CHROMOSOME IDENTIFICATION AND GENE MAPPING IN POTATO BY PACHYTENE, TRISOMIC AND HALF-TETRAD ANALYSIS

CHROMOSOOMIDENTIFICATIE EN GEN-KARTERING BIJ DE AARDAPPEL DOOR MIDDEL VAN PACHYTEEN-TRISOMEN- EN HALFTETRADEN-ANALYSE

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CHROMOSOME IDENTIFICATION AND GENE MAPPING IN POTATO BY PACHYTENE, TRISOMIC AND HALF-TETRAD ANALYSIS

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ABSTRACT

The research described in this thesis deals with chromosome identification and gene mapping. In contrast to results from literature, in this study only three chromosomes (1, 2 and 12) could unambiguously be identified in mitotic cells using conventional staining, and four (1, 2, 3 and 4) in case of Giemsa C-banding. With both methods the chromosomes 1 and 2 could unambiguously be identified and are homologous to the chromosomes 1 and 2 as identified by pachytene analysis. Reliable chromosome identification in potato can be achieved by pachytene analysis.

It was found in this study by using non-radioactive *in situ* hybridization that one basic chromosome of the potato contains rRNA genes. In contrast to a report in the literature about detection of one chromosome with gene(s) for patatin using a cDNA clone, hybridization with a genomic DNA clone used in this study detected more than one basic chromosome carrying genes related to patatin.

The bivalents in *S. phureja* Juz. et Buk. were morphologically very similar to those of *S. tuberosum* L. ssp. *tuberosum* Hawkes cv. Gineke as identified by pachytene analysis. An interchange in *S. phureja* is described and the involvement of the chromosomes 3 and 12 in this interchange could clearly be demonstrated by pachytene analysis and the meiotic behaviour in F_1 hybrids. Trisomic descendants selected in the first selfed generation of the interchange heterozygote were primary trisomic being homozygous for the interchange or tertiary trisomic.

Meiotic behaviour in 11 primary trisomics was investigated and female transmission of the extra chromosome determined. Triple synapsis of pachytene chromosomes was often found in the euchromatic parts of the chromosomes. In this study a significant correlation between the relative chromosome or euchromatin length and the coefficient of realization of a trivalent at metaphase I was found in the primary trisomics of the potato. In spite of this result no relationship could be established between female transmission and the length of the extra chromosome.

By means of half-tetrad analysis the map distance relative to the centromere could be estimated of each of three dominant genes involved in resistance to potato viruses X and Y and to pathotype Ro1 from *Globodera rostochiensis*, and of the recessive gene for yellow leaf-margin. The gene for yellow margin was localized on chromosome 12 and that for topiary on chromosome 3 by means of trisomic analysis.

Stellingen

1. Associatie van drie of meer homologe chromosomen is mogelijk zonder dat partnerwisseling optreedt.
   Comings, D.E. and Okada T.A. (1971). Nature 231:119-121.
   Dit proefschrift.

2. De door Lee *et al.* en Kessel en Rowe geïdentificeerde 'secondary trisomics' zijn primaire trisomen.
   Lee, H.K., Kessel, R., and P.R. Rowe (1972). Can. J. Genet. Cytol. 14:533-543.
   Kessel, R. and Rowe P.R. (1974). Can. J. Genet. Cytol. 16:515-528.
   Dit proefschrift.

3. Het is hoogst onwaarschijnlijk dat pre-meiotische verdubbeling van het aantal chromosomen in aardappel, rogge en *Allium tuberosum* de vorming van 2*n* gameten verklaart.
   Lam, S.L. (1974). J. Heredity 65:175-178.
   Lelley, T., Mahmoud, A.A., Lein, V. (1987). Genome 29:635-638.
   Gohil, R.N., Kaul, R., (1981). Chromosoma 82:735-739.

4. Bij het samenstellen van RFLP-kaarten op basis van genetische recombinatiepercentages in kruisingspopulaties is onderzoek van de meiose van de ouderplanten gewenst.

5. Het uitvinden van de grote doorbraak in de biologische stikstofbinding kost veel geld en creativiteit, maar levert tenslotte een pyrrhusoverwinning op.
   Bron: *Charles Crombach, dagblad Trouw, 19-12-1990.*

6. Het toekennen van de wereldvoedselprijs (1990) aan John Niederhauser onderstreept het belang van resistentie-onderzoek van de schimmel *Phytophthora infestans* als middel ter bestrijding van honger in de wereld.

