandkoming van dit proefschrift en de plezierige herinneringen die ik aan het werken op de vakgroep Plantenveredeling heb overgehouden.

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In het kader van het project 'sexuele polyploidisatie en inductie van apomixie bij *Solanum*' heb ik talrijke studenten en stagiaires mogen begeleiden c.q. mede-begeleiden: Carien Denissen, Marietta van der Werff, Anjo Elgersma, Heleen Bastiaansen, Juliëtte Hermans, Marjolein Hulscher, Ronald Hutten, Wim Beekman, Henk van Kooten, Rien Nijboer, Jan van Oeveren, Marzenna Osiecka, Ehwald Pfeiffer, Wim Sangster, Stefan Schuurmans-Stekhoven, Petra Stam, Marie Claire Suignard, Mien van de Ven, Harry van de Vijver, Ellen Wisman en Coco van der Wolk. Een aantal van hen hebben in het in dit proefschrift opgenomen deel van dat onderzoek geparticipeerd en zullen hun werk ongetwijfeld in de navolgende hoofdstukken terugvinden. De inzet van allen heb ik zeer gewaardeerd.

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Aan een niet in dit proefschrift opgenomen veldproef tenslotte, is veel werk verricht door ir. Stefan van der Heyden (Cebeco Handelsraad), Jacob Eysing (Hettema Zonen) en de sectie akkerbouw van de 'ir. A.P. Minderhoudhoeve', met name Johan Jorink, Onne van der Wal,

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- Erik -

CONTENTS

ACCOUNT

This thesis is a compilation of articles that have been published before or have been accepted or submitted for publication. Other papers that are relevant to the subjects of this thesis but have not been included herein are listed below.

Hermsen JGTh, Ramanna MS and Jongedijk E (1985) Apomictic approach to introduce uniformity and vigour into progenies from true potato seed (TPS). In: Page OT (Ed.) Present and future strategies for potato breeding and improvement; Report of the 26th Planning Conference of the International Potato Center, December 12-14, 1983, Lima, Peru (1985). Training and Communications Department of the International Potato Center, Lima, Peru. pp. 99-114.

Jongedijk E (1986) The present state of research into the induction of apomixis in potato. In: Beekman AGB, Louwes KM, Delleart LMW & Neele AEF (Eds.) Potato research of tomorrow: drought tolerance, virus resistance and analytic breeding methods; Proceedings of an International Seminar, October 30-31, 1985, Wageningen, The Netherlands. Pudoc, Wageningen. pp. 120-123.

Jongedijk E (1987) Desynapsis and FDR 2n-egg formation in potato; its significance to the experimental induction of diplosporic apomixis in potato. In: Jellis GJ & Richardson DE (Eds.) The production of new potato varieties: technological advances; Proceedings EAPR/EUCARPIA Breeding and Variety Assessment Meeting, December 16-20, 1985, Cambridge, England (1987). Cambridge University Press, Cambridge, England. pp. 225-228.

Suurs LCJM, Jongedijk E and Tan MMC (1989). Polyacrylamide gradient-gel electrophoresis: a routine method for high resolution isozyme electrophoresis of *Solanum* and *Lycopersicon* species. Euphytica 40: 181-186.

INTRODUCTION

The cultivated potato, *Solanum tuberosum* L., is a highly heterozygous autotetraploid (2n=4*x*=48) plant species of South American origin. Following its introduction into Europe in the 16th century it has been spread globally and developed into one of the world's major food crops. The traditional and still most widely applied method in potato breeding basically consists of intercrossing superior parental clones and subsequent selection of the best F1 hybrids under continued vegetative propagation. Though apparently simple, this procedure since the early twenties has yielded only a relatively slow progress in varietal improvement. This has been attributed to the generally minor chances of finding superior F1 hybrids in consequence of (i) the tetrasomic inheritance and the extreme heterozygosity of parental clones employed, (ii) the narrow genetic base of the cultivated potato as a result of the limited number of accessions originally imported in Europe and (iii) further reduction of genetic variation through severe diseases.

As proposed by Chase (1963) these limitations might be largely avoided by the adoption of the so-called "Analytic Breeding" scheme, which basically consists of (i) haploidization of the tetraploid potato, (ii) subsequent breeding at the diploid level, taking advantage of disomic inheritance and the convenient use of the vast array of diploid *Solanum* species, and (iii) eventual return to the potentially better performing tetraploid level. Essentially two factors have rendered this approach feasible for practical potato breeding. Firstly, methods enabling routine extraction of dihaploids (2n=2*x*=24) from tetraploid potato through either pseudogamous seed development following pollination with marked *S.phureja* genotypes (Hougas *et al.*, 1964; Hermsen and Verdenius, 1973) or anther culture (Mix, 1982; Wenzel and Foroughi-Wehr, 1984) have now been well established. Secondly, adverse effects of increased levels of homozygosity on the performance of tetraploids following mitotic tetraploidization (colchicine) of enhanced diploids may now be circumvented by employing either somatic hybridization techniques (Wenzel *et al.*, 1979; Puite *et al.*, 1986; De Vries *et al.*, 1987) or meiotic (sexual) polyploidization via numerically unreduced (2n) gametes (Mendiburu *et al.*, 1974; Peloquin, 1982; Hermsen, 1984*a*; Veilleux, 1985). As yet routine production of extensive tetraploid populations by somatic hybridization is hampered by the strong genotype dependence and a number of practical imperfections of the technique. However, the frequent occurrence of genetically determined 2n-gamete formation in diploid *S.tuberosum* and related diploid species has enabled routine transfer of enhanced diploid germplasm to tetraploids by means of unilateral (4*x*-2*x* crosses) or, in a few cases, bilateral (2*x*-2*x* crosses) sexual polyploidization.

Although 2n-gametes may result from a number of meiotic modifications, depending on its genetic consequences only two distinct modes of formation are currently being distinguished: first division restitution (FDR) and second division restitution (SDR). Basically FDR 2n-gametes may be considered to originate from an equational division of the entire (i.e., numerically unreduced) chromosome complement after the completion of prophase I and thus include non-sister chromatids, whereas SDR 2n-gametes, in a strict sense, can be regarded to result from chromosome doubling in the haploid nuclei that are formed after the completion of the first meiotic division and therefore comprise sister chromatids. The actual mode of 2n-gamete formation (i.e., FDR or SDR) in the diploid parental clones is of practical significance in breeding schemes employing sexual polyploidization. Whereas FDR 2n-gametes are expected to preserve a relatively large proportion of the parental heterozygosity and epistasis and thus to strongly resemble each other and the parental clone from which they derive, SDR is expected to yield a relatively heterogeneous population of highly homozygous 2n-gametes (Mendiburu *et al.*,1974; Peloquin, 1983; Hermsen, 1984*b*). As multi-allelism, allowing for higher order intra-locus interactions and complex types of epistasis in tri- and tetra-allelic genotypes, has been inferred to contribute considerably to the performance of autopolyploid crops (Demarly, 1963; Dudley,1964; Busbice and Wilsie, 1966; Lundqvist, 1966; Mendoza and

Haynes, 1974), FDR is generally considered by far superior to SDR. This superiority of FDR has been well established in 4*x*.2*x* crosses (Mok and Peloquin, 1975; Mendiburu and Peloquin, 1977; De Jong and Tai, 1977; McHale and Lauer, 1981). In this respect mutant synaptic genes are of special interest. Owing to reduced gene recombination they would provide an opportunity to maximize the ability of FDR 2n-gametes to pick up the genetic constitution of parental clones, including complex types of favourable epistasis, with a minimum amount of reassortment (Peloquin, 1982, 1983; Iwanaga, 1984; Hermsen, 1984*b*). Maximum performance and uniformity would thus be attained in 2*x*FDR-2*x*FDR crosses when genetic recombination is largely lacking in both parental diploids.

Except for its use in breeding 2*x*FDR-2*x*FDR crosses are particularly important for the production of true potato seed (TPS) varieties. The technology of growing potatoes from true seeds has received considerable attention in response to the urgent need for cheap and disease-free plant material in developing countries and requires production of vigorous tetraploid seedling populations that are sufficiently uniform to be released as varieties. In this perspective also the possibility for identical or near-identical reproduction of superior genotypes through the experimental induction of apomictic seed production has received considerable attention (Hermsen, 1980).

Apomixis sensu stricto (agamospermy) is the asexual formation of maternal embryos or seeds and is subdivided into gametophytic apomixis and adventitious embryony on the basis of presence or absence of a gametophytic stage respectively. In gametophytic apomixis unreduced embryosacs and thus 2n-egg cells are formed that can be of either aposporic or unreduced embryosacs develop directly from vegetative, usually nucellar cells of the ovule through mitotic divisions. In diplospory unreduced embryosacs derive from generative archesporial cells of the ovule, either directly by mitotic divisions or indirectly by modified meiosis. In the latter case neither reduction in chromosome number nor substantial crossing-over occurs during meiosis and functional unreduced megaspores develop into embryosacs through successive mitotic divisions. Both in aposporic and diplosporic apomixis fertilization of the secondary embryosac nucleus cell may or may not be required for the endosperm formation and subsequent parthenogenetic development of the unreduced egg cell (pseudogamous and autonomous apomixis respectively).

The occurrence of latent tendencies for the formation of unreduced gametophytes (Harlan and De Wet, 1975) and parthenogenesis (Kimber and Riley, 1963) in many Angiosperm plant species is consistent with the recent hypothesis that gametophytic apomixis consists of a number of distinct and genetically controlled elements which are within the reproductive potentialities of sexual plant species (Petrov, 1970) and has resulted in several attempts and propositions for its introduction in cultivated and largely sexual crops (Knapp, 1975; Petrov et al.,1979; Asker, 1980; Hermsen, 1980; Matzk, 1982; Savidan, 1986; Hanna and Bashaw, 1987). As to potato Hermsen (lc) suggested that apomictic reproduction might be achieved by combining either mutant genes for asynapsis/desynapsis and FDR 2n-megaspore formation (diplospory), or induced aposporic mutants, with genes for pseudogamous seed development.

The investigations on diploid potato hybrids described in this thesis were originally started to test the feasibility of inducing gametophytic apomixis in potato. Because initial studies revealed that unreduced embryosacs of aposporic origin had never been detected unambiguously in *Solanaceae* the research was primarily focussed on the possibilities for inducing diplospory and thus (i) the identification and characterization of mutant synaptic genes that are expressed in female meiosis and (ii) identification of potato clones with consistent FDR 2n-megaspore formation and elucidation of the mechanisms of FDR 2n-megaspore formation. Special attention is paid to the use of mutant synaptic genes for maximizing the ability of FDR 2n-megaspores to pick up the genetic constitution of parental clones with a minimum amount of reassortment and its application in potato breeding, including the production of true potato seed varieties through 2*x*FDR-2*x*FDR crosses, and the

experimental induction of diplosporic apomixis.

Chapter 1 describes the normal course of female meiosis and its implications for the origin of FDR (and SDR) 2n-megaspore formation and the experimental induction of gametophytic apomixis. Chapters 2 and 3 deal with the optimization and development of cytological techniques required for large-scale screening and detailed observations of female meiosis. Chapters 4 and 5 report on the identity and expression of mutant synaptic genes and the effect of one of these genes, *ds-1*, on chiasma frequencies in both male and female meiosis. In chapter 6 the formation of consistent FDR 2n-megaspore formation in *ds-1* mutants through direct equational division of univalent chromosomes and subsequent omission of the second meiotic division (pseudo-homotypic division) is reported. In addition, data suggesting that both FDR and SDR 2n-megaspore formation are closely interrelated are provided. In chapter 7 the effect of the *ds-1* gene on genetic recombination in male and female meiosis and its effect on the the ability of FDR 2n-megaspores and 2n-pollen to preserve the genetic constition of parental clones is reported. Finally, some of the genetic markers used herein are characterized in chapter 8.

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CHAPTER 1

THE PATTERN OF MEGASPOROGENESIS AND MEGAGAMETOGENESIS IN DIPLOID *SOLANUM* SPECIES HYBRIDS; ITS RELEVANCE TO THE ORIGIN OF 2N-EGGS AND THE INDUCTION OF APOMIXIS

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INDEX WORDS

Solanum, megasporogenesis, megagametogenesis, archesporium, 2n-gametes, apomixis.

SUMMARY

Megasporogenesis and megagametogenesis in diploid tuber-bearing *Solanum* species hybrids were studied and illustrated in detail. Megagametogenesis followed the normal or *Polygonum* type of embryo sac development. In megasporogenesis a few regularly occurring deviations from what is usually considered to be the normal course of megasporogenesis were detected.

Firstly, the development of a surplus of adjacent or non-adjacent megaspore mother cells in a single ovule was frequently observed. As they eventually may give rise to normally reduced, sexual embryo sacs, it is concluded that the archesporium in potato cannot be delimited to a single cell or even a group of adjacent cells in the ovule. Secondly, the micropylar daughter cell which is formed after completion of the first division was often found to degenerate before the onset or completion of the second division giving rise to a triad instead of a tetrad of megaspores. The spatial arrangement of megaspores within the ovule was found to vary according to the variation in the relative orientation of second division spindles.

It is concluded that these deviations should be considered random legitimate variations of megasporogenesis rather than systematic abnormal events.

The implications of the overall pattern of megasporogenesis for the formation of 2n-eggs and the attempts to induce diplosporic or aposporic apomixis in potato are discussed.

INTRODUCTION

During the last decade, considerable attention has been paid to the potential use of 2n-gametes (i.e. gametes with the unreduced, somatic chromosome number) in the cultivated potato, *Solanum tuberosum* L. ($2n = 4\times = 48$), both in relation to the development of alternative and more efficient breeding strategies (MENDIBURU et al., 1974; MENDIBURU & PELOQUIN, 1977; PELOQUIN, 1981, 1982; HERMSEN, 1984b, c) and the new technology of growing potatoes from true seeds (HERMSEN, 1977; PELOQUIN, 1983a). As to the latter, in recent years the possibility of inducing either diplosporic apomixis (HERMSEN, 1980; HERMSEN et al., 1985) or aposporic apomixis (HERMSEN, 1980; IWANAGA, 1980, 1982) has been promoted.

Apomixis sensu stricto (or agamospermy) may be defined as the formation of mater-

nal embryos or seeds by various types of asexual reproduction (RUTISHAUSER, 1967). It is subdivided into gametophytic apomixis and adventitious embryony (STEBBINS, 1950), on the basis of presence or absence of a gametophytic stage respectively. In gametophytic apomixis an unreduced embryo sac is formed that can be of either diplosporic or aposporic origin (RUTISHAUSER, 1967).

In apospory an unreduced embryo sac develops directly from a somatic (vegetative) cell of the ovule through mitotic divisions. In diplospory an unreduced embryo sac derives from an archesporial (generative) cell of the ovule, but neither reduction in chromosome number (apomeiosis) nor crossing over occurs during megasporogenesis. The functional megaspore develops into the unreduced embryo sac through successive mitotic divisions. Both apospory and diplospory involve the formation of female 2n-gametes. In apospory 2n-egg formation is a consequence of the absence of megasporogenesis, whereas in diplospory 2n-eggs result from 'abnormal' megasporogenesis (RUTISHAUSER, 1967).

To decide whether an unreduced embryo sac is of diplosporic or aposporic origin and to be able to recognize abnormalities in megasporogenesis giving rise to 2n-eggs, an accurate knowledge of the delimitation of the archesporium in developing ovules and of the usual pattern of megasporogenesis and megagametogenesis is a prerequisite.

In this respect, surprisingly little work has been carried out in the tuber-bearing wild and cultivated potato species. The only thorough study, as a matter of fact, is that by REES-LEONARD (1935) on the cultivated potato, *Solanum tuberosum* L.

The aims of this study in diploid *S. phureja-S. tuberosum* hybrids, in which it is tried to induce apomixis, were to determine to what extent the archesporium actually is delimited, to establish a standard for the usual course or female meiosis and embryo sac development in these species hybrids and to discuss its implications in relation to 2n-egg formation and the induction of apomixis in potato.

MATERIALS AND METHODS

Ovule development, female meiosis and embryo sac development were studied in 20 diploid genotypes ($2n = 2x = 24$) that were obtained from eight crosses, involving the diploid parental clones USW 5293-3 (A). USW5295-7 (B), USW 5337-3 (C), USW 7589-2 (D) and 77-2102-37 (E): AB(1), BC(3), CB(3), BE(3), CE(3), EC(1), DE(3) and ED(3), with in parentheses the respective number of genotypes from each cross.

The USW clones were selected by Dr S. J. Peloquin and associates at the University of Wisconsin (Madison, USA) from crosses between *Solanum phureja* and dihaploid *S. tuberosum*. The clone 77-2102-37 was selected by Dr E. Jacobsen at the Max-Planck Institute (Cologne, FRG) from the cross $VH^3 \times$ USW 5337-3 (JACOBSEN, 1978) and has *S. vernei, S. phureja* and *S. tuberosum* in its ancestry.

For cytological studies ovaries were collected from flower buds representing the different stages of ovule development. They were fixed in CRAF V (BERLYN & MIKSCHE, 1976) for 24 hours and next rinsed and stored in ethanol (70%). The material was dehydrated in a tertiary buthanol-ethanol series (GERLACH, 1977) and embedded in paraplast plus (m.p. 56–57°C, BDH Chemicals). Sections were cut at 8–16 μm. and stained with safranin-fast green (GERLACH, 1977 with slight modifications) and mounted in Canada balsam.

The ovary is usually composed of two carpels and is two-chambered. Each carpel forms an enlarged placenta on which many ovules are born.

The ovule initials differentiate as groups of cells from the sub-epidermal tissue (Fig. 1A) and as they protrude, the placental epidermis looses its initial smooth surface (Fig. 1B). Ultimately many anatropous-amphitropous ovules, each with a single integument, are formed.

The integument primordia arise as a ring of meristematic tissue near the base of the tenuinucellate nucellus. As megasporogenesis proceeds the single layer of nucellar tissue degenerates and an integumental tapetum surrounding the embryo sac is established from the inner integumental epidermis.

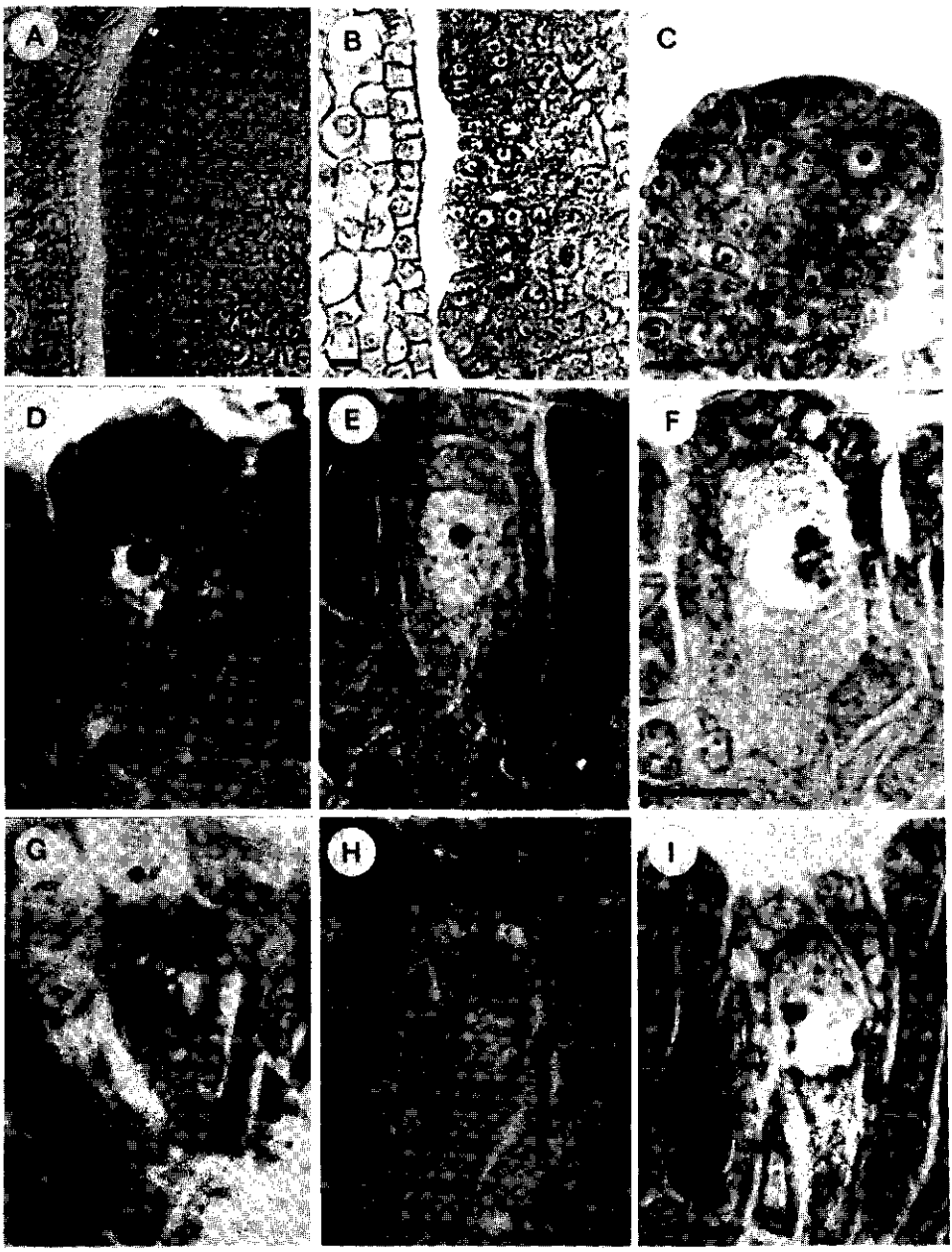
The archesporial cells are of hypodermal origin and mostly differentiate from a group of sub-epidermal cells (Fig. 1C). With the start of meiosis, the archesporial cell is regarded as the megaspore mother cell (MMC). The earliest meiotic activity is seen as the appearance of granular chromatin within the nucleus (Fig. 1D), which like the MMC itself enlarges distinctly. The chromosomes spiralise increasingly and the MMC passes through the successive stages of prophase I (Figs. 1E-I). It should be emphasized that in potato a distinct despiralisation-respiralisation of completely paired bivalents occurs between pachytene and diplotene stage (diffuse stage).

At metaphase I the two centromeres of a bivalent are co-oriented to opposite spindle poles and the bivalents are regularly arranged at the equatorial plate (Fig. 2A). Homologous chromosomes separate disjunctionally and move to opposite poles (Fig. 2B). This movement is usually synchronized, but irregularities may occur in low frequencies. A cell plate is formed across the persisting phragmoplast at telophase (Fig. 2C), resulting in a dyad of reduced(n) daughter cells.

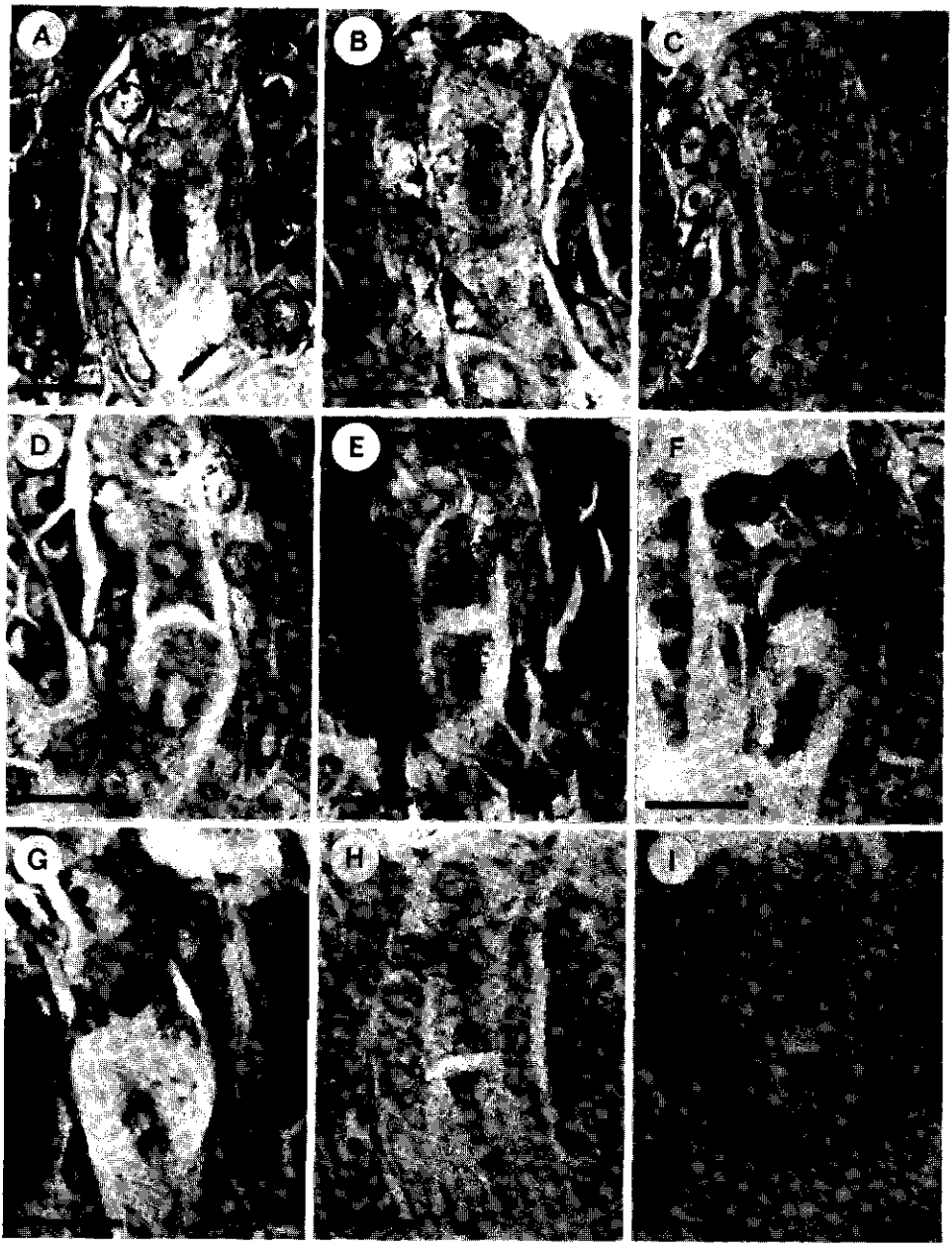
During interkinesis despiralisation of univalent chromosomes is often far from complete since the daughter cells quickly enter into prophase II (Fig. 2D). The haploid chromosome complement in both daughter cells divides equationally and sister chromatids move to opposite poles (Figs. 2E-H), resulting in a tetrad of reduced megaspores after completion of equational cell wall formation at telophase II (Figs. 2I, 3A).

Though it is generally believed that a linear tetrad of megaspores is formed, this does not have to be necessarily so. As cell plate formation in both daughter cells is across the persisting phragmoplasts, the spatial arrangement of megaspores within the ovule will correspond with the relative orientation of the second division spindles to each other. Because the spindles may be oriented at different angles (Figs. 2E-H) the spatial arrangement of the four megaspores actually varies from truly linear (if both spindles aline) to T shaped (perpendicular spindle orientation). This is especially apparent in young tetrads; In older tetrads the three non-functional megaspores have degenerated and may be forced in a more or less linear arrangement by the excessive growth of the ovule.

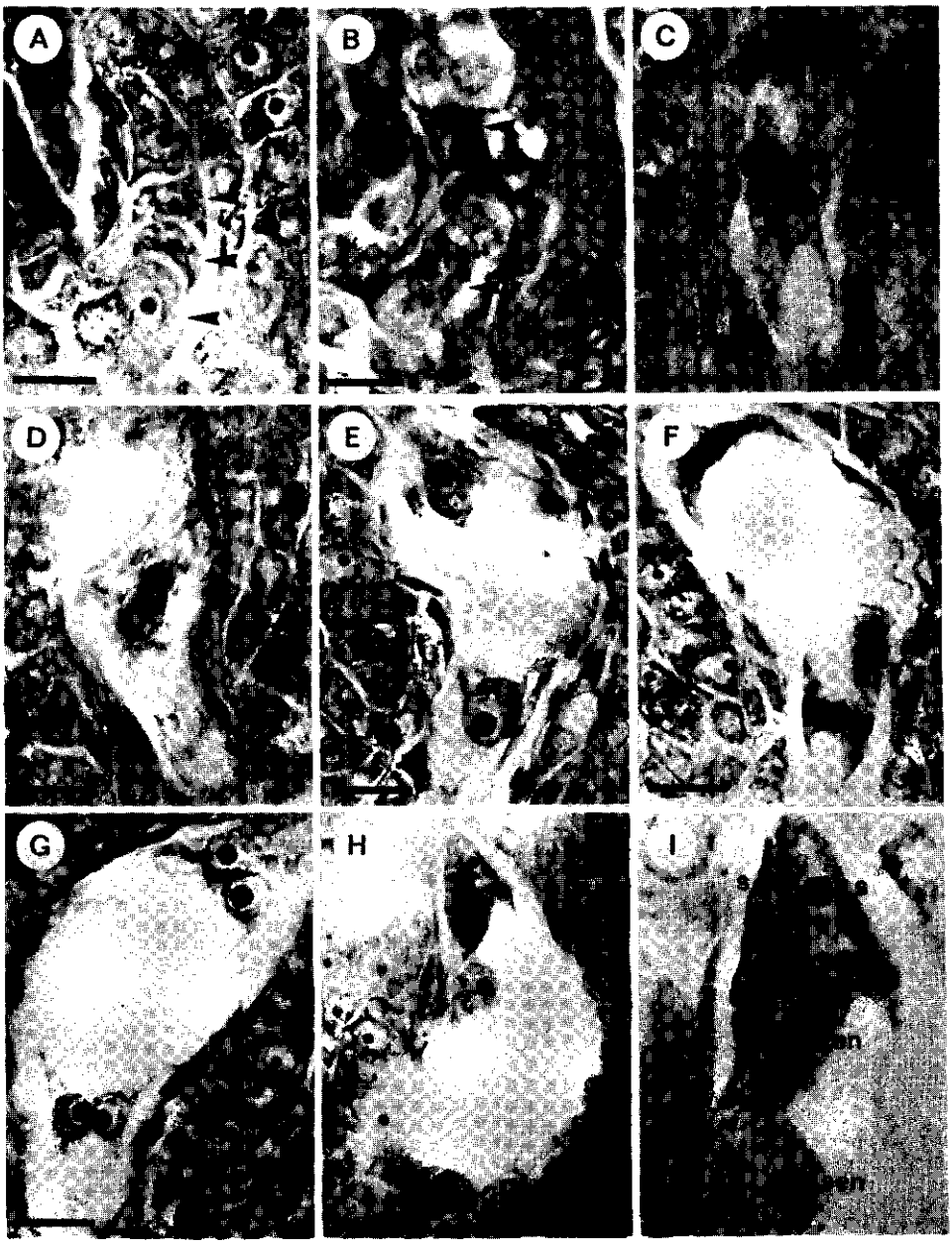
A tetrad of megaspores need not always be formed. In many ovules the micropylar dyad cell was found to degenerate before the onset or completion of the second division (Figs. 4G-H). The chalazal dyad cell in these cases divided normally and after cell wall formation a triad (two degenerating cells + one developing chalazal megaspore)



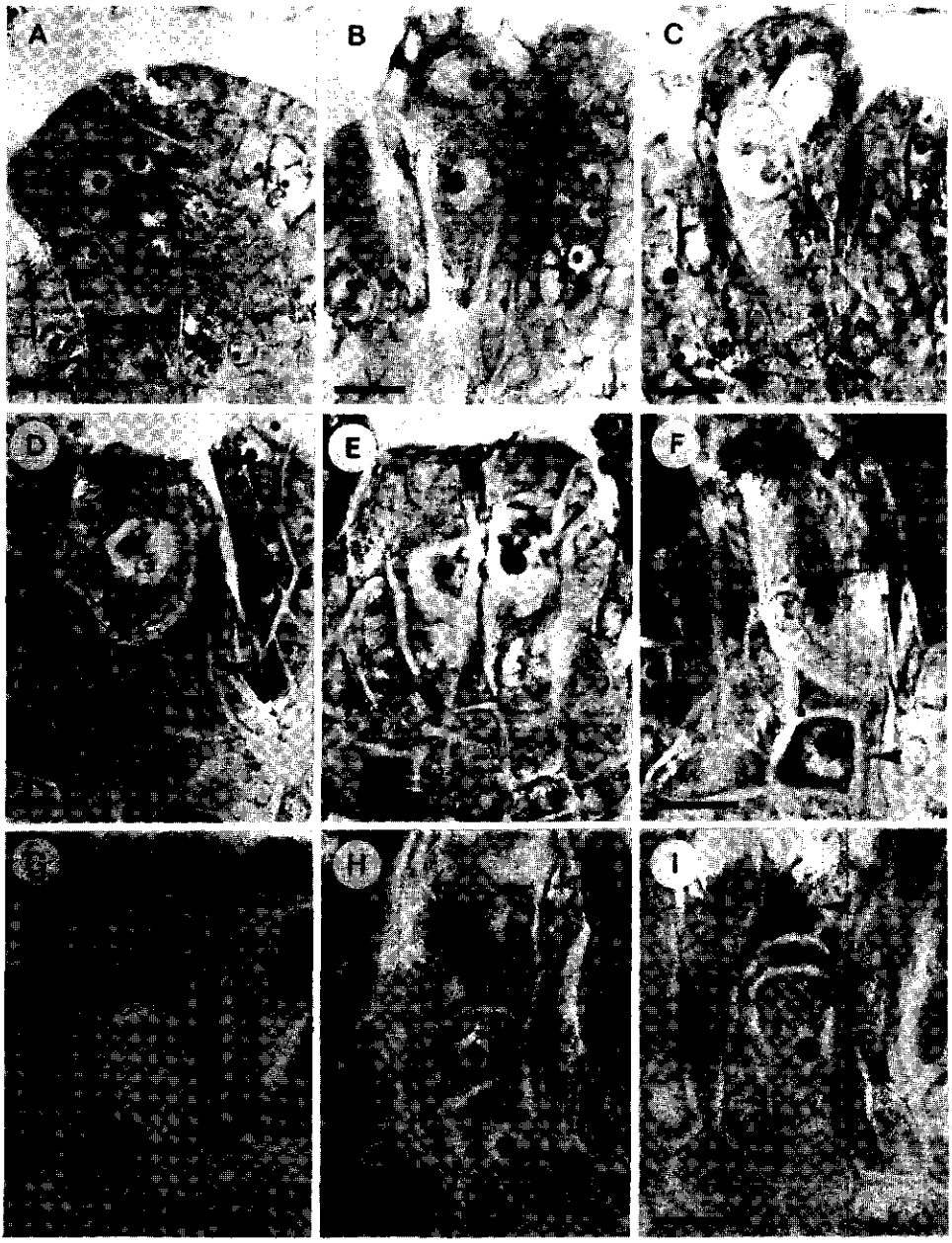
Figs. 1A-I. Megasporogenesis in diploid potato. Micropylar end at the top, bars represent 10 μm. (A) Differentiation of ovule initials. (B) Differentiation of ovules. (C) Archesporial cell. (D) Megaspore mother cell. (E) Leptotene-zygotene. (F) Zygotene-pachytene. (G) Pachytene. (H) Diffuse stage. (I) Diakinesis.



Figs. 2A-I. Megasporogenesis in diploid potato. Micropylar end at the top, bars represent 10 μm. (A) Metaphase I. (B) Anaphase I. (C) Telophase I, cell plate formation. (D) Prophase II (E-H) Metaphase II-anaphase II; varying spindle orientations. (I) Telophase II; cell plate formation.



Figs. 3A-I. Megagametogenesis in diploid potato. Micropylar end at the top, bars represent 10 μm. (A) Tetrad of young megaspores. (B) Tetrad; functional chalazal megaspore. (C) 1-nucleate embryosac. (D) 1st mitotic division. (E) 2-nucleate embryo sac. (F) 2nd mitotic division. (G) 4-nucleate embryo sac. (H) Mature 7-nucleate embryo sac. (I) Egg-apparatus. s = synergid, en = egg cell nucleus, sen = secondary embryo sac nucleus.



Figs. 4A-I. Development of a surplus of MMCs (A-F) and the formation of a triad at sporad stage (G-I) in diploid potato. Micropylar end at the top, bars represent 10 μm. (A) Differentiation of two adjacent archesporial cells. (B) Two adjacent MMCs; early leptotene. (C) Two adjacent MMCs; early pachytene. (D) Three adjacent MMCs; two degenerating. (E) Two non-adjacent MMCs; broad nucellar tissue. (F) Two non-adjacent MMCs; one MMC (early pachytene) differentiated within the chalazal tissue. (G-H) Early degeneration of the micropylar daughter cell. (I) Triad: functional chalazal megaspore.

instead of a tetrad of megaspores was formed (Fig. 4I). This deviation from what is considered to be the normal course of megaspore formation was frequently observed in all hybrids.

Embryo sac development in potato is of the normal or *Polygonum* type. The functional chalazal megaspore (Fig. 3B) shows an excessive growth and a pronounced vacuolization near both ends of the cell (Fig. 3C). After the first mitotic division (Fig. 3D) the resulting nuclei with most of the surrounding cytoplasm move to opposite ends of the embryo sac and are separated by a large central vacuole (Fig. 3E). Both nuclei divide simultaneously, which results in a 4-nucleate embryo sac (Figs. 3F-G), and after a third mitotic division an 8-nucleate embryo sac with four nuclei at both ends is formed.

Cell wall formation occurs only after the successive mitotic divisions have been completed. At the chalazal end three antipodal cells are formed, at the micropylar end two synergids and the egg cell can be distinguished. The remaining polar nuclei, one from each end, fuse to form the secondary embryo sac nucleus, the nucleolus of which usually is relatively large. In the mature 7-nucleate embryo sac (Fig. 3H) the latter is usually located near the egg cell and synergids. Together they form the egg-apparatus (Fig. 3I).

Though in most ovules only one archesporial cell differentiated, the differentiation and development of two or more adjacent archespores is not exceptional (Figs. 4A-C). This phenomenon was observed to a certain extent in all hybrids, in some of them even in 10–15% of all ovules.

The number of ovules with more than more MMC beyong pachytene is generally low due to early degeneration of the surplus of MMCs (Fig. 4D, arrows). In some clones, however, it was noticed sometimes that two adjacent MMCs developed up to the megaspore stage. Other deviations occuring in lower frequency include the development of a broad nucellus enclosing two MMCs that are themselves separated by nucellar tissue (Fig. 4E) and the additional development of an archesporial cell within the chalazal tissue of the ovule (Fig. 4F, arrow).

These observations probably explain both the fact that in some instances two adjacent or non-adjacent sexual embryo sacs in a single ovule were observed and the rare occurrence of twin-seedlings in the cross progeny of some clones.

DISCUSSION

The results of this study on the normal course of megasporogenesis and megagametogenesis in *Solanum phureja-* (*S. vernei-*) *S. tuberosum* hybrids are by and large in agreement with earlier reports concerning *S. tuberosum* (REES-LEONARD, 1935; LAMM, 1937; ARNASON, 1948) and *S. demissum* (WALKER, 1955). However, a few regularly occurring deviations from what is usually considered to be the normal course of megasporogenesis in potato were observed: the development of a surplus of MMCs, the formation of a triad instead of a tetrad of megaspores and the non-linear arrangement of megaspores within the ovule. As these deviations were common in all hybrids studied, it seems to be justified to consider them as random legitimate variations of normal megasporogenesis rather than systematic abnormal events. Though they do not have direct genet-

ic or reproductive consequences, some may have important implications for either the determination of the origin (aposporic or diplosporic) of unreduced megaspores and embryo sacs or the recognition and quantitative estimation of 'abnormalities' in megasporogenesis leading to 2n-egg formation.

From the development of a surplus of adjacent and non-adjacent archesporial cells into functional reduced megaspores and mature sexual embryo sacs as reported in the present study, it may be clear that the archesporium in potato cannot be delimited to a single cell or even to a group of adjacent cells in the ovule. The development of adjacent archesporial cells has also been reported in *S. tuberosum* (YOUNG, 1923; REES-LEONARD, 1935; ARNASON, 1948), *S. melongena* (BHADURI, 1932) and *S. lycopersicum* (LESLEY, 1926). However, in contrast to the observations reported in this study, REES-LEONARD (1935) tended to believe that in potato the surplus of MMCs invariably degenerated before a tetrad of megaspores was formed, and thus did not bring about additionally developed mature embryo sacs. The development of non-adjacent MMCs into mature sexual embryo sacs has also been observed by YOUNG (1922) and LAMM (1937). They sometimes noted the presence of two non-adjacent sexual embryo sacs in *S. tuberosum*.

An archesporial cell differentiated within the chalazal tissue has actually been found to develop into a tetrad of reduced megaspores in *S. melongena* (BHADURI, 1932) and a mature embryo sac in *S. phureja-S. tuberosum* hybrids (IWANAGA, 1980) respectively. However, the latter author, believing that the archesporium in potato is delimited to a group of adjacent cells, failed to recognize its sexual origin. He erroneously interpreted it as a case of apospory and, based on this interpretation, later (IWANAGA, 1982) emphasized the remarkable potency of chemical induction of parthenogenetic seed development from aposporic embryo sacs (i.e. aposporic apomixis) in potato.

Even when a non-adjacent and unreduced gametophyte develops additionally, it cannot simply be regarded as apospory. Detailed studies of early developmental stages are needed to elucidate whether its origin is diplosporic or aposporic.

Both apospory and diplospory involve the formation of female 2n-gametes. In apospory 2n-egg formation is a consequence of the absence of megasporogenesis, whereas in diplospory 2n-eggs result from 'abnormal' megasporogenesis. In megasporogenesis, like in microsporogenesis, various 'abnormal' events may lead to 2n-gamete formation. Depending on the genetic consequences, however, only two distinct types of 2n-gametes are distinguished: first-division-restitution (FDR) and second-division-restitution (SDR) gametes. Basically FDR gametes can be regarded to originate from an equational division of the entire (i.e. numerically unreduced) chromosome complement after completion of prophase I, which may vary in appearance from typically meiotic to almost completely mitotic. SDR gametes, in a strict sense, can be regarded to result from chromosome doubling in the haploid nuclei that result after completion of the first meiotic division (RAMANNA, 1983; HERMSEN, 1984a). In both cases a dyad instead of a tetrad of spores will arise.

FDR gametes are expected to preserve a relatively large amount of the favourable heterozygosity and epistasis present in the parental genotype and thus to strongly resemble each other and the parental clone from which they derive. SDR, in contrast,

is expected to yield a heterogeneous population of highly homozygous 2n-gametes (MENDIBURU et al., 1974; PELOQUIN, 1983b; HERMSEN, 1984a).

Chromosome pairing and/or crossing over (i.e. gene recombination) at pachytene may be influenced by the action of mutant synaptic genes. Synaptic mutants are characterized by an increased frequency of univalent chromosomes at metaphase I as a consequence of either the falling apart of normally paired, homologous chromosomes in prophase I due to their inability to generate or retain chiasmata (desynapsis) or a lack of chromosome pairing and thus crossing over in prophase I (asynapsis). Increased frequencies of univalent chromosomes in metaphases I due to a reduction in chromosome pairing and crossing over need not necessarily be under genetic control. It may also be a consequence of anorthoploidy or a lack of chromosome homology (i.e. structural differences between genomes) as is frequently observed in interspecific and intergeneric hybrids. In these cases SDR and reduced gametes, if produced, are expected to be predominantly sterile due to chromosome imbalance, whereas FDR gametes are expected to be mostly balanced and thus functional (RAMANNA, 1983; JONGEDIJK, 1983; Fig. 5b). If crossing over does not occur at all, such functional FDR gametes are identical and preserve the parental genotype intact like is the case with diplospory.

In potato, cell wall formation during microsporogenesis is of the simultaneous type and in normal synaptic plants besides SDR and reduced pollen, FDR pollen may be formed through either fusion (RAMANNA, 1979) or paralled orientation (MOK & PELOQUIN, 1975) of the second division spindles. In megasporogenesis however cell wall formation is of the successive type. Here fusion or parallel orientation of second division spindles cannot occur and 2n-megaspores if formed in a normal synaptic plant will therefore always be of SDR origin (Fig. 5a). In principle balanced FDR gametes can only be formed through a direct equational division of individual chromosomes (Fig. 5b). It can so be concluded that in megasporogenesis desynapsis, asynapsis, anorthophoidy or genome divergency actually is a prerequisite for the formation of FDR megaspores and consequently for any attempt to induce diplosporic apomixis. In diplosporic apomictic plant species chromosome pairing and crossing over usually lack to a great extent and apomeiosis (FDR-gamete formation!) in the MMC may range from almost meiotic (pseudo-homotypic and semi-heterotypic division) to almost mitotic (mitotized meiosis) (GUSTAFSSON, 1935, 1946).

For the induction of diplospory an attempt to combine FDR megaspore formation with asynapsis might be preferred (HERMSEN, 1980) but also desynapsis, anorthoploidy or genome divergency can be sufficient if crossing over is strongly reduced and predominantly restricted to the chromosome ends.

Whereas such a reduction in crossing over can easily be demonstrated in genetical research, reduction in chromosome pairing, especially if small, will be more difficult to detect. Because ovule development within an ovary is highly asynchronous, leptotene-zygotene stages, early and late pachytene stages and the diffuse stage could wrongly be regarded as pachytene stages with reduced chromosome pairing. Even in microsporogenesis, which is usually more synchronous, careful observations are needed to avoid misinterpretation (RAMANNA, 1983).

As stated before, in a plant with strongly reduced crossing over only FDR gametes are expected to survive. In a normal plant, on the other hand, a mixture of reduced

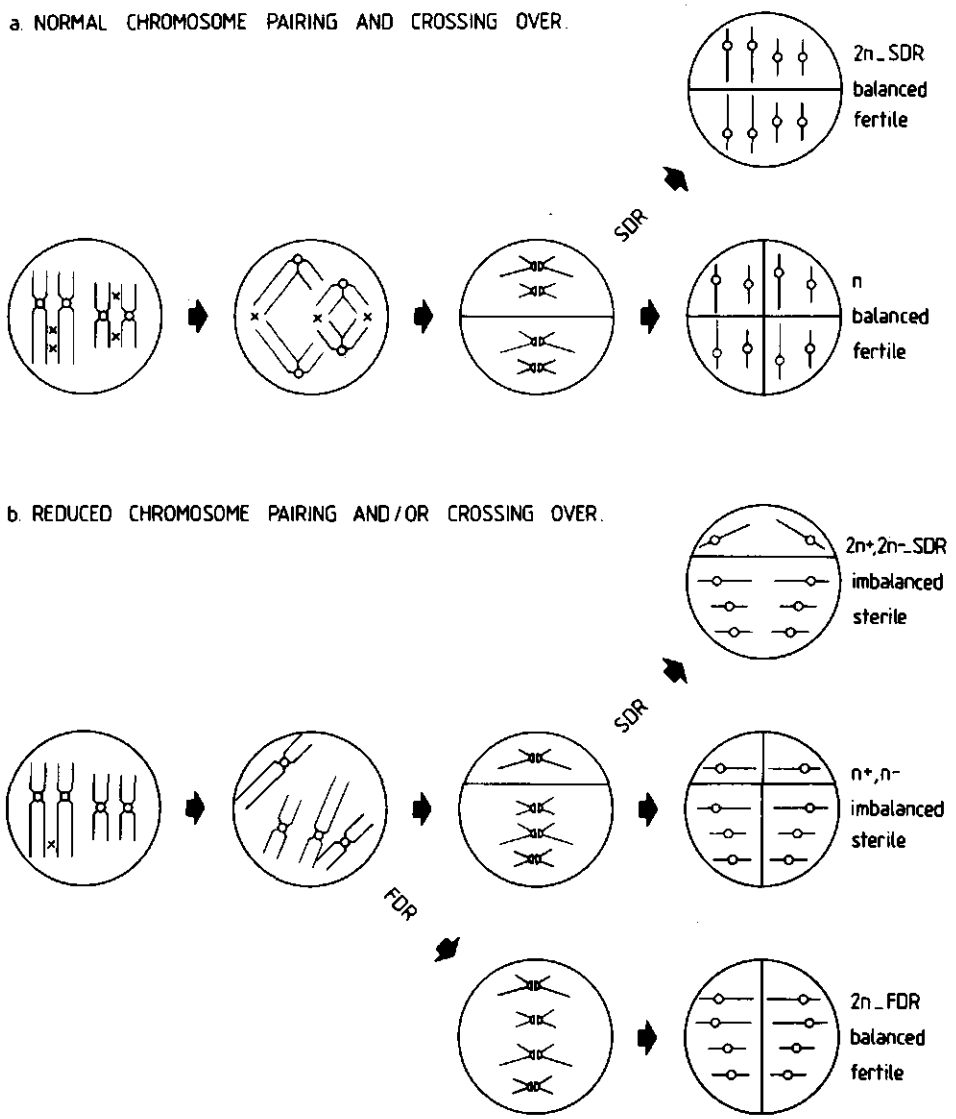


Fig. 5. Schematic representation of FDR and SDR 2n-gamete formation in megasporogenesis (two pairs of hom(e)ologous chromosomes; genetic implications of crossing over not included in the figure). (a) Formation of SDR and reduced megaspores in plants with normal chromosome pairing and crossing over. (b) Reduced chromosome pairing and/or crossing over as a prerequisite for FDR megaspore formation. Note that only FDR megaspores are expected to be balanced and functional.

and SDR megaspores may be expected. As it is generally believed that in potato the result of normal meiosis always is a tetrad of megaspores (REES-LEONARD, 1935; IWANAGA & PELOQUIN, 1979; HERMSEN, 1984a), it is obvious to estimate the frequency of SDR megaspore formation by the ratio unreduced megaspores (dyads): reduced megaspores (tetrads).

To avoid a serious under- or overestimation, triads should be taken into account as well. If they result from degeneration of the micropylar dyad cell before the onset or completion of the second meiotic division, they should be included in the group of reduced megaspores. Triads may also be formed when SDR occurs through omission of the second division or omission of cytokinesis after second division and fusion of the two nuclei (PFEIFFER & BINGHAM, 1983) in the chalazal dyad cell only. They then should be included in the group of unreduced megaspores. Detailed studies of triads and preceding stages of megasporogenesis are necessary to decide about their actual origin.

Finally, it should be mentioned that functional 2n-megaspores may also be formed through pre- or post-meiotic doubling. In normal synaptic plants premeiotic doubling can be followed by either random chromosome pairing or autobivalent formation. In both cases megasporogenesis is expected to give rise to a tetrad of 2n-megaspores. With random chromosome pairing normal tetrasomic inheritance will occur. If on the other hand auto-bivalents are formed, crossing over will not have genetic consequences and the resulting non-segregating population of 2n-megaspores may be regarded as of FDR origin.

This mechanism of 2n-megaspore formation followed by parthenogenetic seed formation has been found in some tetraploid species of the genus *Allium* (HAKANSSON & LEVAN, 1957; GOHIL & KAUL, 1981). Though it strictly does not meet the definition of apomixis it is generally indicated as a form of diplosporic apomixis (RUTISHAUSER, 1967).

In desynaptic or asynaptic plants premeiotic doubling is not expected to produce functional 2n-megaspores. However, if a strong reduction in crossing over is a consequence of structural differentiation of the genomes, it will probably be followed by autosyndytic bivalent formation. The production of functional 2n-megaspores, which can be regarded as of FDR origin, may then again be expected. From an evolutionary point of view it might be interesting to identically double a number of diplosporic species to elucidate whether the usually observed strong reduction in crossing over is a consequence of genetically determined asynapsis (or desynapsis) or genome divergency.

Pre-meiotic doubling of MMCs, if present in potato, can reliably be detected as early as diakinesis or metaphase I. Post meiotic doubling of reduced megaspores taking place during megagametogenesis, if at all present, may be more difficult to detect cytologically. As for the induction of apomixis, post-meiotic doubling is not an interesting mechanism of 2n-megaspore formation, because it will yield a heterogeneous population of highly or even complete homozygous 2n-megaspores. The same holds true for any mechanism of SDR megaspore formation.

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CHAPTER 2

A rapid methyl salicylate clearing technique for routine phase-contrast observations on female meiosis in *Solanum*

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KEYWORDS. *Solanum*, clearing technique, methyl salicylate, phase-contrast microscopy, megasporogenesis, megagametogenesis.

SUMMARY

A rapid clearing technique, involving methyl salicylate clearing and phase-contrast microscopy, that enables large-scale observations on meiosis and embryo sac development in intact ovules of potato, was developed. With high resolution phase-contrast optics the details observed in cleared ovules were as well defined as in microtome sections and 'abnormalities' associated with desynapsis and 2n-megaspore formation were readily detected.

In potato this ovule clearing technique offers certain advantages over earlier clearing and staining–clearing techniques and permits bulk preparation of ethanol (70%) stored ovaries shortly (1·5–2 h) before observation.

INTRODUCTION

Conventional embedding–sectioning techniques have proven to be powerful tools in embryological research. Though they generally yield high-contrast preparations with excellent clarity and nicely maintain the topography of the tissue, they have some major constraints. Firstly, the production of microscopic slides is laborious. Secondly, the (quantitative) interpretation of the investigated phenomena is tedious because three-dimensional structures are often distributed over several sections and thus require reconstruction of the full image.

Ovule clearing techniques have received renewed attention since a considerable improvement was achieved by Herr (1971). He developed a clearing-squash technique, involving a clearing fluid composed of lactic acid (85%), chloral hydrate, phenol, clove oil and xylene (2:2:2:2:1, w/w), suitable for phase-contrast observations in intact ovules of a wide range of plant species. In spite of the use of special optics, however, contrast and resolution have not been optimal for all species. In some, additional squashing of ovules (Herr, 1971; Smith, 1973; Rembert, 1977), changes in the clearing fluid formula and components (Herr, 1973a, b; George *et al.*, 1979; Franke, 1981), changes in the pretreatment of ovules (Herr, 1973a, 1985; Farence & Smith, 1975) or additional embedding–sectioning techniques (Lazarte & Palser, 1979) have been necessary to facilitate detailed observations, especially on megasporogenesis.

A second ovule clearing technique that could be used for observations on embryo sac development of Zephyranthaceae (Crane, 1978) and several grasses (Young *et al.*, 1979)

involves methyl salicylate as the clearing agent and differential interference-contrast microscopy (Crane, 1978). Using methyl salicylate, staining–clearing techniques suited for normal bright field observations of female meiosis were recently put forth for *Medicago* (Pfeiffer & Bingham, 1983) and *Solanum* (Stelly *et al.*, 1984). These latter staining–clearing techniques do not require special optics and permit the production of high-contrast specimens. They still have, however, some serious drawbacks: it takes several days before preparations from ethanol-stored ovaries are available for quantitative analysis of meiotic stages, and the analysis itself is seriously hampered by frequently occurring overstaining of ovules as a consequence of size differences between ovaries and ovules within ovaries (Jongedijk, unpublished).

In potato these drawbacks could be overcome by the adoption of a simple methyl salicylate clearing technique, which includes the use of high resolution phase-contrast optics for observation.

MATERIALS AND METHODS

To evaluate the present clearing technique, observations on megasporogenesis and megagametogenesis in stained microtome sections and cleared ovules of diploid *Solanum tuberosum–S. phureja* hybrids (Jongedijk, 1985) were compared. To determine its potential for the detection of 'abnormalities' in megasporogenesis genotypes with 2n-megaspore formation and desynaptic mutants (Jongedijk, 1983; Ramanna, 1983) were included.

Both for clearing and sectioning, ovaries with different stages of ovule development were fixed in CRAF V (Berlyn & Miksche, 1976) for 24 h and next rinsed and stored in 70% ethanol.

For clearing, the ovaries were dehydrated through two 30 min rinses in absolute ethanol and then directly transferred into pure methyl salicylate. After 30–45 min the material was completely cleared. With the help of a dissecting microscope the ovary wall was removed, ovules were scraped off the placenta and mounted in a drop of methyl salicylate. If necessary, preparations could be made semi-permanent and kept for at least 2 years by sealing the coverslip edges with Canada Balsam (dissolved in xylene).

For microtome sectioning, the ovaries were dehydrated in a tertiary butanol–ethanol series (Gerlach, 1977) and embedded in Paraplast Plus (BDH). Sections were stained with safranin–fast green and mounted in Canada Balsam according to Gerlach (1977).

Microtome sections and cleared ovules were examined and photographed with bright field Köhler illumination using a Planapochromatic 40/1·0 oil immersion objective (Zeiss) and a Planapochromatic 63 PH3H/1·4 oil immersion objective (Zeiss) respectively. All photographs were taken with a Zeiss Photomicroscope II, equipped with an achromatic–aplanatic phase-contrast and interference-contrast condensor (N.A. 1·4) on Kodak Technical Pan Film 2415 using a blue or green filter.

RESULTS AND DISCUSSION

Depending on its developmental stage (the larger the ovules, the more easily they could be scraped off the placental tissue) cleared ovaries yielded 150–300 ovules suitable for analysis. As the risk of overstaining of ovules does not exist with the present technique, megasporogenesis and megagametogenesis could easily be analysed (Figs. 1A–I and 2A–E) in the majority of cases.

The successive stages of megasporogenesis and megagametogenesis (Figs. 1A, B, D, F, G, H and 2A, B, D respectively) could easily be recognized and deviations from the normal pattern associated with desynapsis (Figs. 1C, E, I) or 2n-megaspore formation (Fig. 2C) were readily detected. It is noteworthy to point out that even the origin (sexual or somatic) of additionally developing cells within the chalazal tissue of an ovule could be established (Fig. 2E). This is of particular importance for investigations on gametophytic apomixis, as

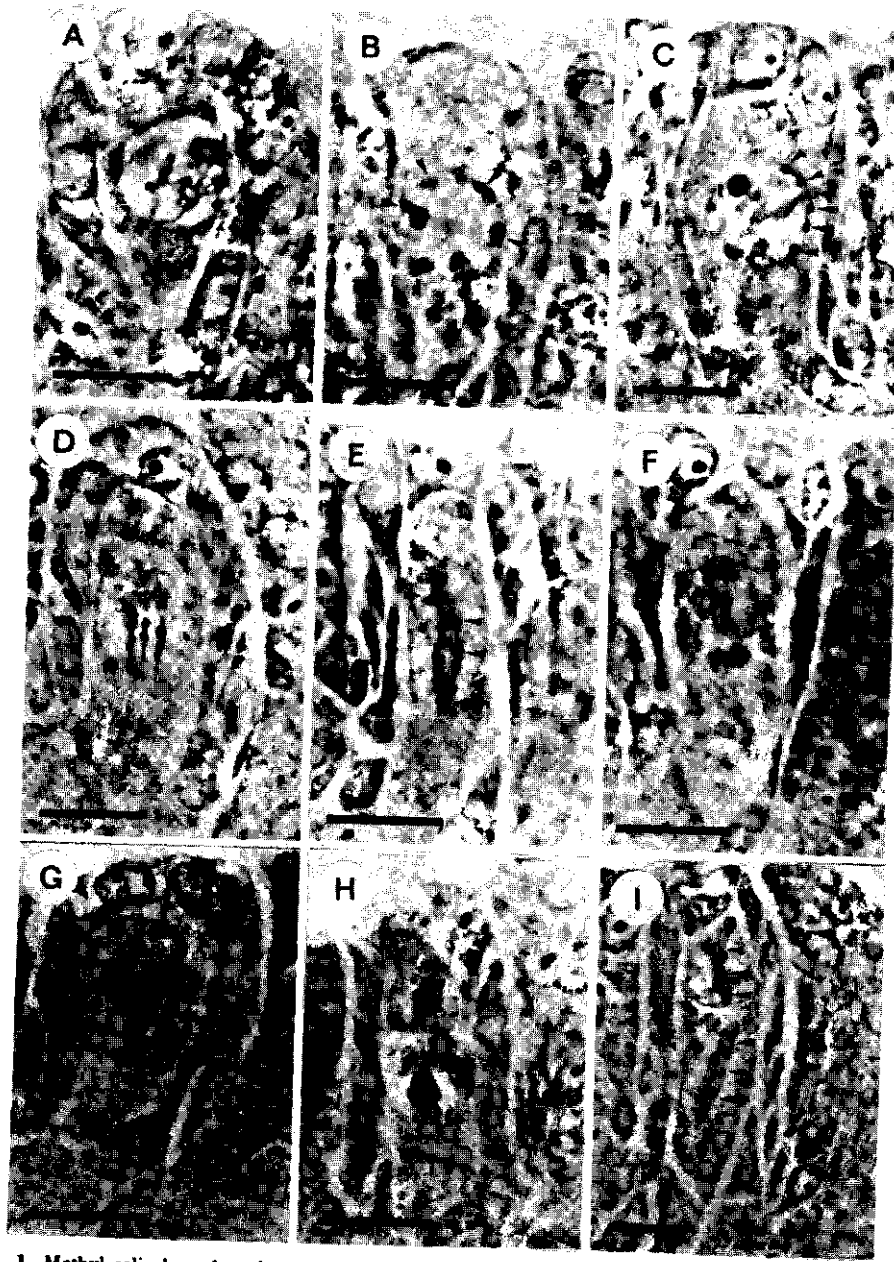

Fig. 1. Methyl salicylate cleared ovules showing different stages of megasporogenesis in diploid potato. Micropylar end at the top, bars represent 10 μm. (A) Pachytene. (B) Normal diakinesis; bivalents (arrows). (C) Desynaptic diakinesis; univalents (arrows). (D) Normal metaphase I; bivalents. (E) Desynaptic metaphase I; univalents (arrows) scattered. (F) Anaphase I. (G) Telophase I; cell plate formation. (H) Metaphase II. (I) Irregular metaphase II in a desynaptic plant.

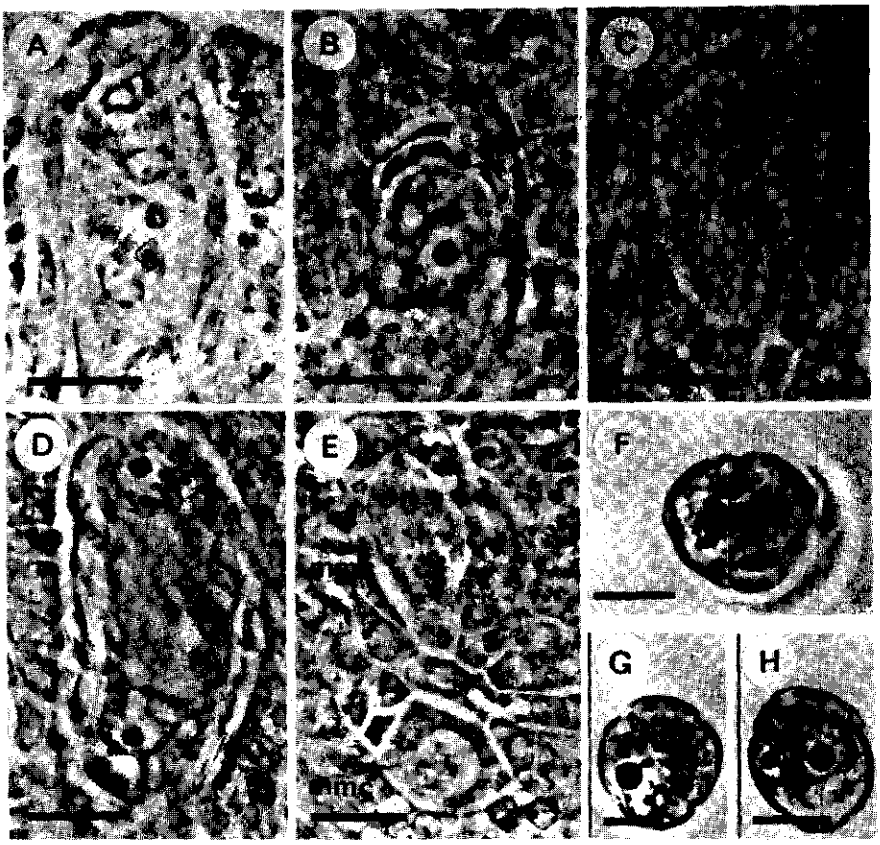

Fig. 2. Methyl salicylate cleared ovules (A–E) and pollen (F–H) showing different stages of megasporogenesis, embryo sac development and pollen development. Micropylar end at the top, bars represent 10 μm. (A) Telophase II; cell plate formation. (B) Tetrad of reduced (n) megaspores. (C) Dyad of unreduced (2n) megaspores. (D) 2-nucleate embryo sac. (E) Two non-adjacent megaspore mother cells (mmc); one mmc (sexual, pachytene) differentiated within the chalazal tissue. (F) First pollen mitosis; anaphase. (G) Uninucleate pollen; mitotic prophase. (H) Binucleate pollen.

it offers an opportunity to reliably trace the origin (sexual with diplospory and somatic with apospory) of additionally developing unreduced embryo sacs within the chalazal tissue.

Though shrinkage effects as a consequence of rapid dehydration and methyl salicylate infiltration as applied in this study may hamper cytological observations on megasporogenesis and megagametogenesis in many plant species, it did not do so in potato. In fact, even in embryo sac stages, which usually are most sensitive, shrinkage effects appeared to be minimal (Fig. 2D).

In Figs. 3A–F some stage of megasporogenesis and megagametogenesis as observed in microtome sections are shown. When these are compared to the phase-contrast images from cleared ovules it is obvious that the high contrast realized with the present technique competes well with that obtained in stained microtome sections. The same holds true for the maintainance of ovule topography. It proved to be the combination of excellent clearing properties of methyl salicylate, the maintenance of ovule topography and in particular the high contrast and resolution specifically obtained with the 63 PH3H/1·4 objective which

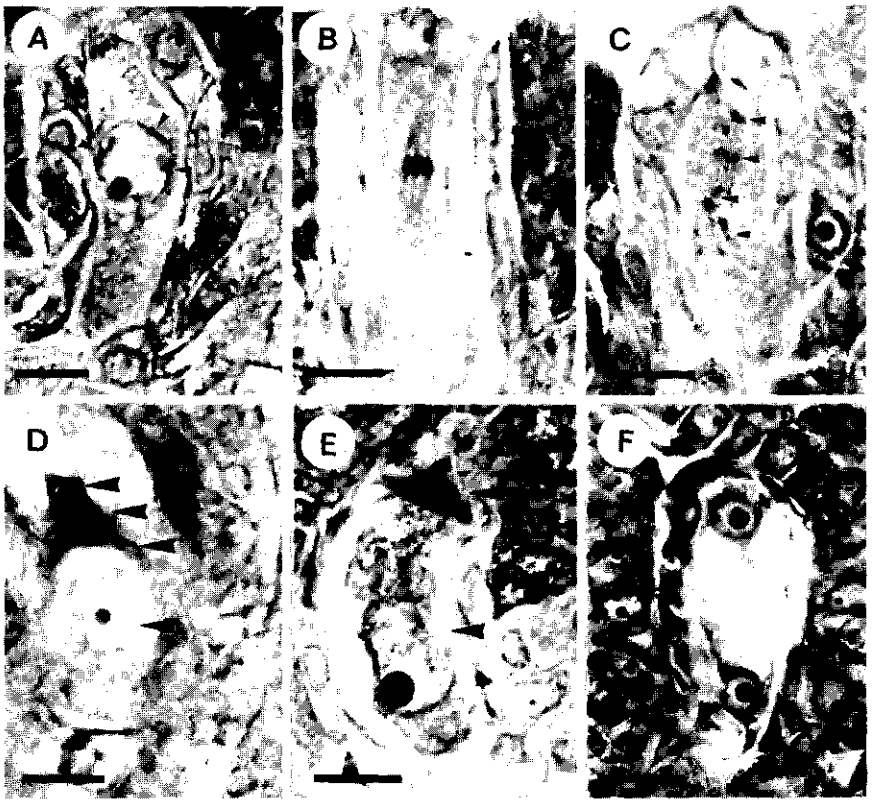

Fig. 3. Safranin–fast green stained sections of paraplast embedded ovules showing different stages of megasporogenesis and embryo sac development in diploid potato. Micropylar end at the top, bars represent 10 μm. (A) Diakinesis; bivalents (arrows). (B) Normal metaphase I; bivalents. (C) Desynaptic metaphase I; univalents (arrows) scattered. (D) Tetrad of reduced (n) megaspores. (E) Dyad of unreduced (2n) megaspores. (F) 2-nucleate embryo sac.

made the classification of megasporogenesis and megagametogenesis as easy in cleared potato ovules as in microtome sections. It should be mentioned that microsporogenesis and pollen development (Figs. 2F–H) could easily be analysed as well. The technique thus facilitates simultaneous study of micro- and megasporogenesis within a single flower bud.

The clearing effect of chemical agents may be caused by their high refractive index, their ability to dissolve the cell content or a combination of both (Gardner, 1975). The clearing effect of methyl salicylate is a consequence of its high refractive index (nD20=1·536–1·538), which apparently closely matches that of the cell walls (nD20 (cellulose)=1·55) in potato ovules. The relatively low refractive index of Herr's (1971) '4½-clearing fluid' might thus partly explain the poor results, which did not even approach those obtained by Herr (1971), when it was applied to potato ovules (Jongedijk, unpublished). Moreover, lactic acid, phenol and chloral hydrate are known to destroy the cytoplasm (Gardner, 1975) and thus negatively affect ovule topography and hamper cytological interpretation. Finally, the traces of water in the tissue (with Herr's technique the dehydration of ovules is far from complete) were found to reduce the clarity and contrast of preparations. With incomplete dehydration methyl salicylate cleared potato

ovules were seriously blurred as well. Since the good contrast and resolution obtained with the 63 PH3H/1·4 objective was by far superior to that obtained with other phase-contrast objectives and differential interference-contrast microscopy, it seems justified to emphasize that the quality of the optical equipment is an essential part of clearing techniques and therefore deserves to receive serious consideration.

In potato the clearing technique adopted in this study overcomes the drawbacks imposed by earlier developed clearing and staining-clearing methods. The particular combination of methyl salicylate clearing and appropriate phase-contrast optics permits routine observations on megasporogenesis and megagametogenesis and bulk preparation of specimens from ethanol (70%) stored potato ovaries shortly (1·5–2 h) before observation.

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CHAPTER 3

A QUICK ENZYME SQUASH TECHNIQUE FOR DETAILED STUDIES ON FEMALE MEIOSIS IN *SOLANUM*

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ABSTRACT. **A simple enzyme squash technique that enables detailed studies of meiosis in potato ovules has been developed. Fixation of ovules in iron-propionic-ethanol followed by enzymatic maceration and squashing in acetocarmine yielded numerous well preserved megasporocytes with nicely spread chromosomes. Resolution was sufficient, allowing detailed analysis of chromosome pairing and chiasma formation and readily permitting distinction between normal and desynaptic mutant plants. Whereas the use of previously developed ovule squash techniques has been restricted to cytogenetic analyses of plant species with relatively large megasporocytes and large chromosomes, the present technique is potentially more useful for analyses of species with small megasporocytes and small chromosomes.**

Information on meiotic chromosome pairing and chiasma formation in angiosperms is largely based on analyses of microsporocytes. The relative paucity of cytogenetic information on megasporocyte meiosis may be attributed to the following: (*i*) microsporocytes are produced in much larger quantities than megasporocytes and (*ii*) the technical difficulties of preparing and analyzing megasporocytes. Preparation and analysis of megasporocyte meiotic specimens by conventional embedding-sectioning techniques is laborious, and cytogenetic analyses are complicated because three-dimensional structures are often distributed over several sections, thus requiring reconstruction of the full image. Recently developed staining-clearing (Stelly *et al.* 1984) and clearing (Herr 1971, Jongedijk 1987) techniques facilitate preparation of intact ovules but fail to yield specimens amenable to detailed analyses of karyotype, chromosome pairing, or chiasma formation.

Most ovule squash techniques developed to date basically consist of hydrochloric acid maceration and staining with Feulgen (Hillary 1940), acetocarmine (Bradley 1948, D'Cruz and Reddy 1967), acetic-lacmoid (Haque 1954) or acetic-orcein stains (Darlington and La Cour 1966). Though they have successfully been applied to study early megagametophyte development in a variety of plant species, their use for detailed observations on chromosome pairing and chiasma formation in megasporogenesis has been restricted to a limited number of plant species with large megasporocytes and large chromosomes (Darlington and La Cour 1966, Sharma and Sharma 1972). Improved squash techniques applicable to species with small chromosomes would thus be desirable. A favorable cytological effect of enzymatic maceration was first noted by Emsweller and Stuart (1944), who used 1% clarase to improve the spreading of chromosomes in microsporocyte squashes of tetraploidized *Lilium longiflorum*. Enzymatic maceration has since been used to enable

detailed chromosome studies in microsporocytes (Narayan 1976, Stack 1982, Loidl 1984), root tips (McKay and Clarke 1946, Chayen and Miles 1954, Setterfield *et al.* 1954, Schwarzacher *et al.* 1980, Pijnacker and Ferwerda 1984) and megagametophytes (Forbes 1960) when appropriate spreading could not be achieved by "standard" techniques. Its use to enable cytogenetic analyses of megasporocyte meiosis in species with small chromosomes, however, has not been reported.

In this article a simple technique for preparing squashes of enzyme-macerated ovules that enables detailed cytogenetic analysis of megasporocyte meiosis in a genus with small chromosomes (*Solanum*) is reported.

Materials and Methods

To evaluate the merits of the present enzyme squash technique, megasporogenesis was studied in diploid (2n = 2x = 24) *Solanum tuberosum-S.phureja* hybrids (Jongedijk 1985). To determine its potential for the detection of "abnormalities" in chromosome pairing and chiasma formation both genotypes with normal chromosome synapsis (*Ds.*) and desynaptic mutants (*dsds*) (Jongedijk 1983, Ramanna 1983) were included.

Fixation. Flower buds were fixed in a freshly prepared solution of either propionic acid (saturated with ferric acetate) and ethanol (1:3, v/v) or glacial acetic acid and ethanol (1:3, v/v-Carnoy) for at least 48 hr (4–5 C) up to several months (−20 C). Removal of the calyx, corolla and anthers improved the penetration of the fixative.

Maceration. Under the dissecting microscope intact placentas were removed from the fixed buds and isolated in a drop of fixative. The placentas were rinsed twice for 20 min in a 0.1 M citric acid-sodium citrate buffer (pH 4.4–4.8) and macerated in a solution 10% with respect to pectinase (Sigma P-5146) and 1.5% with respect to cellulase (Onozuka R-10) in citrate buffer (pH 4.4–4.8) at 37 C for 4–5 min. The enzyme solution was subsequently removed from the tissue by two 20 min rinses with the citrate buffer.

Preparation of slides. With a Pasteur pipette ⅓–⅕ of a single placenta was transferred to a clean slide, excess buffer removed and a drop of 2% aceto-carmine added. The tissue was next carefully divided into small pieces, slightly warmed over a gas flame and left for 1–2 min. A coverslip was then added and the tissue was gently squashed until the cells were well separated and evenly distributed over the slide. After this the slides were heated without boiling over a gas flame, left for 1–2 min on a plate at 30–40 C and further squashed by pressing the coverslip under filter paper without slipping until satisfactory spreading of megasporocytes was achieved. When necessary, preparations were made permanent by keeping them overnight in a mixture of *n*-butyl alcohol and glacial acetic acid (3:1, v/v), followed by a 30–60 sec immersion of the detached coverslip and the slide in absolute *n*-butyl alcohol and remounting in Euparal.

Besides the pressure applied to the coverslip, the degree of spreading of megasporocytes depends on the amount of stain used and the amount of

material on the slide. Removing as much debris as possible while isolating placentas, avoiding excess stain and especially squashing only small pieces of a placenta at a time significantly improved spreading. The staining of chromosomes has been consistently better after iron-propionic-ethanol fixation.

All stages of megasporogenesis were examined and photographed with bright-field Köhler illumination using a Zeiss Planapochromatic 63 PH3H/1.4 oil immersion objective. Photographs were taken with a Zeiss Photomicroscope II equipped with an achromatic-aplanatic phase-contrast and interference-contrast condenser (N.A. 1.4) on Kodak Technical Pan Film 2415 using a blue or green filter.

Results and Discussion

Ovule squash techniques developed to date have not permitted detailed studies of chromosome pairing and chiasma formation in megasporocytes of species with small chromosomes. Small ovules are difficult to handle and megasporocytes generally fragment or severely distort upon squashing before cells and chromosomes are sufficiently spread. Although many of the megasporocytes were inevitably lost for analysis with the present enzyme squash technique as well, a considerable number of well preserved and sufficiently spread megasporocytes per slide were obtained. Up to 40% of all megasporocytes could be analyzed in the best preparations.

Meiotic cells, especially those with first division stages, were easily distinguished from somatic cells by their relatively large size and characteristic appearance (Fig. 1A). They frequently were found associated with respective groups of nucellar cells, which were held together by the undigested cuticle.

The different stages of megasporogenesis were readily recognized, and the extent of chromosome pairing and chiasma formation easily determined. Comparison of corresponding stages from a normal synaptic plant and a desynaptic mutant (Figs. 1B–D and 2A–C, respectively) demonstrated that in megasporogenesis, desynapsis is characterized by normal chromosome pairing through pachytene and a falling apart of bivalents by the time of diakinesis. A similar pattern of expression was previously noted in microsporogenesis (Ramanna 1983).

When compared to first division stages, second division stages are more transient and not as easily detected because of the smaller cell size. In addition, the interpretation of second division stages is seriously hampered as the two daughter cells formed after completion of the first meiotic division only rarely remain side by side. This presented a problem in deciding whether or not such cells are derived from the same megasporocyte. In plant species with tetrasporic embryo sac formation (*Lilium*, *Fritillaria* and others) such problems will not arise, however, as no cell wall is formed after completion of the first meiotic division.

As far as quantitative analysis of chromosome pairing and chiasma formation is concerned, it should be emphasized that in plant species with multiovular ovaries (such as potato) female meiosis generally is highly asynchronized. To

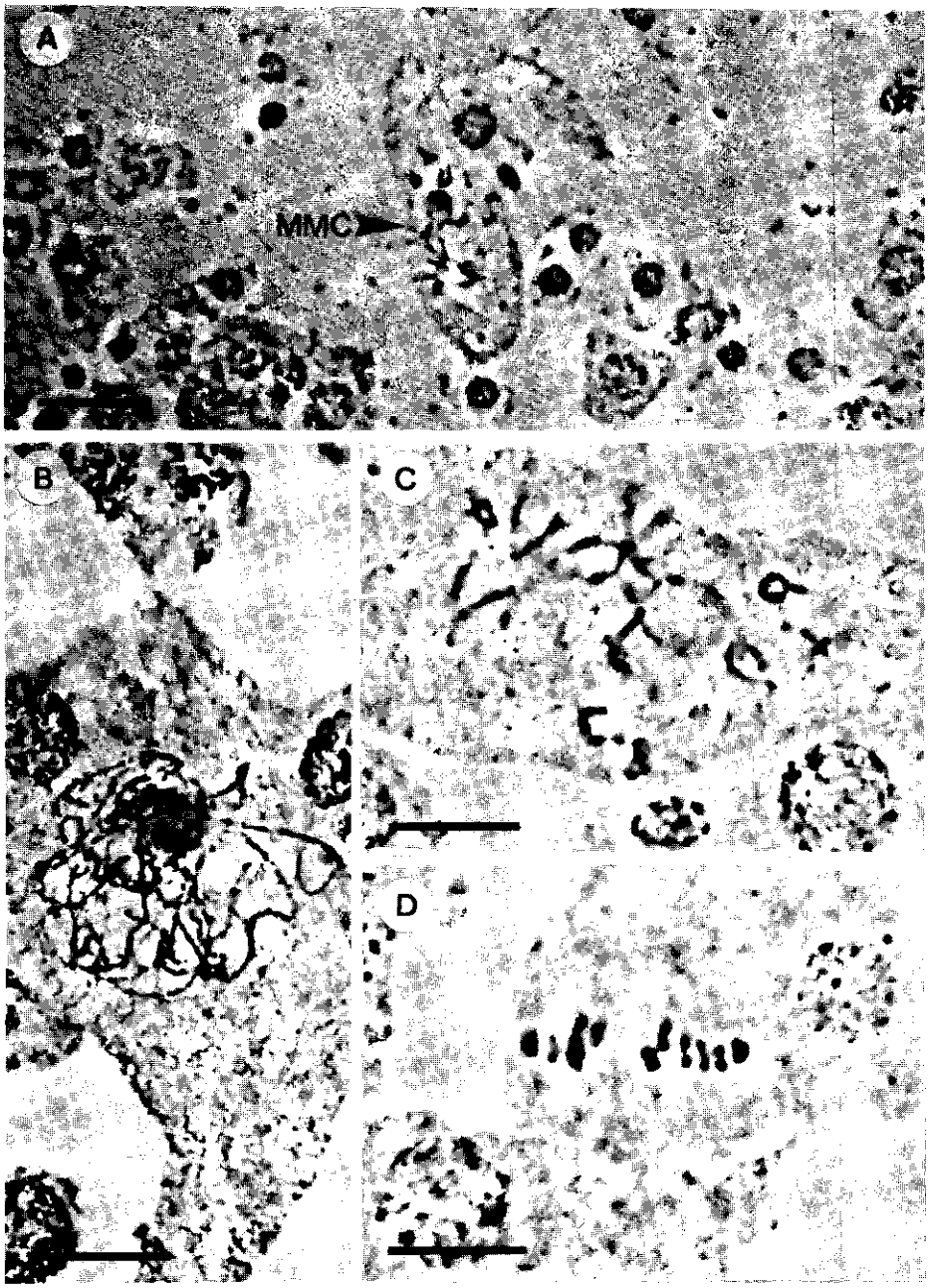

FIG. 1A–D. Acetocarmine squashes of enzyme digested ovules showing different stages of megasporogenesis in a diploid potato clone with normal synapsis. A) Typical megaspore mother cell (MMC) at diakinesis. B) Pachytene; normal chromosome pairing. C) Diakinesis; ring and rod bivalents. D) Metaphase I; ring and rod bivalents congregated at equatorial plate. Bars represent 10 μm.

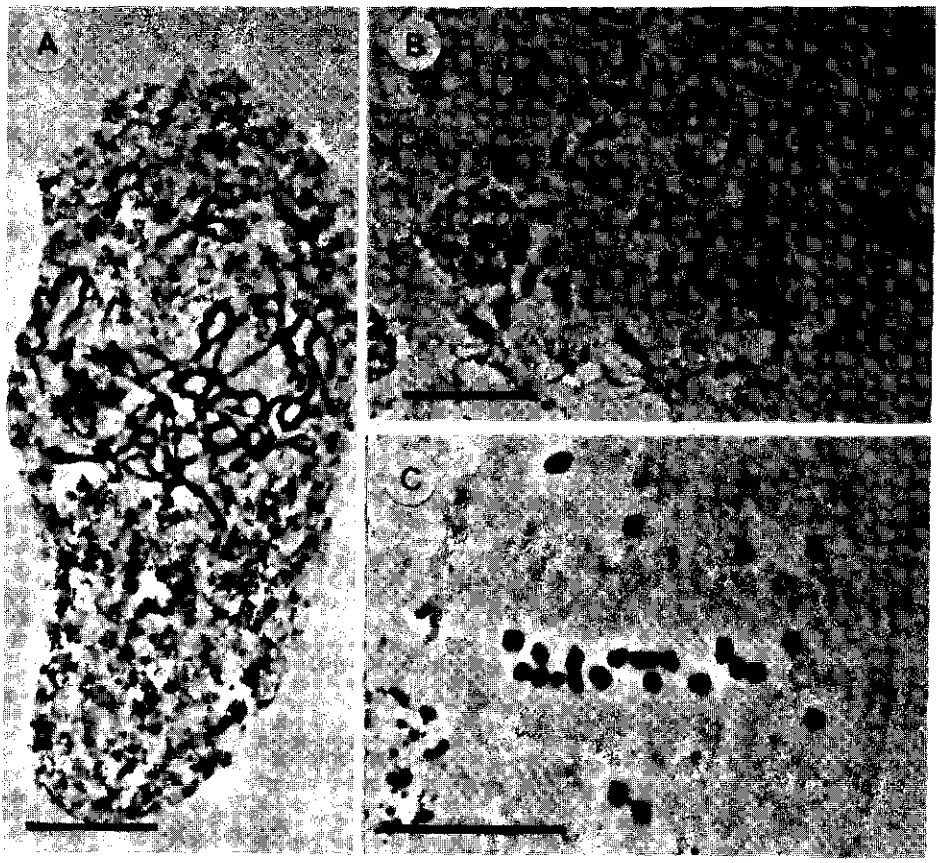

FIG. 2A–C. Acetocarmine squashes of enzyme digested ovules showing different stages of megasporogenesis in a desynaptic, diploid potato clone. A) Pachytene; normal chromosome pairing. B) Diakinesis; predominantly univalents. C) Metaphase I; predominantly univalents, note the predominant congregation of univalents at equatorial plate. Bars represent 10 μm.

obtain a sufficient number of meiotic cells at a particular stage it may therefore be necessary to prepare several placentas, especially if the meiotic stage to be analyzed tends to be transient.