7. Genoverdracht via transformatie bij een ras kan leiden tot verlies van raseigenschappen.

8. De opvatting van de VCOGEM dat zgn. brugkruisingen niet tot de traditionele kweekmethoden moeten worden gerekend, is onjuist.
   Bron: *Concept-richtlijnen voor de vervaardiging van en de handelingen met genetisch gemodificeerde organismen, uitgave VCOGEM d.d. 12 juli 1993.*

9. Veertig kilometer rijden op een liter slaolie in een Mercedes is mogelijk dankzij het geheim van de Elsbettmotor; het milieu zou bijzonder gediend zijn met grootschalige toepassing van deze krachtbron.

10. Het toekennen van het vlinderlogo aan zogenaamd "milieubewust" geteelde groenten en fruit misleidt zowel de consument als de producent.

11. Het bouwen van een computer met gevoel zal een utopie blijven.

12. Het feit dat Eva gevonden is betekent nog niet dat Adam dichtbij is.

Stellingen behorende bij het proefschrift "Chromosome identification and gene mapping in potato by pachytene, trisomic and half-tetrad analysis", door Marinus Wagenvoort in het openbaar te verdedigen op 24 november 1993 in de aula van de Landbouwuniversiteit te Wageningen.

Voor Betsie

"*Aude ac perge*" betekent "Waag en (ga) voort"

(Deze zinspreuk is een aanmoediging en is bedacht door Prof. dr. H. Wagenvoort, hoogleraar in de latijnse taal aan de Rijksuniversiteit te Utrecht. Bron: "De Wagenschouw", dec.1986, uitgave van de Stichting Familie Wagenvoort).

Front cover:

The twelve basic chromosomes of the potato are shown as pachytene bivalents except for chromosome 7 which is shown in a trivalent configuration.
Photo made by the TFDL-DLO Centrale fotodienst, established at ATO-DLO.

VOORWOORD

De verschijning van dit proefschrift vormt de afsluiting van het project "Cytogenetisch onderzoek in aardappel". Fundamenteel cytogenetisch onderzoek in aardappel werd aan het eind van de jaren 60 geïnitiëerd door o.a. Dr. Ir. W. Lange (Stichting voor Plantenveredeling, SVP, in 1990 opgegaan in het Centrum voor Plantenveredelingsonderzoek, CPO, en in 1991 in het Centrum voor Plantenveredelings- en Reproduktieonderzoek, CPRO-DLO). De gezamenlijke interesse van IVP- (Instituut voor Plantenveredeling, Landbouwuniversiteit, Wageningen) en SVP onderzoekers in de toepassing van "trisomen" voor gen-localisatie in aardappel en het verblijf van deze onderzoekers op dezelfde "campus" leidde er in 1970 toe dat de werkgroep "Trisomie in aardappel" werd opgericht. Deze werkgroep onder leiding van mijn promotor, co-promotor en Dr. Ir. W. Lange begeleidde grotendeels het onderzoek zoals beschreven in dit proefschrift. Het practische werk werd uitgevoerd bij de SVP en het CPO in de periode tussen 1968 en 1991.

Aan de totstandkoming van dit boekje hebben velen een bijdrage geleverd. Zonder anderen te kort te willen doen, wil ik enkele personen met name noemen. Mijn promotor Dr. Ir. J.G.Th. Hermsen ben ik veel dank verschuldigd voor de samenwerking gedurende een lange periode. Uw milde wijze van kritiek leveren op de manuscripten was er altijd op gericht de schrijver overeind te houden en bepaalde teksten ten onder te laten gaan. Hiervan ging een stimulerende invloed uit en dit heeft zonder twijfel tot verbetering van de kwaliteit van de meest recent geschreven publicaties geleid. Mijn co-promotor Dr. M.S. Ramanna ben ik zeer erkentelijk voor de leerzame discussies, gevoerd tijdens de uitvoering van het onderzoek en de geuite positieve kritiek op de manuscripten. *I shall never forget your enthousiasm and your shouts of surprise when viewing another pachytene configuration, not earlier found by me. Your extreme kindness and patience as well as your sincere interest in the subject was the basis for a protacted and good connection whereof I express my heartfelt thanks.* Vele oud-SVP medewerkers hadden invloed op mijn ontwikkeling als assistent en later als onderzoeker in het veredelingsonderzoek. Mijn eerste activiteiten werden begeleid door de helaas te vroeg overleden Ir. Nico van Suchtelen geassisteerd door zijn rechterhand Ing. Wietze Bouma (periode 1964-1967). In die periode werd onderzoek verricht aan dihaploide aardappelen en de schimmel *Phytophthora infestans*, veroorzaker van de aardappelziekte. Mijn eerste chromosoom-preparaten vervaardigde ik onder de bezielende leiding van Ing. G.J. Speckmann, waarvoor ik hem van harte bedank. Aan mijn wetenschappelijke vorming heeft Dr. Ir. W. Lange een belangrijk aandeel gehad. Gedurende een reeks van jaren was hij mijn directe begeleider, die mij o.a. de beginselen bijbracht van

het schrijven van wetenschappelijke artikelen. Wouter had bijna altijd wel een oplossing bij de hand als de resultaten niet geheel overeen kwamen met wat we verwachtten. Op deze plaats wil ik hem graag bedanken voor de jarenlange en prettige samenwerking.

Bij de uitvoering van het onderzoek werd ik geassisteerd door Maria Gut-Simicek, Jacqueline de Haas-Buurman, Greet Kuiper, Wietske van der Molen, Karin Nelson en Marleen de Vries en werden de stagiaire Frans de Bruin, de KUN-studenten Theo van der Lee en A.H.M. Vermeer en de LU-studente Ellen Wisman door mij begeleid. De inzet van allen heb ik zéér gewaardeerd, in het bijzonder die van Greet Kuiper die in een zeer woelige periode van fusies van instituten en opheffing van afdelingen toch de moed erin hield en de zoveelste variant van het protocol voor *in situ* hybridisatie uitprobeerde. Dr. H.J. Huizing, Dr. A.F. Krens en Drs. G.J.A. Rouwendal wil ik bedanken voor de practische adviezen gegeven in de laatste fase van het onderzoek, evenals Mevr. Dr. J. (Coosje) Hoogendoorn voor het kritisch lezen van de meest recente manuscripten en Drs. Paul Keizer voor het uitvoeren van enkele statistische analyses.

Collega-medewerkers van SVP, CPO en CPRO die op één of andere wijze een bijdrage hebben geleverd aan dit proefschrift wil ik hierbij van harte bedanken. Voor het vele typewerk kon ik rekenen op de ondersteuning van de afdeling tekstverwerking van het CPRO-DLO. Alle medewerksters van harte bedankt, met name Mevr. Ans Corver en Mevr. Jannie Kramp die eveneens (met een 'beetje' hulp van Robert van Loo) de "lay out" verzorgden en een bijzondere prestatie hebben geleverd.

Het ontwikkelen en afdrukken van de originele (micro)-foto's werd uitgevoerd door Peter Stad en zijn naaste medewerkers van de TFDL-DLO Centrale fotodienst. Hartelijk dank voor de geleverde diensten en de bijzondere kwaliteit.

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CONTENTS

ACCOUNT

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

Hermsen, J.G.Th., M. Wagenvoort & M.S. Ramanna, 1970. Aneuploids from natural and colchicine induced autotetraploids of *Solanum*. Can. J. Genet. Cytol. 12: 601-613.

Lange, W. & M. Wagenvoort, 1973. Meiosis in triploid *Solanum tuberosum* L. Euphytica 22: 8-18.

Wagenvoort, M. and W. Lange, 1975. The production of aneudihaploids in *Solanum tuberosum* L. Group *Tuberosum* (The common potato). Euphytica 24: 731-741.

Ramanna, M.S. and M. Wagenvoort, 1976. Identification of the trisomic series in diploid *Solanum tuberosum* L. Group *Tuberosum*. I. Chromosome identification. Euphytica 25: 233-240.

Wagenvoort, M. and M.S. Ramanna, 1979. Identification of the trisomic series in diploid *Solanum tuberosum* L. Group *Tuberosum*. II. Trivalent configurations at pachytene stage. Euphytica 28: 633-642.

Wagenvoort, M. and W. Lange, 1980. Fertility, plant morphology and transmission rates of the extra chromosome in single and double trisomics of *Solanum tuberosum* L. Group *Tuberosum*. Euphytica 29: 281-293.

Wagenvoort, M., 1986. The effect of temperature on 2n-pollen formation in *Solanum phureja*. In: A.G.B. Beekman, K.M. Louwes, L.M.W. Dellaert and A.E.F. Neele (Eds.) Potato research of tomorrow: Drought tolerance, virus resistance and analytic breeding methods pp. 124-137.

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RFLP's (Restrictie Fragment Lengte Polymorfieën) voor de constructie van genenkaarten. Prophyta 42: 275-278.