The success of the present technique for potato probably results from the enzymatic maceration procedure. In most earlier ovule squash techniques hydrochloric acid was used for maceration. Hydrochloric acid, in dissolving the pectic salts of the middle lamella, ruptures the connection between cells, but cell walls, though softened, and the elasticity of the cytoplasm are largely maintained. With the pectinase-cellulase solution used for maceration in this study both middle lamellas and cell walls are digested and the elasticity of the cytoplasm is largely destroyed. Cell walls and cytoplasmic elasticity are known to thwart the flattening of cells and spreading of chromosomes upon squashing (Emsweller and Stuart 1944, Narayan 1976). While hydrochloric acid maceration methods may suffice for plant species with large chromosomes, the

enzyme squash technique reported here seems much more appropriate for species with small chromosomes, since flatter preparations are needed for equivalent cytogenetic observations.

Finally, it should be mentioned that the present technique is expected to be of limited usefulness in the analysis of megagametophyte development. Megagametophytic stages generally are so large and the cytoplasm is so highly vacuolated that even gentle squashing causes their collapse. In that case, however, the use of recent "protoplast" techniques for the isolation of intact mature megagametophytes (Zhou and Yang 1982, Zhou 1985, Hu *et al.* 1985) might be considered.

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CHAPTER 4

Synaptic mutants in potato, *Solanum tuberosum* L. I. Expression and identity of genes for desynapsis

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For tuber-bearing *Solanum* species, six monogenic recessive synaptic mutants, designated *sy-1*, *sy-2*, *sy-3*, *sy-4*, *ds*, and ds_c have been reported in the literature. In the present investigation no indication for the existence of the mutant *sy-1*, affecting megasporogenesis only, was found. The mutant *ds* was confirmed to display typical desynaptic behaviour in microsporogenesis and shown to similarly affect megasporogenesis. It furthermore proved to be allelic to the mutants *sy-3* and ds_c. It is proposed that the mutants *sy-3*, *ds*, and ds_c be uniformly designated *ds-1*, whereas the remaining mutants *sy-2* and *sy-4* (possibly identical) may be designated simply as synaptic mutant until their actual identity has been established. The observed F_1 segregations generally support monogenic recessive inheritance of *ds-1*. However, in one cross progeny the expected mutant phenotype was not clearly expressed in contrast with its reciprocal, which might indicate cross-specific influence of the cytoplasm on *ds-1* expression. The potential value and limitations of desynaptic (*ds-1ds-1*) mutants for potato breeding and true potato seed production are discussed.

Key words: *Solanum*, (de)synaptic mutants, microsporogenesis, megasporogenesis, 2*n* gametes.

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La littérature a fait état, en ce qui a trait aux espèces de *Solanum* qui sont productrices de tubercules, de six mutants synaptiques, monogéniques récessifs, désignés *sy-1*, *sy-2*, *sy-3*, *sy-4*, *ds* et ds_c. Dans la présente recherche, aucune indication portant sur l'existence du mutant *sy-1*, lequel n'affecte que la gynosporogenèse, n'a été décelée. Le mutant *ds* a été confirmé; il affecte typiquement le comportement désynaptique au cours de l'androsporogenèse et, de façon similaire, celui de la gynosporogenèse. De plus, il s'est avéré être allélique des mutants *sy-3* et ds_c. La proposition est dès lors avancée que les mutants *sy-3*, *ds* et ds_c devraient être uniformément désignés *ds1* et les autres mutants *sy-2* et *sy-4* (possiblement identiques) désignés mutant synaptique, jusqu'à ce que leur véritable identité soit établie. Les ségrégations observées chez les F_1 appuient généralement l'hérédité monogénique récessive du *ds-1*. Toutefois, chez un descendant de croisement, le phénotype mutant attendu ne s'est pas exprimé clairement, ce qui peut être l'indice d'une influence du cytoplasme spécifique au croisement sur l'expression du *ds-1*. La valeur potentielle et les limitations de mutants désynaptiques (*ds-1ds-1*) pour l'amélioration de la pomme de terre et la production de véritables graines de semence chez cette espèce sont discutées.

Mots clés : *Solanum*, mutants (dé)synaptiques, microsporogenèse, mégasporogenèse, gamètes 2*n*.

[Traduit par la revue]

Introduction

Meiotic mutants affecting homologous gene recombination have been reported in many higher plant species (Baker *et al.* 1976; Gottschalk and Kaul 1980*a*, 1980*b*; Koduru and Rao 1981). They are mostly monogenic recessive and generally give rise to increased univalent frequencies at metaphase I, either as a consequence of complete or partial failure of homologous chromosome pairing (asynapsis), or through the inability of normally synapsed homologues to generate or retain chiasmata (desynapsis). Distinction between asynaptic and desynaptic mutants, which in practice requires detailed analysis of early prophase I stages, cannot always be made unequivocally. Following Riley and Law (1965), both are therefore frequently referred to as "synaptic mutants."

Synaptic mutants, when studied in comparison to normal sibs, provide favourable material to gain a better insight into the (molecular) regulation of meiotic chromosome pairing and recombination. So far, however, this has not led to any clear result and their use has been restricted to the production of aneuploid series, which are frequently applied in gene localization research. Recently, breeding schemes employing synaptic mutants in combination with first division restitution (FDR) 2*n* gamete formation to efficiently transfer diploid germ plasm to tetraploids have been proposed for the cultivated potato, *Solanum tuberosum* L., in response to the need for both more efficient breeding strategies (Mendiburu *et al.* 1974; Peloquin 1981, 1982; Hermsen 1984*b*, 1984*c*) and true potato seed (TPS) technology (Peloquin 1983*a*; Hermsen *et al.* 1985).

Basically, FDR 2*n* gametes originate from an equational division of the entire (i.e., numerically unreduced) chromosome complement (Ramanna 1979, 1983; Jongedijk 1985). They are expected to preserve a relatively large amount of original heterozygosity and consequently favourable intra- and inter-locus interactions (epistasis). FDR 2*n* gametes are thus expected to genotypically resemble each other and the parental genotype from which they derive (Mendiburu *et al.* 1974; Peloquin 1983*b*; Hermsen 1984*a*) and to significantly contribute to both hybrid vigour and homogeneity of tetraploid progeny obtained via unilateral or bilateral ($4x-2x$ and $2x-2x$ crosses, respectively) sexual polyploidization.

Mutant synaptic genes are of particular importance, since they were shown to be a prerequisite for consistent FDR 2*n* megaspore formation (Jongedijk 1985) and cause the exclusive

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occurrence of functional FDR 2*n* gametes (Ramanna 1983; Jongedijk 1985). Owing to reduced gene recombination, they would also simultaneously maximize the intact transfer of heterozygosity (Peloquin 1982, 1983*b*; Iwanaga 1984; Hermsen *et al.* 1985). Maximum possible performance and uniformity could thus be attained in tetraploid progeny from 2xFDR − 2xFDR crosses when genetic recombination is completely lacking in both diploid parents. With respect to TPS technology, complete uniformity might also be achieved by aposporic or diplosporic apomixis (Hermsen 1980; Iwanaga 1983; Hermsen *et al.* 1985). Since induction of diplosporic apomixis seems to offer the best prospects (Jongedijk 1986), the association of mutant synaptic genes with FDR 2*n* megaspore formation is of crucial importance (Jongedijk 1985).

In potato, monogenic recessive synaptic mutants have been identified among dihaploids derived from the *S. tuberosum* varieties Chippewa (ds_c[2]: Matsubayashi 1979) and Atzimba (*sy-4:* Iwanaga 1984), in diploid *S. commersonii* (*sy-2:* Johnston *et al.* 1986), and in the progeny of diploid *S. tuberosum* − *S. phureja* hybrids (*sy-1:* Iwanaga and Peloquin 1979; *sy-3:* Okwuagwu and Peloquin 1981; and *ds:* Hermsen and Ramanna 1981; Ramanna 1983). The mutants *sy-2* and *sy-4* were reported to be expressed in both micro- and mega-sporogenesis, whereas *sy-1* would affect megasporogenesis only. The mutants *sy-3*, *ds*, and ds_c have been exclusively studied in microsporogenesis. The *ds* and ds_c mutants were clearly shown to be desynaptic (Matsubayahsi 1979; Ramanna 1983). The mutants *sy-1*, *sy-2*, *sy-3*, and *sy-4*, on the other hand, can best be described as synaptic, since no unequivocal classification into either desynaptic or asynaptic could yet be made.

In the framework of our attempts to experimentally induce diplosporic apomixis in potato, mutant synaptic genes represent one of the central research themes. In the present paper the expression of the *ds* mutant in both micro- and mega-sporogenesis, its allelism to *sy-3* and ds_c, and earlier misclassification of *sy-1* expression are reported. Additional data on the overall pattern of expression and inheritance of the *ds* mutant are given.

Materials and methods

Plant material

The inheritance and expression of the synaptic mutants were studied in nine diploid progenys (Table 1) that were obtained from intercrossing the diploid parental clones USW5292.7 (coded B), USW5337.3 (coded C), USW7589.2 (coded D), and 77.2102.37 (coded E). The USW clones were originally selected by Dr. S.J. Peloquin and associates (University of Wisconsin, Madison, WI) and are derived from *S. phureja* and dihaploid *S. tuberosum*. In addition, the clone 77.2102.37, selected by Dr. E. Jacobsen at the Max Planck Institute (Cologne, Federal Republic of Germany), has *S. vernei* in its ancestry.

The four parental clones were reported to be heterozygous at the *Ds/ds* locus that controls the occurrence of recessive desynapsis in microsporogenesis (Ramanna 1983). The clones USW5295.7 and USW5337.3 were reported to be heterozygous also at the *Sy-1/sy-1* and *Sy-3/sy-3* loci, which were expressed at megasporogenesis only (Iwanaga and Peloquin 1979) and microsporogenesis (Okwuagwu and Peloquin 1981), respectively.

To test for allelism of *ds* and ds_c, 'Chippewa' was crossed with the diploid desynaptic (*dsds*) clone CE10, which is highly male fertile because of the predominant production of FDR 2*n* pollen (Ramanna 1983). The resulting tetraploid progeny from this interploidy (4*x*.2*x*) cross was screened for the occurrence of desynaptic segregants.

All crosses were made on plants grafted onto tomato root stock. To exclude selfing, the flowers of the seed parents were emasculated. All plants were grown in a conditioned greenhouse during summer.

Cytological analyses

The ploidy level of the progeny was checked by either establishing the mean number of chloroplasts in the stomatal guard cells (Frandsen 1968) or, in case of doubt, by counting chromosome numbers in root tip meristems, and verified in meiotic preparations.

Microsporogenesis was studied in young anthers by a routine acetocarmine (2%) squash method, following fixation in a 1:3 (v/v) mixture of propionic acid (saturated with ferric acetate) and ethanol. For large scale screening and detailed studies of megasporogenesis in young ovaries, a routine methyl salicylate clearing technique (Jongedijk 1987*a*) and an enzyme squash technique (Jongedijk 1987*b*) were used, respectively.

All photographs were taken with a Zeiss Photomicroscope II, using a Zeiss Planapochromatic 63 PH3H/1.4 oil immersion objective on Kodak Technical Pan Film 2415.

Results

Screening for sy-1, sy-3, *and* ds

Among the four diploid parental clones, USW5295.7 and USW5337.3 were reported to be heterozygous at three apparently distinct loci affecting homologous gene recombination: *Sy-1sy-1*, *Sy-3sy-3*, and *Dsds*. Despite the fact that chiasma formation was claimed to be absent in *sy-3sy-3* mutants (Peloquin 1982) and clearly demonstrated in *dsds* mutants (Ramanna 1983), these mutant genes obviously are allelic, as both were reported to segregate 3:1 in microsporogenesis of USW5295.7 × USW5337.3 cross progeny (Okwuagwu and Peloquin 1981; Peloquin 1982; Ramanna 1983). Although Iwanaga and Peloquin (1979) claimed *sy-1* to affect megasporogenesis only and to represent a distinct locus, its actual existence may be questioned. Preliminary data on megasporogenesis and female fertility of *ds* mutants suggested its expression in megasporogenesis as well. The reported data on *sy-1* mutants would be incompatible with this. With the gene *ds* expressed in both micro- and mega-sporogenesis and assuming *dsds* to be epistatic over the contrast *Sy-1*. − *sy-1sy-1*, 3/16 of the plants would be expected to show the mutant condition in megasporogenesis only (i.e., *Ds.sy-1sy-1*) and 4/16 in both micro- and mega-sporogenesis (i.e., 3/16 *dsdsSy-1*. + 1/16 *dsdssy-1sy-1*). The chance of exclusively obtaining the former type of desynapsis among four mutant individuals, as reported by Iwanaga and Peloquin (1979), would then amount to only $(3/7)^4 \approx 3\%$.

To establish whether the mutant *ds* is indeed expressed in megasporogenesis as well and whether the mutant *sy-1* actually exists, microsporogenesis was studied in 1017 F_1 plants from nine different crosses. In 455 of these, megasporogenesis was examined in detail.

Although the extent of chromosome association at metaphase I varied in different plants, it could be used as a criterion to unambiguously classify mutants in the majority of cases (Figs. 1C, 1F, 2C, and 2F). Whenever such a classification was doubtful because of variation between cells from different anthers or ovaries, several fixations were studied and, following Ramanna (1983), additional criteria such as lack of metaphase I orientation, unbalanced anaphase I separation, and relatively high degree of sterility were used for classification. The results are presented in Table 1. They fit the obvious hypothesis of allelism of *ds* and *sy-3* (Table 1, crosses 1 and 2)

[2]These mutants originally were not assigned a gene symbol, but are here referred to as ds_c.

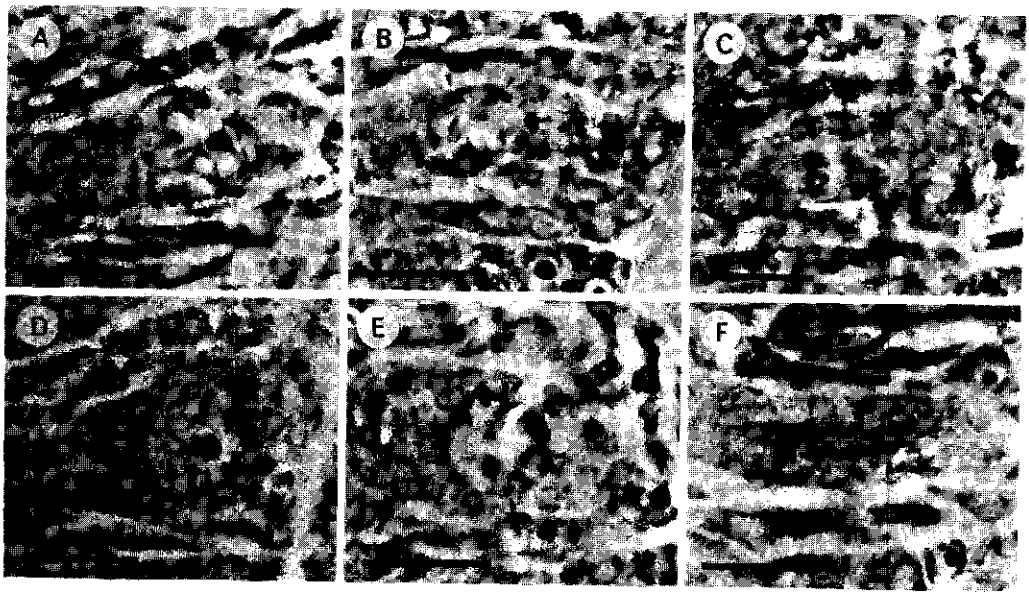

FIG. 1. Methylsalicylate cleared ovules from normal synaptic (A–C) and desynaptic (D–F) diploid potato clones. (A) Pachytene. (B) Normal diakinesis; arrowheads point to bivalents. (C) Normal metaphase I showing bivalents. (D) Pachytene. (E) Desynaptic diakinesis; arrowheads point to univalents. (F) Desynaptic metaphase I; arrowheads point to univalents scattered over the spindle. Micropylar end at the right, bars represent 10 μm.

TABLE 1. Number of plants with normal and desynaptic microsporogenesis in the diploid progeny of nine crosses

Cross	Code	F_1 segregation			χ^2 (3:1)	χ^2 (contingency)
		Normal	Desynaptic	Total		
1. USW5295.7 × USW5337.3	BC	79 (36)	19 (13)	98 (49)	1.646	0.473
2. USW5337.3 × USW5295.7	CB	65 (26)	20 (16)	85 (42)	0.098	
3. USW5295.7 × 77.2102.37	BE	76 (39)	25 (23)	101 (62)	0.003	1.375
4. 77.2102.37 × USW5295.7	EB	29 (27)	15 (15)	44 (42)	1.939	
5. USW7589.2 × 77.2102.37	DE	61 (27)	24 (23)	85 (50)	0.475	0.151
6. 77.2102.37 × USW7589.2	ED	58 (36)	20 (16)	78 (52)	0.017	
7. USW7589.2 × USW5295.7	DB	36 (26)	18 (12)	54 (38)	2.000	—
8. USW5337.3 × 77.2102.37	CE	234 (55)	92 (24)	326 (79)	1.804	51.298*
9. 77.2102.37 × USW5337.3	EC	146 (41)	0 (0)	146 (41)	48.667*	
Subtotal (crosses 1–8)		638 (272)	233 (142)	871 (414)	1.414	6.269
Total (crosses 1–9)		784 (313)	233 (142)	1071 (455)	2.357	57.466*

NOTE: The data on microsporogenesis from Ramanna (1983) are included in the F_1 segregation. The numbers in parentheses refer to the number of plants also classified in megasporogenesis.
*Significant at the 1% level.

and unambiguously demonstrate that *ds* (= *sy-3*) equally affects both micro- and mega-sporogenesis (Table 1, crosses 1–9). In addition, there is no indication of the existence of a gene *sy-1*, with expression on the female side only (Table 1, crosses 1 and 2), which was claimed to be present in the material used (Iwanaga and Peloquin 1979).

Characterization of desynaptic (dsds) *mutants*

The mutant *dsds* condition could not be detected in early prophase I stages, as micro- and mega-sporogenesis proceeded apparently normally through pachytene (Figs. 1A, 1D, 2A, and 2D). Only as early as diakinesis, the appearance of varying univalent frequencies allowed its detection (Figs. 1B, 1E, 2B, and 2E). In both normal and mutant plants the paired chromosomes were extended strongly between pachytene and diplotene, giving rise to a diffuse diplotene stage. Like early and late pachytene stages, this diffuse stage might easily be mistaken for pachytene with, reduced chromosome pairing (i.e., asynapsis) and vice versa. In megasporogenesis this holds true even for late leptotene–zygotene stages, as megasporocyte development in ovules from a single ovary is highly asynchronous. Critical examination and comparison of early

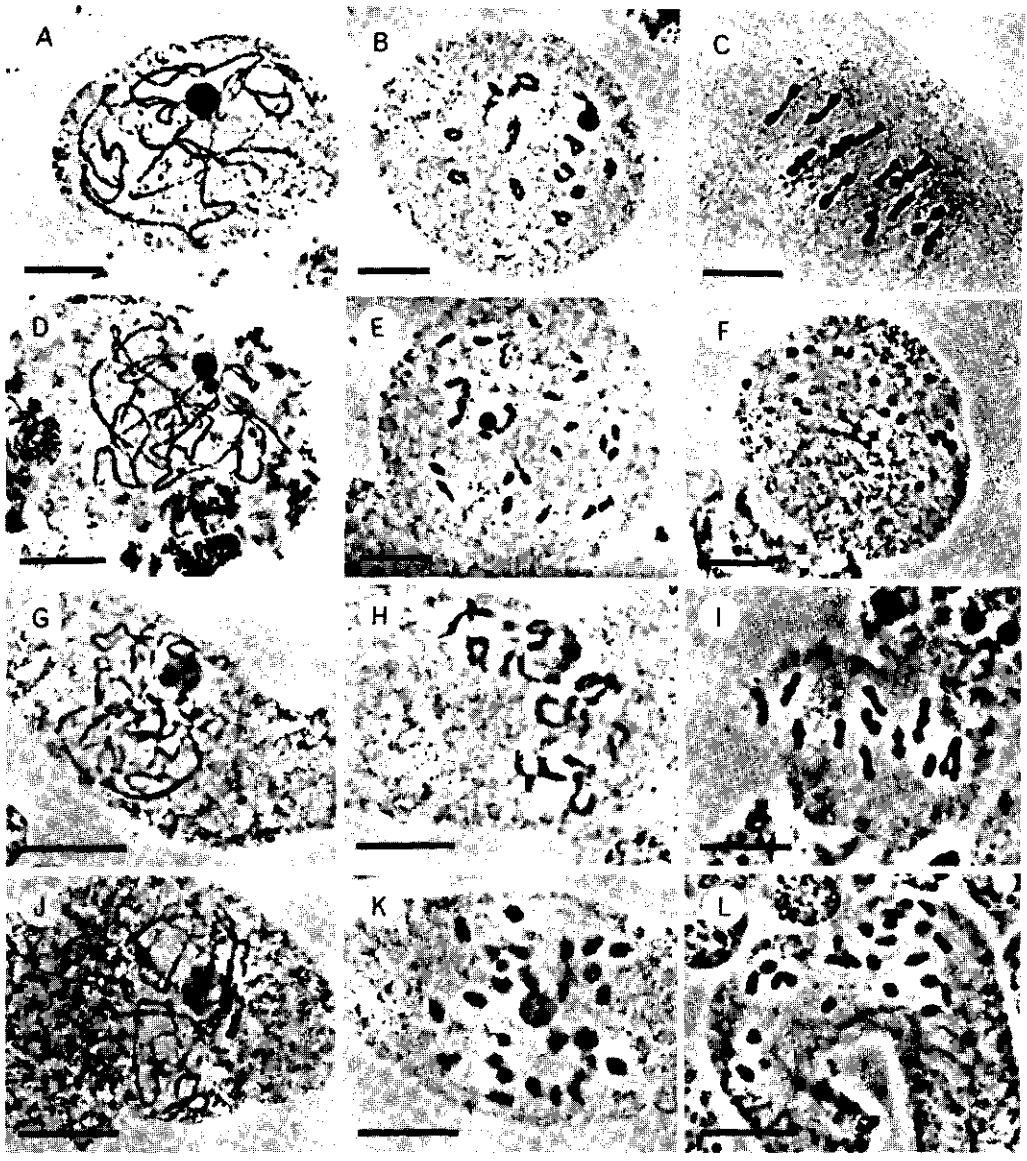

FIG. 2. Acetocarmine squashes of pollen mother cells (A–F) and megaspore mother cells (G–L) from normal synaptic (A–C and G–I) and desynaptic (D–F and J–L) diploid clones. (A, D, G, J) Pachytene; normal chromosome pairing. (B, H) Normal diakinesis; bivalents. (E, K) Desynaptic diakinesis; predominantly univalents. (C, I) Normal metaphase I; bivalents. (F, L) Desynaptic metaphase I; predominantly scattered univalents. Bars represent 10 μm.

prophase I stages in normal and mutant plants indicated that *ds* is characterized by normal chromosome pairing through pachytene and a falling apart of most bivalents at diakinesis in both microsporogenesis (Ramanna 1983; Figs. 2A–2F) and megasporogenesis (Jongedijk 1987*b*; Figs. 1A–1F and 2G–2L), and thus should be regarded as a clear case of desynapsis.

Reciprocal difference for ds *expression*

As all parental clones were reported to be heterozygous *Dsds* (Ramanna 1983), a 3:1 normal to mutant ratio was to be

TABLE 2. Number of plants with normal and desynaptic microsporogenesis in the diploid progeny from different seed lots of the crosses CE and EC

Cross	Code	Seed lot	F_1 segregation			χ^2 (3:1)	χ^2 (contingency)
			Normal	Desynaptic	Total		
USW5337.3 × 77.2102.37	CE	1981	53 (45)	19 (17)	72 (62)	0.074	0.149
		1985	181 (10)	73 (7)	254 (17)	1.895	
77.2102.37 × USW5337.3	EC	1981	64 (11)	0 —	64 (11)	21.333*	0.000
		1983	82 (30)	0 —	82 (30)	27.333*	

NOTE: The data on microsporogenesis from Ramanna (1983) are included in the F_1 segregation. The numbers in parentheses refer to the number of plants also classified in megasporogenesis.
*Significant at the 1% level.

TABLE 3. Number of plants with normal and desynaptic microsporogenesis in the dihaploid and tetraploid progeny of the crosses 'Chippewa' × *S. phureja* and 'Chippewa' × CE10, respectively

Cross	Progeny				χ^2 (5:1)	χ^2 (3:1)	χ^2 (contingency)
	Ploidy	Normal	Desynaptic	Total			
'Chippewa' × *S. phureja*[a] (*DsDsdsds*)	2*x*	28	10	38	2.553	0.035	
'Chippewa' × CE10 (*DsDsdsds*) (*dsds*)	4*x*	27 (13)	13 (8)	40 (21)	7.210*	1.200	
Total		55 (13)	23 (8)	78 (21)	9.231*	0.838	0.361

NOTE: The data on microsporogenesis from Matsubayashi (1979) were used for the cross 'Chippewa' × *S. phureja*. The numbers in parentheses refer to the number of plants also classified in megasporogenesis.
*Significant at the 1% level.

expected in all nine crosses. In eight progeny, the observed segregations fit this ratio (Table 1, crosses 1–8). In the 77.2102.37 × USW5337.3 progeny (EC), a weak expression of *ds* was sometimes suspected. However, no plain mutants could be identified (Table 1, cross 9). The resulting reciprocal difference between the CE and EC progeny (Table 1) could not be ascribed to accidental interchange of seed lots (Table 2) or contamination, and thus appears to be real.

Allelism of ds *and* ds_c

Studying microsporogenesis in 'Chippewa' derived dihaploids, Matsubayashi (1979) concluded this variety to be duplex for monogenic recessive desynapsis. The tetraploid progeny derived from the testcross Chippewa × CE10 (*dsds*, FDR 2*n* pollen) proved to segregate into normal and mutant plants, indicating allelism of *ds* and ds_c (Table 3).

As expected when *ds* and ds_c are allelic the mutant condition was again found to affect both micro- and mega-sporogenesis (Table 3). The observed segregation in this study fit that reported by Matsubayashi (1979) and the overall data are compatible with his suggestion that the common potato might show preferential pairing between the chromosomes involved in the *Ds/ds* segregation (Table 3, χ^2 (random pairing) = 9.231, χ^2 (preferential pairing) = 0.838).

Discussion

Extensive observation on male and female meiosis (Table 1) indicates that the *ds* gene is expressed in both micro- and mega-sporogenesis. This appears to be true for most synaptic mutants in higher plants in which desynapsis has been investigated so far (Baker *et al.* 1976; Koduru and Rao 1981).

Mutant synaptic genes, though potentially valuable, may have certain limitations for their use in breeding. When expressed in both micro- and mega-sporogenesis, they have to be manipulated in heterozygous condition, since mutants will either be largely sterile or reproduce functional FDR 2*n* gametes only, resulting into polyploidization upon crossing. If, on the other hand, expression is limited to either micro- or mega-sporogenesis only, mutants may successfully be crossed as female or male parent, respectively. Therefore, the expression of *ds* in micro- and mega-sporogenesis as well as the fact that there is no indication of a gene *sy-1* affecting megasporogenesis only, are rather unfortunate.

The mutant gene *ds* was shown to be allelic to *sy-3* and ds_c. Okwuagwu and Peloquin (1981) and Peloquin (1982) reported a complete lack of chiasma formation and consequently of gene recombination in *sy-3* mutants, and on this basis emphasized the exceptional opportunity of transmitting all parental heterozygosity intact to polyploid offspring, if *sy-3* would be combined with FDR 2*n* gamete formation. However attractive, this assumption does not seem justified, since the present observations as well as those independently reported by Matsubayashi (1979) and Ramanna (1983) unambiguously demonstrated the occurrence of at least some chiasmata in both sexes. Although chiasma frequency appears to be reduced, this need not necessarily be the case. In the mutant at issue, desynapsis might also result from the inability of normally paired homologues to retain chiasmata as a consequence of changes in chromatid adhesion. For this reason and because of preferential survival of progeny resulting from meiocytes with reasonably balanced segregation, even increased recombination frequencies for marked loci have been reported in other plant species (see reviews by Baker *et al.* 1976; Koduru and Rao 1981). Therefore, definite conclusions on the actual extent of gene recombination in the *ds* mutant and thus its potential for both sexual polyploidization and experimental induction of diplo-

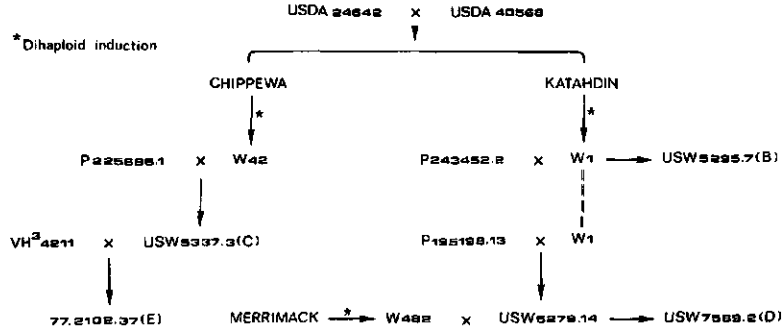

FIG. 3. Pedigree of the diploid potato clones USW5295.7 (B), USW5337.3 (C), USW7589.2 (D), and 77.2102.37 (E). P, *S. phureja*; V, *S. vernei*, and H, *S. tuberosum* dihaploid.

sporic apomixis has to await further (cyto)genetic analysis.

The established allelism of *ds* and ds_c was not totally unexpected. As can be seen from Fig. 3, the parental clones USW 5337.3 and 77.2102.37 are closely related and derive from var. Chippewa. 'Katahdin', a full sib of 'Chippewa', is a common ancestor of the half sib parental clones USW5295.7 and USW7589.2. It thus seems plausible that the *ds* gene originally derives from these two varieties. If this holds true, 'Katahdin' is expected to be triplex (*DsDsDsds*), since in 23 tetraploid progeny from the cross 'Katahdin' × CE10, no mutants were detected (unpublished results).

Because 'Chippewa' and especially 'Katahdin' have been extensively used in potato breeding programs, the *ds* gene might be present in a significant number of other potato varieties as well. It therefore would not be surprising if also the synaptic mutant *sy-4*, which was identified among 'Atzimba' dihaploids and, when compared to *ds*, similarly expressed in micro- and mega-sporogenesis (Iwanaga 1984), would prove to be allelic upon tests yet to be performed. With respect to the synaptic mutant *sy-2*, identified in diploid *S. commersonii* (Johnston *et al.* 1986), allelism with *ds* is not expected because preliminary cytological data of Johnston *et al.* (1986) on pachytene and diakinesis of *sy-2* mutants seemed to indicate a different pattern of expression. Again, tests for allelism will nevertheless be needed.

In view of the present data (Table 1), it is puzzling how the mutant *sy-1* could ever have been reported. It might be explained by the assumption that Iwanaga and Peloquin (1979) studied only megasporogenesis and concluded for normal microsporogenesis on the basis of either earlier data from identical progeny (Mok and Peloquin 1975*b*) or misleading pollen fertility data (i.e., apparently normal male fertility of mutants, but due to FDR 2*n* pollen formation). Having doubts about nonexpression of *sy-1* in microsporogenesis after *ds* and *sy-3* had been discovered in identical material, Stelly and Peloquin (1986) indicated that nonexpression of *sy-1* was indeed based on pollen fertility data, thus suggesting similarity of *sy-1* and *sy-3* mutants. However, in the original report (Iwanaga and Peloquin 1979) microsporogenesis was claimed to be investigated and found not to be affected. Regardless of the reasons for this controversy, it is obvious that a tentative classification of synaptic mutants affecting microsporogenesis by pollen screening, as suggested by Peloquin (1982), should be handled with caution. In addition, the reliability of the cytological conclusions on the meiotic mutant *ps* (controlling FDR 2*n* pollen formation) and its inheritance (Mok and Peloquin 1975*a*, 1975*b*) can be questioned, at least as far as the involved progeny from USW5295.7 × USW5337.3 are concerned. The reason is that in this cross no synaptic mutants were detected by that time (Mok and Peloquin 1975*a*, 1975*b*), although all microsporogenesis stages from metaphase I onwards were reported to have been intensively studied.

The monogenic recessive inheritance of desynapsis as earlier and independently reported by Matsubayashi (1979), Okwuagwu and Peloquin (1981), and Ramanna (1983) was supported by F_1 segregation ratios obtained in all crosses but 77.2102.37 × USW5337.3 (Table 1). At present, the nondetection of desynaptic segregants in this progeny cannot be explained. As stated before, it could not be ascribed to accidental interchange of seed lots or contamination. Because of the normal 3:1 segregation ratios observed in the reciprocal cross combination and other progeny, it could not be explained either by abortion of *ds* carrying gametes caused by supposed (virtually absolute) linkage of the *ds* gene to *S* alleles (gametophytic self-incompatibility system), of which one must be commonly present in both parental clones, or by gamete eliminator genes (Rick 1966; 1971) causing lethality of microspores and (or) megaspores in the parental clones USW5337.3 and 77.2102.37. Although it might be tempting to assume apparently cross-specific, cytoplasmic influence on *ds* expression, further investigations are needed to elucidate the actual cause of this unexpected phenomenon.

From this study it may be concluded that of the six synaptic potato mutants reported, one (*sy-1*) has apparently been misclassified earlier, whereas the remaining five represent at most three different genes or loci, each affecting both micro- and mega-sporogenesis. Since they were shown to be the allelic and to display typical desynaptic behaviour, we propose to further designate the mutant genes *ds*, *sy-3*, and ds_c by the symbol *ds-1*. At present, the mutants *sy-2* and *sy-4* may both be designated simply as synaptic mutant because different numbers suggest different identities, but in these cases no tests for allelism with any other mutant gene have actually been made.

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CHAPTER 5

Synaptic mutants in potato, *Solanum tuberosum* L. II. Concurrent reduction of chiasma frequencies in male and female meiosis of *ds-1* (desynapsis) mutants

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JONGEDIJK, E., and RAMANNA, M. S. 1989. Synaptic mutants in potato, *Solanum tuberosum* L. II. Concurrent reduction of chiasma frequencies in male and female meiosis of *ds-1* (desynapsis) mutants. Genome, **32**: 1054–1062.

Chiasma frequencies in pollen mother cells and megaspore mother cells from both normal and desynaptic (*ds-1ds-1*) diploid potato clones were estimated on the basis of chiasmate chromosome arm association in metaphase I. In desynaptic mutants both the mean chiasma and bivalent frequencies per cell and the mean chiasma frequency per bivalent proved to be significantly lower. Despite significant differences in within-cell chiasma frequency variation among and particularly between normal and desynaptic clones, no clear effects of the *ds-1* gene on the distribution of chiasmata over chromosomes in a cell were detected. The distribution of chiasmata over chromosomes appeared to be more or less random in both normal and desynaptic plants, which suggests that the *ds-1* gene similarly affects chiasma frequencies in all chromosomes. Genetic data reported in the literature indicate that the *ds-1* gene affects both the overall chiasma frequency and the chiasma distribution along individual chromosomes rather than chiasma maintenance. Sex differences in chiasma formation were not observed among normal plants or among desynaptic mutants, which indicates that chiasma formation in male and female meiosis of potato is governed by a single control system that is similarly expressed in both sexes.

Key words: *Solanum*, desynapsis, chiasma frequency, male meiosis, female meiosis.

JONGEDIJK, E., et RAMANNA, M. S. 1989. Synaptic mutants in potato, *Solanum tuberosum* L. II. Concurrent reduction of chiasma frequencies in male and female meiosis of *ds-1* (desynapsis) mutants. Genome, **32** : 1054–1062.

Les fréquences de chiasmas chez les androsporocytes et les gynosporocytes, tant normaux que désynaptiques (*ds-1ds-1*), des clones de pommes de terre diploïdes ont été évaluées sur la base de l'association de bras de chromosomes chiasmatiques à la métaphase I. Chez les mutants désynaptiques, tant la moyenne des chiasmas que la fréquence de bivalents par cellule et la fréquence moyenne de chiasmas par bivalent ont été significativement inférieures. Malgré les différences significatives des variations de fréquences de chiasmas à l'intérieur des cellules parmi, et particulièrement entre, les clones désynaptiques et normaux, aucun effet précis du gène *ds-1* sur la distribution des chiasmas n'a été décelé dans les chromosomes. La distribution des chiasmas chez les chromosomes a semblé se faire plus ou moins au hasard aussi bien chez les plantes normales que chez les désynaptiques, ce qui suggère que le gène *ds-1* affecte la fréquence des chiasmas de façon similaire chez tous les chromosomes. Les données génétiques rapportées dans la littérature indiquent que le gène *ds-1* affecte à la fois l'ensemble de la fréquence des chiasmas et la distribution des chiasmas le long des chromosomes individuels, plutôt que le maintien des chiasmas. Chez les plantes normales, pas plus que chez les mutants désynaptiques, des différences de sexe par formation de chiasmas n'ont été observées, ce qui indique que la formation de chiasmas dans les méioses mâle et femelle de la pomme de terre est gouvernée par un seul système de contrôle qui s'exprime de la même façon chez les deux sexes.

Mots clés : *Solanum*, désynapsis, fréquence de chiasmas, méiose mâle, méiose femelle.

[Traduit par la revue]

Introduction

For the cultivated potato, *Solanum tuberosum* L., breeding schemes employing synaptic mutants and first division restitution (FDR) 2*n* gamete formation have been proposed. These breeding schemes are expected to improve the efficiency of current breeding methods (Mendiburu *et al.* 1974; Peloquin 1982; Hermsen 1984) and to provide sufficiently uniform tetraploid populations for true potato seed (TPS) technology (Peloquin 1983; Hermsen *et al.* 1985). With regard to the latter, the induction of diplosporic apomixis by combining genes for desynapsis or asynapsis, FDR 2*n* megaspore formation, and (pseudogamous) parthenogenesis in a single genotype has received considerable attention (Hermsen 1980; Hermsen *et al.* 1985; Jongedijk 1985, 1987*a*). The level of uniformity of the apomictic progeny to be established depends on the extent of genetic recombination in female meiosis and thus may vary with different synaptic mutants employed.

According to the cytological behaviour of chromosomes at prophase I, meiotic mutants affecting genetic recombination are divided into asynaptic and desynaptic mutants. The former are characterized by complete or partial failure of homologous chromosome pairing, whereas in the latter normally synapsed homologues are unable to generate or retain chiasmata. Both types of mutants generally are monogenic recessive and often express in both sexes (Baker *et al.* 1976; Gottschalk and Kaul 1980*a*, 1980*b*; Koduru and Rao 1981; Kaul and Murthy 1985). Asynaptic mutants, particularly those with a virtually complete absence of homologous chromosome pairing, obviously are the most attractive candidates to engineer diplosporic apomixis. Desynaptic mutants on the other hand may suffice if genetic recombination is sharply reduced.

For tuber-bearing *Solanum* species six monogenic recessive synaptic mutants, designated *sy-1*, *sy-2*, *sy-3*, *sy-4*, *ds*, and ds_c, have been reported in the literature (Matsubayashi 1979; Iwanaga and Peloquin 1979; Okwuagwu and Peloquin 1981; Ramanna 1983; Iwanaga 1984; Johnston *et al.* 1986). Recently the mutant *sy-1* was concluded to be nonexistent, and the mutants *sy-3*, *ds*, and ds_c were shown to be allelic (gene sym-

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bol *ds-1*) and to display typical desynaptic behaviour in both male and female meiosis (Jongedijk and Ramanna 1988). Thus far, cogent cases of asynapsis are still lacking.

As far as gross bivalent frequencies and genetic recombination in male meiosis are concerned, *ds-1* mutants have been characterized reasonably well (Matsubayashi 1979; Ramanna 1983; Douches and Quiros 1988). However, with respect to female meiosis, no information on chiasma formation and genetic recombination is yet available. Although chiasma frequency and distribution need not necessarily be different in the two sexes (Darlington and La Cour 1940; Brock 1954; Bennett *et al.* 1973; Davies and Jones 1974; Kitada and Omura 1984), considerable sex differences have been demonstrated in a number of higher plant species (Fogwill 1958; Carniel 1960; Ved Brat 1969; Barlow and Vosa 1970; Vosa 1972; Vosa and Barlow 1972). Thus, to reliably evaluate the potential of (de)synaptic mutants for engineering diplosporic apomixis in potato, a study of chiasma formation and genetic recombination in female meiosis of normal synaptic plants and (de)synaptic mutants is essential. A comparative analysis of male and female meiosis may establish whether sex differences in the expression of *ds-1* occur in potato.

In the present paper cytogenetic data on male and female meiosis of *ds-1* mutants and normal synaptic plants are provided.

Materials and methods

Plant material

Chiasma formation was analysed in male and female meiosis of 17 diploid potato hybrids that were obtained from intercrossing the diploid parental clones USW 5295-7 (code B), USW 5337-3 (code C), USW 7589-2 (code D), and 77-2102-37 (code E). All four parental clones were reported to be heterozygous at the *Ds-1/ds-1* locus (*Ds-1ds-1*), which controls the recessive character "desynapsis" in both male and female meiosis (Jongedijk and Ramanna 1988). The hybrid progeny was derived from nine different crosses and included both normal (*Ds-1.*) and desynaptic (*ds-1ds-1*) segregants. Detailed information on the parental clones and hybrid progeny has been provided earlier (Jongedijk and Ramanna 1988).

All plant material was grafted onto tomato rootstock to induce flowering and was grown in a conditioned greenhouse.