Wagenvoort, M. & G.J.A. Rouwendal, 1989. *In situ* hybridisatie: Een snelle methode voor de localisatie van specifieke DNA en RNA sequenties. Prophyta 43: 107-108.

Jacobs, J.J.M.R., F.A. Krens, W.J. Stiekema, M. van Spanje, M. Wagenvoort, 1990. Restriction fragment length polymorfism analysis in *Solanum* spp. for the construction of a genetic map of *Solanum tuberosum* L.: a preliminary study. Potato Research 33: 171-180.

Wagenvoort, M. and A.P.M. den Nijs, 1992. Implications of 2*n* pollen for breeding tetraploid perennial ryegrass. In: A. Mariani and S. Tavoletti (Eds.). Gametes with Somatic Chromosome Number in the Evolution and Breeding of Polyploid Polysomic Species: Achievements and Perspectives pp. 5-14. Tipolitografia Porziuncola - Assisi (PG) Italy.

GENERAL INTRODUCTION

The cultivated potato *Solanum tuberosum* L. ssp. *tuberosum* Hawkes, and its relatives belong to the subgenus Potatoe (G. Don) D'Arcy, section Petota of the genus *Solanum* (Hawkes, 1990). Cultivars of this important food crop are predominantly tetraploid ($2n=4x=48$) and belong to the taxonomic series *Tuberosa*. This series also includes the cultivated diploid ($2n=2x=24$) primitive species *S. phureja* Juz. et Buk. and *S. stenotomum* Juz. et Buk., both with genome formula AA (Hawkes, 1958). The cultivated potato encompasses the two subspecies *andigena* (Juz. et Buk.) Hawkes and *tuberosum* both with genome formula AAA^tA^t according to Matsubayashi (1991). It is believed on taxonomic grounds that the tetraploid potato arose through hybridization of the primitive species *S. stenotomum* with a wild diploid species, e.g. *S. sparsipilum* (Bitt.) Juz. et Buk.. Further evolution took place with at least two other wild species, *S. acaule* Bitt. and *S. megistacrolobum* Bitt., bringing genes for frost resistance into the cultivated gene pool (Hawkes, 1988). Interspecific hybridization, including species from different series, resulted in a polyploid series. Alternatively, Matsubayashi (1991) on the basis of tuber characters concluded that *S. phureja* might have crossed with *S. stenotomum* giving rise to ssp. *andigena*. *S. phureja* has been described as a cultivated diploid species derived from *S. stenotomum* by gene mutations and selections (Hawkes 1988; Matsubayashi 1991). It shows a close similarity to *andigena* in pachytene morphology (Matsubayashi, 1991). *S. tuberosum* ssp. *tuberosum* is generally felt to have evolved by long-day adaptation and selection from *S. tuberosum* ssp. *andigena*. However, some differences have been pointed out for cytoplasmic factors (non-chromosomal genes in mitochondria and chloroplasts) of ssp. *tuberosum* on the one hand and ssp. *andigena*, *S. phureja* and *S. stenotomum* on the other (Grun, 1979; Hosaka *et al.*, 1984). Some cytoplasmic sensitive factors cause abnormalities of the reproductive organs due to an interaction with different dominant nuclear genes. For example, the diploid hybrids between *S. tuberosum* ssp. *tuberosum* and *S. phureja* described in this thesis showed various kinds of flower abnormalities and were mostly male sterile.

The basic chromosome number of potato is assumed to be 12 on the basis of chromosome behaviour in dihaploids and monohaploids from *S. tuberosum* (Chavez and De Sosa, 1972; Van Breukelen *et al.*, 1975, 1977) and monoploids ($2n=x=12$) from the diploid species *S. verrucosum* Schlechtd. (Irikura, 1976). According to Kawakami and Matsubayashi

(1957), and Matsubayashi (1981), *S. tuberosum* is rather a segmental allotetraploid than an autotetraploid. Two reasons in particular led to this conclusion. Firstly, the meiotic behaviour and fertility of some dihaploid *tuberosum* clones are remarkably variable and consequently these clones are meiotically unstable as compared to certain natural diploid species (Matsubayashi, 1960; Yeh *et al.*, 1964., Sosa and Sosa, 1971). Secondly, structural differences occur, such as heteromorphic short arms of the nucleolar chromosome in some *andigena* dihaploids (Yeh and Peloquin, 1965), loops between paired chromosomes in *tuberosum* dihaploids (Ramanna and Wagenvoort, 1976) and, sporadically unpaired segments in bivalents (Matsubayashi, 1991). In spite of this, regular meiosis and good pollen fertility were observed in many diploid hybrids even from crosses between species from different taxonomic series. This suggests, that differences exist at the genic level rather than at the chromosomal level (Swaminathan and Howard, 1953; Howard, 1960; Matsubayashi and Misoo, 1979; Matsubayashi, 1983; Peloquin *et al.*, 1983; and others). Therefore, other workers consider *S. tuberosum* an autotetraploid rather than an allotetraploid.