Cytological analyses

Male meiosis was studied in random samples of at least 150 pollen mother cells (PMCs) by routine acetocarmine (2%) squashing of young anthers, following fixation in a mixture of propionic acid (saturated with ferric acetate) and ethanol (1:3, v/v). A recently developed enzyme squash technique (Jongedijk 1987*b*) was applied to analyse female meiosis in samples of 25 and 75 megaspore mother cells (MMCs) of normal and desynaptic plants, respectively.

Chiasma frequencies were estimated on the basis of chiasmate chromosome arm association in metaphase I, i.e., assuming that 0, 1, and 2 chiasmata had been formed per univalent pair, rod, and ring bivalent, respectively. Metaphase I stages in MMCs generally occurred by the time PMCs had developed into microspores. To minimize bias generated by environmental differences, fixations for male and female meiosis were made from a single plant at the same time.

All photographs were taken with a Zeiss photomicroscope II, using a Zeiss Planapochromatic 40/1,0 or a 63 PH3H/1,4 oil-immersion objective on Kodak Technical Pan film 2415.

Results

Sampling of male and female meiosis

In potato diplotene and diakinesis are inaccessible for quantitative analysis of chiasma formation. Thus, direct and exact determination of the number and location of chiasmata is not possible and data on chiasma formation have to be inferred from metaphase I chromosome configurations. Because metaphase I bivalents of potato are highly condensed, it could only be determined whether no or at least one chiasma was present in a chromosome arm (Figs. 1C–1N). Chiasma frequencies were therefore estimated on the basis of chiasmate chromosome arm association in metaphase I, i.e., assuming only one chiasma per bound chromosome arm. Chiasma frequencies thus obtained may obviously underestimate the number of chiasmata that actually occurred. Too low chiasma frequency estimates resulting from a loss of chiasmata in late metaphase I – early anaphase I stages may largely be avoided by recording chromosome configurations in early–mid metaphase I stages only.

Due to the generally synchronous development of PMCs within an anther, relatively large samples of PMCs at the proper metaphase I stage were easily obtained for both normal and desynaptic plants (Figs. 1A–1B). Female meiosis in the multiovular potato ovaries on the other hand is highly asynchronous (Jongedijk 1985). In MMCs of normal plants, the degree of stretching of metaphase I bivalents at the equatorial plate was a helpful criterion for the selection of early–mid metaphase I stages. In desynaptic mutants, where bivalent frequencies were strongly reduced and often no bivalents were formed at all, this criterion could not be relied on. In the latter case, univalent behaviour was used to determine how far metaphase I had developed. MMCs with many univalents situated at the spindle poles are indicative for late metaphase I – anaphase I stages. Univalents predominantly gathered at the equatorial plate, at least in male meiosis, may occur as a consequence of reorientation of initially scattered univalents (Ramanna 1983). Both types of MMCs were systematically avoided and only MMCs with univalents predominantly scattered over the spindle were included.

The lack of synchrony in female meiosis generally limited the size of MMC samples that could be obtained. In normal synaptic plants metaphase I is transient and occurs in less than 5% of the ovules. Since a considerable number of megasporocytes is inevitably lost for analysis after enzyme squashing (Jongedijk 1987*b*), screening of many preparations was needed to establish sample sizes of approximately 25 MMCs. In desynaptic mutants the duration of metaphase I appeared to be prolonged and samples of about 75 MMCs could be obtained with some effort.

Chromosome configurations at metaphase I were recorded in 9 normal and 8 desynaptic plants derived from 9 different, partly reciprocal crosses. For the sake of brevity, the data concerning normal plants as well as desynaptic mutants derived from reciprocal crosses were pooled. Among reciprocal hybrids within each category, only insignificant differences were observed (P ($\chi^2_{\text{homogeneity}}$) $\geq$ 0.01). The results on chiasma formation in PMCs and MMCs of normal and desynaptic plants are summarized in Tables 1–3.

Chiasma frequency

In male as well as female meiosis only small differences in the mean chiasma frequency per cell and per bivalent and in the mean bivalent frequencies per cell occurred among normal plants and among desynaptic mutants. However, when compared with normal plants, the mean chiasma and bivalent frequencies per cell proved to be sharply reduced in both male and female meiosis of desynaptic mutants (Table 1). In the

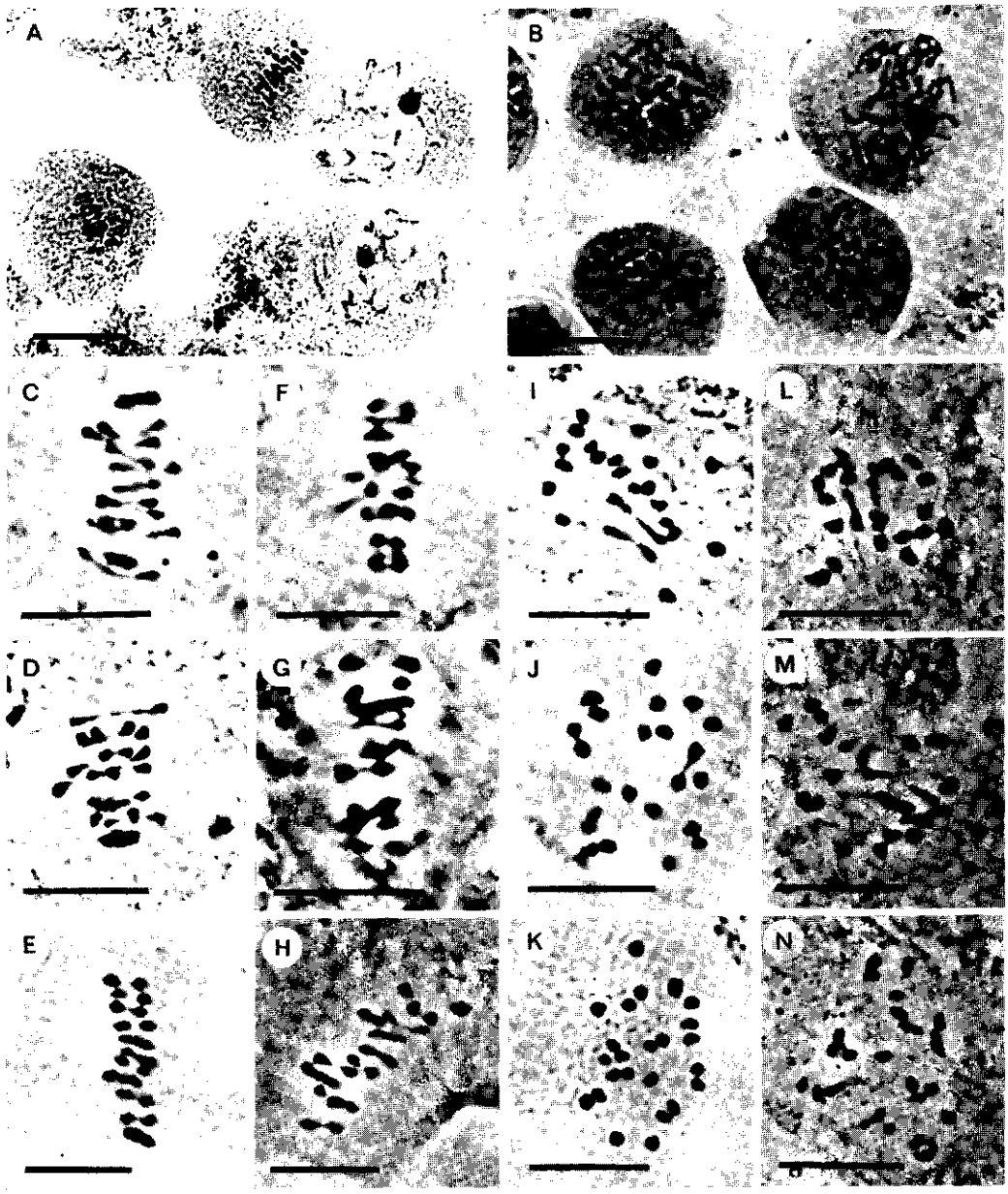


FIG. 1. Acetocarmine squashes of pollen mother cells (A–E, I–K) and enzyme-digested megaspore mother cells (F–H, L–N) from normal synaptic (A, C–H) and desynaptic (B, I–N) diploid potato clones, showing different early–mid metaphase I configurations. Bars represent 10 μm.

TABLE 1. Mean number of chiasmata per cell (X_c), bivalents per cell (B_c), and chiasmata per bivalent (X_b) estimated on the basis of chiasmate chromosome arm association and their mutual relationship in male and female meiosis of normal (*Ds-1.*) and desynaptic (*ds-1ds-1*) hybrids representing five reciprocal sets of crosses

Reciprocal crosses (codes)[a]	Hybrids		Meiosis		X_c		B_c		X_b		Correlation coefficient		
	Ds-1/ds-1	No.	Sex	No. of PMCs or MMCs	Mean[b]	σ	Mean[b]	σ	Mean[b]	σ	$r(X_c, B_c)$	$r(X_c, X_b)$	$r(B_c, X_b)$
BC/CB	*Ds-1.*	2	♂	320	14.14*x*	1.64	11.97*x*	0.27	1.18*x*	0.13	0.25*	0.98*	0.08
			♀	50	14.02*x*	1.41	11.98*x*	0.14	1.17*xy*	0.11	0.31*	1.00*	0.21
	ds-1ds-1	2	♂	320	1.51*y*	1.49	1.36*y*	1.25	1.11*y*	0.19	0.97*	0.34*	0.20*
			♀	150	1.19*y*	1.73	1.02*y*	1.33	1.17*xy*	0.21	0.98*	0.64*	0.48*
BE/EB	*Ds-1.*	2	♂	310	13.23*x*	1.35	11.92*x*	0.30	1.11*x*	0.11	0.27*	0.97*	0.03
			♀	50	13.30*x*	1.09	11.96*x*	0.20	1.11*x*	0.11	0.25*	0.98*	0.05
	ds-1ds-1	2	♂	330	1.36*y*	1.34	1.19*y*	1.10	1.14*x*	0.26	0.96*	0.46*	0.12*
			♀	150	1.03*y*	1.35	0.93*y*	1.12	1.11*x*	0.19	0.97*	0.58*	0.31*
CE/EC	*Ds-1.*	2	♂	320	13.28*x*	1.12	11.96*x*	0.22	1.11*x*	0.09	0.29*	0.98*	0.09
			♀	50	13.70*x*	1.34	11.98*x*	0.14	1.14*x*	0.14	0.30*	0.99*	0.19
	ds-1ds-1	1[c]	♂	160	1.19*y*	1.28	1.14*y*	1.17	1.04*y*	0.14	0.99*	0.37*	0.16*
			♀	75	1.05*y*	1.31	0.96*y*	1.13	1.09*xy*	0.19	0.96*	0.62*	0.27*
DE/ED	*Ds-1.*	2	♂	320	14.13*x*	1.40	11.98*x*	0.16	1.18*x*	0.11	0.47*	0.99*	0.06
			♀	50	13.86*x*	1.21	11.98*x*	0.14	1.16*x*	0.10	0.34*	0.99*	0.23
	ds-1ds-1	2	♂	320	1.17*y*	1.12	1.09*y*	0.99	1.07*y*	0.17	0.96*	0.45*	0.16*
			♀	144	0.95*y*	1.39	0.89*y*	1.22	1.07*y*	0.12	0.98*	0.54*	0.40*
DB	*Ds-1.*	1	♂	160	13.58*x*	1.34	11.98*x*	0.17	1.13*x*	0.11	0.19*	0.99*	0.06
			♀	25	13.40*x*	1.19	11.96*x*	0.20	1.12*x*	0.09	0.42*	0.99*	0.27
	ds-1ds-1	1	♂	160	1.11*y*	1.22	1.04*y*	1.07	1.07*xy*	0.16	0.95*	0.53*	0.23*
			♀	75	0.91*y*	1.03	0.88*y*	0.96	1.03*y*	0.09	0.99*	0.51*	0.32*

[a]For explanation of codes, see section Plant material.
[b]Per reciprocal set of crosses different letters (*x*, *y*) denote a significant difference (based upon the range of the 95% confidence intervals).
[c]*ds-1ds-1* clone from CE; EC does not segregate (Jongedijk and Ramanna 1988).
*Significant at the 5% level.

TABLE 2. Total (MS_t), between-cell (MS_b), and within-cell (MS_w) bivalent chiasma frequency mean squares in male (PMC) and female (MMC) meiosis of normal (*Ds-1.*) and desynaptic (*ds-1ds-1*) hybrids representing five reciprocal sets of crosses

Ds-1/ds-1 genotype	Cross code	MS_t			MS_b			MS_w		
		PMC	MMC	Mean	PMC	MMC	Mean	PMC	MMC	Mean
Ds-1.	BC/CB	0.152	0.144	0.148	0.240	0.165	0.203	0.144	0.142	0.132
	BE/EB	0.104	0.103	0.104	0.153	0.099	0.126	0.100	0.104	0.102
	CE/EC	0.102	0.125	0.114	0.104	0.151	0.128	0.101	0.123	0.112
	DE/ED	0.150	0.135	0.143	0.163	0.122	0.143	0.149	0.136	0.143
	DB	0.119	0.110	0.115	0.150	0.118	0.134	0.116	0.109	0.113
ds-1ds-1	BC/CB	0.134	0.119	0.127	0.184	0.249	0.217	0.129	0.107	0.118
	BE/EB	0.129	0.094	0.112	0.150	0.151	0.151	0.127	0.089	0.108
	CE	0.099	0.096	0.098	0.136	0.144	0.140	0.096	0.091	0.094
	DE/ED	0.100	0.083	0.092	0.108	0.160	0.134	0.100	0.077	0.089
	DB	0.096	0.074	0.085	0.125	0.088	0.107	0.093	0.073	0.083
Mean		0.119	0.108	0.113	0.151	0.145	0.148	0.112	0.105	0.110
Mean *Ds-1.*		0.125	0.123	0.124	0.162	0.131	0.147	0.122	0.123	0.122
Mean *ds-1ds-1*		0.112	0.093	0.102	0.141	0.158	0.150	0.109	0.087	0.098

latter, the mean chiasma frequency per bivalent also tended to be lower.

Significant sex differences were observed neither in normal plants nor in desynaptic mutants. However, in desynaptic mutants the mean chiasma and bivalent frequency per cell appeared to be slightly but systematically lower in female meiosis (Table 1). As indicated earlier, deciding whether a particular MMC was in the proper (early–mid) metaphase I stage was most difficult in female meiosis of desynaptic mutants. Thus, in the analysis of desynaptic mutants late metaphase I – early anaphase I stages may well have been included and led to an underestimation of the mean chiasma and bivalent frequencies per cell. In fact, comparison of the observed distributions of chiasmata and bivalents per cell in male and female meiosis (Fig. 2) suggested that, where judgement of female metaphase I stages had been reasonably good in normal plants, in desynaptic mutants a considerable amount of late metaphase I – early anaphase I stages had been recorded.

TABLE 3. Analysis of variance of total (MS_t), between-cell (MS_b), and within-cell (MS_w) bivalent chiasma frequency mean squares in male and female meiosis of normal (*Ds-1.*) and desynaptic (*ds-1ds-1*) hybrids

Source of variation	df	MS_t		MS_b		MS_w	
		MS[a]	*F*	MS[a]	*F*	MS[a]	*F*
Hybrids	9	8.572	7.38**	23.959	2.02ns	8.334	5.95**
Ds-1. vs. *ds-1ds-1*	1	24.200	20.83**	0.450	0.04ns	29.282	20.90**
Within *Ds-1.*	4	7.691	6.62**	20.448	1.72ns	7.321	5.23*
Within *ds-1ds-1*	4	5.546	4.78*	33.348	2.81ns	4.177	2.98ns
Sexes	1	5.202	4.48ns	2.178	0.18ns	5.408	3.86ns
Hybrids × sexes (error)	9	1.162	—	11.879	—	1.401	—

[a]Mean squares value × 10^4.
*Significant at the 5% level.
**Significant at the 1% level.

The simultaneous reduction of the mean chiasma frequency per cell and per bivalent and the mean bivalent frequency per cell in both male and female meiosis of desynaptic mutants suggest that the *ds-1* gene similarly affects all chromosomes in both sexes.

Chiasma distribution

With regard to chiasma distribution, two different aspects are generally considered, viz., chiasma frequency variation among cells and bivalents and chiasma distribution along chromosome arms, which is positional in nature.

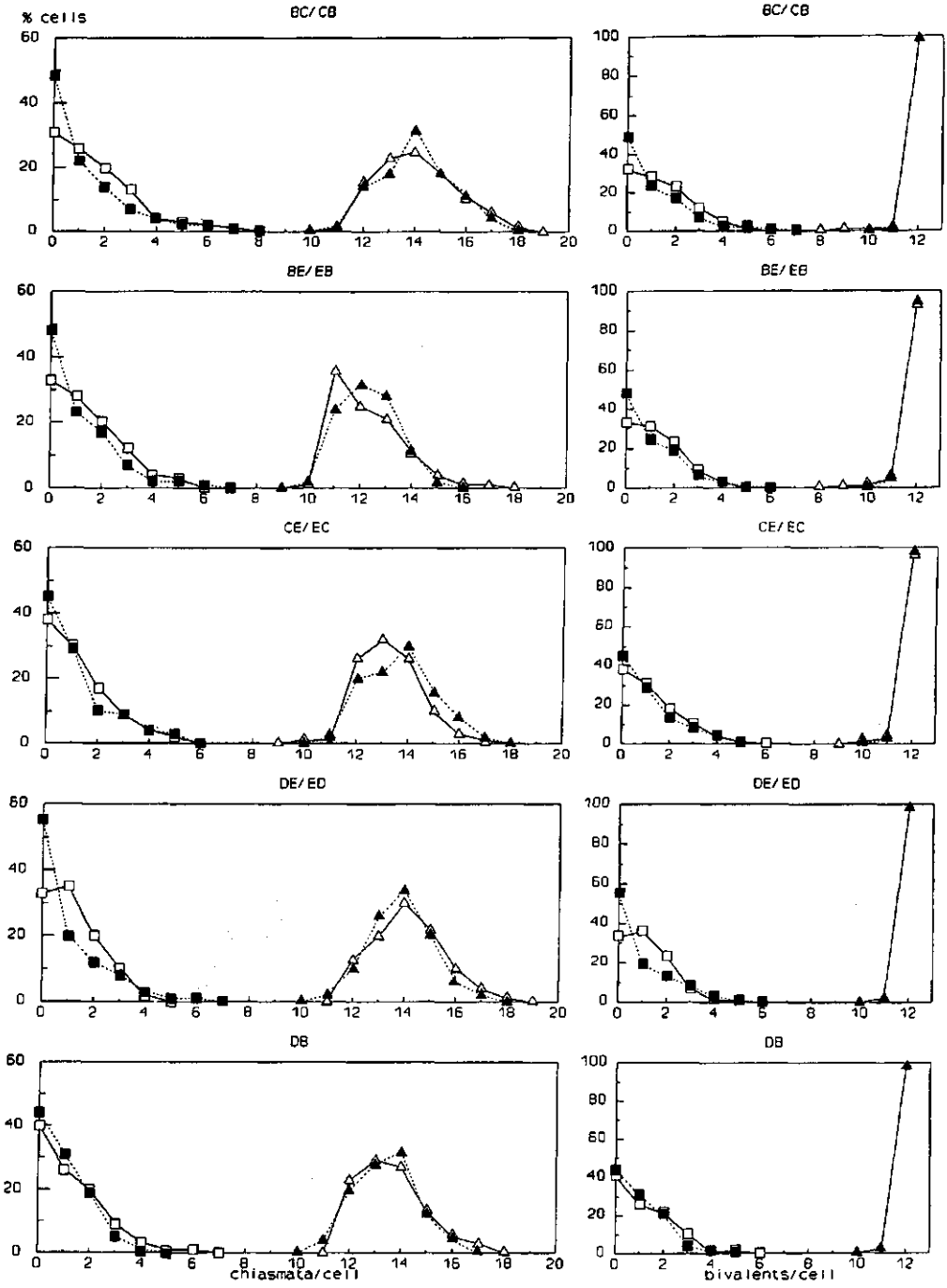
To evaluate variation in chiasma frequency among cells and bivalents, the total mean square of all bivalent chiasma frequencies in a sample (MS_t) and its between-cell (MS_b) and within-cell (MS_w) components (measuring the total between-bivalent chiasma variation and the amount of chiasma variation between cells in a sample and between bivalents in a cell, respectively) were calculated according to Mather (1936; Table 2) and compared between hybrids and sexes (Table 3). Between hybrids significant differences in total and within-cell chiasma frequency variation were observed. In both cases they could be traced to differences among and particularly between normal and desynaptic plants (Table 3). Since such differences did not occur for the between-cell variation in chiasma frequency, the MS_t and MS_w values may actually be considered to measure the same kind of chiasma variation, i.e., between bivalents. Chiasma variation between bivalents proved to be considerably lower in desynaptic plants (Table 2). No differences in chiasma frequency variation were observed between sexes (Table 3).

Where the preceding analysis showed that normal and desynaptic plants differ with respect to between-bivalent chiasma variation, comparison of the amount of between-cell and within-cell chiasma variation may provide some information regarding the nature of the chiasma distribution over whole bivalents in a cell. A significantly larger within-cell chiasma variation generally indicates negative interchromosome correlation with respect to chiasma frequencies. Such a negative correlation may be due to either positive interference between chiasmata formed in different chromosomes (Mather 1936) or intrinsic differences in chiasma formation between chromosomes (Sybenga 1967). In both cases the distribution of chiasmata over bivalents in a cell is nonrandom. However, in the hybrids studied here, the between-cell chiasma variation generally was larger than the variation within cells (Table 2), suggesting a more or less random distribution of chiasmata over bivalents within a cell in both normal and desynaptic plants. The significantly lower within-cell chiasma variation in desynaptic mutants thus seems to be due to the extremely low frequencies of rod and particularly ring bivalents in these mutants rather than to differences between desynaptic and normal plants with respect to the nature of chiasma distribution over chromosomes in a cell.

A more or less random distribution of chiasmata over chromosomes within cells is also suggested by the consistently positive correlations between the number of chiasmata per cell (X_c) on the one hand and the number of bivalents per cell (B_c) and chiasmata per bivalent (X_b) on the other (Table 1). In desynaptic mutants, which are characterized by extremely low numbers of chiasmata and low numbers of predominantly rod bivalents per cell, an increase in the number of chiasmata per cell generally resulted in an increase of particularly rod bivalents (correlation coefficients: $r(X_c, B_c) = 0.95-0.99$ and $r(X_c, X_b) = 0.34-0.64$). In normal plants, where the number of bivalents per cell approaches the maximum number of bivalents possible, increasing chiasma frequencies per cell were largely attended with an increase of ring bivalents (correlation coefficients: $r(X_c, B_c) = 0.19-0.47$ and $r(X_c, X_b) = 0.97-1.00$). Negative correlations between B_c and X_b, which indicate the occurrence of interchromosomal compensatory chiasma formation or intrinsic differences in chiasma formation between chromosomes, were detected neither in normal nor in desynaptic clones.

Although chiasma distribution over whole chromosomes in a cell appeared to be more or less random in normal and desynaptic plants, this need not necessarily be the case with respect to chiasma distribution along individual chromosomes. As to the latter, longer chromosome arms are for instance likely to be bound more frequently and to generate more chiasmata than shorter ones. Nonrandom distribution of chiasmata along chromosomes or chromosome arms may further occur with positive or negative chiasma interference (Sybenga 1975). Chiasma distribution along individual chromosomes and chromosome arms was not analysed because the small and

FIG. 2. Frequency distributions for the number of chiasmata per cell and bivalents per cell in male and female meiosis of normal synaptic (*Ds-1.*) and desynaptic (*ds-1ds-1*) diploid potato clones derived from five different reciprocal sets of crosses. □—□, ♂, *ds-1ds-1*; ■·····■, ♀, *ds-1ds-1*; △—△, ♂, *Ds-1.*; ▲·····▲, ♀, *Ds-1.*


% cells
BC/CB
BC/CB
BE/EB
BE/EB
CE/EC
CE/EC
DE/ED
DE/ED
DB
DB
chiasmata/cell
bivalents/cell

highly condensed metaphase I bivalents of potato do not allow the detection of more than one chiasma per chromosome arm or the detection of arm length differences.

Discussion

Effect of the ds-1 *gene on chiasma frequency and distribution*

Where in male meiosis a complete lack of bivalent formation (Okwuagwu and Peloquin 1981; Peloquin 1982) and low, but significant bivalent frequencies (Matsubayashi 1979; Ramanna 1983) have been reported for *ds-1* mutants, no systematic comparison of bivalent and particularly chiasma frequencies with those of normal sibs nor comparative data concerning female meiosis were available. The data on male and female meiosis of both normal plants and *ds-1* mutants provided here (Table 1; Figs. 1 and 2) clearly demonstrate that the *ds-1* gene reduces chiasma and bivalent frequencies per cell and generally also chiasma frequencies per bivalent equally strongly in both sexes (mean reduction of chiasmata per cell, bivalents per cell, and chiasmata per bivalent in male and female meiosis were 90.7, 90.3, 4.8 and 92.5, 92.2, 4.0%, respectively).

Ramanna (1983) studied frequencies of bivalent formation in male meiosis of 51 *ds-1* mutants in detail and in some clones noted a highly variable behaviour hampering clear-cut classification. Five clones tentatively classified by Ramanna (1983) as desynaptic (CB19, CB42, BC21, DE7, and DE28 with mean bivalent numbers per cell of 4.9, 5.9, 8.3, 5.8, and 6.4, respectively) proved to be largely normal upon subsequent observation in 3 successive years (E. Jongedijk and M. S. Ramanna, unpublished results). The mean bivalent frequencies per cell of the remaining 46 *ds-1* mutants (Ramanna 1983; 0.6–3.9 bivalents per cell) as well as those reported for 10 *ds-1* mutants derived from the variety Chippewa (Matsubayashi 1979: 0.93–2.21 bivalents per cell) correspond reasonably well with the data presented here (0.88–1.36 bivalents per cell). In contrast to what was claimed by Okwuagwu and Peloquin (1981) and Peloquin (1982) these observations unambiguously demonstrate the formation of low, but significant bivalent frequencies in both sexes of *ds-1* mutants. The simultaneous reduction of the mean number of chiasmata and bivalents per cell and chiasmata per bivalent in desynaptic mutants and the consistent positive correlations between these variables in all *ds-1* mutants (Table 1) suggest that the *ds-1* gene similarly affects all chromosomes in a cell. Comparable genes for desynapsis have been reported in a variety of other plant species (Beadle 1933; Soost 1951; Enns and Larter 1960; Thomas and Rajhathy 1966; Singh *et al.* 1977; Giraldez and Lacadena 1978; Gottschalk and Kaul 1980*b*; Kitada and Omura 1983).

In general, mutant synaptic genes give rise to increased univalent frequencies at metaphase I. For desynaptic mutants theoretically two distinct mechanisms leading to this phenomenon may be considered. First, genes for desynapsis may affect chiasma formation either by generating less chiasmata, which are distributed as in normal plants, or by altering chiasma distribution among or along chromosomes, irrespective of the reduction in initial chiasma frequency (Baker *et al.* 1976; Koduru and Rao 1981; Kaul and Murthy 1985). Second, increased univalent frequencies may be due to the inability of normally synapsed homologues to retain chiasmata once initiated, i.e., due to changes in chromatid adhesion (Maguire 1978). Since chiasmata are considered to be the cytological equivalent of genetic recombination events, in the former cases equally reduced or differentially altered recombination frequencies among chromosomal sites of exchange may be expected. In the latter case, however, the apparent reduction of chiasma frequency is not caused by defective crossing-over and normal wild-type recombination rates may be expected at all chromosomal sites of exchange. With respect to the *ds-1* mutants examined here, genetic studies have demonstrated a differential, and mostly severe, reduction of genetic recombination for several marker loci in male and (or) female meiosis (Jongedijk 1987*a*; Douches and Quiros 1988). However, for the marker locus *Got-2*, which is located very close to the centromere, no reduction in recombination rate could be detected (E. Jongedijk, R. C. B. Hutten, J. M. A. S. A. van der Wolk, and S. E. J. Schuurmans-Stekhoven, in preparation). The *ds-1* gene thus appears to affect the overall chiasma frequency and chiasma distribution along individual chromosomes rather than chiasma maintenance. Overall reduction of chiasma frequencies, but normal or even increased recombination rates in specific chromosome segments, due to differential changes in chiasma distribution or preferential survival of progeny resulting from meiocytes with reasonably balanced chromosome disjunction, has been reported in (de)synaptic mutants of several plant species (see reviews by Baker *et al.* 1976; Koduru and Rao 1981; Kaul and Murthy 1985). With regard to the distribution of chiasmata over chromosomes within a single cell, no clear effects of the *ds-1* gene could be detected. Despite the observed differences in chiasma frequency variation between bivalents within cells (Table 3), the distribution of chiasmata over chromosomes seemed to be more or less random in both normal and desynaptic clones.

As indicated earlier, metaphase I bivalents of potato do not allow the detection of more than one chiasma per chromosome arm. Although chiasma frequencies thus are likely to have been underestimated, this need not necessarily be so. In synaptonemal complexes of normal tomato plants, Stack and Anderson (1986) recently noted a precipitous loss of recombination nodules in early pachytene that typically leaves only one or two recombination nodules per synaptonemal complex. Since recombination nodules are assumed to correspond with potential sites of recombination (Carpenter 1975, 1979*a*, 1979*b*), this might indicate that in tomato only one or two chiasmata are generated per chromosome. To establish whether a similar situation occurs in potato a detailed study of the number and distribution of recombination nodules in synaptonemal complexes of potato is required. Obviously, a comparative study of synaptonemal complexes in normal plants and (de)synaptic mutants, as has been reported for *Drosophila* (Carpenter 1979*a*, 1979*b*), may provide a more direct estimate of the effect of mutant synaptic genes on chiasma frequency and distribution.

Control of chiasma formation in male and female meiosis

Among angiosperm plant species comparative studies of male and female meiosis are scarce. They have, however, demonstrated that chiasma frequency and chiasma distribution may be very different in the two sexes, the mean chiasma frequencies generally being significantly higher in female meiosis (Fogwill 1958; Carniel 1960; Ved Brat 1969; Barlow and Vosa 1970; Vosa 1972; Vosa and Barlow 1972). Such sex differences in chiasma formation have been supposed to result from independent meiotic control systems operating in male and female meiosis. According to Davies and Jones (1974), however, they might also result from a differential response of

a joint control system to different conditions in male and female meiocytes.

Despite the occurrence of plant differences in chiasma formation within and particularly between the two categories of clones (i.e., normal and desynaptic) studied here, sex differences in chiasma and bivalent frequency per cell, chiasma frequency per bivalent, and chiasma frequency distribution within anthers and ovaries, between meiocytes and between bivalents proved to be consistently absent. Although theoretically the absence of sex differences might result from two independent, sex-specific control systems acting convergently to produce the same effect (Davies and Jones 1974), the consistently identical expression of obviously genetically determined variation in chiasma formation in male and female meiosis reported here, instead suggests meiosis, or at least chiasma formation, in the two sexes to be jointly controlled.

Similar findings have been reported for lilies (Darlington and La Cour 1940; Brock 1954), barley (Bennett *et al.* 1973), rye (Davies and Jones 1974), and rice (Kitada and Omura 1984). For the potato material studied here, a joint control system operating in male and female meiosis is further supported by the recent observation that, even though the *ds-1* gene differentially altered genetic recombination rates for a number of marker loci, significant sex differences in genetic recombination for these loci did occur neither in normal plants nor in *ds-1* mutants (E. Jongedijk, R. C. B. Hutten, J. M. A. S. A. van der Wolk, and S. E. J. Schuurmans-Stekhoven, in preparation).

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CHAPTER 6

FORMATION OF FIRST DIVISION RESTITUTION (FDR) 2N-MEGASPORES THROUGH PSEUDO-HOMOTYPIC DIVISION IN *ds-1* (DESYNAPSIS) MUTANTS OF DIPLOID POTATO: ROUTINE PRODUCTION OF TETRAPLOID PROGENY FROM 2XFDR-2XFDR CROSSES

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Summary

The level and mode of 2n-megaspore formation was studied in full-sib diploid potato clones with both normal and desynaptic (*ds-1ds-1*) meiosis. Cytological analysis revealed that functional 2n-megaspores produced by normal and desynaptic clones originate exclusively from 'second division restitution (SDR)' and 'first division restitution (FDR)' respectively. SDR 2n-megaspores resulted from the omission of the second meiotic division following chromosome doubling after anaphase I, whereas FDR 2n-megaspores resulted from a direct equational division of univalent chromosomes at anaphase I (pseudo-homotypic division). Comparative data strongly indicated that the observed mechanisms of SDR and FDR 2n-megaspore formation are extremes of a continuum that is being brought about by common genes for precocious chromosome division. Depending on the relative timing of cell cycle and chromosome division this precocious chromosome division may impose post-reductional (SDR) or pre-reductional (FDR) 'restitution' of the sporophytic chromosome number under normal synaptic and desynaptic conditions respectively. The observed frequencies of 2n-megaspores closely correlated with seed set following pollination by tetraploid varieties and by desynaptic diploid clones with exclusive FDR 2n-pollen formation. Up to 54.0 and 21.5 seeds/fruit were obtained from normal synaptic (SDR) and desynaptic (FDR) progeny respectively. The high frequency of segregants with either SDR or FDR 2n-megaspore formation (78.0 and 45.2 % respectively) supports the hypothesis that sexual polyploidization is the driving force behind the origin and evolution of polyploid *Solanum* species. The present identification of diploid potato clones with consistent FDR 2n-megaspore formation extends the opportunities for direct transfer of enhanced diploid germplasm to tetraploids and particularly advocates the feasibility of 2*x*(*ds-1*;FDR).2*x*(*ds-1*;FDR) breeding schemes in cultivar development and the production of relatively vigorous and uniform true potato seed (TPS) varieties. Its potential value and limitations for breeding and the experimental induction of diplosporic apomixis are discussed.

Key words: *Solanum*, 2n-megaspores, desynapsis, pseudo-homotypic division, sexual polyploidization, apomixis.

Introduction

Sexual polyploidization via numerically unreduced or 2n-gametes has been identified as the driving force behind the origin and evolution of polyploid plant species (Harlan and de Wet, 1975). As to potato the frequent occurrence of 2n-gamete formation in many diploid species has substantiated their evolutionary significance in the origin of the polyploid complexes found in some taxonomic series of the tuber-bearing *Solanums* (Den Nijs and Peloquin, 1977). More important, they also enable the adoption of relatively efficient breeding schemes, which basically consist of direct transfer of enhanced diploid germplasm to tetraploids through

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unilateral (4*x*-2*x* crosses) and bilateral (2*x*-2*x* crosses) sexual polyploidization, for both cultivar development and the production of true potato seed (TPS) varieties (Mendiburu *et al.*, 1974; Peloquin, 1982; Hermsen, 1984*a*).

Unreduced gametes may result from a number of different meiotic 'abnormalities'. According to the genetic consequences, however, only two distinct types, first division restitution (FDR) and second division restitution (SDR), are distinguished. Where FDR 2n-gametes essentially derive from an equational division of the entire chromosome complement and thus include 'non-sister' chromatids, SDR 2n-gametes result from chromosome doubling following reductional chromosome division and comprise 'sister chromatids'. For breeding schemes employing sexual polyploidization the actual mode of 2n-gamete formation in the diploid parental clones is of practical significance. FDR 2n-gametes are by far superior in preserving parental heterozygosity and thus significantly contribute to both vigour and homogeneity of tetraploid progeny recovered by sexual polyploidization (Mendiburu *et al.*, 1974; Peloquin, 1982, Hermsen, 1984*b*). In addition, mutant synaptic genes are known to cause the exclusive occurrence of functional FDR 2n-gametes (Ramanna, 1983; Jongedijk, 1985) and, owing to reduced gene recombination, to particularly increase the ability of FDR 2n-gametes to maintain the genetic constitution of parental clones, including complex types of favourable epistasis, with a minimum amount of reassortment (Douches and Quiros, 1988*a*; Jongedijk *et al.*, 1991). Maximum possible performance and uniformity might thus be attained in tetraploid progeny from 2*x*FDR-2*x*FDR crosses when genetic recombination is largely lacking in both parental clones. As to TPS technology nearly complete uniformity might also be achieved by diplosporic apomixis. The latter could possibly be obtained by combining genes for asynapsis/desynapsis, FDR 2n-megaspore formation and pseudogamous seed development (Hermsen, 1980; Hermsen *et al.*, 1985; Jongedijk, 1985).

For the efficient use of 2n-gametes in breeding, knowledge of the cytological mechanisms of SDR and FDR and their inheritance is indispensable. Whereas the mechanisms and genetic control of SDR and FDR 2n-pollen formation have been well established (Ramanna, 1974, 1979, 1983; Mok and Peloquin, 1975; Veilleux *et al.*, 1982), cytological data concerning 2n-megaspore formation are relatively scarce. So far genetic and cytological studies have indicated the predominant occurrence of SDR with normal synapsis and revealed that the prevailing mechanism of SDR 2n-gamete formation consists of 'omission of the second meiotic division' (Stelly and Peloquin, 1986a, 1986b; Werner and Peloquin, 1987, 1991; Douches and Quiros, 1988*b*). Similar mechanisms of SDR 2n-megaspore formation have been reported for *Datura*, maize and barley (Satina and Blakeslee, 1935; Rhoades and Dempsey, 1966; Finch and Bennett, 1979). According to Werner and Peloquin (1987, 1991) SDR 2n-megaspores may also be formed through the failure of cytokinesis after the second meiotic division and subsequent fusion of daughter nuclei prior to embrysac development or by nuclear restitution following irregular second meiotic divisions. The former mechanism has previously been noted to cause SDR 2n-megaspore formation in alfalfa (Pfeiffer and Bingham, 1983). As expected with mutant synaptic conditions being required for consistent FDR 2n-megaspore formation (Jongedijk, 1985), the latter has only been inferred to occur in some of the synaptic mutants tested so far. Although cytological studies of female meiosis in these mutants revealed occasional nuclear restitution following typically desynaptic metaphases (Iwanaga and Peloquin, 1979; Werner and Peloquin, 1987, 1991), conclusive evidence for FDR 2n-megaspore formation by the formation of restitution nuclei including all chromosomes and their subsequent division in the second meiotic division has not yet been provided.

In this paper consistent FDR 2n-megaspore formation in desynaptic mutants through direct equational division of univalent chromosomes and subsequent omission of the second meiotic division (pseudo-homotypic division) is reported. In addition comparative data on SDR and FDR 2n-megaspore formation are provided which suggest that both are caused by common genes for precocious chromosome division.

Materials and methods

Plant material

The formation of 2n-megaspores was studied in the diploid parental clones USW5295-7 (coded B), USW 5337-3 (coded C), USW 7589-2 (coded D), 77-2102-37 (coded E) and derived F_1 hybrids (two letter codes). Detailed information on the origin and pedigree of the parental clones has been summarized earlier (Jongedijk and Ramanna, 1988). The F_1 hybrids included both normal (*Ds-1*.) and desynaptic (*ds1-ds-1*) segregants (Jongedijk and Ramanna, 1988, 1989). Levels of 2n-megaspore formation were estimated on the basis of seed set following pollination by the tetraploid potato cultivars 'Gineke', 'Libertas', 'Chippewa' and 'Katahdin' (2*x*.4*x* testcrosses) and by diploid desynaptic clones with exclusive FDR 2*n* pollen formation (2*x*.2*x*(*ds-1*;FDR) testcrosses) and with high seed set in 4*x*.2*x* crosses (Table 1). At least 50 flowers of each clone were pollinated with 4-6 different male parents. To exclude selfing, flowers were emasculated well before anthesis. Mature fruits were collected 6-8 weeks after pollination. To obtain a value measuring the average seed set on a particular clone the data concerning different pollen parents were pooled. Only seed-containing berries were included in the analysis. Relevant data concerning the parental genotypes at the *Ds-1/ds-1* locus and parental '2*x*.4*x* crossability' are summarized in Table 2.

Cytological analyses

Ploidy distributions in testcross progenies were checked by establishing the mean number of chloroplasts in stomatal guard cells (Frandsen, 1968) or, in cases of doubt, by chromosome counts in root tip meristems. For large scale screening and detailed observations of megasporogenesis in young ovaries a routine methyl salicylate clearing technique (Jongedijk, 1987*a*) and an enzyme squash technique (Jongedijk, 1987*b*) were used respectively. Frequencies of 2n-megaspores were estimated in random samples of about 500 ovules from 3-4 different ovaries with predominantly sporad stages. Frequencies of desynaptic and (partially) pseudo-homotypic metaphase stages in *ds-1* mutants were estimated in random samples of about 150 megaspore mother cells from 2-3 different ovaries. All photographs were taken with a Zeiss Photomicroscope II, using a Zeiss Planapochromatic 63 PH3H/1.4 oil immersion objective on Kodak technical Pan Film 2415.

Results

Seed set in 2x.4x and 2x.2x(ds-1ds-1/FDR) testcrosses

In the absence of premeiotic and postmeiotic doubling, 2n-megaspores from normal synaptic potato clones are expected to arise through SDR, whereas consistent FDR 2n-megaspore formation requires mutant synaptic conditions (Jongedijk, 1985). In the latter case

Table 1. Percentage of stainable and 2n-pollen in diploid (2*x*) desynaptic pollen parents, and seed set following pollination of tetraploid (4*x*) seed parents.

Pollen parent	% stainable pollen[a]	% 2n pollen[a]	Seeds/fruit from 4*x*.2*x* crosses
CE-10	69.7	96.7	128.77
CE-101	69.1	94.8	97.73
BE-62	45.9	93.6	80.97
BE-67	63.1	96.2	65.72

[a] Data from Ramanna (1983).

Table 2. Average seed set on diploid (2*x*) normal synaptic (*Ds-1ds-1*) parental clones from 2x.4x and 2x.2x(*ds-1*;FDR) testcrosses.

Diploid parent (code)	Genotype	Average seed set[a]			No. poll.
		f/p	s/f	s/p	
USW 5295-7 (B)	*Ds-1ds-1*	0.75	12.29	9.22	69
USW 5337-3 (C)	*Ds-1ds-1*	0.17	1.13	0.19	84
USW 7589-2 (D)	*Ds-1ds-1*	0.54	25.33	13.68	144
77-2102-37 (E)	*Ds-1ds-1*	0.71	1.81	1.28	160

[a] f=fruits, s=seeds, p=pollination.

Table 3. Mean number of fruits/pollination (f/p), seeds/fruit (s/f) and seeds/pollination (s/p) obtained from 2*x*.4*x* and 2*x*.2*x*(*ds-1*;FDR) testcrosses and their relation in normal synaptic (*Ds-1.*) and desynaptic (*ds-1ds-1*) diploid progenies from five sets of (reciprocal) crosses.

Hybrids (2n=2x=24)				Hybrids with seed set										
					f/p		s/f		s/p		Correlation coefficient		
Parentage[a]	*Ds-1/ds-1*	No.	No. poll.	% clones	Mean	Range	Mean	Range	Mean	Range	r(f/p,s/f)	r(f/p,s/p)	r(s/f,s/p)
BC/CB	*Ds-1.*	22	2186	54.5	0.25	0.01-0.82	2.46	1.00- 6.13	0.84	0.01- 5.04	0.66*	0.89*	0.83*
	ds-1ds-1	30	2098	56.7	0.15	0.02-0.49	6.47	1.00-21.53	1.29	0.02- 6.50	0.38	0.78*	0.78*
BE/EB	*Ds-1.*	17	1285	76.5	0.26	0.01-0.78	14.47	2.38-36.86	4.86	0.08-26.67	0.46	0.87*	0.77*
	ds-1ds-1	33	2212	39.4	0.11	0.01-0.50	4.43	1.00-13.78	0.75	0.02- 3.35	0.30	0.70*	0.83*
CE/EC	*Ds-1.*	42	2116	80.9	0.38	0.01-0.90	4.42	1.00-25.64	1.79	0.03-11.97	0.08	0.51*	0.82*
	ds-1ds-1[b]	19	2108	47.4	0.15	0.01-0.44	4.88	1.50-10.85	0.96	0.02- 3.78	0.49	0.83*	0.81*
DE/ED	*Ds-1.*	29	2249	86.2	0.57	0.06-1.00	15.92	1.17-54.00	9.08	0.29-54.00	0.27	0.54*	0.91*
	ds-1ds-1	34	2576	44.1	0.20	0.01-0.60	3.33	1.00-12.53	0.99	0.01- 5.52	0.50	0.76*	0.86*
DB	*Ds-1.*	13	678	92.3	0.56	0.08-0.94	9.89	1.00-36.67	6.93	0.13-27.50	0.53	0.62*	0.98*
	ds-1ds-1	10	527	30.0	0.50	0.03-0.83	2.71	0.03- 6.00	1.92	1.00- 5.00	0.79	0.82	0.99
TOTAL	*Ds-1.*	123	8514	78.0	0.42	0.01-1.00	9.21	1.00-54.00	4.63	0.01-54.00	0.30*	0.58*	0.86*
	ds-1ds-1	126	9521	45.2	0.17	0.01-0.83	4.73	1.00-21.53	1.14	0.01- 6.50	0.27*	0.77*	0.75*

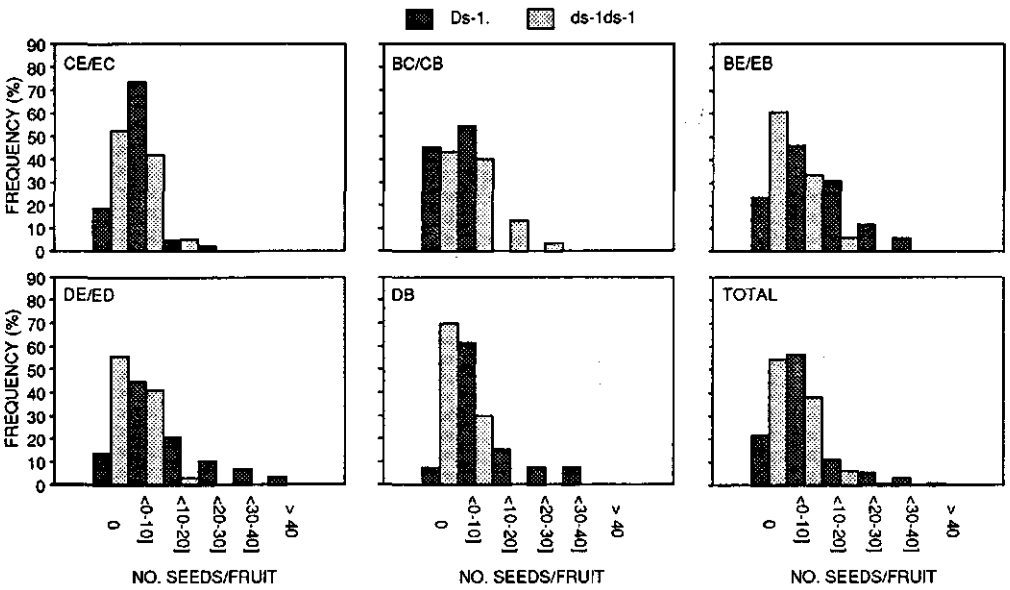
[a] For explanation of codes, see section Plant material.
[b] *ds-1ds-1* hybrids from CE only; EC does not segregate (Jongedijk and Ramanna, 1988).
* Significant at the 5% level.

both SDR and 'reduced' megaspores may be formed, but abort due to chromosome imbalance (Ramanna, 1983; Jongedijk, 1985). Because of the nearly complete 'triploid block' (Marks, 1966) and the near absence of premeiotic and postmeiotic doubling in potato (Stelly and Peloquin, 1986a; Werner and Peloquin, 1987, 1991), the average seed set in 2*x*.4*x* and 2*x*.2*x*(*ds-1*;FDR) testcrosses may therefore be assumed to give rise to tetraploid offspring and to be a measure of the seed parents' ability to produce either SDR or FDR 2n-megaspores in case of normal synapsis and desynapsis respectively.

Seed set following 2*x*.4*x* and 2*x*.2*x*(*ds-1*;FDR) testcrosses was estimated for 123 normal synaptic (*Ds-1.*) and 126 desynaptic (*ds-1ds-1*) diploid F_1 hybrids from nine different, partly reciprocal crosses (Table 3). Data about seed set on normal synaptic segregants as well as on desynaptic segregants from reciprocal crosses were pooled. Among reciprocal hybrids within each category, only insignificant differences were observed ($P(X^2_{homogeneity}) \geq 0.01$). The populations differed substantially in the percentage of diploid hybrids with seed set. In addition, this frequency was generally higher among the normal synaptic segregants than among the desynaptic segregants. On the whole 78.0 % of the normal synaptic progeny and 45.2 % of the desynaptic mutants tested formed SDR and FDR 2n-megaspores respectively (Table 3). Either between or within different populations the average numbers of fruits/pollination (f/p), seeds/fruit (s/f) and seeds/pollination (s/p) produced by normal synaptic and desynaptic segregants varied considerably, the averages and ranges usually being smaller among *ds-1* mutants (Table 3). Significant positive correlations were observed between the numbers of seeds/pollination on the one hand and the numbers of fruits/pollination and seeds/fruit on the other in all (sub)populations (correlation coefficients: r(f/p, s/p) = 0.51-0.89 and r(s/f, s/p) = 0.77-0.99). However, the number of fruits/pollination generally did not significantly correlate with the number of seeds/fruit (Table 3). Similar amounts of seeds/pollination thus may result from relatively high berry set but moderate numbers of seeds/fruit and from moderate berry set but relatively high numbers of seeds/fruit.

Normal synaptic F_1 hybrids with numbers of seeds/fruit exceeding that of the highest parental clone were observed in all crosses but BC/CB. As to the desynaptic seed parents

Figure 1. Average numbers of seeds/fruit following testcrossing of synaptic (*Ds-1.*) and desynaptic (*ds-1ds-1*) diploid potato clones.



such F_1 hybrids were observed among BC/CB and CE/EC progeny only (Fig. 1). Following testcrossing up to 54.0 and 21.5 seeds/fruit were obtained from normal synaptic and desynaptic clones respectively. Seed parents with medium-high numbers of seeds/fruit generally produced sufficient berries to allow for routine production of extensive testcross progeny. The average number of seeds/pollination produced by normal synaptic plants with <0-10], <10-20], <20-30], <30-40] and >40 seeds/fruit amounted to 1.4, 6.5, 13.0, 27.6 and 54.0 respectively, whereas *ds-1* mutants representing the lower three classes produced on the average 0.6, 3.2 and 6.5 seeds/pollination.

Ploidy levels of testcross progeny

In order to make sure that seed set following the 2*x*.4*x* and 2*x*.2*x*(*ds-1*;FDR) testcrosses does accurately measure the seed parents' ability to produce 2n-megaspores, ploidy levels of testcross progenies that involved normal synaptic and desynaptic seed parents with low-high seed set were checked. For comparison, ploidy levels among progeny derived from additional 4*x*.2*x* and 2*x*.2*x*(*Ds-1.*) crosses were established. Normal synaptic pollinators used in these latter crosses included the parental clones USW5295-7 (B), USW5337-3 (C), 77-2102-37 (E) and BE-44 which produce substantial amounts of predominantly FDR 2n-pollen (Mok and Peloquin, 1975; Jacobsen, 1978; Jongedijk *et al.*, 1991).

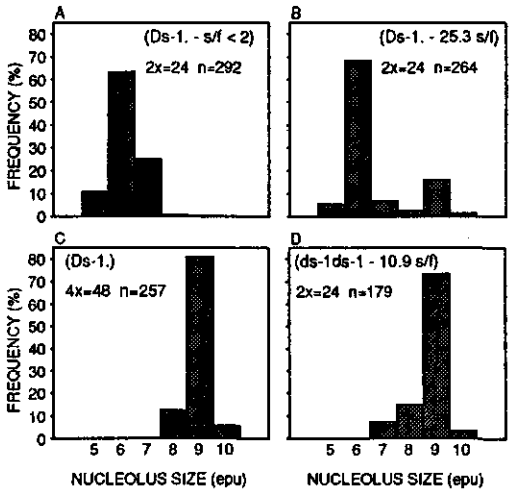
As expected with a nearly complete triploid block, the frequency of tetraploids among progeny derived from either 2*x*-4*x* crosses or 2*x*-2*x* crosses involving at least one desynaptic parent was extremely high (Table 4). However, due to the formation of reduced gametes in normal synaptic parents, the frequency of tetraploid progeny from 2*x*(*Ds-1.*).2*x*(*Ds-1.*) crosses was relatively low: even in crosses between normal synaptic parental clones with relatively high levels of 2n-gamete formation only up to 35.1 % tetraploids were observed (Table 4). Desynapsis conferred by the *ds-1* gene may thus be concluded to act as an effective sieve against the formation of functional reduced (and SDR) gametes. Not surprisingly in that case,

Table 4. Ploidy levels of progeny from 2*x*.4*x* and 2*x*.2*x*(*ds-1*;FDR) testcrosses and, for comparison, those of progeny from 4*x*.2*x* and 2*x*.2*x*(*Ds-1*) crosses.

Mating type	No. crosses	No. progeny	Ploidy level (%) 2*x*	3*x*	4*x*
2*x* x 4*x*:					
2*x Ds-1* x 4*x tbr*	22	1385	0.9	0.4	98.7
2*x ds-1* x 4*x tbr*	17	1784	0.4	0.0	99.6
4*x* x 2*x*:					
4*x tbr* x 2*x Ds-1*	10	1227	0.8	0.2	99.0
4*x tbr* x 2*x ds-1*	11	1119	0.1	0.0	99.9
2*x* x 2*x*(*ds-1*;FDR):					
2*x Ds-1* x 2*x ds-1*	19	1900	1.0	0.2	98.8
2*x ds-1* x 2*x ds-1*	22	1624	0.2	0.1	99.7
2*x* x 2*x*(*Ds-1*):					
2*x Ds-1* x 2*x Ds-1*	36	5009	85.7	0.1	14.2[a]
2*x ds-1* x 2*x Ds-1*	21	1465	1.4	0.1	98.5

[a] Range (depending on cross combination): 0.0-35.1 %.

Figure 2. Average nucleolus sizes in 1-4 nucleate embryosacs from normal synaptic (*Ds-1.*) diploid potato clones with (A) low and (B) high levels of SDR, (C) a normal synaptic tetraploid potato variety and (D) a desynaptic (*ds-1ds-1*) diploid potato clone with FDR. 1 epu (eye piece unit) ≈ 0.5 μm. n = no. embryosacs.



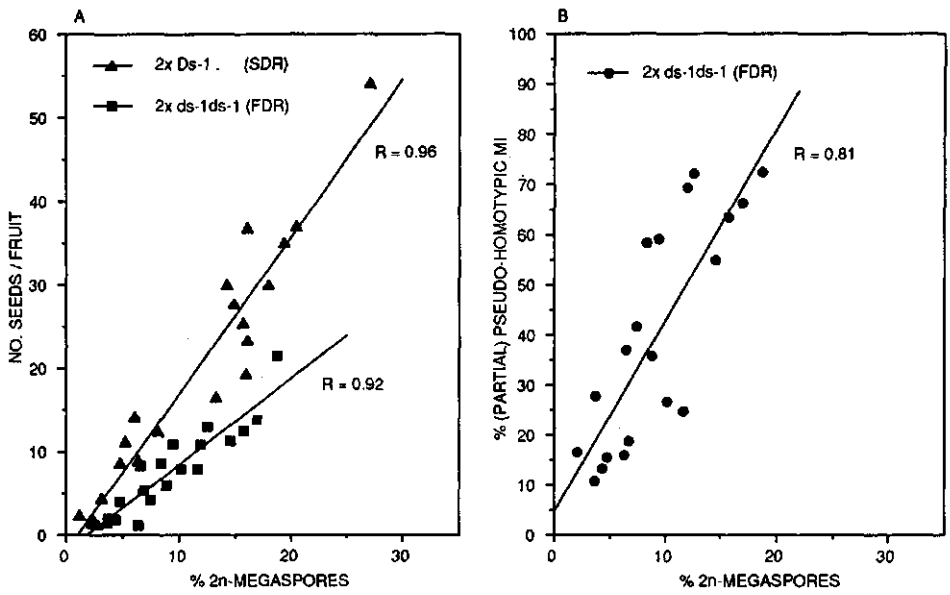
the highest frequencies of tetraploids (99.6-99.9 %) were observed in progenies involving only desynaptic diploid parents. The rare occurrence of diploid (and triploid) progeny from 2*x*-2*x* crosses involving at least one desynaptic parent (Table 4) might be explained by the occasional formation of largely balanced reduced gametes in *ds-1* mutants. The diploid progeny from 2*x*-4*x* crosses might result from pseudogamous parthenogenetic development of (un)reduced diploid egg cells.

Mechanisms of SDR and FDR 2n-gamete formation

Female meiosis was studied in detail in 20 normal synaptic plants and 20 desynaptic mutants with consistent seed set following 2*x*.4*x* and 2*x*.2*x*(*ds-1*;FDR) testcrosses. Normal synaptic clones and desynaptic ones without any seed set collectively served as experimental controls. To avoid bias generated by asynchronous development of reduced and unreduced megaspores, cytological quantification of 2n-megaspore formation was based on the frequencies of dyad megaspores and, whenever present, well developed 1-4 nucleate embryosacs with average nucleolar sizes exceeding 3.5 μm (Stelly and Peloquin, 1985,; cf. Figs. 2A-2D). The frequencies of 2n-megaspores so established closely correlated with seed set following 2*x*.4*x* and 2*x*.2*x*(*ds-1*;FDR) testcrosses (Figure 3A).

The normal course of female meiosis and embryosac formation in potato has been described extensively (Rees-Leonard, 1935; Jongedijk, 1985). Briefly, in female meiosis cytokinesis is of the successive type. Following disjunctional separation of homologous chromosomes at anaphase I, a cell plate is formed across the persisting phragmoplast. The haploid chromosome complements of the resulting daughter cells subsequently divide equationally, giving rise to a tetrad of reduced megaspores. Megagametogenesis in the functional chalazal megaspore follows the *Polygonum* type of embryosac development. (Figs.

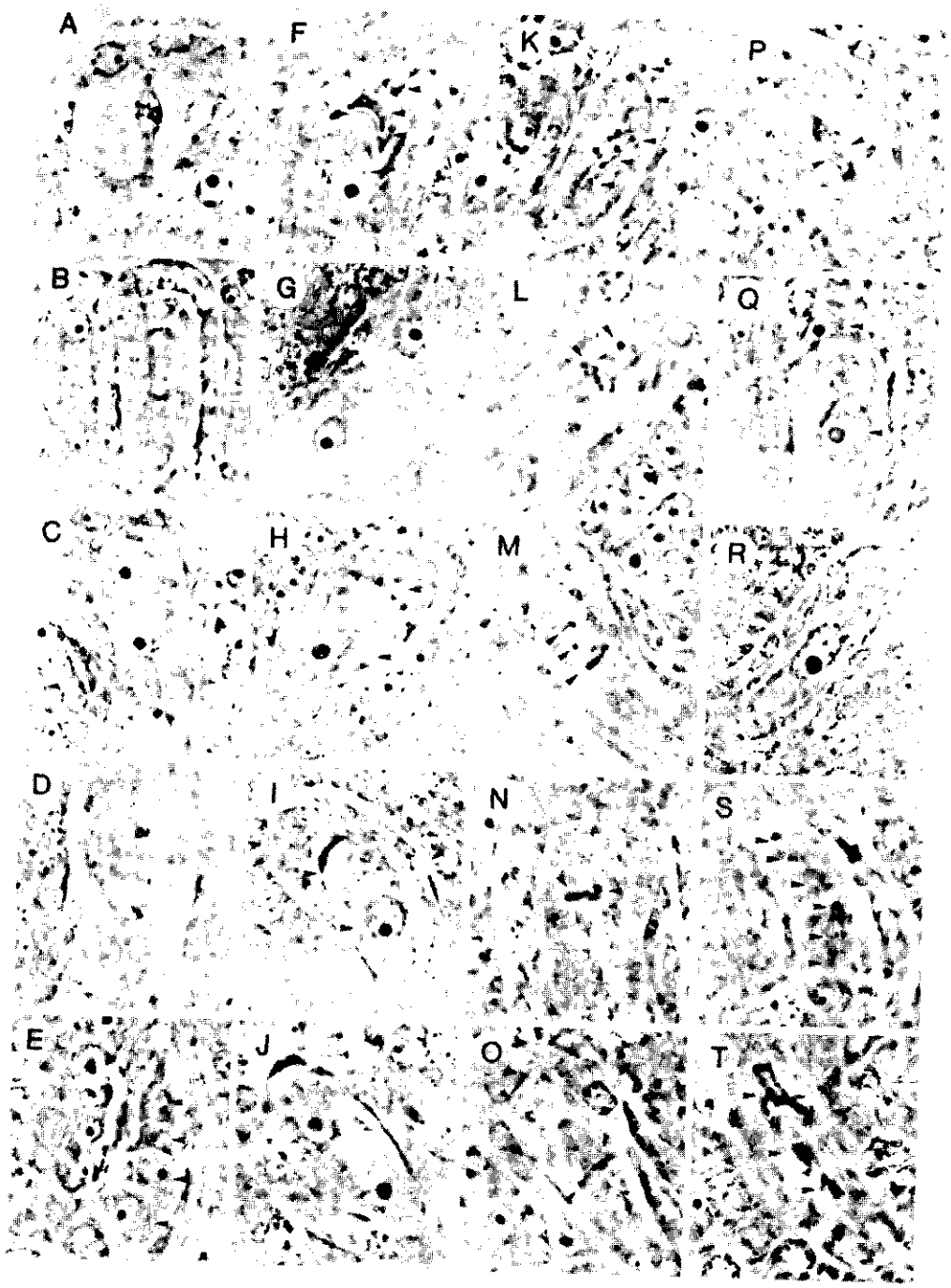
Figure 3. Relationship between the frequency of 2n-megaspores and (A) the number of seeds/fruit following testcrossing in synaptic (*Ds-1*.;SDR) and desynaptic (*ds-1ds-1*;FDR) diploid potato clones (B) the frequency of (partial) pseudo-homotypic metaphase I stages in desynaptic (*ds-1ds-1*;FDR) diploid potato clones.



4A-4G).

In normal synaptic clones with consistent 2n-megaspore formation the developmental sequence differed from normal in the occasional occurrence of irregular chromosome movement at late anaphase I and the relatively frequent incidence of dyad formation, unreduced embryosacs (Figs. 4H-4J) and irregular metaphase II-anaphase II stages (Fig. 4S). These aberrations were interpreted to result from precocious separation of sister chromatids at late anaphase I- prophase II. When all chromosomes are involved, such a precocious chromosome division obviously results in the omission of the second division and thus in the formation of dyads of numerically unreduced megaspores which are genetically equivalent to SDR. If incomplete, however, the daughter cells may enter into metaphase II resulting in random segregation of univalent sister chromatids and subsequent abortion of predominantly aneuploid megaspores. Where irregular second division stages were virtually absent in normal synaptic clones lacking 2n-megaspore formation, their incidence among clones with consistent 2n-megaspore formation amounted to 3.7-25.9 % of all cases, high levels of 2n-megaspore formation invariably being associated with high frequencies of irregular metaphase II stages. The low and insignificant correlation between the frequencies of second division irregularities and the overall levels of female sterility (r=0.39) indicated the latter to be largely determined by factors other than the observed second division irregularities. High levels of female sterility (19.4-43.6 %) were observed in both normal synaptic plants with and without consistent 2n-megaspore formation. Premeiotic and postmeiotic doubling or doubling by failure of cytokinesis in chalazal megaspores followed by fusion of reduced nuclei prior to megagametophyte development, were not observed.

Desynaptic mutants are characterized by normal chromosome pairing throughout pachytene and a falling apart of bivalents at diakinesis (Ramanna, 1983; Jongedijk and Ramanna, 1988).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In *ds-1* mutants lacking 2n-megaspore formation female sterility was nearly complete, not a single pollen parent being succesful in inducing seed set. Meiotic abnormalities further included the occurrence of disfigured spindles (Fig. 4K), random distribution of univalents,occasional univalent division (Fig. 5B), the formation of micro-nuclei and abortion of megaspore mother cells cells before the onset or completion of the second meiotic division.

As expected with the *ds-1* gene being an effective sieve against the formation of functional reduced (and SDR) gametes, among *ds-1* mutants with 2n-megaspore formation highly significant positive correlations were observed between the level of female fertility (0.9-19.3 %) on the one hand and the frequency of 2n-megaspores (r=0.98) and seed set following 2*x*.4*x* and 2*x*.2*x* (*ds-1*;FDR) testcrosses (r=0.94) on the other. Meiotic studies in these mutants typically revealed substantial frequencies of megaspore mother cells with an exceptionally strong tendency to univalent division at late metaphase I-early anaphase I. Univalent division was often, but not necessarily, preceded by an approximate orientation of most univalents at the equatorial region (Figs. 4L-4O), suggesting amphitelic orientation of sister chromatid kinetochores and thus centromere division taking place at early metaphase I-anaphase I. Amphitelic orientation of sister chromatid kinetochores was confirmed in acetocarmine squashes of enzyme digested megaspore mother cells (Figs. 5C-5G) and proved to be particularly apparent in male meiosis of the *ds-1* mutants under consideration (Fig. 5H). As indicated by the "bivalent like" structure of equationally dividing univalents and the frequent occurrence of "chromatin tails" at anaphase I (Fig. 4P) sister chromatid cohesiveness typically persisted beyond metaphase I. Second division stages, if present, were highly irregular (Fig. 4S). Obviously, the equational division of the entire chromosome complement at late metaphase I-early anaphase I (pseudo-homotypic division) resulted in the omission of the second division and thus the formation of dyads of numerically unreduced megaspores, which are genetically equivalent to FDR. However, if incomplete (i.e. partial pseudo-homotypic division), preponderantly aneuploid daughter cells arise which either abort directly or , again, enter into an abortive second division with randomly segregating, univalent sister chromatids. The percentage of 2n-megaspores closely correlated with the frequency of megaspore mother cells with (partial) pseudo-homotypic metaphase I stages (Fig. 3B). Either megaspore mother cells with largely mitotic divisions (mitotized meiosis) or restitution nucleus formation following desynaptic or (partial) pseudo-homotypic anaphase I and subsequent division of univalent chromosomes in the second division (semi-heterotypic division) were not observed in any clone.

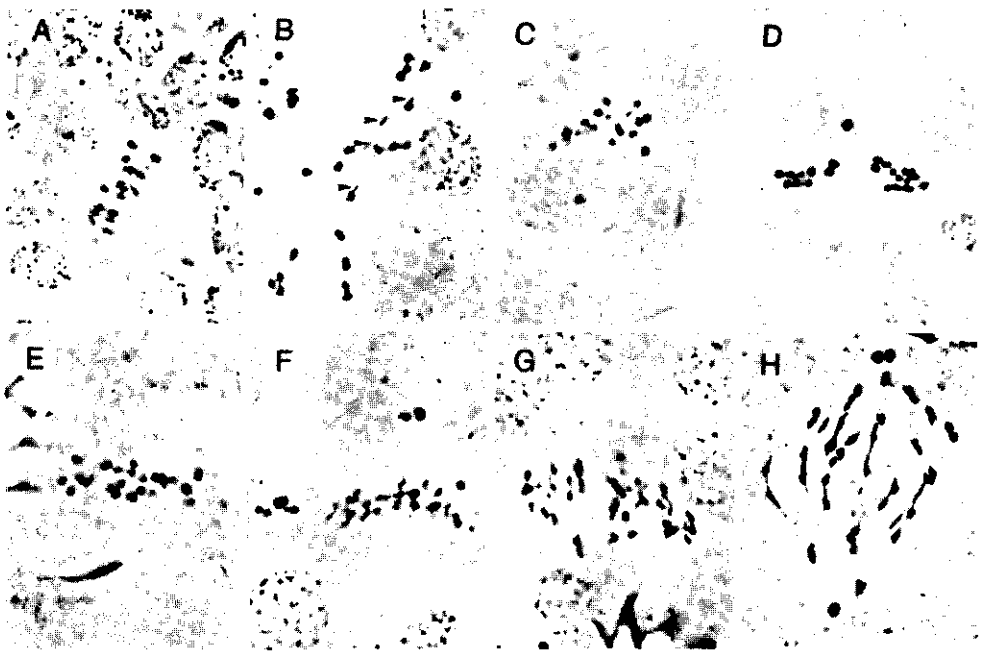
Discussion

Mechanisms of SDR and FDR 2n-megaspore formation

Studying the normal sequence of female meiosis in diploid potato, Jongedijk (1985) inferred that functional 2n-megaspores produced under normal synaptic and under mutant synaptic conditions are likely to originate through SDR and FDR respectively. Cytological and genetical analyses of 2n-megaspore formation have since shown that 2n-megaspore formation in normal synaptic potato clones is by SDR and that the prevailing mechanism of SDR 2n-megaspore formation consists of omission of the second meiotic division following chromosome doubling

Figure 4. Methyl salicylate cleared ovules with typical stages of female meiosis in normal synaptic SDR clones (A-J) and desynaptic FDR clones (K-T). (A) Metaphase I; bivalents. (B) Anaphase I. (C) Interkinesis. (D) Metaphase II. (E-F) Tetrad of reduced megaspores. (G) reduced 2-nucleate embryosac. (H-I) Dyad of SDR 2n-megaspores. (J) unreduced (SDR) 2-nucleate embryosac. (K) Desynaptic metaphase I: univalents. (L-O) Pseudo-homotypic metaphase I; predominant congregation of univalents at equatorial plate. (P) Pseudo-homotypic anaphase I; persisting chromatid cohesiveness. (Q) Dyad of FDR 2n-megaspores. (R) unreduced (FDR) 1-nucleate embryosac. (S) Typical irregular metaphase II; scattered sister chromatids. (T) Degenerating megaspores. (± x700).

Figure 5. Acetocarmine squashes of enzyme digested megaspore mother cells (A-E) and pollen mother cells (F). (A) Metaphase I; bivalents. (B) Desynaptic metaphase I; univalents scattered, two dividing univalents (arrows). (C-E) Pseudo-homotypic metaphase I; predominant congregation of univalents at equatorial plate. (F-H) Pseudo-homotypic anaphase I; (predominant) univalent division, persisting chromatid cohesiveness. (± x1000).



after anaphase I (Stelly and Peloquin, 1986*a*, 1986*b*; Werner and Peloquin, 1987, 1991; Douches and Quiros, 1988*b*). The present study confirms that SDR 2n-megaspore formation in normal synaptic, diploid potato is caused by the precocious separation of sister chromatids at late anaphase I-prophase II. Precocious chromosome division at late anaphase I-prophase II associated with occasional SDR 2n-megaspore formation and high ovule sterility due to second division irregularities has previously been observed in *pc* mutants of tomato (Clayberg, 1959). Frequent second division irregularities in normal synaptic, diploid potato clones with substantial SDR 2n-megaspore formation through omission of the second division was previously observed by Werner and Peloquin (1991). However, presuming subsequent nuclear restitution in both daughter cells they considered it an independent abnormality of the second division and thus a distinct mechanism of SDR 2n-megaspore formation. Also their observation that in typically delayed megaspore mother cells some equational chromosome division may occur at anaphase I ('delayed meiotic division'), might be attributed to division precocity.

'Sub-sexual' processes resulting in FDR 2n-megaspores typically occur in diplosporic apomictic plant species (Gustafsson, 1946; Rutishauser, 1967; Nogler, 1984). One essentially consists of omission of female meiosis in archesporial cells, which instead directly develop into mature gametophytes through successive mitotic divisions (mitotized meiosis or mitotic diplospory). The others typically involve a nearly complete lack of chromosome association at metaphase I and basically consist of (i) equational division of the entire chromosome complement in the second meiotic division following nuclear restitution at anaphase I (semi-heterotypic division) or (ii) direct equational division of univalent chromosomes at metaphase

I and subsequent omission of the second meiotic division (pseudo-homotypic division). The latter mechanism has been claimed to occur in *Taraxacum* , *Erigeron*, *Archieracium* and *Chondrilla* (Gustafsson, 1935; Bergman, 1944) and, as reported here, was often noted to be preceded by an approximate orientation of initially scattered univalents at the equatorial plate. The actual existence of the pseudo-homotypic division has however been disputed and still is considered to be 'very improbable' (Rutishauser,1967; Nogler, 1984).

Studying 2n-megaspore formation in synaptic mutants of potato, Iwanaga and Peloquin (1979) and Werner and Peloquin (1987, 1991) noted the occasional formation of a restitution nucleus including all chromosomes following anaphase I. However, conclusive evidence for FDR 2n-megaspore formation by subsequent equational division of all chromosomes in the second meiotic division was not provided. In the *ds-1* mutants studied here nuclear restitution following either typical desynaptic or (partial) pseudo-homotypic anaphase I stages was consistently absent. The close correlation between the observed percentage of 2n-megaspores on the one hand and the percentage of (partial) pseudo-homotypic metaphases and seed set following 2*x*.4*x* testcrosses on the other (Figs. 3A and 3B) thus imply that FDR 2n-megaspores in these mutants do originate through pseudo-homotypic division. In male meiosis of the same plant material an exceptionally strong tendency for functional FDR 2n-pollen formation through univalent division at late metaphase I-early anaphase I and consistent absence of nuclear restitution following (partial) pseudo-homotypic metaphases has previously been reported by Ramanna (1983). Recently both 2n-pollen and 2n-megaspore formation through pseudo-homotypic division has also been found in desynaptic tomato lines (Ramanna *et al.*, 1991). As far as the authors are aware these are the first and only documented cases of functional FDR 2n-megaspore formation through pseudo-homotypic division in otherwise typically sexual plant species. The exceptionally strong tendency for univalent division, however, is by no means unique. Though generally associated with extreme sterility, it has previously been noted in (a)synaptic mutants of *Brassica campestris* (Stringham, 1970), *Zea mays* (Golubovskaya and Mashnenkov, 1975), *Crepis capillaris* (Richardson, 1935), *Oenothera decipiens* (Catcheside, 1939) and *Alopecurus myosuroides* (Johnsson, 1944) and in interspecific hybrids of several plant species (Meurman, 1931; Lamm, 1941; Maan and Sasakuma, 1977).

The striking similarities of chromosome behaviour in normal synaptic SDR and desynaptic FDR clones suggests the observed mechanisms of SDR and FDR 2n-megaspore formation to be closely interrelated. Both basically consist of precocious chromosome division. Depending on whether or not all chromosomes are involved this precocious chromosome division results either in the omission of the second division and subsequent dyad formation or in the abortion of largely aneuploid megaspores following random segregation of sister chromatids in the second division. In fact the simultaneous occurrence of normal synaptic and of desynaptic progeny both with and without consistent 2n-megaspore formation through precocious chromosome division in all crosses (Table 3; Fig. 1) indicates division precocity per se to be controlled by a common genetic factor, with the gene or genes involved being independent of *ds-1* and exerting a similar effect on chromosome division in both categories of clones. However, where in normal synaptic plants SDR 2n-megaspores consisting of 'sister' chromatids result from post-reductional division precocity, division precocity in *ds-1* mutants is basically pre-reductional and 'non-sister' chromatids are included in FDR megaspores. This apparent difference in the relative timing of division precocity might simply be attributed to the mutant synaptic condition; Synaptic abnormalities including those conferred by the *ds-1* gene have been noted to considerably prolong the duration of metaphase I-anaphase I (Clayberg, 1958; Wagenaar, 1961a, 1961b; Jongedijk and Ramanna, 1989). Such prolongation when associated and with chromosome division largely proceeding along the normal time lines, generally causes a loss of synchrony between cell cycle and chromosome division (Wagenaar, 1968; Golubovskaya, 1979; Koduru and Rao, 1981; Kaul and Murthy, 1985). According to Wagenaar (1968) under such conditions "time is a limiting factor for chromosome division" and

"metabolic processes imposing interphase on dividing cells" interrupt and effectively terminate chromosome division by the breakdown of the spindle and the formation of nuclear membranes enclosing chromosomal material wherever located in the cell. Obviously, genes causing precocious chromosome division as early as late anaphaseI-prophaseII in case of normal synapsis, if similarly expressed in *ds-1* mutants, are likely to take effect in the typically prolonged metaphase I-anaphase I. In *ds-1* mutants they might thus allow for univalent orientation and for largely regular but basically pre-reductional univalent division (pseudo-homotypic division) before the cell is interrupted by "imposition of interphase". In this perspective the observed mechanisms of SDR (i.e., 'omission of the second division') and FDR (i.e., pseudo-homotypic division) 2n-megaspore formation should be considered extremes of a continuum that is being brought about by common genes for division precocity, which depending on the relative timing of cell cycle and chromosome division may impose post-reductional (SDR) or pre-reductional (FDR) 'restitution' of the sporophytic chromosome number under normal and mutant synaptic conditions respectively.

Studying chromosome behaviour in the semi-dominant meiotic mutants *ord* and *mei-S332* and wild type *Drosophila*, Goldstein (1980) and Lin and Church (1982) concluded that the predominantly post-reductional division precocity in the former mutants resulted from reduced chromatid cohesiveness rather than from kinetochore properties. The unmistakably amphitelic orientation of sister chromatid kinetochores but frequent persistence of some degree of sister chromatid cohesion in (partial) pseudo-homotypic metaphases from *ds-1* mutants (Figs. 5C-H) indicates that the same holds true for the precocious chromosome division in normal and desynaptic potato clones reported here. Among plant species reduced sister chromatid cohesiveness has previously been inferred to cause division precocity in *pc* mutants of tomato (Clayberg, 1959). In addition it might also be involved in SDR 2n-megaspore formation in the *dy*, *el* and *tri* mutants from *Datura*, maize and barley respectively (Satina and Blakeslee, 1935; Rhoades and Dempsey, 1966; Finch and Bennett, 1979) and in FDR 2n-megaspore formation through pseudo-homotypic division observed in some diplosporous apomicts (Gustafsson, 1946; Rutishauser, 1967).

Genetic basis of 2n-megaspore formation

Where desynapsis is controlled by a single recessive gene and similarly affects male and female meiosis (Ramanna, 1983: Jongedijk and Ramanna, 1988, 1989: Jongedijk *et al.*, 1991), the expression and genetic basis of precocious chromosome division and thus 2n-megaspore formation appears to be more complicated. As indicated by the substantial frequencies of megaspore mother cells with largely normal meiosis in SDR clones and of typical desynaptic meiosis in FDR clones, expression is only partial. In addition, levels of precocious chromosome division in different cells from a single clone and the overall frequency of cells affected in male and in female meiosis may differ considerably (unpublished results). The reason for these sex differences in the expression pattern are as yet obscure. However, sex differences in the overall meiotic sequence (e.g. simultaneous (♂) versus successive (♀) cytokinesis), in the timing of meiosis and meiotic stages (e.g. earlier onset and relatively synchronous course of male meiosis) and/or in the fine tuning of the cycles of cell division and chromosome division might be of crucial importance.

Recently, Werner and Peloquin (1991) claimed that SDR 2n-megaspore formation through omission of the second division in normal synaptic potato clones is controlled by a single recessive gene, *os*. Obviously the *os* gene may be presumed to cause post-reductional division precocity, which if affecting all chromosomes results in the formation of SDR 2n-megaspores. However, in view of the present results from testcrossing of normal synaptic segregants (Table 3; Fig. 1), monogenic control of SDR 2n-megaspore formation is questionable. Firstly, the large variation in seed set within single cross progenies suggests involvement of additional genetic factors that control the frequency and the level of post-reductional division precocity and thus functional SDR 2n-megaspore formation. Secondly,

assuming homozygosity (*osos*) of the high seed set parents B and D (12.3 and 25.3 seeds/fruit respectively) and heterozygosity (*Osos*) of the low seed set parents C and E (1.1 and 1.8 seeds/fruit respectively) only 50% of the normal synaptic BE/EB and DE/ED progeny and 25% of the normal synaptic CE/EC progeny is expected to form some SDR 2n-megaspores. Assuming single gene control, the observed large excess of normal synaptic SDR clones in the latter crosses (Table 3) would indicate semi-dominant expression of *os* rather than recessiveness. Regardless of the exact genetic basis of 2n-megaspore formation the seed set data demonstrate the possibility to select for increased rates of SDR and consistent FDR 2n-megaspore formation in normal synaptic and desynaptic cross progeny respectively.

FDR 2n-megaspore formation in evolution and breeding

The high frequency of 2n-megaspore producing clones among the normal synaptic and the desynaptic progeny supports the hypothesis that sexual polyploidization is the driving force behind the origin and evolution of polyploid *Solanum* species (Den Nijs and Peloquin, 1977). The evolutionary significance of SDR 2n-megaspores has only recently been recognized (Stelly and Peloquin, 1986*a*, 1986*b*; Douches and Quiros, 1988*b*). The present results suggest that also FDR 2n-megaspore formation, though admittedly less frequent, may be more important than commonly thought.

Whereas diploid potato clones with consistently high levels of SDR 2n-megaspores formation have previously been identified among a variety of *Solanum* species (Stelly and Peloquin, 1986*b*; Werner and Peloquin, 1987, 1991; Douches and Quiros, 1988*b*), levels of FDR 2n-megaspore formation reported so far are quite low (Iwanaga and Peloquin, 1979; Werner and Peloquin, 1991). Obviously, this may be attributed to the low number of synaptic mutants included in studies on 2n-megaspore formation so far. In the present study systematic screening of desynaptic mutants revealed FDR 2n-megaspore formation in 45.2% of all *ds-1* mutants. Although the majority of *ds-1* mutants on the average formed less than 5 seeds/fruit following testcrossing, 14% produced FDR 2n-megaspores in frequencies that resulted in consistent seed set within the 5-25 seeds/fruit range and allowed for routine production of nearly exclusive tetraploid progeny from 2*x*(*ds-1*;FDR).4*x*, 2*x*(*ds-1*;FDR).2*x*(*Ds-1*;SDR/FDR) and 2*x*(*ds-1*;FDR).2*x*(*ds-1*;FDR) crosses. The present availability of diploid potato clones with consistent FDR 2n-megaspore formation thus extends the opportunities for direct transfer of enhanced diploid germplasm to tetraploids. In particular it advocates the application of 2*x*(*ds-1*;FDR).2*x*(*ds-1*;FDR) breeding schemes in cultivar development and in the production of relatively vigorous and uniform true potato seed (TPS) varieties, because FDR 2n-gametes from *ds-1* mutants are relatively efficient in preserving the genetic constitution of the parental clone (Jongedijk *et al.*, 1991).

It should be recognized that mutant synaptic genes also impose certain limitations on their use in breeding. They have to be manipulated in heterozygous condition because they are generally expressed in both male and female meiosis and thus are either largely sterile or produce only functional FDR 2n-gametes resulting in polyploidization upon crossing. As outlined by Hermsen *et al.* (1985) breeding schemes that consist of (i) introducing mutant synaptic genes and genes for FDR 2n-gamete formation in advanced diploids through backcrossing and (ii) subsequent selection of improved mutant synaptic segregants with FDR 2n-gamete formation following intercrossing of advanced heterozygotes, would be appropriate but laborious. Furthermore it should be realized that mutant synaptic conditions are actually required for FDR 2n-megaspore formation. Since heterozygous diploid clones are normal synaptic and thus at best form SDR 2n-megaspores the question remains how to predict whether or not such clones carry genes that will cause substantial FDR 2n-megaspore formation in derived synaptic mutants. If indeed SDR and FDR 2n-megaspore formation are caused by common genes for division precocity, the occurrence of SDR in normal synaptic heterozygotes, particularly if associated with substantial precocious chromosome division as

early as anaphase I, might be a helpfull criterion.

As to the proposed breeding for diplosporous apomixis in potato by combining genes for asynapsis/desynapsis, FDR 2n-megaspore formation and pseudogamous seed development (Hermsen, 1980; Hermsen *et al.*, 1985; Jongedijk, 1985) largely similar limitations are encountered. Nevertheless, some desynaptic clones formed substantial amounts of FDR 2n-megagametophytes of diplosporic origin and pseudogamous seed development from FDR (and SDR) 2n-eggs could be induced using marked *S.phureja* pollinators (unpublished results). These observations strongly support the hypothesis that gametophytic apomixis comprises of a number of distinct and genetically controlled elements (Petrov, 1970; Asker, 1980; Hermsen, 1980; Matzk,1982). In addition, they demonstrate the feasibility of their combination to attain pseudogamous diplosporic apomixis allowing approximately identical reproduction in largely sexual plant species. The application of this approach to produce uniform true potato seed varieties obviously requires breeding for increased levels of FDR 2n-megaspore formation in synaptic mutants and either introduction of genes for pseudogamy in these clones or the development of an efficient system for pseudogamous seed production using marked *S.phureja* pollinators.