Tetrasomic inheritance, a high degree of heterozygosity of cultivars, and the high number of small and morphologically very similar chromosomes, seriously hamper genetic and cytogenetic research in this crop. Consequently, methods were developed for the production of dihaploids from tetraploid *tuberosum* and *andigena* clones. This has been accomplished via crosses between the autotetraploids and pollinator clones of *S. phureja*, where dihaploids are thought to originate from unfertilized eggs through pseudogamy. The dihaploids can be detected using the seedling marker hypocotyl colour (Peloquin and Hougas, 1959) or the more efficient seed marker embryo-spot (Hermsen and Verdenius, 1973). A second cycle of pseudogamous haploid induction accomplished by crossing dihaploids with the same pollinator clones gave rise to monohaploids (Van Breukelen *et al.*, 1975, 1977; Uijtewaal *et al.*, 1987). Mono(hap)ploids are a very useful tool for karyotyping the potato genome. However, recently it has been suggested that some of these dihaploids could not be of parthenogenetic origin since aneuploid cells and *S. phureja*-specific DNA sequences have been detected in these plants using cytological and molecular methods including randomly amplified polymorphic DNA (RAPD)(Clulow *et al.*, 1991; Waugh *et al.*, 1992). It is felt that dihaploids also can arise by interspecific pollination followed by normal double fertilisation and preferential elimination of *S. phureja* chromosomes during early stages of embryo development. At present, it has not yet been shown whether chromosomes from *S. tuberosum* are also eliminated during the early growth stages of the *tuberosum-phureja* hybrids.

Identification of somatic chromosomes of the potato by conventional staining is difficult because their size is small and the morphology of the twelve basic chromosomes is very similar. Although Pijnacker and Ferwerda (1984) developed a Giemsa C-banding technique for identification of somatic chromosomes, accurate identification of the chromosomes 5-12 is difficult, if not impossible. In tomato, a species closely related to potato, chromosome identification can be performed on pachytene chromosomes (Barton, 1950; Ramanna and Prakken, 1967). Morphological traits such as chromosome size, arm ratio, proportion of chromatic parts and presence and size of achromatic parts of the chromosomes were used for identification. Similarly, the morphology of the pachytene chromosomes of diploid *S. tuberosum* ssp. *andigena* (Yeh and Peloquin, 1965) and of diploid *S. tuberosum* ssp. *tuberosum* (Ramanna and Wagenvoort, 1976) has been described. Recently, Lapitan *et al.*, (1989) karyotyped the somatic chromosomes of tomato by *in situ* hybridization of a satellite 162 bp telomeric DNA repeat to metaphase chromosomes. Variation in the spatial and quantitative distribution of this repeat, created distinct patterns on most of the chromosomes, which along with other morphological characteristics such as chromosome length and arm length ratio, allowed the identification of each of the 12 mitotic chromosomes of the tomato. Assignment of somatic chromosomes, identified by the telomeric repeat, to the previously established linkage groups was accomplished via *in situ* hybridization to mitotic spreads of primary trisomics. However, this repeat is lacking in potato.

In diploid potatoes chromosomal interchanges and inversions are rare, as has appeared from cytological investigations of a vast number of different interspecific hybrids during several decades. In the diploid hybrids regular chromosome pairing at pachytene, diakinesis and metaphase I was most commonly observed (Magoon *et al.*, 1958a, 1958b; Dvořák, 1983). The interchange described in this thesis originated spontaneously in *S. phureja*.