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CHAPTER 7

SYNAPTIC MUTANTS IN POTATO, *SOLANUM TUBEROSUM* L. III. EFFECT OF THE *Ds-1/ds-1* LOCUS (DESYNAPSIS) ON GENETIC RECOMBINATION IN MALE AND FEMALE MEIOSIS

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Summary

By applying half-tetrad analysis to segregating tetraploid progenies that had been raised from 2*x*-4*x* and 2*x*-2*x* crosses 5 marker loci (*ym*, *y*, *Got-1*, *Got-2* and *ds-1*) were mapped to their respective centromeres in male and female meiosis of both normal synaptic and desynaptic (*ds-1ds-1*) diploid potato clones. Significant sex differences in genetic recombination for these loci did not occur in either normal plants or desynaptic mutants, which suggests that genetic exchange in both the sexes is governed by a the same system. In desynaptic mutants a severe reduction in crossing-over was observed for *ym* and *y* (83.7 and 89.1 % reduction respectively), whereas recombination rates for *Got-2* appeared to have systematically, although not significantly, increased. The *ds-1* gene was concluded to substantially reduce the overall chiasma frequency and to differentially alter chiasma distribution along individual chromosomes. Based on segregation ratios in progenies from different types of test-crosses, first division restitution (FDR) and second division restitution (SDR) 2n-gametes formed by normal synaptic plants were estimated to transmit on average about 82.7 and 36.1 %, respectively, of the parental heterozygosity to tetraploids respectively. With desynapsis the average amount of heterozygosity transmitted by FDR 2n-gametes amounted to 94.1 %. SDR 2n-gametes from desynaptic mutants are sterile as a result of aneuploidy. The *ds-1* gene was demonstrated to particularly enhance the ability of FDR 2n-gametes to preserve the genetic constitution of diploid parental clones with a minimum amount of reassortment. The potential value and limitations of the *ds-1* gene for the production of true potato seed varieties and the experimental induction of diplosporic apomixis are discussed.

Key words: *Solanum*, genetic markers, gene-centromere mapping, desynapsis, reduced recombination.

Introduction

The frequent occurrence of numerically unreduced or 2n-gametes in diploid potato species enables direct transfer of enhanced diploid germplasm to tetraploids by means of unilateral (4*x*-2*x* crosses) or bilateral (2*x*-2*x* crosses) sexual polyploidization (Mendiburu *et al.*, 1974; Peloquin, 1982; Hermsen, 1984*a*). Two genetically distinct modes of 2n-gamete formation are generally distinguished: first division restitution (FDR) and second division restitution (SDR). Basically FDR 2n-gametes are formed by equational division of the entire (i.e., numerically unreduced) chromosome complement, whereas SDR 2n-gametes result from chromosome doubling in the haploid nuclei that are formed after completion of the first meiotic division. In both cases two of the four chromatids of a homologous pair of chromosomes are recovered in a single spore. Whereas FDR 2n-gametes comprise "non-sister chromatids", in SDR 2n-

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gametes the "sister chromatids" are included (Peloquin, 1983*a*; Hermsen, 1984*a*).

For breeding schemes employing sexual polyploidization, the combination of FDR 2n-gameteformation with mutant synaptic genes is of particular significance. FDR 2n-gametes are expected to typically preserve a relatively large amount of the parental heterozygosity (Mendiburu *et al.*, 1974; Peloquin, 1983*a*; Hermsen, 1984*b*) and thus significantly contribute to both vigour and homogeneity of tetraploid progeny recovered by sexual polyploidization (Mok and Peloquin, 1975*a*; De Jong and Tai, 1977; Mc Hale and Lauer, 1981). Mutant synaptic conditions are known to be required for consistent FDR 2n-megaspore formation (Jongedijk, 1985) and to favour the exclusive occurrence of functional FDR 2n-gametes in both male and female meiosis (Ramanna, 1983; Jongedijk, 1985). Moreover, mutant synaptic genes generally reduce the overall amount of recombination (Baker *et al.*, 1976; Koduru and Rao, 1981; Kaul and Murthy, 1985) and thus provide a means to maximize the ability of FDR 2n-gametesto preserve the genetic constitution of the parental clone from which they derive (Peloquin, 1982; Iwanaga, 1984; Hermsen *et al.*, 1985; Douches and Quiros, 1988*a*).

When the production of vigorous and uniform true potato seed (TPS) varieties through either 2*x*FDR-2*x*FDR crosses (Peloquin, 1982, 1983*b*) or the induction of diplosporic apomixis (Hermsen, 1980; Hermsen *et al.*, 1985; Jongedijk, 1985) is considered, combination of FDR 2n-megaspore formation with mutant synaptic genes, which substantially reduce gene recombination, is essential. Asynaptic mutants, particularly those with a virtually complete absence of homologous chromosome pairing, obviously are the most attractive to be used. As cogent cases of asynapsis have not yet been identified in potato, desynaptic mutants, characterized by precocious separation of bivalents following normal chromosome pairing, provide the best alternative currently available.

Recently a number of hitherto reported synaptic mutants were shown to be allelic and to display typical desynaptic behaviour in both male and female meiosis (Jongedijk and Ramanna, 1988). Supplementary studies of these *ds-1* mutants have indicated a concurrent reduction of chiasma frequencies in male and female meiosis (Jongedijk and Ramanna, 1989) and substantially reduced genetic recombination rates in male meiosis (Douches and Quiros, 1988*a*). In the case of female meiosis, data concerning the effect of the *ds-1* gene on genetic recombination are still lacking. To establish the potential of *ds-1* mutants for the production of uniform TPS varieties through either bilateral sexual polyploidization or the engineering of diplosporic apomixis, a study of genetic recombination in female meiosis of normal synaptic plants and (de)synaptic mutants is required. Comparative analysis of male and female meiosis, in addition, may reveal whether sex differences in the effect of mutant synaptic genes on genetic recombination occur in potato.

Genetic recombination rates at specific chromosome segments are generally estimated from observed recombination frequencies between physically linked marker loci. Whereas this approach provides reliable estimates in normal synaptic plants, in synaptic mutants such estimates may be biased as a consequence of preferential survival of progeny resulting from meiocytes with reasonably balanced chromosome disjunction. However, such a bias is avoided when genetic marker loci are mapped relative to their respective centromeres by means of half-tetrad analysis (HTA). HTA employs the feature that two of the four chromatids from a homologous pair of chromosomes are recovered in 2n-spores. With the identification of the mode of 2n-gamete formation (i.e., the manner in which a pair of chromatids is recovered in 2n-gametes) in diploid clones heterozygous at a marker locus, the frequency of crossing-over between that locus and the centromere may be estimated from the frequency of tetraploid progeny classes obtained from 4*x*-2*x* or 2*x*-2*x* testcrosses (Mendiburu and Peloquin, 1979).

At present the availability of diploid potato clones that combine either normal synapsis and exclusive SDR 2n-megaspore formation or desynapsis and exclusive FDR 2n-megaspore formation (Stelly and Peloquin, 1986; Douches and Quiros, 1988*b*; Jongedijk *et al.*, 1991) enables routine production of tetraploid offspring from 2*x*-4*x* and 2*x*-2*x* testcrosses and thus

permits reliable gene-centromere mapping of marker loci in normal synaptic and desynaptic female meiosis. In the present paper comparative data on genetic recombination in male and female meiosis of both normal synaptic potato clones and *ds-1* mutants are provided.

Materials and methods

Comparative gene-centromere mapping

Assuming that double or higher order crossing-over does not occur in potato, the ratio of genotypic classes among tetraploid progeny from 2*x*(Aa)-4*x*(aaaa) and 2*x*(Aa)-2*x*(aa) testcrosses is expected to equal (Mendiburu and Peloquin, 1979):

$$f(AAaa) : f(Aaaa) : f(aaaa) = \tfrac{1}{4}px + \tfrac{1}{2}(1-p)(1-x) : x + p - 3/2.px : \tfrac{1}{4}px + \tfrac{1}{2}(1-p)(1-x),$$

where f is the frequency of genotypic classes, x and 1- x are the frequencies of FDR and SDR 2n-gametes from the heterozygous diploid parent ($0 \leq x \leq 1$) and p and 1- p are the frequencies of meiocytes with single and no crossing-over between the A/a locus and the centromere ($0 \leq p \leq 1$). With codominant alleles, allowing discrimination between duplex and simplex heterozygotes, the gene-centromere map distance (z) in case of either exclusive FDR (i.e., x=1) or exclusive SDR (i.e., x=0) 2n-gamete formation may thus be estimated by the maximum likelihood estimators:

$$z_{FDR} = \tfrac{1}{2}p = [1 - f(simplex)].100 \text{ cM and } z_{SDR} = \tfrac{1}{2}p = [\tfrac{1}{2}.f(simplex)].100 \text{ cM}.$$

For traits exhibiting dominance (A>a), that is when only two progeny classes (heterozygotes versus homozygotes) can be distinguished, these gene-centromere map distances may be estimated as:

$$z_{FDR} = \tfrac{1}{2}p = 2.f(nulliplex).100 \text{ cM and } z_{SDR} = \tfrac{1}{2}p = [\tfrac{1}{2} - f(nulliplex)].100 \text{ cM}.$$

Estimates of gene-centromere map distances in male and female meiosis of closely related normal synaptic and desynaptic diploid potato clones were compared by calculating binomial confidence intervals $[f_i ; f_j]$ for either f(simplex) or f(nulliplex) according to Fruend (1971):

$$[f_i ; f_j] = n/(n+u^2) \,.\, [f + u^2/2n \pm u.\sqrt{\{f(1-f)/n + u^2/4n^2\}}],$$

where $u = u_{\alpha/2}$ and n is the total number of tetraploid progeny. The corresponding confidence intervals for estimated gene-centromere map distances $[z_i ; z_j]$ were subsequently derived by substituting f_i and f_j in the relevant mapping formulas. Segregation data concerning similar tetraploid families were pooled, whenever they proved homogeneous ($P [X^2(\text{homogeneity})] \geq 0.05$).

Genetic markers

Five monogenic marker loci were included: (i) yellow margin (*ym*), yellow margin recessive to normal and characterized by relatively small, roundish leaflets with glossy appearance and yellow or sometimes reddish margins (Dodds and Paxman, 1962; Hermsen *et al.*, 1978); (ii) tuber flesh colour (*y*), white flesh recessive to yellow, with modifying genes of relatively small effect that determine the degree of yellowness (Howard, 1970; Jongedijk *et al.*, 1990); (iii) desynapsis (*ds-1*), desynapsis recessive to normal synapsis and characterized by normal chromosome pairing through pachytene and falling apart of bivalents at diakinesis in both male and female meiosis (Ramanna, 1983; Jongedijk and Ramanna, 1988); and (iv and v) glutamate oxaloacetate transaminase (*Got-1* and *Got-2*), dimeric isozymes controlled by two independently segregating loci, each with several codominantly expressed alleles (Oliver and

Martinez-Zapater, 1985; Jongedijk *et al.*, 1990).

Segregation for yellow margin among tetraploid cross progeny was recorded from the four- to five-leaf seedling stage onwards. Tuber flesh colour was scored on a graduated scale, according to Jongedijk *et al.* (1990). Mature, field grown tubers were sampled in 2 successive years and segregation ratios were based on calculated mean scores, the tuber flesh colour classes 4-5 (white-creamy white) and 6-8 (pale yellow-deep yellow) being considered as white and yellow respectively. Employing a routine acetocarmine (2%) squash method and a routine methyl salicylate ovule clearing technique (Jongedijk, 1987), segregation ratios for desynapsis were assessed on the basis of the extent of metaphase I chromosome association in male and (or) female meiosis. GOT isozymes (E.C.2.6.1.1.) were assayed in freshly prepared leaf samples by polyacrylamide gradient-gel electrophoresis (PAGE) as previously described (Suurs *et al.*, 1989).

Plant material

The diploid parental clones included USW5295-7 (coded B), USW5337-3 (coded C), USW7589-2 (coded D), 77-2102-37 (coded E) and derived F_1 hybrids (two-letter codes). Detailed information concerning the origin and pedigree of these clones has been summarized earlier (Jongedijk and Ramanna, 1988). All diploid clones were selected on the basis of both their marker genotype (Jongedijk *et al.*, 1990) and their ability to produce relatively high proportions of either SDR or FDR 2n-pollen and (or) 2n-eggs (Ramanna, 1983; Jongedijk *et al.*, 1991).

The tetraploid parental clones included the white fleshed potato varieties Astarte and Blanka (coded 4*xy*) and the yellow margin *Solanum phureja* selections 76-1-15, (76-1-15 x CE101)-1,2 and (76-1-15 x PM7)-1 (coded 4*xym*). The latter had been selected on the basis of flowering ability and female-male fertility. The clones 76-1-15 (4*x*) and PM7 (2*x*) were kindly provided by

Table 1. Testcrosses used for HTA-mapping of *y*, *ym*, *ds-1*, *Got-1* and *Got-2* in male and female meiosis of normal synaptic (*Ds-1.*) and desynaptic (*ds-1ds-1*) diploid potato clones. Tetraploid white fleshed (*yyyy*) and yellow margin (*ymymymym*) clones are designated 4*xy* and 4*xym* respectively.

Marker locus	2x-2x and 2x-4x testcrosses			
	Parental genotypes ♀	♂	Relevant mapping features	Cross combination
y[a]	*Yy* (8)	*yy* (4)	♀: *Ds-1.* - SDR	BC43 x CE10
			♀: *ds-1ds-1* - FDR	CB21 x CE10 ; CE8 x CE10 ; CE8 x B
	yyyy (4)	*Yy* (8)	♂: *Ds-1.* - FDR	4*xy* x C
			♂: *ds-1ds-1* - FDR	4*xy* x CB50 ; 4*xy* x CE8
ym	*Ymym*	*ymymymym*	♀: *Ds-1.* - SDR	BE44 x 4*xym* ; E x 4*xym* ; ED126 x 4*xym*
			♀: *ds-1ds-1* - FDR	CE8 x 4*xym* ; CE13 x 4*xym* ; CE129 x 4*xym*
				CE149 x 4*xym* ; DE35 x 4*xym* ; EB104 x 4*xym*
	ymymymym	*Ymym*	♂: *Ds-1.* - FDR	4*xym* x BE44 ; 4*xym* x E
			♂: *ds-1ds-1* - FDR	4*xym* x CE10 ; 4*xym* x CE101
Got-2	2^s2^n	2^s2^s	♀: *Ds-1.* - SDR	ED126 x BE62
			♀: *ds-1ds-1* - FDR	CE8 x E ; ED38 x BE62
	2^s2^s	2^s2^n	♂: *Ds-1.* - FDR	CE13 x C
			♂: *ds-1ds-1* - FDR	CE120 x CE10 ; EB104 x CE10
Got-1	1^s1^f	1^f1^f	♀: *Ds-1.* - SDR	CE120 x B ; CE120 x CE10 ; DE28 x CE10
				ED126 x BE62
	1^f1^f	1^s1^f	♂: *Ds-1.* - FDR	CE8 x E
ds-1	*Ds-1ds-1*	*ds-1ds-1*	♀: *Ds-1.* - SDR	BC43 x CE10 ; D x CE10
	ds-1ds-1	*Ds-1ds-1*	♂: *Ds-1.* - FDR	CE13 x B ; CE13 x E ; CE8 x E

[a] Control populations:
- *yy (4)* [♀: *Ds-1* . - SDR] x *yy (4)* [♂: *ds-1ds-1* - FDR] : D x CE10
- *yy (5)* [♀: *ds-1ds-1* - FDR] x *yy (4)* [♂: *Ds-1* . - FDR] : CE13 x B

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Crosses were made on plants grafted onto tomato rootstock. To prevent selfing, the flowers of the seed parents were emasculated well before anthesis. Tetraploid cross progeny was selected by establishing the mean number of chloroplasts in the stomatal guard cells (Frandsen, 1968) and, in cases of doubt, by counting of chromosome numbers in root tip meristems. All crosses and the marker genotypes of the respective parental clones are summarized in Table 1.

Results

Mode of 2n gamete formation

Precise gene-centromere mapping by means of half-tetrad analysis requires accurate identification of the mode of 2n-gamete formation in the heterozygous testcross parent. Functional 2n-pollen and 2n-megaspores produced by synaptic mutants have been shown to originate through FDR exclusively (Ramanna, 1983; Jongedijk, 1985; Jongedijk *et al.*, 1991). Similarly 2n-megaspore formation in normal synaptic clones may be considered to result from SDR, because (i) consistent FDR 2n-megaspore formation requires mutant synaptic conditions (Jongedijk, 1985) and (ii) extensive cytological studies of megasporogenesis have indicated that 2n-megaspore formation by premeiotic or postmeiotic doubling only rarely, if ever, occur in potato (Stelly and Peloquin, 1986; Werner and Peloquin, 1987; Jongedijk *et al.*, 1991). With normal synaptic male meiosis, on the other hand, functional 2n-pollen may arise through both FDR and SDR. As it is virtually impossible to cytologically monitor SDR 2n-pollen formation (Ramanna, 1979) and thus to accurately establish the actual FDR/SDR ratio, the pollen parents employed for gene-centromere mapping in normal synaptic male meiosis were selected for predominant production of FDR 2n-pollen. Following Ramanna (1979), the latter were identified by screening for a close correspondence between the frequencies of fused spindles in metaphase II (fs) and dyads (Table 2; Fig. 1). Parallel orientation of metaphase-anaphase II spindles may, but need not necessarily result in FDR 2n-pollen formation (cf. Mok and Peloquin, 1975*b* and Ramanna, 1979) and therefore was not taken into account. In normal synaptic clones with dyad frequencies exceeding the frequency of fused spindles, the relative contribution of FDR to the pool of 2n-pollen should thus be considered minimum estimates.

Classification of marker genotypes

Although tuber flesh colour is generally accepted to be controlled by a single gene, with yellow dominant to white, classification usually was not that straightforward. In most segregating populations a gradual rather than a clearcut distinction between white and yellow was observed (Figs. 2A-D). Following Jongedijk *et al.* (1990) we therefore adopted a standardized classification method, which basically consists of scoring tuber flesh colour on a graduated scale, with the ratings 4-5 (white-creamy white) and 6-8 (pale yellow-deep yellow) being considered white (*yyyy*) and yellow (*Y...*) respectively, and minimizing bias generated by differences in genetic background (cf. Figs. 2E and 2F) through the use of parental clones that represent the extremes of the potential range of flesh colour ratings. Doing so, the range of

Pollen parent	Metaphase II		Sporads		% FDR pollen
	No. cells	% fs	No. cells	% dyads	(fs/dyads)
B	378	37.6	498	41.6	90.4
C	401	17.7	607	20.3	87.2
E	333	48.9	444	57.0	85.8
BE-44	267	69.7	513	81.9	85.1

Table 2. Relative contribution of FDR to the pool of 2n-pollen in parental clones employed for gene-centromere mapping in normal synaptic male meiosis.

Figure 1. Acetocarmine squashes of pollen mother cells from the diploid potato clone BE-44. (A) Metaphase II; predominantly fused spindles. (B) Sporad stage; predominantly dyads. Bars represent 10 μm.

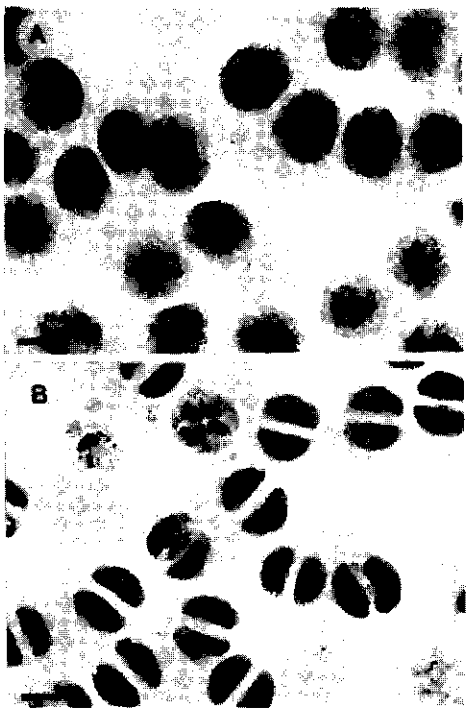
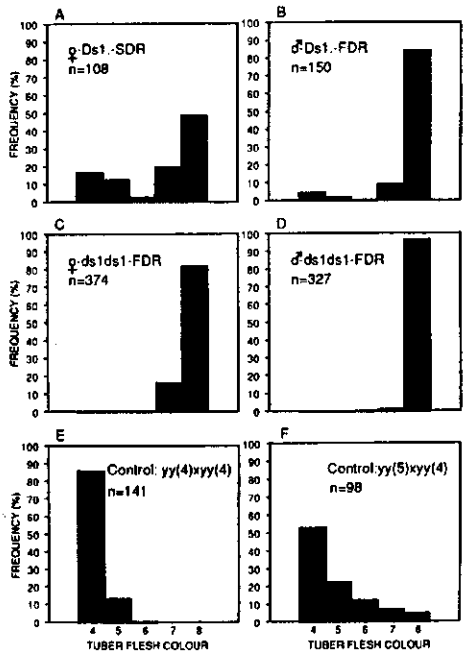


Figure 2. Overall frequency distributions for tuber flesh colour ratings in tetraploid mapping populations (A-D) and control progeny (E-F). (A) *Yy(8)* x *yy(4)*; ♀-*Ds-1*.-SDR. (B) *yyyy(4)* x *Yy(8)*; ♂-*Ds-1*.-FDR. (C) *Yy(8)* x *yy(4)*; ♀-*ds-1ds-1*-FDR. (D) *yyyy(4)* x *Yy(8)*; ♂-*ds-1ds-1*-FDR. (E) *yy(4)* [♀-*Ds-1* .-SDR] x *yy(4)* [♂-*ds-1ds-1*-FDR]. (F) *yy(5)* [♀-*ds-1ds-1*-FDR] x *yy(4)* [♂-*Ds-1* .-FDR].

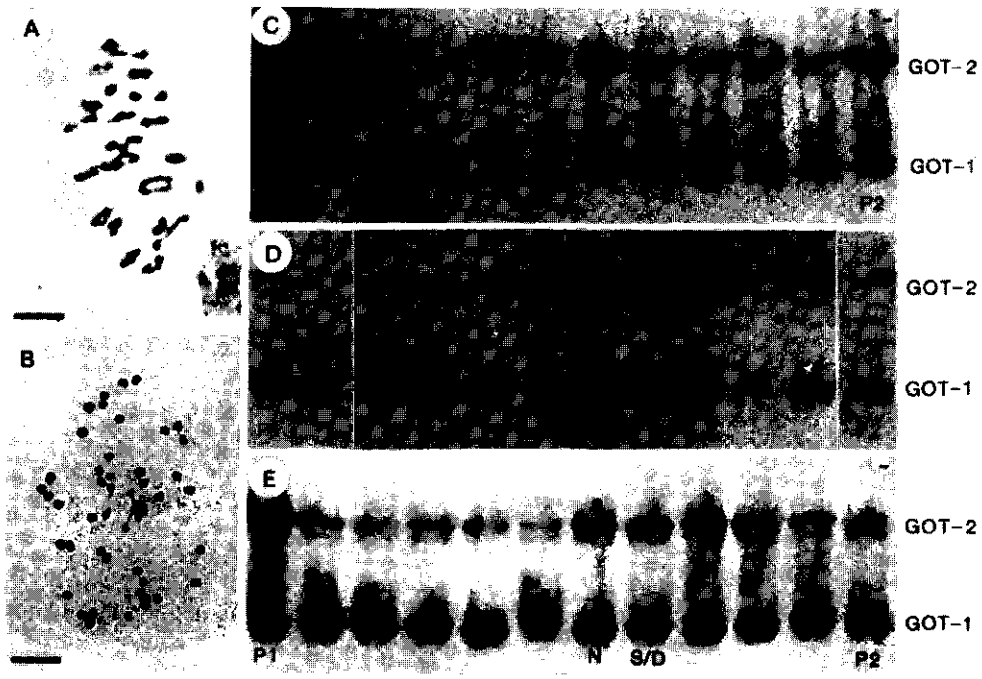


tuber flesh colour ratings observed in standardized control (white-4 x white-4) crosses proved to be by and large consistent with single gene control (Fig. 2E).

Among tetraploid progeny segregation for yellow margin (*ym*) and desynapsis (*ds-1*) could easily be scored. As to desynapsis the extent of metaphase I chromosome association allowed for unambiguous classification of mutant genotypes in most cases (cf. Figs. 3A and 3B). If not, additional criteria such as lack of metaphase I orientation, unbalanced anaphase I separation and relatively high degree of pollen sterility were used for classification.

The codominant expression of *Got-1* and *Got-2* allozymes enabled clearcut identification of homozygous and heterozygous segregants. The good resolution of *Got-2* allozymes moreover allowed differential banding intensities, which result from differences in gene dosage, to be the basis for discrimination between simplex ($2^s2^s2^s2^n$) and duplex ($2^s2^s2^n2^n$) heterozygotes (Figs. 3C-3E) and thus the relatively accurate mapping of the *Got-2* locus. Heterozygosity at the *Got-1* locus was generally detected by the occurrence of a broad, indiscrete zone of enzyme activity (Figs. 2D and 2E), which hampered the detection of differential banding intensities and thus complete classification of heterozygous progeny. As was the case with the marker loci *y*, *ym*,and *ds-1*, gene-centromere map distances for *Got-1* were therefore solely based on the frequency of homozygous nulliplex ($1^f1^f1^f1^f$) progeny.

Figure. 3. Segregation for (A and B) *ds-1* and (C-E) *Got-1* and *Got-2* in tetraploid mapping populations. (A) Normal metaphase I; bivalents and multivalents. (B) Desynaptic metaphase I; predominantly scattered univalents. (C) ED-38 x BE62; *Got-2* mapping in ♀-*ds-1ds-1*-FDR. (D) ED-126 x BE-62; *Got-1* and *Got-2* mapping in ♀-*Ds-1*.-SDR. (E) CE-120 x CE-10; *Got-1* and *Got-2* mapping in ♀-*Ds-1*.-SDR and ♂-*ds-1ds-1*-FDR respectively. P1 and P2, female and male parental phenotype; N, nulliplex phenotype ($1^1 1^1 1^1 1^1$ and $2^s 2^s 2^s 2^s$); S, simplex phenotype ($2^n 2^s 2^s 2^s$); and S and D, simplex or duplex phenotype ($1^s 1^1 1^1 1^1$; $1^s 1^s 1^1 1^1$); -, cathodal end. Bars represent 10 μm. Note: The 2^n allele encodes an 2^1 allozyme subunit capable of forming heterodimeric but no homodimeric active molecules (Oliver and Martinez-Zapater, 1985; Jongedijk *et al.*, 1990).



Comparative gene-centromere mapping

Gene-centromere map distances measure genetic recombination frequencies in a chromosome segment between a locus and its centromere. Consistent differences in gene-centromere linkage of marker loci between normal synaptic plants and (de)synaptic mutants and between male and female meiosis, may thus be considered to result from mutant-specific and sex-specific recombination rates in the marked chromosome segments respectively. To establish whether such specificities occur in potato, *y*, *ym* and *Got-2* were mapped relative to their respective centromeres in both male and female meiosis of closely related normal synaptic (*Ds-1*.) and desynaptic (*ds-1ds-1*) diploid clones (Table 3). Because half-tetrad analysis does not allow the mapping of *ds-1* in *ds-1* mutants and because heterozygosity for *Got-1* was not observed among *ds-1* mutants that exhibited sufficiently high levels of (FDR) 2n-gameteformation, these latter loci were mapped in male and female meiosis of normal synaptic diploid potato clones only (Table 3).

Significant sex differences in genetic recombination rates at the marked chromosome segments were not observed either among normal synaptic clones or among desynaptic mutants (Table 3). Based on the combined data of male and female meiosis, under normal synaptic conditions average gene-centromere distances of 34.97, 23.70, 20.93, 4.39 and 5.31

Table 3. Gene-centromere map distances for *y*, *ym*, *ds-1*, *Got-1* and *Got-2* in male and female meiosis of normal synaptic (*Ds-1.*) and desynaptic (*ds-1ds-1*) diploid potato clones as estimated by HTA-mapping.

Marker locus	Parental genotypes ♀	Parental genotypes ♂	Relevant mapping features	Tetraploid progeny: nulliplex	duplex	simplex	total	Gene-centromere map distance[a] (cM)	95% binomial confidence interval	No. families[b]
y	*Yy* (8)	*yy* (4)	♀: *Ds-1.* - SDR	32	—— 76 ——		108	20.37[x]	[11.18;28.16]	1
			♀: *ds-1ds-1* - FDR	4	—— 370 ——		374	2.14[y]	[0.83; 5.43]	3
	yyyy (4)	*Yy* (8)	♂: *Ds-1.* - FDR	16	—— 134 ——		150	21.33[x]	[13.35;33.25]	1
			♂: *ds-1ds-1* - FDR	4	—— 323 ——		327	2.45[y]	[0.95; 6.21]	2
ym	*Ymym*	*ymymymym*	♀: *Ds-1.* - SDR	33	—— 204 ——		237	36.08[x]	[30.47;39.58]	3
			♀: *ds-1ds-1* - FDR	28	—— 816 ——		844	6.46[y]	[4.61; 9.51]	6
	ymymymym	*Ymym*	♂: *Ds-1.* - FDR	55	—— 267 ——		322	34.16[x]	[26.73;43.15]	2
			♂: *ds-1ds-1* - FDR	7	—— 354 ——		361	3.88[y]	[1.88; 7.90]	2
Got-2	2^s2^n	2^s2^s	♀: *Ds-1.* - SDR	44	40	8	92	4.35[x]	[2.24; 8.12]	1
			♀: *ds-1ds-1* - FDR	8	10	182	200	9.00[x]	[5.67;13.78]	2
	2^s2^s	2^s2^n	♂: *Ds-1.* - FDR	6	3	144	153	5.88[x]	[3.13;10.80]	1
			♂: *ds-1ds-1* - FDR	11	9	175	195	10.26[x]	[6.74;15.31]	2
Got-1	1^s1^f	1^f1^f	♀: *Ds-1.* - SDR	195	—— 237 ——		432	4.86[x]	[0.15; 9.49]	4
	1^f1^f	1^s1^f	♂: *Ds-1.* - FDR	1	—— 91 ——		92	2.17[x]	[0.38;11.81]	1
ds-1	*Ds-1ds-1*	*ds-1ds-1*	♀: *Ds-1.* - SDR	53	—— 156 ——		209	24.64[x]	[18.33;30.06]	2
	ds-1ds-1	*Ds-1ds-1*	♂: *Ds-1.* - FDR	22	—— 172 ——		194	22.68[x]	[15.22;33.15]	2

[a] For each marker locus, different letters denote a significant difference.
[b] All families within a mapping group are homogeneous at the 5% level.

map units were obtained for *ym*, *ds-1*, *y*, *Got-1* and *Got-2* respectively. In desynaptic mutants *ym*, *y* and *Got-2* were mapped at an average of 5.69, 2.28 and 9.62 map units from their respective centromeres. Whereas the genetic recombination rates in the chromosome segments marked by the quite distal loci *y* and *ym* were significantly reduced in both male and female meiosis of *ds-1* mutants (mean reduction of 83.73 and 89.96 % for *ym* and *y* respectively), the linkage of the proximal locus *Got-2* in *ds-1* mutants was not significantly reduced (Table 3). However, because the more tightly a locus is linked to its centromere the more difficult it is to detect significant differences in gene-centromere linkage, it is likely that genetic recombination rates for *Got-2* in *ds-1* mutants have actually increased.

Transmission of heterozygosity by FDR and SDR 2n gametes

The segregation ratios observed among tetraploid progeny from the different types of test-crosses provide an opportunity to compare the amount of parental heterozygosity transmitted by FDR and SDR 2n-gametes under normal synaptic conditions and to quantify the increase in transmission of heterozygosity by FDR 2n-gametes attained in *ds-1* mutants. As genetic recombination rates at the marked chromosome segments do not differ between sexes, the frequencies of simplex segregants among progeny from 2*x*(*Ds-1.*;SDR) x 2*x*/4*x* and 2*x*/4*x* x 2*x*(*Ds-1.*;FDR) testcrosses may be taken as a measure for the efficiency in transmission of heterozygosity by SDR and FDR 2n-gametes respectively. Similarly, changes in heterozygosity transmission by FDR 2n-gametes in *ds-1* mutants may be assessed by comparing the frequencies of simplex segregants among 2*x*/4*x* x 2*x*(*Ds-1.*;FDR) and 2*x*(*ds-1ds-1*;FDR) x 2*x*/4*x* testcross progeny. According to the half-tetrad rationale nulliplex and duplex segregants are expected to occur in approximately equal frequencies among all testcross progeny. In the case of *y*, *ym*, *Got-1* and *ds-1*, which do not allow discrimination between simplex and duplex heterozygotes, the proportion of simplex segregants may thus be estimated to amount to f(simplex) = 1-2.f(nulliplex). The segregation patterns obtained for *Got-2* enabled direct estimation of the proportion of simplex segregants. For this locus the observed frequencies

Table 4. Transmission of parental heterozygosity by SDR and FDR 2n-gametes under normal synaptic and mutant (de)synaptic conditions calculated on the basis of (estimated) frequencies of simplex progeny in different categories of testcrosses.

Marker locus	Percentage of simplex testcross progeny		
	SDR/*Ds-1*.	FDR/Ds-1.	FDR/*ds-1ds-1*
y	40.7	78.7	97.7
ym	72.2	65.8	94.2
Got-2	8.7	94.1	90.4
Got-1	9.7	97.8	–
ds-1	49.3	77.3	–
Means:			
y - ds-1	36.1	82.7	–
y - Got-2	(40.5)	79.5	94.1

of nulliplex and duplex genotypes (Table 3) were confirmed to be very similar (P $[X^2(1:1)] \geq$ 0.34-0.69).

For each marker locus the proportion of simplex segregants among the marked categories of testcross progeny was determined and the overall amount of heterozygosity transmitted by the corresponding types of 2n-gametes was subsequently estimated as the mean proportion of simplex segregants over all loci within each category. Under normal synaptic conditions FDR proved to be by far superior to SDR in transmitting heterozygosity at the quite proximal loci *ds-1*, *y*, *Got-1* and *Got-2*. Transmission of heterozygosity at the relatively distally positioned *ym* locus, however, was slightly more efficient with SDR. For the five marker loci sampled the overall fraction of heterozygosity transmitted by SDR and FDR gametes was estimated to be 36.1 and 82.7 % respectively (Table 4). For the marker loci *y*, *ym* and *Got-2* the overall fraction of heterozygosity transmitted by FDR gametes was estimated to amount to 79.5 and 94.1 % in normal and desynaptic meiosis respectively. Where the *ds-1* gene obviously did not significantly affect transmission of heterozygosity at the *Got-2* locus, the transmission rates of heterozygosity for *y* and *ym* increased from 78.7 to 97.7 % and from 65.8 to 94.2 % respectively. In the case of the *ym* locus the FDR transmission rate of heterozygosity attained in *ds-1* mutants considerably exceeded that with SDR (Table 4). The significance of the *ds-1* gene for maximizing heterozygosity transmission by FDR gametes is most strikingly illustrated when the overall preservation of parental heterozygosity is considered. For the marker loci *y*, *ym* and *Got-2*, parental heterozygosity at all three loci would be preserved in 48.7 and 83.2 % of the FDR gametes under normal and mutant synaptic conditions respectively. With SDR, which requires normal synapsis, this would have been the case in only 2.6 % of all 2n-gametes.

Discussion

Validity of gene-centromere map distance estimates

In half-tetrad analysis the proportion of recombinant testcross progeny is estimated as 2.[f(duplex)+f(nulliplex)] and f(simplex) in case of FDR and SDR respectively. If the assumption that double or higher order crossing-over between a locus and its centromere does not occur holds true, these estimates accurately measure the proportion of 'single-exchange tetrads' and thus may be equated to the frequency of crossing-over between a locus and its centromere. Whenever multiple exchanges between a locus and its centromere occur in significant frequencies, the frequency of recombinant testcross progeny will depend on (i) the *mean* number and distribution of chiasmata between the locus and its centromere, (ii) the mutual relation between the strands involved in successive chiasmata, and (iii) the type of strand separation, reductional or equational, at the centromere. With random strand assortment at

any chiasma, the proportions of reductional and equational strand separation at a particular locus after n=N chiasmata may be expressed as (Mather, 1935):

$$R_n = \tfrac{1}{2}E_{n-1} = \tfrac{1}{2} - (\tfrac{1}{2})^2 + (\tfrac{1}{2})^3 - (\tfrac{1}{2})^4 \ldots (\tfrac{1}{2})^n.(1 - R_0) \text{ and}$$

$$E_n = 1 - R_n = 1 - \tfrac{1}{2} + (\tfrac{1}{2})^2 - (\tfrac{1}{2})^3 + (\tfrac{1}{2})^4 \ldots (\tfrac{1}{2})^n.(1 - R_0).$$

In the case of SDR, which consists of chromosome doubling in haploid nuclei formed after completion of anaphase I and thus involves reductional strand separation at the centromere (i.e., $R_0=1$ and $1 - R_0=0$), it can be derived that (Fig. 4A):

$$R_n(SDR) = 1/3.[1 - (-\tfrac{1}{2})^{n-1}] = f(\text{nulliplex}) + f(\text{duplex}) = 2.f(\text{nulliplex}) \text{ and}$$

$$E_n(SDR) = 1 - R_n(SDR) = 2/3.[1 - (-\tfrac{1}{2})^n] = f(\text{simplex}).$$

As FDR basically consists of an equational division of the entire chromosome complement and thus involves equational strand separation at the centromere (i.e., $R_0=0$ and $1 - R_0=1$), it can similarly be derived that (Fig. 4A):

$$R_n(FDR) = 1/3.[1 - (-\tfrac{1}{2})^n] = f(\text{nulliplex}) + f(\text{duplex}) = 2.f(\text{nulliplex}) \text{ and}$$

$$E_n(FDR) = 1 - R_n(FDR) = 2/3.[1 - (-\tfrac{1}{2})^{n+1}] = f(\text{simplex}).$$

Assuming absence of chiasma interference, which implies a Poisson distribution of chiasmata ($P[\underline{n}=n] = e^{-\alpha}.\alpha^n/n!$), the relation between the frequency of SDR and FDR derived nulliplex and simplex testcross progeny at the one hand and the *mean* number of chiasmata (x) formed between a locus and its centromere (i.e., the cytological map distance) on the other may thus be expressed as (Fig. 4B):

$$f^{SDR}(\text{simplex}) = \Sigma_n\, e^{-x}.\, x^n/n!.\, 2/3.\, [1-(-\tfrac{1}{2})^n],$$

$$f^{SDR}(\text{nulliplex}) = \Sigma_n\, e^{-x}.\, x^n/n!.\, 1/6.\, [1-(-\tfrac{1}{2})^{n-1}],$$

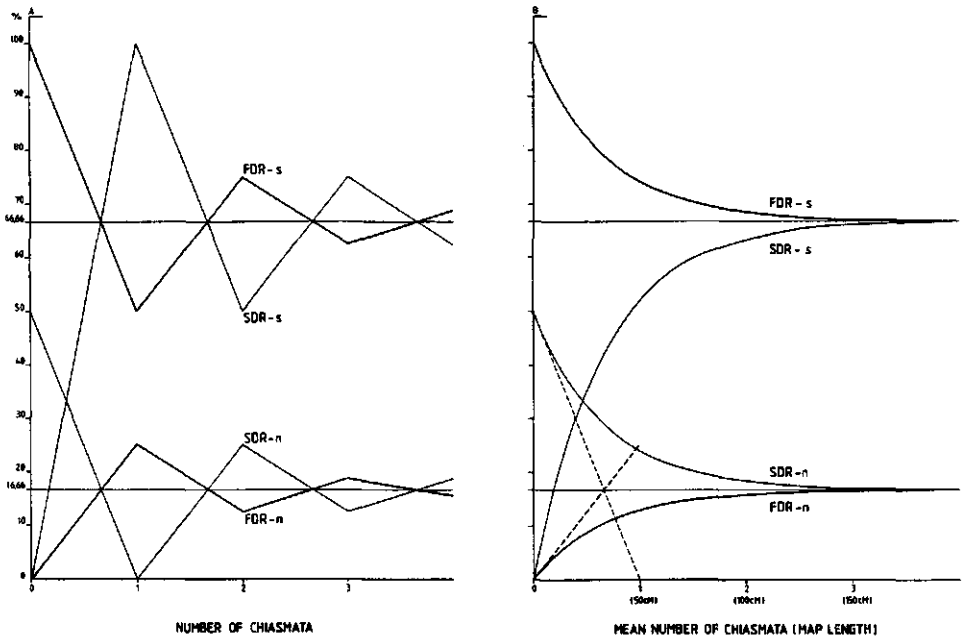
$$f^{FDR}(\text{simplex}) = \Sigma_n\, e^{-x}.\, x^n/n!.\, 2/3.\, [1-(-\tfrac{1}{2})^{n+1}] \text{ and}$$

$$f^{FDR}(\text{nulliplex}) = \Sigma_n\, e^{-x}.\, x^n/n!.\, 1/6.\, [1-(-\tfrac{1}{2})^n].$$

Obviously, the random ratio of genotypic classes among SDR and FDR derived testcross progeny, which theoretically is only achieved with an infinitely large number of chiasmata between a locus and its centromere and thus amounts to f(duplex) : f(simplex) : f(nulliplex) = 1/6 : 4/6 : 1/6 with both SDR and FDR, will be closely approximated after the formation of only relatively few chiasmata (Fig. 4B). Thus, if higher order crossing-over and largely random strand assortment at each chiasma do frequently occur, uncorrected gene-centromere linkages exceeding 33.3 cM would only rarely, if ever, be detected. With considerable positive chiasma interference favouring the formation of single rather than multiple exchanges at marked chromosome segments and/or considerable chromatid interference (Hearne and Huskins, 1935; Kayano, 1959) gene-centromere linkages within the 33.3-50 cM range may be revealed.