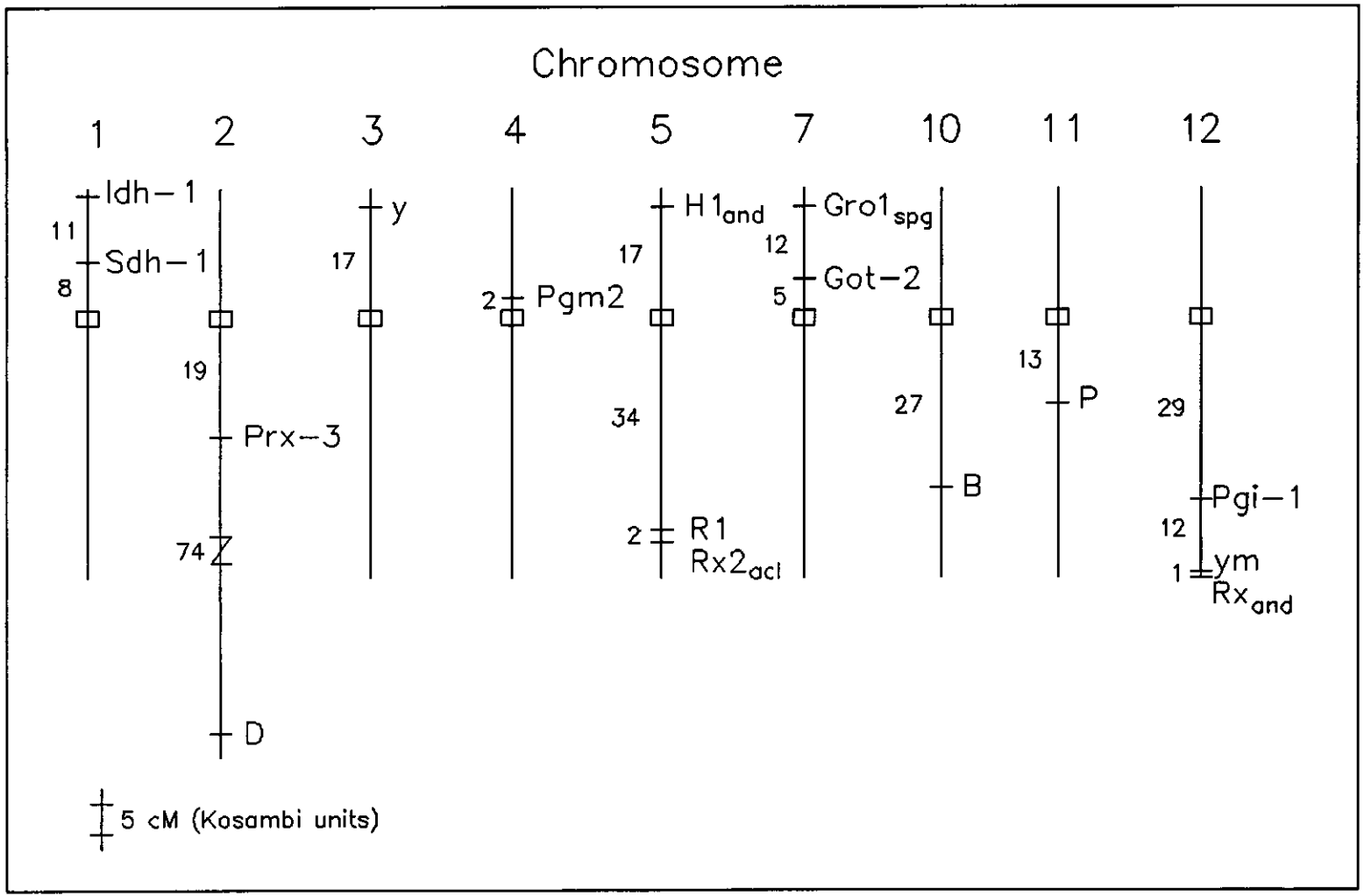
Trisomics contain one chromosome in addition to the diploid complement and have been a useful tool for assigning genes or linkage groups to chromosomes in a variety of species. The trisomic method of identifying chromosomes with their respective linkage groups has also been successfully applied in several crops, such as maize (McClintock and Hill, 1931); tomato (Rick and Barton, 1954; Rick *et al.*, 1964); *Antirrhinum* (Rudorf-Lauritzen, 1958); barley (Tsuchiya, 1959); spinach (Janick *et al.*, 1959); perennial ryegrass (Lewis *et al.*, 1980); rice (Iwata *et al.*, 1984); and others. Gene dosage effects could be detected when trisomics were compared to normal individuals (Tanksley, 1984; Young *et al.*, 1987). In potato, trisomics ($2n=2x+1=25$) were isolated from parthenogenetic aneuhaploid offspring