In potato the near absence of marker genes with centromere linkages beyond the theoretical limit of 33.3 cM (Douches and Quiros, 1987) suggests that random multiple exchanges might frequently be formed in potato chromosome arms. Since the 95% confidence intervals for centromere linkage of *ym* in normal synaptic male and female meiosis both include the theore-

Figure 4. Relations between the proportion of FDR and SDR derived simplex and nulliplex progeny (FDR-s, FDR-n, SDR-s, and SDR-n) in tetraploid mapping populations and (A) the absolute number of chiasmata between a locus and its centromere or (B) the mean number of randomly distributed chiasmata between a locus and its centromere (cytological map distance). In Fig. 4B the relation between the frequency of FDR and SDR derived nulliplex progeny and the map distance with absence of double or higher order crossing-over are indicated by the broken lines.



tical maximum linkage of 33.3 cM (Table 3), it cannot be concluded that the *ym* locus is indeed linked to its centromere. In view of the relatively small discrepancies in the frequency of genotypic classes with gene-centromere map distances up to 20 map units (Fig. 4B), the average gene-centromere linkages obtained for *y*, *Got-1* and *Got-2* in normal synaptic plants as well as those obtained for *y*, *ym* and *Got-2* in desynaptic mutants (Table 3) may be considered to be largely correct. As to the *ds-1* locus, its actual linkage to the centromere is supported by the fact that it was found to be linked to *Got-1* at a map distance of approximately 28.3 cM (Jongedijk *et al.*, 1990). Depending on whether or not both loci are positioned on opposite chromosome arms, the *ds-1* locus may thus be inferred to actually map about 23.9-32.7 cM apart from its centromere.

Since the formation of at least some SDR 2n-pollen in normal synaptic, male parental clones cannot be excluded, it should be pointed out that the centromere linkage estimates in normal synaptic male meiosis might in fact be slightly biased. The observed segregation ratios for *Got-1* and *Got-2*, which would allow for the occurrence of only insignificant amounts of SDR, as well as the close correspondence between the gene-centromere map distance estimates for all five marker loci in normal synaptic male and female meiosis seem to indicate, however, that SDR 2n-pollen formation may be considered to have had only a marginal effect.

Effect of the ds-1 gene on genetic recombination

Studying chiasmate chromosome association at metaphase I in *ds-1* mutants and normal

sibs, Jongedijk and Ramanna (1989) noted a concurrent reduction of overall chiasma frequencies in male and female meiosis of *ds-1* mutants, which on average amounted to approximately 91%, and concluded that the *ds-1* gene similarly affected chiasma frequencies in all chromosomes.

Comparison of the present data on gene-centromere linkage of *ym*, *y* and *Got-2* in normal plants and *ds-1* mutants demonstrate that the *ds-1* gene differentially affects chiasma formation at the corresponding chromosome segments in both male and female meiosis. An average reduction in crossing-over of (at least) 83.7 and 89.1 % was observed in both sexes for the chromosome segments marked by the distal loci *ym* and *y*, whereas genetic recombination rates for the proximal *Got-2* locus appeared to have increased consistently, although not significantly (Table 3). In male meiosis of *ds-1* mutants a differential but generally severe reduction in genetic recombination has previously been observed for *Sdh-1*, *y*, *Prx-3*, *Idh-1* and *6Pgdh-3* (Douches and Quiros, 1988*a*). Genetic recombination rates for these loci, which under normal synaptic conditions were mapped at a distance of 8.3, 16.8, 18.0, 18.4 and 30.5 cM from their respective centromeres (Douches and Quiros, 1987), were estimated to have decreased with about 94.1, 82.9, 76.9, 98.9 and 92.4 % respectively (Douches and Quiros, 1988*a*). The *ds-1* gene can thus be concluded to substantially reduce the overall amount of chiasma formation and to differentially alter chiasma distribution along individual chromosomes. An overall reduction of both chiasma frequencies and genetic recombination, but normal or even increased recombination rates in specific chromosome segments due to differential changes in chiasma distribution as is reported here, have been observed in (de)synaptic mutants of several plant species (see reviews by Baker *et al.* ,1976; Koduru and Rao, 1981; Kaul and Murthy, 1985).

In some plant species it has been noted that genetic recombination rates may be very different in the two sexes (Rhoades, 1941; Nel, 1975; Moran *et al.*, 1983). Sex differences in chiasma formation have been suggested to result from either independent meiotic control systems operating in male and female meiosis or a differential response of a joint control system to different conditions in male and female meiocytes (Davies and Jones, 1974). Based on the consistent absence of sex differences in overall chiasma frequencies and chiasma distribution among chromosomes, Jongedijk and Ramanna (1989) concluded that chiasma formation in male and female meiosis is likely to be governed by the same control system. Their conclusion is supported by the consistent lack of sex differences in genetic recombination among normal synaptic plants and *ds-1* mutants.

Transmission of heterozygosity by SDR and FDR 2n gametes

Considering all five marker loci included in the present study, SDR and FDR 2n-gametes produced by normal synaptic plants were estimated to transmit on average about 36.1 and 82.7 % of the parental heterozygosity. Sampling a number of 5 and 11 different loci similar rates of overall heterozygosity transmission by SDR and FDR gametes have been reported by Douches and Quiros (1987, 1988*b*). With largely random chiasma formation the difference in heterozygosity transmission by SDR and FDR gametes is expected to be particularly large for loci that are positioned relatively proximally to the centromere and rapidly decreases with increasing gene-centromere map distances (Fig. 4B). Assuming the loci mapped to constitute a random sample of loci in the potato genome, the differences in heterozygosity transmitted by FDR and SDR gametes suggest that most loci map within 50-75 cM from their respective centromeres. This is supported by extensive RFLP analyses which indicate that the potato linkage map is relatively short (Bonierbale *et al.*, 1988; Gebhardt *et al.*, 1989).

FDR gametes formed by *ds-1* mutants transmitted on average 94.1 % of the parental heterozygosity to tetraploids. In comparison, the overall fraction of heterozygosity transferred by FDR pollen under normal synaptic conditions amounted to approximately 79.5 % (Table 4). Sampling the marker loci *y*, *Sdh-1*, *Prx-3*, *Idh-1* and *6Pgdh-3* Douches and Quiros (1988a) estimated that FDR pollen produced by the *ds-1* mutant M6 transmitted on average 98 % of

M6's heterozygosity to tetraploids, being 16.4 % more than expected with normal synapsis. Considering the clear absence of sex differences in genetic recombination rates reported here and the gene-centromere linkage estimates for *ym*, *y*, *Got-2*, *Sdh-1*, *Prx-3*, *Idh-1* and *6Pgdh-3* in normal synaptic plants and *ds-1* mutants (Table 3; Douches and Quiros, 1987, 1988*a*), parental heterozygosity at all loci is expected to be preserved in 20.9 and 77.4 % of all FDR gametes with normal synapsis and and desynapsis respectively. With SDR this would have been the case in only 0.4 % of all 2n-gametes. Therefore, where under normal synaptic conditions FDR is by far superior to SDR in the preservation of parental heterozygosity, the *ds-1* gene may be concluded to particularly increase the ability of FDR gametes to pick up the genetic constitution of parental clones, including complex types of favourable epistasis, with a minimum amount of reassortment.

The similar expression of the *ds-1* gene in both sexes and the present availability of diploid *ds-1* mutants with sufficiently high levels of FDR pollen and (or) reasonable amounts of FDR megaspore formation (Peloquin, 1982; Ramanna, 1983; Jongedijk *et al.*, 1991) advocates the feasibility of 2*x*(*ds-1*/FDR)-2*x*(*ds-1*/FDR) breeding schemes to produce highly vigorous and uniform TPS varieties. Because of the potential for limited genetic recombination throughout the potato genome, the use of the *ds-1* gene in the development of diplosporic apomixis seems less obvious. However, since mutant synaptic genes without genetic recombination have not been identified in potato so far, it may be considered the best alternative that is currently available. In fact, unexpectedly high levels of genetic diversity have been reported to occur within populations of apomictic plant species like *Erigeron annuus* (Hancock and Wilson, 1976) and *Taraxacum officinale* (Lyman and Ellstrand, 1984; Mertens-King and Schaal, 1990). Although such a diversity is generally assumed to result from residual sexuality and/or high somatic mutation rates, genetically variable apomictic progeny might, at least in the case of diplospory, also be explained on the basis of incomplete asynapsis or incomplete genome divergence allowing low but significant amounts of recombination in otherwise largely "apomeiotic" megaspore mother cells.

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CHAPTER 8

Analysis of glutamate oxaloacetate transaminase (GOT) isozyme variants in diploid tuberous *Solanum;* inheritance and linkage relationships to *ds1* (desynapsis), *y* (tuber flesh colour), *cr* (crumpled) and *yc* (yellow cotyledon)

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Key words: Genetic markers, GOT isozymes, inheritance, linkage analysis, tuberous *Solanum*

Summary

Employing polyacrylamide gradient-gel electrophoresis, the genetic control of leaf glutamate oxaloacetate transaminase (GOT) isozyme variants and their linkage to *ds1* (desynapsis), *y* (tuber flesh colour), *cr* (crumpled) and *yc* (yellow cotyledon) were studied in diploid tuberous *Solanum* species hybrids.

Leaf GOT isozymes proved to be dimeric and under the control of two independently segregating loci (*Got-1* and *Got-2*) with two (1^s, 1^f) and three (2^s, 2^f, 2^n) alleles respectively. The 2^n allele was concluded to encode a 2^f allozyme subunit capable of forming heterodimeric but no homodimeric active molecules. As expected with *Got-1* and *Got-2* isozymes being localized in different cellular compartments, no intergenic heterodimer formation was observed.

In contrast to earlier studies, a gradual rather than discrete transition between the tuber flesh colour classes white and yellow was observed in segregating progenies. Adopting a standardized classification method, however, monogenic recessive inheritance of white tuber flesh was confirmed.

Got-1, ds1 and *cr* were found to represent a single linkage group. Among the presently studied marker loci no further linkages were detected. As to linkage analysis, the difficulties imposed by gametophytic incompatibility in nearly all diploid potato species and by the frequent occurrence of recessive (sub)lethal genes are discussed.

Introduction

Genetic linkage maps of genes that affect morphological characters have been developed for many plant species. Though valuable in basic genetic research, their application in plant breeding has been restricted, because morphological markers mostly are recessive and frequently have undesirable phenotypes. Today linkage maps of some of the crop plants are being saturated more rapidly with phenotype neutral, codominantly expressed isozyme and DNA markers (Tanksley & Orton, 1983; Bernatzky & Tanksley, 1986; Helentjaris et al., 1986a, 1986b; Young et al., 1987; Landry et al., 1987), which are claimed to offer an unique opportunity to improve the efficiency of current plant breeding procedures (Tanksley, 1983; Beckmann & Soller, 1986a, 1986b).

Among Solanaceous crops, by far the most extensive genetic map has now been established for

tomato (Mutschler et al., 1987; Young et al., 1987). In potato, *Solanum tuberosum* L., on the other hand, the number of reported marker genes that can be used in genetic and cytological mapping is still limited. This is obviously due to its autotetraploidy, the gametophytic imcompatibility in nearly all related diploid species, and its vegetative propagation, which promotes the accumulation of recessive (sub)lethal genes (Dodds & Paxmann, 1962; Simmonds, 1966; Hermsen et al., 1978; Wagenvoort, 1982; Douches & Quiros, 1988b). In addition, gene localization has been hampered by the genetic heterogeneity of the trisomic series and relatively difficult chromosome identification (Ramanna & Wagenvoort, 1976; Wagenvoort & Ramanna, 1979). Despite these difficulties, a rapid development of the genetic map of potato may be expected with the recent initiation of isozyme marker analysis (Staub et al., 1984; Martinez-Zapater & Oliver, 1984; Quiros & McHale, 1985; Douches & Quiros, 1988b), the exploration of DNA-markers and attempts to simplify gene-localization through the development of a homogeneous trisomic series and in situ hybridization techniques.

In potato four monogenic recessive traits have so far been localized by means of trisomic analysis: *a* (albinism), *v* (virescens), *ym* (yellow margin) and *tp* (topiary) marking the chromosomes 3 and 12 (Lam & Erickson, 1971; Hermsen et al., 1973; Wagenvoort, 1982, 1988). Linkage analyses have yielded eight small linkage groups: *Nx(tbr), Nx (chc), Ny(chc), Na(tbr)* and *Nc(tbr),* all dominant genes for hypersensitive resistance to potato viruses X, Y, A and C respectively (Cockerham, 1970); *B, I, F, Ow* and *Pf,* all dominant genes affecting anthocyanin distribution (Dodds & Long, 1956; De Jonge & Rowe, 1972; De Jong, 1987); *dr* (droopy) and *S* (gametophytic incompatibility) (Simmonds, 1966); *v* (virescens) and *t* (*S*-bearing translocation) (Hermsen, 1978); *Ld* (pollen lobedness) and *Tr* (tetrad sterility) (Abdalla & Hermsen, 1971); *Prx-2* and *Prx-3* (Quiros & McHale, 1985); *Idh-1* and *Sdh-1* and *6Pgdh-3* and *Dia-1* (Douches & Quiros, 1988b). Except for the linked loci *v* and *t* on chromosome 12, none of them could yet be assigned to a specific chromosome. Finally, approximate gene-centromere map distances have recently been established via half-tetrad-analysis for a variety of morphological and isozyme marker loci (Mendiburu & Peloquin, 1979; Stelly & Peloquin, 1986; Douches & Quiros, 1987, 1988a; Jongedijk et al., 1989).

In this paper data on the inheritance of glutamate oxalocetate transaminase (GOT) isozyme variants as revealed by polyacrylamide gradient-gel electrophoresis are provided and a linkage group involving *Got-1, ds1* (desynapsis) and *cr* (crumpled) is reported.

Materials and methods

Plant material

The segregation of GOT isozymes and morphological markers was analysed in nine diploid F1 progenies (including four reciprocal sets of crosses), derived from four diploid tuberous *Solanum* species hybrids: USW 5295-7 (coded B), USW 5337-3 (coded C), USW 7589-2 (coded D) and 77-2102-37 (coded E). Detailed information on the origin and ancestry of these parental clones is provided by Jongedijk & Ramanna (1988). The genetics of GOT isozymes was additionally verified in diploid backcross populations of two F1 plants with their respective parental clones. All crosses and backcrosses are listed in the Tables 2 and 3.

Crosses were made on plants grafted onto tomato rootstock. To exclude selfing, the flowers of the seed parents were emasculated well before anthesis. All seeds obtained were sown in petri-dishes. Upon germination they were put into seedling trays and 3–4 weeks after emergence the seedlings were transplanted into plastic pots. The ploidy level of progeny was checked by establishing the mean number of chloroplasts in the stomatal guard cells (Frandsen, 1968). In cases of doubt, chromosome numbers in root tip meristems were counted. All plant material was grown in a conditioned greenhouse during summer.

Morphological markers

Four monogenic recessive 'morphological' markers were included:
- 'Crumpled (*crcr*)'; sublethal mutant. Stunted plantlets with contorted stems and crumpled leaves. Generally dying 1–2 weeks after emergence (Simmonds, 1966; Fig. 2a).
- 'Yellow cotyledon (*ycyc*[1])'; lethal mutant. Plantlets developing to the cotyledon stage, then turning yellow and dying (Hermsen et al., 1978).
- 'Desynapsis (*ds1ds1*); meiotic mutant. Micro- and megasporogenesis characterized by normal chromosome pairing through pachytene and a falling apart of most bivalents at diakinesis (Ramanna, 1983; Jongedijk & Ramanna, 1988).
- 'Tuber flesh colour'; white tuber flesh (*yy*) recessive to yellow (*Y.*), with modifying genes of relatively small effect that determine the degree of yellowness (Howard, 1970).

The parental genotypes for each of these marker genes are indicated in the Tables 2 and 3.

In order not to miss any of the 'crumpled' or 'yellow cotyledon' plantlets in the early stages of plant development, each seedling was numbered upon emergence and assessed at 3-days intervals over a period of 4 weeks. The tuber flesh colour was scored as indicated in Table 1. Mature tubers were sampled in 2–3 successive years and segregation ratios were based on calculated mean scores, whereby the tuber flesh colour classes 4–5 (white-creamy white) and 6–8 (pale yellow-deep yellow) were considered white and yellow respectively. The data concerning desynapsis are from Jongedijk & Ramanna (1988).

1. This marker was originally assigned the gene symbol l_2 by Hermsen et al. (1978), but with approval of these authors is here re-designated as *yc*.

GOT isozyme analysis

Glutamate oxaloacetate transminase (GOT; E.C. 2.6.1.1) isozymes were assayed in freshly prepared samples of actively growing leaves by polyacrylamide gel electrophoresis (PAGE), using 5–20% polyacrylamide pore size gradient-gels. Sample preparation and electrophoretic procedures were as described by Suurs et al. (1989).

GOT isozyme staining was essentially according to Vallejos (1983), however to improve the clarity of gels, 1% soluble polyvinyl pyrrolidone (PVP) was added to the staining solution. Gels were photographed with a Polaroid MP-4 camera on Pac film (type 665) using a Kodak 23A filter.

Statistical analyses

Chi square tests were used to assess the goodness of fit to expected Mendelian ratios for single loci, independent segregation of pairs of loci and homogeneity of monogenic and digenic segregation ratios. For genetic analyses, monogenic and digenic segregation data concerning reciprocal F1 progenies were pooled whenever they proved homogeneous ($P[\chi^2 \text{ homogeneity}] \geq 0.05$).

Maximum likelihood estimates (MLE) of recombination frequencies between linked loci were obtained using the 'MaxLik' computer program developed by Dr. P. Stam (Department of Genetics,

Table 1. Graduated scale and standard potato varieties used for scoring tuber flesh colour (according to the Dutch Descriptive List of Varieties, 1988)

Rating	Flesh colour	Standard varieties
4	white	Astarte, Aurora, Blanka, Element
5	creamy white	Draga, Renova, Romano, Senator
6	pale yellow	Alcmaria, Bintje, Désirée, Radosa
7	yellow	Climax, Eersteling, Prevalent, Saturna
8	deep yellow	Doré, Eba, Elvira, Gloria

Agricultural University Wageningen). The corresponding map distances were calculated according to Kosambi's (1944) mapping function.

Results

(Sub)lethal mutants

In populations segregating for the (sub)lethal mutants 'crumpled (*crcr*)' or 'yellow cotyledon (*ycyc*)' observations concerning desynapsis (*Ds1/ds1* locus), tuber flesh colour (*Y/y* locus) and GOT-isozymes could be obtained for normal plant only, since both *ycyc* and *crcr* mutants neither flowered nor tuberized and even died before usable leaf samples for isozyme electrophoresis could be collected. In these populations deviations from expected Mendelian segregation ratios for desynapsis, tuber flesh colour and GOT isozyme variants among the normal progeny might thus indicate linkage of these markers with the *Cr/cr* or the *Yc/yc* locus.

Segregation for 'crumpled (*crcr*)' and 'yellow cotyledon (*ycyc*)' could readily be scored. Simultaneous segregation of these traits was not observed. The parental genotypes and observed segregation ratios are summarized in Table 2. No deviations from expected monogenic ratios were observed.

Desynapsis and tuber flesh colour

Segregation for desynapsis and tuber flesh colour were recorded in F1 progenies only (Table 2). The results on desynapsis were summarized from Jongedijk & Ramanna (1988). Their data concerning the ExC progeny were omitted here, as in this progeny they unexpectedly observed no (plain) segregation. This was sugested to be due to cross-specific cytoplasmic influence on *ds1* expression. The observed segregation ratios (Jongedijk & Ramanna 1988; Table 2) supported monogenic recessive inheritance of *ds1*.

Tuber flesh colour ratings proved to be rather constant over years. Small differences within a single genotype were only occasionally detected. In studies employing tuber flesh colour as a genetic marker, usually only two classes are distinguished: white (*yy*) and yellow (*Y.*) (Mok et al., 1976; Stelly & Peloquin, 1986; Douches & Quiros, 1987, 1988b). Though apparently simple, such a classification actually was difficult to make in the present study. Indeed, yellow tuber flesh colour is general-

Table 2. Monogenic segregation ratios of *yc* (yellow cotyledon), *cr* (crumpled), *ds1* (desynapsis) and *y* (tuber flesh colour) in F1 and backcross progenies. The figures *(1)* and *(2)* are codes used in Table 4 to indicate the genotypes for desynapsis and tuber flesh colour. Parental genotypes: B = USW5295-7 = *YcYc/CrCr/Ds1ds1/yy(4);* C = USW5337-3 = *Yc./Crcr/Ds1ds1/Yy(8);* D = USW7589-2 = *Ycyc/CrCr/Ds1ds1/yy(4);* E = 77-2102-37 = *YcYc/Crcr/Ds1ds1/yy(4);* (D × E)3 = *Ycyc/CrCr/Ds1./yy(4);* (D × E)18 = *Ycyc/CrCr/Ds1./yy(4)*

(Back)cross combination	*yc* segregation				*cr* segregation				*ds1* segregation[a]				*y* segregation			
	Yc.	*ycyc*	Exp.	χ^2	*Cr.*	*crcr*	Exp.	χ^2	*Ds1* *(1)*	*ds1ds1* *(2)*	Exp.	χ^2	*Y.* *(1)*	*yy* *(2)*	Exp.	χ^2
B × C/C × B	340	0	1:0	0,00	340	0	1:0	0,00	114	39	3:1	1,33	73	68	1:1	0,18
B × E/E × B	286	0	1:0	0,00	286	0	1:0	0,00	105	40	3:1	0,52	0	113	0:1	0,00
D × E/E × D	307	0	1:0	0,00	307	0	1:0	0,00	119	44	3:1	0,35	0	140	0:1	0,00
C × E/E × C	812	0	1:0	0,00	604	208	3:1	0,16	234	92[b, c]	3:1	1,80	174	188[c]	1:1	0,54
D × B	148	0	1:0	0,00	148	0	1:0	0,00	36	18	3:1	2,00	–	–	–	–
(D × E)3 × D	78	32	3:1	0,98	110	0	1:0	0,00	–	–	–	–	–	–	–	–
(D × E)3 × E	109	0	1:0	0,00	109	0	1:0	0,00	–	–	–	–	–	–	–	–
(D × E)18 × D	95	29	3:1	0,17	124	0	1:0	0,00	–	–	–	–	–	–	–	–
(D × E)18 × E	126	0	1:0	0,00	126	0	1:0	0,00	–	–	–	–	–	–	–	–

[a] Data collected by Jongedijk & Ramanna (1988).
[b] Data on C × E progeny only (see text).
[c] Segregation ratio among *Cr.* progeny.

ly agreed to be governed by a dominant major gene, but in addition modifying minor genes are known to almost invariably give rise to different gradations of white and yellow (Howard, 1970). Consequently a gradual transition rather than clear-cut distinction between the two phenotypes was observed in segregating populations (Fig. 1c, d). Therefore classification of tuber flesh colour as either white or yellow is dependent on arbitrary limits that are set to each class. In view of the observed tuber flesh colour ratings in F1 progeny from control (white × white) crosses (Fig. 1a, b) and the fact that no standardized criteria for classifying different flesh colour gradations as either white or yellow have yet been reported in the literature, we here assessed the classes 4–5 (white-creamy white) and 6–8 (pale yellow-deep yellow) as white and yellow respectively. Tuber flesh colour segregation ratios thus obtained fitted remarkably well to the ratios expected on the basis of single gene control (Table 2).

Segregation for *ds1* and *y* in the C × E/E × C progeny could be scored among non lethal (i.e. *Cr.*) plants only. Nevertheless no deviations from expected monogenic ratios were observed, which indicates that both *ds1* and *y* segregate independently of *cr*.

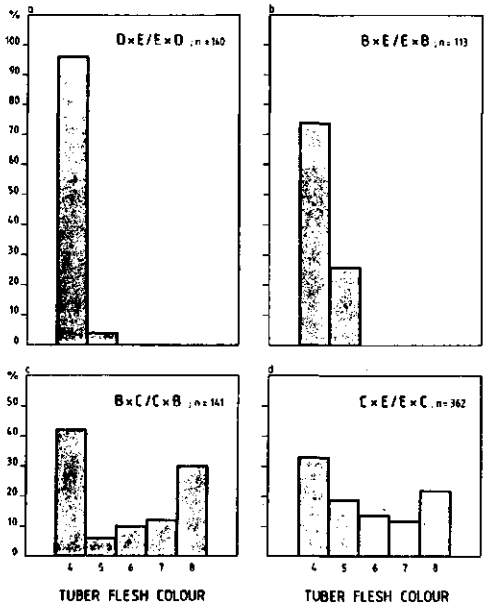

Fig. 1a–d. Overall frequency distribution of tuber flesh colour ratings in the diploid progeny of four sets of reciprocal crosses. Parental genotypes: B = USW5295-7 = *yy*(4), C = USW5337.3 = *Yy*(8), D = USW7589-2= *yy*(4) and 77-2102-37 = *yy*(4).

GOT zymograms

GOT isozyme electrophoresis of potato leaf extracts revealed two distinct zones of enzymatic activity, designated *Got-1* and *Got-2* in sequential order from anode (Fig. 2d–g).

For *Got-1* two different single-banded phenotypes and one three-banded phenotype were observed, which suggested *Got-1* isozymes to be governed by a single locus with two alleles that produce a single homodimeric slow (1^s) and fast (1^f) migrating allozyme in 1^s1^s and 1^f1^f homozygotes respectively and an additional heterodimeric allozyme of intermediate mobility in 1^s1^f heterozygotes (Fig. 2b). As a consequence of only small migrational differences between these assumed *Got-1* allozymes, the latter were generally detected by the occurrence of a relatively broad, indiscrete zone of enzyme activity (Fig. 2e–g).

Better band resolution was obtained for *Got-2*. Here five different phenotypes were observed (Fig. 2c). The single-banded and three-banded phenotypes might be explained with *Got-2* representing dimeric enzymes, governed by a single locus with two alleles (2^s and 2^f) as well. The detection of two banded phenotypes (Fig. 2d–g) and empty lanes (Fig. 2c, g), however, seemed to indicate segregation for an additional 'null' allele (2^n), which would encode a 2^f allozyme subunit capable of forming heterodimeric but no homodimeric active molecules. Under this hypothesis a total of five different *Got-2* phenotypes might be expected: three-banded (2^s2^f), two-banded (2^s2^n), single-banded slow (2^s2^s), single-banded fast (2^f2^f and 2^f2^n) and no bands at all (2^n2^n).

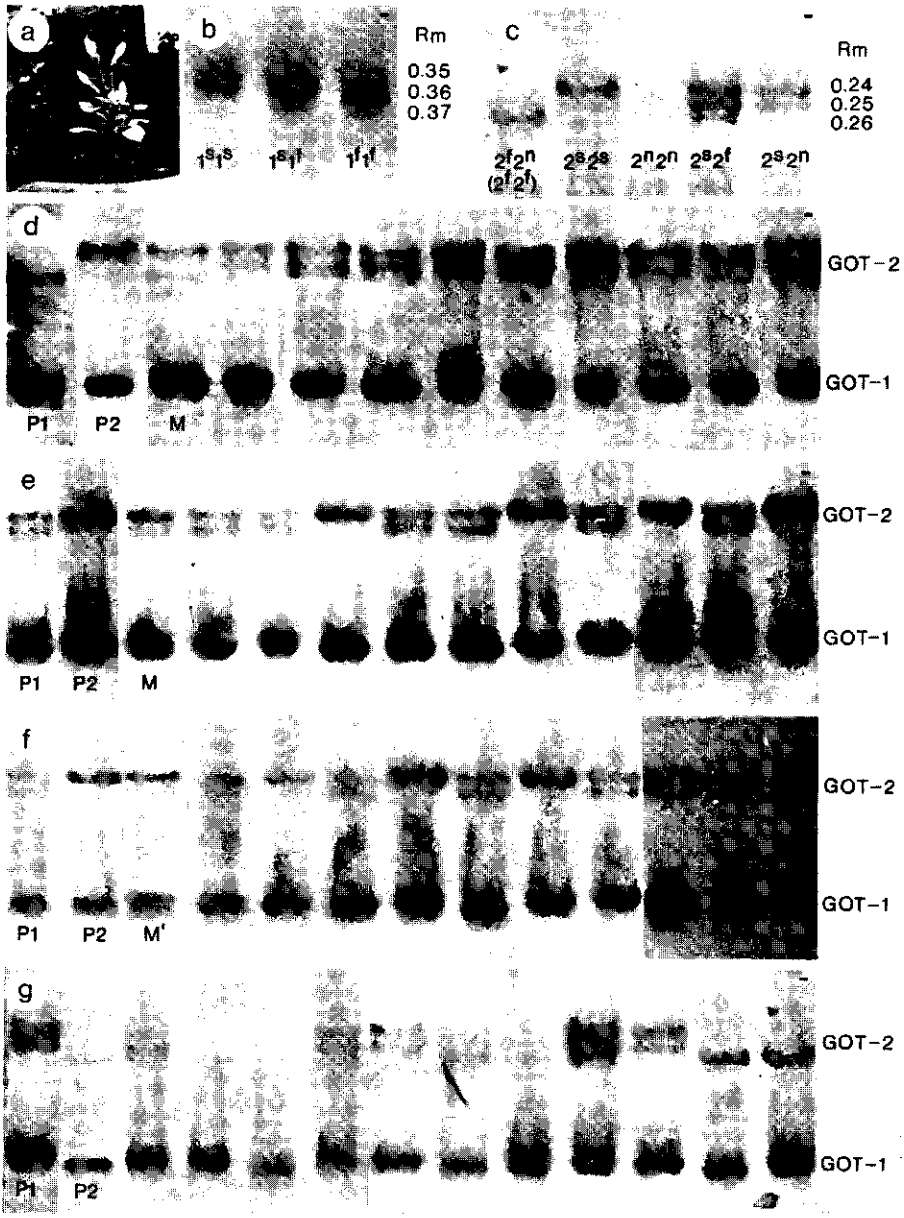

Fig. 2. Crumpled (*crcr*) plantlet (a), observed *Got-1* (b) and *Got-2* (c) zymogram phenotypes and segregation for *Got-1* and *Got-2* in D × B (d), C × E (e), (D × E)18 × E (f) and (D × E)(18 × D (g). Rm = relative mobility; P_1/P_2 = female/male parental phenotype, M = $P_1 + P_2$, M' = progeny mixture, − = cathodal end.

Genetic control of Got-1 and Got-2 isozymes; linkage of Got-1 and cr

The segregation ratios observed in F1 and backcross progenies (Table 3) generally supported the hypotheses of *Got-1* and *Got-2* isozymes being distinct dimeric enzymes, each controlled by a single locus (*Got-1* and *Got-2*) with two (1^s and 1^f) and three (2^s, 2^f and 2^n) alleles respectively.

The absence of deviations from expected *Got-2* segregation ratios, even in crosses segregating for *yc* and *cr* (Table 3), indicates *Got-2* to segregate independently of both (sub)lethal markers. *Got-1* proved to segregate independently of *yc* as well. In crosses segregating for *cr* (Table 3, C × E/E × C), however, a significant deviation from the expected segregation ratio was observed. As seed germination in these crosses was virtually complete (~96%) and GOT isozymes were assessed among a random sample of *Cr.* progeny, the relative lack of 1^s1^f progeny is likely to result from linkage of *Got-1* and *cr*, with the parental genotypes C and E being

$$\frac{1^f\ cr}{1^f\ Cr} \text{ and } \frac{1^s\ cr}{1^f\ Cr} \text{ respectively.}$$

Assuming a recombination frequency r(*Got* − *1*/*cr*) = r_1 in both parental clones a ratio of $(1 + r_1)/3$ 1^s1^f: $(2 - r_1)/3$ 1^f1^f (expected) = 148 : 246 (observed) among the *Cr.* progeny is then most likely to occur with r_1 (MLE) = 0,127 ± 0.073 (χ^2[0df] = 0,00).

Digenic segregation ratios; linkage of Got-1/cr and ds1

Segregation for *Got-1* and *Got-2* on the one hand and *ds1* and *y* on the other could not always be recorded in identical subpopulations within a F1 progeny. Firstly, because not all plants sampled for GOT isozyme analysis flowered or tuberized and secondly, because progeny previously classified for *ds1* and *y* was not always available for GOT isozyme analysis. Nevertheless, digenic segregation ratios based upon sufficiently large populations could be calculated in the majority of cases (Table 4).

When the linkage of *Got-1* and *cr* ($r_1 = 0.127$) was taken into account, the overall results (Table 4) clearly indicate independent segregation of *Got-1*/*Got-2*, *Got-1*/*y*, *Got-2*/*ds1*, *Got-2*/*y* and *ds1*/*y* and linkage of *Got-1* with *ds1*. If not (i.e. $r_1 = 0.50$), additional pseudo linkages would have been detected for *Got-1*/*Got-2* and *Got-1*/*y* in the C × E/E × C progeny, which segregated for *cr* (Table 4). Obviously, *Got-1*, *ds1* and *cr* represent a linkage

Table 3. Monogenic segregation ratios of *Got-1* and *Got-2* in F1 and backcross progenies. The figures *(1)* – *(5)* are codes used in Table 4 to indicate the genotypes for *Got-1* and *Got-2*.
Parental genotypes: B = USW5295-7 = $1^f1^f/2^s2^s$; C = USW5337-3 = $1^f1^f/2^s2^n$; D = USW7589-2 = $1^s1^f/2^f2^n$; E = 77-2102-37 = $1^s1^f/2^s2^s$; (D × E)3 = $1^f1^f/2^s2^n$; (D × E)18 = $1^s1^f/2^s2^n$

(Back)cross combination	*Got-1* segregation					*Got-2* segregation						
	1^s1^s *(1)*	1^s1^f *(2)*	1^f1^f *(3)*	Exp.	χ^2	2^s2^s *(1)*	2^s2^f *(2)*	2^s2^n *(3)*	2^f2^n *(4)*	2^n2^n *(5)*	Exp.	χ^2
B × C/C × B	0	0	169	0:0:1	0,00	77	0	72	0	0	1:0:1:0:0	0,17
B × E/E × B	0	81	88	0:1:1	0,29	169	0	0	0	0	1:0:0:0:0	0,00
D × E/E × D	0	77	90	0:1:1	1,01	0	86	81	0	0	0:1:1:0:0	0,15
C × E/E × C[a]	0	148	246	0:1:1	24,38*	206	0	188	0	0	1:0:1:0:0	0,82
D × B	0	0	72	0:0:1	0,00	0	34	38	0	0	0:1:1:0:0	0,22
(D × E)3 × D[b]	0	0	71	0:0:1	0,00	0	18	20	19	14	0:1:1:1:1	1,17
(D × E)3 × E	0	34	38	0:1:1	0,22	32	0	40	0	0	1:0:1:0:0	0,89
(D × E)18 × D[b]	0	39	32	0:1:1	0,69	0	16	22	16	17	0:1:1:1:1	0,97
(D × E)18 × E	15	36	21	1:2:1	1,00	33	0	39	0	0	1:0:1:0:0	0,50

[a] Segregation ratios among *Cr.* progeny.
[b] Segregation ratios among *Yc.* progeny.
* Significant at the 0,001 level.

group which segregates independently of both *Got-2* and *y*. In addition *Got-1* and *Got-2* segregate independently of *yc*, whereas the linkage relationship of *yc* to *ds1* and *y* as yet remains unknown because in the present study simultaneous segregation for the respective pairs of loci did not occur.

With respect to the *Got-1/ds1* segregation a relative lack of $I^sI^f/ds1ds1$ and surplus of $I^fI^f/ds1ds1$ progeny was observed in both the C × E (expected $3(1 + r_1)$ $I^sI^f/Ds1.$: $(1 + r_1)$ $I^sI^f/ds1ds1$: $3(2 - r_1)$ $I^fI^f/Ds1.$: $(2 - r_1)$ $I^fI^f/ds1ds1 \sim 86 : 29 : 143 : 48$ ($r_1 = 0.127$) with independent segregation of *Got-1* and *ds1*) and B × E/E × B + D × E/E × D (expected 3 $I^sI^f/Ds1.$: 1 $I^sI^f/ds1ds1$: 3 $I^fI^f/Ds1.$: 1 $I^fI^f/ds1ds1 \sim 37 : 12 : 37 : 12$ with independent segregation of *Got-1* and *ds1*) cross progenies. With the genotypes of the parental clones B, D and C being

$$\frac{I^f\ ds1}{I^f\ Ds1},$$

the genotype of the parental clone E could thus be concluded to be

$$\frac{I^f\ ds1}{I^s\ Ds1}.$$

Assuming a recombination frequency r(*Got-1/ds1*) = r_2 in all parental clones, in the B × E/E × B + D × E/E × D progeny a ratio of $(2\text{-}r_2)/4$ $I^sI^f/Ds1.$: $(r_2)/4$ $I^sI^f/ds1ds1$: $(1 + r_2)/4$ $I^fI^f/Ds1.$: $(1\text{-}r_2)/4$ $I^fI^f/ds1ds1$ (expected) = 40 : 7 : 30 : 21 (observed) is then most likely to occur with r_2 (MLE) = 0,256 ± 0,078 (χ^2 [2df] = 0,70).

Although *Got-1*, *ds1* and *cr* all segregated simultaneously in the C × E progeny, no estimates of r_1 = r(*Got-1/cr*), r_2 = r(*Got-1/ds1*) and r_3 = r(*ds1/cr*) based upon trigenic segregation ratios can be calculated, since both *Got-1* and *ds1* could be scored among non-lethal (i.e. *Cr.*) progeny only. Consequently also the gene order within the *Got-1/ds1/cr* linkage group cannot be determined. Alternatively, indirect estimation of r_3 from the

Table 4. Digenic segregation ratios for *Got-1*, *Got-2*, *ds1* and *y* among non lethal (*Cr.Yc.*) F1 backcross progenies. The pairs of figures (e.g. *2/1*, *2/3* etc.) under the caption 'Digenic segregation ratios' denote the genotypes for the corresponding pairs of characters in column 1 (for explanation of figures see Tables 2 and 3)

Tested loci 1st/2nd	(Back)cross combination	Digenic segregation ratios								Expected ratios	χ^2	
										[r_1 = r (*Got-1/cr*)]	r_1 = 0,50	r_1 = 0,127
Got-1/Got-2	C × E/E × C	83	*2/1*	65	*2/3*	123	*3/1*	123	*3/3*	$1 + r_1 : 1 + r_1 : 2 - r_1 : 2 - r_1$	26,02**	2,19
	D × E/E × D	44	*2/2*	33	*2/3*	42	*3/2*	48	*3/3*	1:1:1:1	----------	2,89 ----------
	(D × E)3 × E	18	*2/1*	16	*2/3*	14	*3/1*	24	*3/3*	1:1:1:1	----------	3,11 ----------
	(D × E)18 × D	8	*2/2*	14	*2/3*	10	*2/4*	7	*2/5*			
		8	*3/2*	8	*3/3*	6	*3/4*	10	*3/5*	1:1:1:1:1:1:1:1	----------	4,83 ----------
	(D × E)18 × E	7	*1/1*	8	*1/3*	18	*2/1*	18	*2/3*			
		8	*3/1*	13	*3/3*					1:1:2:2:1:1	----------	2,44 ----------
Got-1/ds1	C × E	101	*2/1*	19	*2/2*	116	*3/1*	70	*3/2*	$3(1 + r_1) : 1 + r_1 : 3(2 - r_1) : 2 - r_1$	37,79**	21,39**
	B × E/E × B + D × E/E × D[a]	40	*2/1*	7	*2/2*	30	*3/1*	21	*3/2*	3:1:3:1	----------	10,03* ----------
Got-1/y	C × E/E × C	65	*2/1*	68	*2/2*	106	*3/1*	108	*3/2*	$1 + r_1 : 1 + r_1 : 2 - r_1 : 2 - r_1$	18,98**	0,17
Got-2/ds1	C × E + B × C/ C × B[a]	144	*1/1*	53	*1/2*	125	*3/1*	54	*3/2*	3:1:3:1	----------	3,69 ----------
	D × E/E × D	16	*2/1*	7	*2/2*	15	*3/1*	6	*3/2*	3:1:3:1	----------	0,61 ----------
Got-2/y	C × E/E × C + B × C/C × B[a]	113	*1/1*	104	*1/2*	96	*3/1*	104	*3/2*	1:1:1:1	----------	1,39 ----------
ds1/y	C × E + B × C/ C × B[a]	136	*1/1*	130	*1/2*	54	*2/1*	55	*2/2*	3:3:1:1	----------	3,45 ----------

[a] Pooled data; P (χ^2 homogeneity) > 0.50).

*, ** Significant at the 0,025 and 0,001 level respectively.

Table 5. Linkage estimates for *Got1-cr*, *Got1-ds1* and *ds1-cr* with two different gene orders

Gene order	Recombination fraction[a] (map distance[b])		
	r_1	r_2	r_3[c]
ds1/Got1/cr	0,127 ± 0,073 (13,0)	0,256 ± 0,078 (28,3)	0,339 (41,3)
ds1/cr/Got1	0,127 ± 0,073 (13.0)	0,256 × 0,078 (28,3)	0,148 (15,3)

[a] r_1 = r (*Got-1/cr*); r_2 = r (*Got-1/ds1*); r_3 = r (*ds1/cr*).
[b] In cM, according to Kosambi (1944).
[c] Calculated from r_1 and r_2.

monogenic segregation ratio among *Cr.* progeny is dependent on the unknown way in which *ds1* and *cr* are linked (i.e. repulsion versus coupling phase) in the parental clone C, whereas indirect estimation of r_2 from the digenic segregation ratio among *Cr.* progeny in addition is actually dependent on gene order.