following 4*x*-2*x* crosses (Hermsen *et al.*, 1970; Wagenvoort and Lange, 1975) or from the progeny of 3*x*-2*x* crosses (Wagenvoort and Lange, 1975). In triploid (2*n*=3*x*=36) and tetraploid plants having more than two homologues of each chromosome type, aneuploid gametes are produced due to the formation of multivalents at metaphase I and consequently may give rise to 2:1 or 3:1 disjunctions at anaphase I. Among genotypes of triploid *S. tuberosum* the mean frequency of trivalents per metaphase I cell varied from 4.70 to 8.38 (Lange and Wagenvoort, 1973) and in tetraploid *S. tuberosum* the frequency of trivalents + quadrivalents varied from 1.48 to 5.24 (Cadman, 1943; Lamm, 1945; Swaminathan 1954a,b). Trisomics of the potato have been produced at a high frequency in the USA and in the Netherlands, using triploids of *Solanum* species or species hybrids (Vogt and Rowe 1968; Lam and Erickson 1970, 1971a; Lee *et al.*, 1972; Kessel and Rowe 1974; Kessel *et al.*, 1975; Lee and Rowe, 1975; Wagenvoort and Lange, 1975). Although the meiotic behaviour of many of these intraspecific and interspecific trisomics was studied (Hermsen *et al.*, 1970, 1973; Lee and Rowe, 1975, and others), a possible relationship between the coefficient of realization of a trivalent (CRT) at metaphase I and the relative chromosome length or the relative euchromatin length of the chromosome at pachytene could not be detected previously since the identity of the extra chromosome was unknown. For the same reason a possible relationship between CRT and the transmission of the extra chromosome through the female gametes could not be established.

The trisomics described in this thesis were produced from triploid-diploid crosses (Wagenvoort and Lange, 1975). The triploids originated mainly from 4*x*-2*x* crosses using cultivar Gineke and its selfincompatible dihaploid G609 (JGTh Hermsen, personal communication). The diploid male parents in the triploid-diploid crosses were the selfcompatible dihaploid G254, also from Gineke, and a hybrid from the cross G609 x G254. Both methods of aneuhaploid production in potato - the tetraploid-diploid crosses and the triploid-diploid crosses - yielded aneuploid populations that were highly variable as to plant morphology, vitality and fertility. Following a scheme for backcrossing and inbreeding with trisomics from Gineke and clones obtained through inbreeding of selfcompatible dihaploids of the same variety, some trisomic types could be distinguished on the basis of plant morphology in the seedling stage (Wagenvoort and Lange, 1980). All trisomics identified through pachytene analysis by Wagenvoort and Ramanna (1979) proved to be primaries, containing a complete chromosome in triplicate.

Primary trisomics provide the most effective means of associating linkage groups and genes with their respective chromosomes through the modified genetic ratios for genes

located on the triplicate chromosome. Since there are three homologous chromosomes instead of two, the genetic ratio for genes on these chromosomes, the so-called "critical ratio", differs from the 3:1 or 1:1 ratios found in F_2 and BC_1 from a normal diploid that is heterozygous for a recessive gene. Basic information for the use of primary trisomics in genetic and breeding research has been reported by Burnham (1962), Hermsen (1970) and Khush (1973). Romagosa (1982) specifically determined family size needed for isolation of all primary trisomic types and Romagosa and Leiva (1982) estimated the population size needed for primary trisomic analysis. In potato four monogenic recessive traits have so far been localized by means of trisomic analysis, namely *a* (albinism) on chromosome 12 by Lam and Erickson (1971b), *v* (virescens) on chromosome 12 by Hermsen *et al.* (1973), *ym* (yellow margin) on chromosome 12 by Wagenvoort (1982) and *tp* (topiary) on chromosome 3 by Wagenvoort (1988). Linkage analyses have yielded ten small linkage groups: *Nx*(*tbr*), *Nx*(*chc*), *Ny*(*chc*), *Na*(*tbr*) and *Nc*(*tbr*), all dominant genes for hypersensitivity to the potato viruses X, Y, A, and C respectively (Cockerham, 1970); *B*, *I*, *F*, *Ow* and *Pf*, all dominant genes affecting tissue specific expression of anthocyanin (Dodds and Long, 1956; De Jong and Rowe, 1972; De Jong 1987); *E* (red colour in sprouts, stems, inflorescences and tuber periderm) and *M* (restriction of tuber periderm pigmentation to areas around the eyes) (Howard, 1966); *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 and Hermsen, 1971); *Prx-2* and *Prx-3* (Quiros and McHale, 1985); *Idh-1* and *Sdh-1* and *Pgdh* and *Dia-1* (Douches and Quiros, 1988); *Got-1*, *ds1* (desynapsis) and *cr* (crumpled) (Jongedijk *et al.*, 1990). One of these linkage groups was assigned to a specific chromosome by trisomic analysis and five by restriction fragment length polymorphism (RFLP) analysis (Bonierbale *et al.*, 1988; Van Eck *et al.*, 1993). The remaining four groups have not been localized so far. In addition, genes encoding for other isozymes, anthocyanins, morphological and physiological traits or resistance genes along with more than 1000 molecular markers with unknown coding capacity have been mapped on the potato genome (Bonierbale *et al.*, 1988; Gebhardt *et al.*, 1989, 1991, 1993; Barone *et al.*, 1990; Ritter *et al.*, 1991; Leonards-Schippers, 1992; Leonards-Schippers *et al.*, 1992; Van Eck *et al.*, 1993, Tanksley *et al.*, 1992, Kreike *et al.*, 1993). A non-radioactive method for detection of hybridization signals on Southern blots using biotinylated probes or probes labelled with digoxigenin proved to be satisfactory and reliable. This technique appears to be much quicker than the common method using radioactive labelling (Jacobs *et al.*, 1990; Allefs *et al.*, 1990). Finally, approximate gene-centromere map distances have been