When the recombination frequencies between a pair of loci is assumed to be largely similar in all parental clones, the gene order *Got1-ds-cr* is unlikely, since r_1 = r(*Got-1/cr*) and r_2 = (*Got-1/ds1*) were estimated at 0.127 and 0,256 respectively. In that case two different, tentative genetic linkage maps for *Got-1, ds1* and *cr* would remain (Table 5; Fig. 3). Because *ds1* and *cr* were found to segregate independently in C × E, it may be attractive to assume an only loose linkage of these two loci as is the case with the gene order *ds1-Got1-cr*. However, this need not necessarily be the case. In the parental clone E, *ds1* and *cr* could be deduced to be linked in repulsion phase

$$\left(\frac{1^s\ cr}{1^f\ Cr} + \frac{1^s\ Ds1}{1^f\ ds1} \rightarrow \frac{cr\ Ds1}{Cr\ ds1}\right),$$

whereas in C they could be linked in either repulsion or coupling phase. The expected ratio *Ds1. : ds1ds1* among *Cr.* progeny from

$$\frac{cr\ Ds1}{Cr\ ds1}\ (C) \times \frac{cr\ Ds1}{Cr\ ds1}\ (E)$$

and from

$$\frac{Cr\ Ds1}{cr\ ds1}\ (C) \times \frac{cr\ Ds1}{Cr\ ds1}\ (E)$$

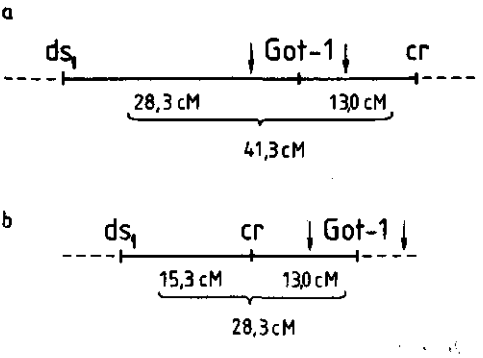

Fig. 3a–b. Two possible tentative linkage maps for the loci *Got-1, ds1* and *cr*. Map length estimates according to Kosambi (1944). Arrows indicate possible centromere position (according to Douches & Quiros (1987) and Jongedijk et al. (1989).

would be $(2 + r_3^2)/3 : (1\text{-}r_3^2)$ and $(2 + r_3\text{-}r_3^2)/3 : (1 - r_3 + r_3^2)/3$ respectively. In neither case the actually observed segregation ratios (Table 2) differ significantly from the calculated ratios based on r_3(*ds1-Got1-cr*) = 0.339 (χ^2[1df] = 0,26 and 2.85 respectively) or r_3(*ds1-cr-Got1*) = 0.148 (χ^2[1df] = 0,94 and 0,13 respectively).

Discussion

Desynapsis and tuber flesh colour

In the crosses C × E and E × C Jongedijk & Ramanna (1988) unexpectedly observed segregation for *ds1* in the former progeny only. They noted that this reciprocal difference might indicate cross-specific cytoplasmic influence on *ds1* expression, since it could not be ascribed to accidental interchange of seed lots, accidental cross or self pollination or (virtually) absolute linkage of the *ds1* gene to either *S* alleles (gametophytic incompatibility) or gamete eliminator genes causing lethality of micro- and/or megaspores in the parental clones C and E respectively. Their conclusion that the E × C progeny did consist of true F1 hybrids is supported in the present study by the segregation ratios found in both C × E and E × C for the markers *Got-1, Got-2, y, cr* and *yc*.

With respect to tuber flesh colour it should be recognized that observed segregation ratios in hybrid progenies in fact strongly depend on both the parental genotypes and phenotypes and on the arbitrary limits that are set for the classes white and yellow. The parental clones B, D and E (tuber flesh colour 4) and C (tuber flesh colour 8) represent the extremes of the observed range of tuber flesh colour gradations. Considering the classes 4–5 (white-creamy white) and 6–8 (pale yellow-deep yellow) as white and yellow respectively, the presently observed ratios fit well to the hypothesis of single gene control, that was reported previously (Howard, 1970). Taking other criteria for classification, significant deviations from that hypothesis become apparent. In the past, researchers may have adopted variable criteria for tuber flesh colour classification, as monogenic inheritance of tuber flesh colour is generally taken for granted in genetic studies (Mok et al., 1976; Stelly & Peloquin, 1986; Douches & Quiros, 1987, 1988b). To achieve uniformity in handling tuber flesh colour as a genetic marker, the classification method adopted in this study is helpful.

Genetic control of GOT isozyme variants

The results of this study clearly demonstrated a dimeric nature of GOT isozymes in potato and their genetic control by two independently segregating loci, with at least two (*Got-1*^{s}, *1*^{f}), and three (*Got-2*^{s}, *2*^{f}, *2*^{n}) alleles respectively. Applying horizontal starch gel electrophoresis, similar conclusions were previously inferred by Martinez-Zapater & Oliver (1984) and Oliver & Martinez-Zapater (1985). Based on the segregation for GOT isozymes in two tetraploid progenies derived from putative heterozygous parental clones and on GOT zymograms obtained from 74 potato varieties, also these authors concluded GOT to be dimeric isozymes. They tentatively identified the loci *Got-A* (identical to *Got-1*) and *Got-B* (identical to *Got-2*) with three (*a, b, c*) and six (*a, b, c, c', d, e*) different alleles respectively. Although both loci segregated simultaneously in one of the tetraploid progenies studied, digenic ratios revealing their linkage relationship unfortunately were not provided.

Oliver & Martinez-Zapater (1985) assumed the *Got-B c'* allele to be a 'null' allele encoding an enzyme subunit capable of forming heterodimeric but no homodimeric active molecules. This suggests that the alleles *2*^{f} and *2*^{n} identified in the present study are identical with *c* and *c'* respectively. In view of the ancentry of the diploid parental clones used in this study (Jongedijk & Ramanna, 1988) and the most frequent occurrence of *Got-A a, b* and *Got-B c, d* in cultivated *Solanum* species (Oliver & Martinez-Zapater, 1984) it is tempting to assume allelism of *Got-A a, b* and *Got-B d* with *1*^{f}, *1*^{s} and *2*^{s} respectively. To unambiguously establish their actual identity, however, tests for allelism are needed.

Applying horizontal starch gel electrophoresis, Douches & Quiros (1988b) recently established 5 different alleles at the *Got-1* locus (1^1, 1^2, 1^3, 1^4 and 1^5). One allele (1^2) was provisionally assumed to express as a doublet phenotype but might actually prove to consist of 'hidden' single banded alleles upon progeny testing which is yet to be performed. Single GOT alleles expressing as doublet phenotypes were neither detected by Oliver & Martinez-Zapater (1985) nor in the present study. As shown here, the occurrence of doublet phenotypes might in fact be explained on the basis of segregation for a 'null' allele. As yet, it is unknown whether the alleles 1^1, 1^3, 1^4 and 1^5 correspond to any of the alleles identified by us and Oliver & Martinez-Zapater (1985).

As expected with *Got-1* and *Got-2* being localized in different cellular compartments (Oliver & Martinez-Zapater, 1985) no intergenic heterodimers were detected.

Linkage analysis

It may be argued that the distorted segregation ratio of *Got-1* in the C × E/E × C progeny might as well be attributed to linkage of *Got-1* and the *S* locus (gametophytic incompatibility). The parental clones C and E are known to have an *S* allele in

common (Jongedijk & Ramanna, 1988). Assuming the genotypes

$$\frac{1^f S_i}{1^f S_j} \text{ and } \frac{1^s S_j}{1^f S_k}$$

for C and E respectively and a recombination frequency r (*Got-1/S*) = r_1 in both parental clones, in the C × E progeny a (r_1) 1^s1^f : (1-r_1) 1^f1^f (expected) = 134 : 227 (observed) ratio might be expected with r_1 (MLE) = 0.371 (χ^2[0df] = 0,00). In the E × C progeny on the other hand a regular 1 : 1 segregation or a lack of 1^s1^f progeny is to be expected with linkage of *Got-1* to *S* or *cr*, respectively. Due to the relatively small sample size in this population, the actually observed ratio (14 1^s1^f : 19 1^f1^f) could be explained with both hypotheses (χ^2 [1df] = 0.33 and 0.78 with linkage to *cr* and *S*, respectively). However, the fact that hardly any lack of 1^s1^f progeny was observed among the (D × E)3 × E progeny (Table 3), which does not segregate for *cr* but also derives from parental clones with a common *S* allele, seemed to indicate linkage of *Got-1* and *cr* rather than *Got-1* and *S*. In the latter case a ratio of (r_1) 1^s1^f : (1-r^1) 1^f1^f ~ *27 : 45* (*r_i = 0,371*) and thus a remarkable deficit of 1^s1^f progeny would have been expected. Nevertheless, the possibility of *Got-1/S* linkage could not be totally ruled out since the observed ratio among (D × E)3 × E progeny (Table 3) still is marginally compatible to that expected with such a linkage (χ^2 [1df] = 3,16).

With assumed linkage of *Got-1* and *S*, *Got-1* and *ds1* would still have been concluded to be linked, as also then the expected digenic ratio among C × E progeny with independent segregation (i.e. 3(r_1) 1^s1^f/*Ds1*. : (r_1) 1^s1^f/*ds1ds1* : 3(1-r_1) 1^f1^f/*Ds1*. : (1-r_1) 1^f1^f/*ds1ds1* ~ 85 : 29 : 144 : 48 (r_1 = 0,371)) deviates significantly from that actually observed (χ^2[3df] = 21,98). In that case independent segregation of *ds1* and *S* and thus the gene order *ds1-Got1-S* would have been obvious, as in the parental clone E the alleles *Ds1* and ds1 then would be linked to 1^s–S_j and 1^f–S_k respectively, while for *ds1* no deviation from the expected monogenic ratio was observed among C × E progeny. In addition no further linkages among the presently studied marker genes would have been detected either.

Distorted segregation ratios for GOT isozymes due to close linkage to the *S* locus have recently been reported in *Malus* (Manganaris & Alston, 1987). In potato deviant *Got-1* ratios have been observed previously by Douches & Quiros (1988b). They noted it might be a clue for linkage of *Got-1* with either loci that are selected against gametophytically (e.g. *S* alleles) or recessive (sub)lethal genes which express during embryo or early plant development. As shown here this may actually have been the case. Such a linkage, if not taken into account in genetic analyses, might lead to the detection of either pseudo linkages involving *Got-1* and other loci, which as a matter of fact segregate independently (Table 4) or, inversely, to the detection of, pseudo independent segregation of loci, which are actually linked with *Got-1*. Therefore, the linkage data on isozyme markers provided by Douches & Quiros (1988b), which were in fact partly based on crosses with distorted ratios for *Got-1* (and other isozyme loci) should be handled with caution. In conclusion it should be pointed out that the wide spread occurrence of gametophytic incompatibility among diploid *Solanum* species and especially the frequent occurrence of recessive (sub)lethal genes imposes a serious problem in establishing gene order within a linkage group, thus hampering linkage analysis in potato.

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Note added in proof

Recently detailed genetic linkage maps comprising of numerous RFLP markers have been established for potato (Bonierbale et al., 1988; Gebhardt et al., 1989). As outlined by Tanksley et al. (1989), these RFLP maps provide an excellent basis for improving the efficiency of current potato breeding methods.

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CONCLUDING SUMMARY

The cultivated potato, *Solanum tuberosum* L., is a highly heterozygous autotetraploid (2n=4x=48) plant species, which after its introduction into Europe in the 16th century has become one of the world's major food crops. The potato has traditionally been grown from tubers. However, as tubers are an excellent substrate for many pathogens and parasites, it is extremely difficult and expensive to produce healthy seed tubers. Most developing countries lack both the knowledge and infrastructure required for the efficient production of healthy seed tubers and the currency for its importation and distribution. As a consequence small farmers in developing countries are generally forced to use diseased tubers from a previous harvest, which may result in dramatic yield losses. In response to the urgent need of cheap but healthy plant material the International Potato Center (CIP) in Peru has, since the Planning Conference on this subject in 1979, propagated the new technology of growing potatoes from true seeds. True potato seeds are relatively easy and cheap to produce and even when harvested from heavily diseased plants generally do not carry any diseases. However one of the major problems in breeding potato varieties that can be maintained and grown from true seeds is the lack of uniformity. True seed progeny of existing varieties or inter-varietal hybrids is mostly highly heterogeneous due to the extreme heterozygosity of the potato.

Several methods to synthesize sufficiently uniform true potato seed varieties have been proposed. One of these methods takes advantage of the frequent occurrence of numerically unreduced (2n) gametes in wild and cultivated diploid potato species, which enables the production of hybrid tetraploid progeny from tetraploid-diploid (unilateral sexual polyploidization) or diploid-diploid (bilateral sexual polyploidization) matings. The vigour and uniformity of tetraploid populations produced in this way, largely depends on the mode of 2n-gamete formation in the selected diploid parents. Depending on the genetic consequences of meiotic abnormalities that result into 2n-gamete formation, two distinct modes, viz. first division restitution (FDR) and second division restitution (SDR), can be distinguished. In general FDR is considered superior to SDR because of its abilitity to preserve a relatively large amount of favourable parental heterozygosity, including complex types of epistasis. In this respect the combination of FDR 2n-gamete formation with mutant synaptic genes that substantially reduce gene recombination, is of particular significance as it would provide a means to enhance the ability of FDR 2n-gametes to pick up the genetic constitution of the selected diploid parents with a minimum amount of reassortment. Using synaptic mutants with a *virtually complete lack of gene recombination maximum performance and nearly complete* uniformity may thus be expected from 2*x*FDR-2*x*FDR crosses.

Complete or nearly complete uniformity might also be achieved by the induction of apomictic seed formation. Apomixis sensu stricto is the asexual development of maternal embryo's and seeds and thus combines the advantages of both vegetative propagation (uniformity) and generative propagation (disease free plant material). Apomictic embryos may arise either directly from somatic cells outside the embryosac (adventitious embryony) or from unreduced and unfertilized (parthenogenesis) cells, usually egg cells, of the embryosac (gametophytic apomixis). In the latter case unreduced embryosacs are formed that may be of either aposporic or diplosporic origin. In apospory it develops directly from a somatic, mostly nucellar cell of the ovule. In diplospory the unreduced embryosac derives from a generative archesporial cell of the ovule, either directly by omission of meiosis or indirectly by modified meiosis in which neither reduction in chromosome number nor (substantial) gene recombination takes place. Fertilization of the secondary embryosac nucleus may or may not be required as a stimulus for endosperm formation and subsequent parthenogenetic development of the unreduced egg cells into mature embryos and seeds (pseudogamous and autonomous apomixis respectively).

Although apomictic seed formation has never been observed in *Solanaceae* its importance

for growing potatoes from true seeds justified an attempt to breed for apomictic reproduction in potato. The research described in this thesis focussed on the perspectives for inducing gametophytic apomixis, in particular pseudogamous diplosporic apomixis, because:

1. The manipulation of gametophytic apomixis (i.e., apospory and diplospory) is to be preferred to adventitious embryony as the presence of embryosacs enclosing apomictic embryos is an efficient barrier against most viruses and thus greatly facilitates the production of virus free true potato seeds.
2. Apospory has never been detected in potato and thus would require an extensive mutation breeding programme. In view of the scanty and in many cases contradictory knowledge about its genetic control (monogenic-polygenic; dominant-recessive) and the lack of efficient screening methods for identifying 'positive' mutations, the outcome of such a trial and error procedure would be highly uncertain.
3. It has been suggested that apomixis consists of a number of distinct and genetically controlled elements that lie within the reproductive potentialities of sexual plants. In case of diplosporic apomixis the elements that can roughly be distinguished are a strongly reduced chromosome pairing and/or reduced gene recombination (asynapsis/desynapsis), the formation of unreduced megaspores and embryosacs through FDR, and parthenogenetic development of unreduced egg cells into mature embryos and seeds. As some of these elements - viz. recessive genes for asynapsis/desynapsis, genes for (SDR) 2n-megaspore formation and genes for parthenogenesis along with pseudogamy - are available in potato, their combination in a single genotype may be feasible.
4. An attempt to induce diplospory requires detailed studies on the, as yet unkown, effects of mutant synaptic genes in female meiosis and the largely obscure causes and mechanisms of 2n-megaspore formation. Such studies might thus provide vital 'spin off' results to be used in pursuing the as yet utopian production of vigorous and uniform tetraploid true potato seed varieties from 2*x*FDR-2*x*FDR crosses.

An accurate knowledge of the normal pattern of female meiosis and embryosac formation is essential for deciding whether or not an unreduced embryosac is of diplosporic origin and for recognizing abnormalities in female meiosis that are associated with the expression of mutant synaptic genes and the formation of 2n-eggs. Therefore, normal meiosis and embryosac formation were studied in several diploid potato clones (Chapter 1). In contrast to results reported in the literature, this study indicated that the archesporium of potato cannot be delimited to a single cell. A surplus of archespores sometimes developed into normal sexual embryosacs. So the occurrence of multiple embryosacs within a single ovule need not necessarily be due to apospory as had previously been suggested by some potato researchers. On the basis of the normal sequence of female meiosis it was inferred that 2n-megaspores, if formed by meiotic abnormalities in normal synaptic plants, are likely to be of exclusive SDR origin, whereas meiotic abnormalities resulting in consistent FDR 2n-megaspore formation, and thus the induction of diplospory, would actually require mutant synaptic conditions. Subsequent research was therefore primarily focussed on (i) the identification and characterization of mutant synaptic genes that express in female meiosis, (ii) the identification of synaptic mutants with consistent FDR 2n-megaspore formation and (iii) the elucidation of the cytological mechanisms of FDR 2n-megaspore formation.

For this purpose, first quick routine methods allowing for large scale screening and detailed studies of female meiosis had to be developed, because conventional embedding-sectioning techniques are very laborious and hamper quantitative interpretation of meiotic processes. Two techniques were developed. One enabled the screening of female meiosis and embryosac development in intact methyl salicylate cleared ovules and permitted bulk preparation of fixed ovaries within 2 hours (Chapter 2). The other, a 30 minute enzyme-squash procedure, allowed for detailed studies on the effect of mutant synaptic genes and genes for 2n-megaspore formation on chromosome behaviour (Chapter 3).

Using these techniques it was established that (at least) three of the six mutant synaptic genes that had been reported for potato were allelic and similarly expressed in both male and female meiosis, whereas a mutant that had been claimed to express in female meiosis only proved to be non-existent. The former three mutants were characterized by normal chromosome behaviour throughout pachytene and a falling apart of bivalent chromosomes at diakinesis and thus displayed a typical desynaptic behaviour. They were therefore reassigned the gene symbol *ds-1* (Chapter 4). Asynaptic mutants with a virtually complete absence of chromosome pairing and hence gene recombination are the most attractive candidates for engineering diplosporic apomixis. However, as cogent cases of asynapsis have not yet been reported for potato, desynaptic mutants are the best alternative that is currently available. Therefore the following questions were raised:

1. Is it possible to select for consistent 2n-megaspore formation in *ds-1* mutants?
2. If so, are these 2n-megaspores of exclusive FDR origin as was predicted?
3. To what extent does crossing-over and hence gene recombination occur?

In chapter 6 the first two questions are addressed. The level of 2n-megaspore formation was determined in 126 *ds-1* mutants using seed set from 2*x*.4*x* and 2*x*.2*x*FDR testcrosses as a criterion. Although the majority formed on the average less than 5 seeds/fruit, 14% of all *ds-1* mutants produced 2n-megaspores in frequencies that resulted in consistent seed set within the 5-25 seeds/fruit range and allowed for routine production of nearly exclusive tetraploid progeny from 2*x*.2*x*FDR crosses. Subsequent cytological analyses revealed that 2n-megaspore formation in *ds-1* mutants resulted from a direct equational division of univalent chromosomes in the first meiotic division (pseudo-homotypic division), an FDR mechanism that had previously been reported to occur in some of the diplosporic apomictic plant species. Additional data on SDR 2n-megaspore formation in full-sib normal synaptic plants indicated that both SDR and FDR 2n-megaspore formation are likely to be caused by common genes for precocious chromosome division. Depending on the relative timing of cell cycle and chromosome division this precocious chromosome division may impose post-reductional (SDR) or pre-reductional (FDR) 'restitution' of the somatic chromosome number under normal and mutant synaptic conditions respectively.

Simply inherited marker traits are required for the analysis of genetic recombination in *ds-1* mutants and had to be identified first. Some of the genetic markers used are characterized in chapter 8. On the basis of extensive analysis of chiasma formation and gene-centromere mapping of a number of simply inherited marker genes in normal synaptic plants and desynaptic mutants it could be concluded that the *ds-1* gene substantially reduced the overall frequency of crossing-over and thus gene recombination in both male and female meiosis (Chapters 5 and 7). In addition, the genetic analyses revealed that FDR 2n-megaspores and FDR 2n-pollen from *ds-1* mutants preserve approximately 94.1 % of the overall parental heterozygosity as opposed to 79.5 % that is preserved by FDR 2n-pollen from normal synaptic plants. The *ds-1* gene was further demonstrated to particularly enhance the ability of FDR 2n-megaspores and 2n-pollen to pick up the genetic constitution of the parental clone, including complex types of favourable epistasis, with a minimum amount of reassortment.

Summarizing, it may be stated that the identification of diploid desynaptic mutants with consistent FDR 2n-megaspore formation extends the opportunities for direct transfer of enhanced diploid germplasm to tetraploids by means of sexual polyploidization and, since FDR 2n-megaspores and 2n-pollen from *ds-1* mutants are relatively efficient in preserving the genetic constitution of selected diploid parents particularly demonstrates the feasibility of 2*x*(*ds-1*;FDR)-2*x*(*ds-1*;FDR) crosses for the production of relatively uniform and vigorous true potato seed varieties.

Because of the potential for limited genetic recombination the use of the *ds-1* gene in the development of diplosporic apomixis seems less obvious. However, as genes for asynapsis have not been identified in potato so far, it may be considered the best alternative that is

currently available. Moreover some genetic diversity in apomictic progeny of diplosporic plant species as a consequence of autosegregation is quite common. The finding that some desynaptic clones formed FDR 2n-eggs through pseudo-homotypic division (≈ diplospory) strongly supports the hypothesis that gametophytic apomixis consists of a number of distinct and genetically controlled elements which may be combined to attain approximately identical reproduction in largely sexual plant species. The application of this approach to produce completely uniform true potato seed varieties obviously requires breeding for increased levels of FDR 2n-egg formation in synaptic mutants completely suppressing genetic recombination, and in addition either introduction of genes for pseudogamous seed development in such clones or the development of an efficient system for pseudogamous seed production.

Finally, it should be recognized that mutant synaptic genes may impose certain limitations. Because they are generally expressed in both male and female meiosis and thus are either largely sterile or produce only functional FDR 2n-gametes resulting in polyploidization upon crossing, they have to be manipulated in heterozygous condition. Breeding schemes that consist of (i) introducing mutant synaptic genes and genes for FDR 2n-gamete formation in advanced diploids through backcrossing and (ii) subsequent selection of improved mutant synaptic segregants with FDR 2n-gamete formation following intercrossing of advanced heterozygotes, would be appropriate but laborious. As to FDR 2n-megaspore formation it should moreover be realized that mutant synaptic conditions are actually required. Since heterozygous diploid clones are normal synaptic and thus at best form SDR 2n-megaspores the question remains how to predict whether or not such clones carry genes that bring about substantial FDR 2n-megaspore formation in derived synaptic mutants. If the hypothesis holds true, that SDR and FDR 2n-megaspore formation are caused by common genes for division precocity (Chapter 6), the occurrence of SDR 2n-megaspore formation through post-reductional precocious chromosome division in normal synaptic heterozygotes, might be a helpful criterion.

AFSLUITENDE SAMENVATTING

De kultuuraardappel, *Solanum tuberosum* L., is een zeer heterozygote, autotetraploide (2n=4*x*=48) plantensoort, die zich na zijn introduktie in Europa in de 16e eeuw heeft ontwikkeld tot een van de belangrijkste voedselgewassen in de wereld. De aardappel wordt van oudsher geteeld uit knollen. Knollen bieden echter een ideaal milieu aan tal van ziekteverwekkers, zodat bij de teelt voortdurend moet worden uitgegaan van gezond pootgoed. De produktie hiervan vereist een grondige kennis en een goede infrastruktuur. Omdat deze in de meeste ontwikkelingslanden ontbreken, moeten zij gezond pootgoed uit ontwikkelde landen importeren. Meestal zijn de hiervoor benodigde deviezen niet voorhanden. Veel, vooral kleine, boeren telen zodoende noodgedwongen zieke poters van eigen grond, hetgeen resulteert in sterke opbrengstredukties. In antwoord op de dringende behoefte aan goedkoop en gezond plantmateriaal houdt het Internationale Aardappel Centrum (CIP) te Peru zich sinds 1979 onder meer bezig met het zoeken naar methoden voor de produktie van zaad als uitgangsmateriaal voor de teelt. Aardappelzaden zijn relatief gemakkelijk en goedkoop te produceren en, zelfs indien geoogst van zieke planten, in de regel vrij van ziekteverwekkers. Eén van de problemen hierbij is echter het gebrek aan de veelal gewenste uniformiteit van een uit zaad geteeld aardappelgewas: wanneer het zaad voor zo'n gewas is verkregen via vrije bestuiving of onderlinge kruising van bestaande rassen, zal dat gewas als gevolg van het hoge niveau van heterozygotie in de aardappel in de regel zeer heterogeen zijn.

Relatief uniforme zaailingpopulaties kunnen in principe op een aantal verschillende manieren worden verkregen. Eén produktiemethode maakt gebruik van het herhaaldelijk voorkomen van ongereduceerde (2n) gameten in diploide aardappels, zowel gekultiveerde en wilde, als daarvan afgeleide vormen. Zij bieden de mogelijkheid tot synthese van tetraploide hybriden via 4*x*-2*x* en 2*x*-2*x* kruisingen (respektievelijk unilaterale en bilaterale seksuele polyploidisatie). De groeikracht en uniformiteit van aldus verkregen hybriden zijn grotendeels afhankelijk van de wijze van 2n-gametenvorming in de geselekteerde diploide ouderklonen. Op basis van de genetische konsekwenties van verschillende tijdens de meiose optredende abnormaliteiten wordt onderscheid gemaakt tussen 'first division restitution (FDR)' en 'second division restitution (SDR)' 2n-gameten. FDR is het meest gewenst daar zij, in tegenstelling tot SDR, een hoog percentage van de in de oorspronkelijke diploide kruisingsouders aanwezige, gunstige genen- en allelencombinaties onveranderd overdragen op de via seksuele polyploidisatie verkregen tetraploide nakomelingschap en zodoende sterk bijdragen tot de uniformiteit daarvan. In dat verband is de kombinatie van FDR 2n-gametenvorming met synaptische mutanten van eminent belang. Omdat de genetische rekombinatie in dergelijke mutanten in de regel veel lager is dan normaal, versterken zij het vermogen van FDR 2n-gameten om het ouderlijk genotype grotendeels intakt over te dragen op de nakomelingen aanmerkelijk. Bij volledige afwezigheid van genetische rekombinatie in diploide FDR klonen zouden via 2*x*FDR-2*x*FDR kruisingen zelfs volledig uniforme, zeer groeikrachtige tetraploide hybriden kunnen worden verkregen.

Komplete of bijna komplete uniformiteit kan in principe ook worden bereikt door de induktie van apomiktische zaadvorming. Apomixis *sensu stricto* is het ongeslachtelijk ontstaan van embryo's en zaden, waardoor alle zaailingen met de moederplant en dus ook onderling genetisch identiek zijn evenals bij de normale vegetatieve vermeerdering uit knollen. Apomiktische embryo's kunnen rechtstreeks ontstaan uit somatische cellen buiten de in het zaadbeginsel gevormde embryozak (adventief-embryonie) of onstaan uit ongereduceerde cellen, meestal eicellen, van de embryozak (gametophytische apomixis) zonder dat bevruchting van deze cellen optreedt (parthenogenese). De eveneens ongereduceerde embryozak kan hierbij onstaan uit een somatische, meestal nucellaire cel (aposporie) of uit een generatieve zogenaamde archespore cel van het zaadbeginsel (diplosporie). In het laatste geval kan sprake zijn van het geheel achterwege blijven van de meiose of van een sterk

gemodificeerde meiose, waarin noch reduktie tot het haploide (n) chromosoomaantal noch noemenswaardige genetische rekombinatie optreedt. Voor de parthenogenetische ontwikkeling van ongereduceerde eicellen tot volwassen embryo's en zaden kan bevruchting van de secundaire embryozakkern als stimulus voor endosperm vorming al dan niet noodzakelijk zijn (pseudogame resp. autonome apomixis).

Alhoewel apomiktische zaadvorming nog nooit is waargenomen in *Solanaceae*, rechtvaardigde het belang ervan voor de produktie van rassen ten behoeve van de teelt van aardappels uit zaad een poging om apomiktische reproduktie in de aardappel te induceren. Het in dit proefschrift beschreven onderzoek richtte zich op de perspektieven voor de induktie van gametophytische apomixis, in het bijzonder van pseudogame diplospore apomixis en wel om de volgende redenen:

1. Gametophytische apomixis (i.e., aposporie en diplosporie) verdient de voorkeur boven adventief-embryonie, omdat bij gametophytische apomixis de embryozak een efficiënte barrière is tegen de meeste aardappelvirussen en zodoende een belangrijke bijdrage levert tot de produktie van virusvrije zaden.
2. Aposporie is nog nooit in de aardappel waargenomen en dus moet voor de induktie daarvan een omvangrijk mutatie-veredelingsprogramma worden opgezet. Op grond van de schaarse en veelal tegenstrijdige literatuurgegevens omtrent de genetika van aposporie (monogeen-polygeen; dominant-recessief) en het ontbreken van efficiënte methoden voor het opsporen van 'positieve' mutanten mag verwacht worden, dat de uitkomsten van een dergelijk programma hoogst onzeker zijn.
3. Apomixis bestaat volgens een recente hypothese uit een aantal afzonderlijke en genetisch bepaalde elementen, die, weliswaar verspreid, ook voorkomen in grotendeels seksuele plantesoorten. De in het geval van diplosporie globaal te onderscheiden elementen zijn: afwezige of sterk gereduceerde chromosoomparing en/of genetische rekombinatie (asynapsis/desynapsis), de vorming van ongereduceerde FDR-megasporen en embryozakken, en pseudogame dan wel autonome parthenogenetische ontwikkeling van de ongereduceerde eicellen tot embryo's en zaden. Daar enkele van deze elementen verspreid in de aardappel voorkomen, moet kombinatie daarvan in één genotype in principe mogelijk zijn.
4. Voor induktie van diplosporie is bestudering van de tot op heden grotendeels onbekende effekten van synaptische mutanten op de vrouwelijke meiose noodzakelijk. Hetzelfde geldt voor de mechanismen c.q. oorzaken van FDR 2n-eicel vorming. Eventuele nevenresultaten kunnen zodoende wellicht gebruikt worden bij het streven naar de produktie van voldoende uniforme tetraploide hybriden uit de eerdergenoemde 2xFDR-2xFDR kruisingen.

Om vast te kunnen stellen of ongereduceerde embryozakken van diplospore afkomst zijn en om met de expressie van synaptische mutanten aan vrouwelijke zijde of 2n-eicelvorming geassocieerde abnormaliteiten te kunnen herkennen, is een gedegen kennis van het normale verloop van de meiose en de embryozakvorming essentieel. Dit normale verloop werd bestudeerd aan verscheidene diploide aardappelklonen (Hoofdstuk 1). In tegenstelling tot wat in de literatuur is vermeld, bleek het archesporium van de aardappel niet altijd beperkt te zijn tot slechts één enkele cel. Een surplus aan archesporen ontwikkelde zich soms tot normale ongereduceerde embryozakken. Uit het zo nu en dan voorkomen van meer dan één embryozak in een zaadbeginsel mag dus niet zonder meer gekonkludeerd worden, dat er sprake is van aposporie, hoewel dat in het verleden door verscheidene onderzoekers is verondersteld. Op basis van het verloop van de vrouwelijke meiose werd bovendien afgeleid dat 2n-eicellen uitsluitend door SDR onstaan, wanneer sprake is van normale chromosoomparing en overkruising tijdens de meiose en voorts, dat asynapsis/desynapsis een noodzakelijke voorwaarde is voor FDR 2n-eicelvorming en dus ook voor de induktie van diplosporie. Het verdere onderzoek concentreerde zich daarom primair op de identifikatie en karakterisering van genen voor asynapsis dan wel desynapsis in de vrouwelijke meiose, de

identifikatie van synaptische mutanten met 2n-eicelvorming en de opheldering van de daarin optredende cytologische mechanismen van FDR 2n-eicelvorming.

Omdat de voor dit type onderzoek traditioneel toegepaste mikrotoomtechnieken te arbeidsintensief zijn en bovendien niet geschikt zijn voor kwantitatieve interpretatie van meiotische processen, moesten eerst snelle en routinematig toepasbare technieken beschikbaar komen. Twee van dergelijke technieken werden in het kader van dit promotie-onderzoek ontwikkeld. De ene techniek maakte gebruik van het feit, dat vooraf gefixeerde zaadbeginsels binnen enkele uren doorzichtig gemaakt kunnen worden met behulp van methyl-salicylaat, zodat zij zich bij uitstek leende voor grootschalige beoordeling van het verloop van de meiose en de embryozakontwikkeling (Hoofdstuk 2). De andere, een techniek voor het maken van squash-preparaten van zaadbeginsels, maakte het mogelijk om het effect van synaptische mutanten en van genen voor 2n-eicelvorming op het chromosoomgedrag gedetailleerd te bestuderen (Hoofdstuk 3).

Gebruik makend van deze beide technieken werd vastgesteld dat (ten minste) drie van zes voor de aardappel gerapporteerde synaptische mutanten identiek zijn en zowel in de mannelijke als vrouwelijke meiose tot expressie komen. Daarnaast kon het bestaan worden weerlegd van een in de literatuur gerapporteerde synaptische mutant, die uitsluitend in de vrouwelijke meiose tot expressie zou komen. Aan de drie eerdergenoemde, identieke synaptische mutanten werd het gensymbool *ds-1* toegekend. Zij werden gekarakteriserd door het voortijdig (tijdens diakinese) uiteenvallen van normaal gepaarde homologe chromosomen en vertoonden dus een typisch desynaptisch gedrag (Hoofdstuk 4). Asynaptische mutanten met volledige afwezigheid van chromosoomparing en dus genetische rekombinatie zijn het meest aantrekkelijk voor de induktie van diplosporie. Omdat dergelijke mutanten tot nu toe nog niet in de aardappel zijn gevonden, vormden de bovengenoemde desynaptische mutanten het beste, thans beschikbare alternatief. De volgende vragen dienden daarom te worden beantwoord:

1. Kunnen desynaptische mutanten worden geselekteerd met een redelijk niveau van 2n-eicelvorming?
2. Zo ja, zijn deze 2n-eicellen, zoals eerder werd voorspeld, inderdaad van FDR afkomst?
3. In welke mate is de overkruising en dus genetische rekombinatie in desynaptische mutanten gereduceerd?

De eerste twee vragen komen aan de orde in hoofstuk 6. Na toetskruising van 126 diploide desynaptische mutanten met tetraploide rassen en diploide FDR-testers, werd het aantal zaden/bes bepaald, zijnde een goed criterium voor het niveau van 2n-eicelvorming in die mutanten. In iets minder dan de helft van deze klonen werd aldus 2n-eicelvorming aangetroffen. 14 % van alle geteste desynaptische klonen leverden na toetskruising gemiddeld 5-25 zaden per bes. Het niveau van 2n-eicelvorming in deze klonen was in de regel voldoende konstant voor min of meer routinematige produktie van vrijwel uitsluitend tetraploide nakomelingen uit 2*x*.2*x* kruisingen. Cytologische analyse toonde aan dat de in desynaptische mutanten gevonden 2n-eicelvorming onstaat door een vroegtijdige equationele deling van univalenten tijdens de eerste meiotische deling (zogenaamde pseudo-homotypische deling). Dit is een FDR-mechanisme dat reeds eerder is waargenomen in enkele plantesoorten met diplospore apomixis. Mede op grond van additionele gegevens omtrent SDR 2n-eicelvorming in normale zusterklonen werd verder gekonkludeerd dat SDR en FDR 2n-eicelvorming wordt veroorzaakt door dezelfde genen, die voortijdige chromosoomdeling bewerken en die, afhankelijk van het moment waarop zij tot expressie komen, aanleiding geven tot post-reduktionele (SDR) dan wel pre-reduktionele (FDR) 'restitutie' van het somatische chromosoomaantal in respektievelijk normale en desynaptische klonen.

Voor de analyse van de in desynaptische mutanten optredende genetische rekombinatie is het noodzakelijk te kunnen beschikken over eenvoudig verervende zogenaamde marker-eigenschappen. De identifikatie van een aantal van de in de genetische analyses gebruikte marker-eigenschappen is beschreven in hoofdstuk 8. Op basis van uitgebreide analyse van

chiasmavorming, het cytologische equivalent van genetische rekombinatie, en schatting van van gen-centromeer afstanden in normale planten en desynaptische mutanten (hoofdstuk 5 en 7) kon worden gekonkludeerd, dat de in laatstgenoemde mutanten optredende genetische rekombinatie over het geheel genomen aanmerkelijk lager was dan normaal. FDR 2n-eicellen en FDR 2n-pollen afkomstig van desynaptische mutanten behielden gemiddeld zo'n 94.1 % van de in de ouderplant aanwezige heterozygotie, 14.6 % meer dan FDR 2n-pollen afkomstig van normale planten. De resultaten gaven bovendien aan dat het vermogen van FDR 2n-gameten om het ouderlijk genotype grotendeels onverandert over te dragen, in desynaptische mutanten aanmerkelijk hoger was dan normaal het geval is.

Samenvattend kan worden gesteld dat de identifikatie van diploide desynaptische mutanten met FDR 2n-eicelvorming de mogelijkheden voor efficiënte overdracht van waardevol diploid materiaal naar tetraploiden via unilaterale en bilaterale seksuele polyploidisatie aanmerkelijk verruimt en dat de produktie van relatief uniforme tetraploide hybriden ten behoeve van de teelt van aardappels uit zaad via 2*x*(*ds-1*;FDR)-2*x*(*ds-1*;FDR) kruisingen haalbaar is gebleken.

Gezien de weliswaar geringe genetische rekombinatie in desynaptische mutanten ligt het gebruik van die mutanten voor de induktie van diplospore apomixis minder voor de hand. Zij vormen echter het beste beschikbare alternatief, zolang asynaptische mutanten in de aardappel nog niet zijn gevonden. Overigens is het voorkomen van enige genetische variabiliteit in apomiktische nakomelingschappen van diplospore plantesoorten als gevolg van zogenaamde 'auto-segregatie' heel normaal. De vorming van FDR 2n-eicellen door pseudo-homotypische deling in desynaptische mutanten (≈ diplosporie) ondersteunt hoe dan ook de hypothese, dat gametophytische apomixis is opgebouwd uit een aantal afzonderlijke en genetisch bepaalde elementen, die verspreid voorkomen in grotendeels seksuele plantesoorten en daarin, indien samengebracht in één plant, identieke reproduktie via zaad kunnen bewerkstelligen. Voor de praktische toepassing van deze benadering ten behoeve van de teelt van aardappels uit zaad dient het niveau van FDR 2n-eicelvorming in bij voorkeur asynaptische mutanten nog aanmerkelijk verhoogd te worden. Daarnaast is het noodzakelijk om genen voor pseudogame parthenogenese in dergelijke klonen te introduceren, of om een efficiënt systeem voor pseudogame zaadproduktie op te zetten. Daarbij kan gebruikt gemaakt worden van reeds lange tijd beschikbare *Solanum phureja* bestuivers, die pseudogame parthenogenese stimuleren.

Ten slotte dient vermeld te worden dat aan het werken met synaptische mutanten ook enkele nadelen verbonden zijn. Omdat genen voor asynapsis en desynapsis meestal in de mannelijke en vrouwelijke meiose tot expressie komen, zijn zij of volledig steriel of ze vormen uitsluitend funktionele FDR 2n-gameten, hetgeen na kruising resulteert in tetraploiden (polyploidisatie). Genen voor asynapsis en desynapsis zullen daarom veelal slechts in heterozygote vorm gehanteerd kunnen worden. Veredelingsschema's die bestaan uit (i) introduktie van genen voor asynapsis/desynapsis en genen voor FDR 2n-gametenvorming in goede diploiden via herhaalde terugkruising en (ii) daaropvolgende selektie van verbeterde synaptische mutanten met FDR 2n-gametenvorming na onderlinge kruising van heterozygote diploiden, zijn in principe bruikbaar, maar zeer arbeidsintensief. Met betrekking tot de vorming van FDR 2n-eicellen dient men zich bovendien te realiseren dat hiervoor in principe asynapsis dan wel desynapsis vereist is. Normaal synaptische, heterozygote diploiden vormen dus hoogstens SDR 2n-eicellen. De grote vraag is zodoende, hoe voorspeld kan worden of dergelijke klonen drager zijn van genen, die FDR 2n-eicelvorming bewerken. Indien de hypothese juist is, dat SDR en FDR 2n-eicelvorming wordt veroorzaakt door dezelfde genen voor voortijdige chromosoomdeling, is het voorkomen van SDR als gevolg van voortijdige post-reduktionele chromosoomdeling in heterozygote klonen mogelijk een bruikbaar criterium.

CURRICULUM VITAE

Erik Jongedijk werd op 18 juli 1957 geboren in Apeldoorn. Na het behalen van het Atheneum-B diploma aan de Koninklijke Scholengemeenschap Apeldoorn (KSA) in 1975, begon hij zijn studie aan de toenmalige Landbouwhogeschool te Wageningen. In 1982 studeerde hij af in de studierichting Plantenveredeling met als keuze-vakken Erfelijkheidsleer en Landbouwplantenteelt. Zijn praktijktijd bracht hij door bij het Koninklijk Kweekbedrijf en Zaadhandel D.J. van der Have b.v. te Rilland-Bath en bij de toenmalige Stichting voor Plantenveredeling (SVP) te Wageningen. Voor de in het kader van zijn studie geschreven verhandeling over de potenties en toepassing van seksuele polyploidisatie in de aardappelveredeling en de teelt van aardappels uit zaad ontving hij in 1984 een door de *'Stichting Broekemafonds 1921' ingestelde scriptieprijs. Vanaf oktober 1982 tot mei 1988* was hij verbonden aan de vakgroep Plantenverdeling van de Landbouwuniversiteit Wageningen, eerst als promotie-assistent in de aardappelonderzoeksgroep van prof.dr.ir. J.G.Th. Hermsen en vanaf augustus 1984 als universitair docent. Sinds mei 1988 is hij werkzaam bij het plantenbiotechnologie-bedrijf MOGEN International n.v. te Leiden.