Chromosome
1
2
3
4
5
7
10
11
12
Idh−1
11
Sdh−1
8
19
Prx−3
74
D
y
17
2
Pgm2
H1and
17
34
R1
2
Rx2acl
Gro1spg
12
Got−2
5
27
B
13
P
29
Pgi−1
12
ym
1
Rxand
5 cM (Kosambi units)

← Fig. 1. Relative map distance to the centromere for various isozyme marker loci and for some morphological and resistance genes on the chromosomes 1, 2, 3, 4, 5, 7, 10, 11 and 12. *Idh-1, Sdh-1, Prx-3, Pgm-2, Got-2, Pgi-1,* loci of various isozymes; *D,* a basic gene for brownish and red colour in stems and inflorescences, according to the notation of Van Eck *et al.* (1993); *y,* yellow tuber flesh, $H1_{and}$, gene from *Solanum andigena* conferring resistance to *Globodera rostochiensis,* pathotype Ro1; *R1,* gene conferring vertical resistance to *Phytophthora infestans;* $Rx2_{acl}$, gene from *S. acaule* conferring extreme resistance to potato virus X; $Gro1_{spg}$, gene from *S. spegazzinii,* conferring resistance to pathotype Ro1 from *G. rostochiensis; B,* gene for tissue-specific expression of anthocyanin; *P,* gene for the production of purple anthocyanin; *ym,* gene for yellow margin. The position of *ym* on the arm of chromosome 12 also involving the markers *Pgi-1* and Rx_{and} is questionable as no linkage with these markers has been reported. *ym* might also be situated on the other arm of chromosome 12. It is expected that Rx_{and} from *S. andigena* conferring extreme resistance to potato virus X corresponds to $Rx1_{and}$ reported in the literature.

established via half-tetrad analysis for a variety of isozyme marker loci and some morphological traits and resistance genes. Fig. 1 shows diagramatically nine chromosomes of the potato involving markers with known relative distance to the centromere. Some other markers or genes not localized in the same mapping populations were placed on these chromosomes based on the use of closely linked common reference markers reported in the literature. Comparisons between potato RFLP maps derived from different genetic backgrounds revealed conservation of marker order, but a significant reduction of map length was observed in interspecific compared to intraspecific crosses (Gebhardt *et al.,* 1991). It has to be noted that the map presented by Gebhardt *et al.,* (1989) shows a difference in marker order for the PAL loci on chromosome 9 compared to the map by Gebhardt *et al.,* (1991).

Two quantitative trait loci (QTLs) involved in resistance to *Globodera rostochiensis* (Kreike *et al.,* 1993) were mapped on the chromosomes 10 and 11 and 19 QTLs involved in resistance to *Phytophthora infestans* on the chromosomes 2, 3, 4, 5, 6, 9 and 12 (Leonards-Schippers, 1992). The investigations on trisomics described in this thesis were originally initiated to establish a complete series of primary trisomics at the diploid level in *S. tuberosum* ssp. *tuberosum.* These studies included (i) production of aneudihaploids and identification of the 12 possible trisomics using pachytene analysis (ii) assessment of fertility,

plant morphology and transmission rates of the extra chromosome in single and double trisomics.

Outline of research

The aim of the present research was to demonstrate homology of pachytene chromosomes with somatic chromosomes of the potato, to map recessive genes by trisomic analysis, to determine the gene-centromere map distances of recessive and dominant genes by half-tetrad analysis and finally to explore the use of a series of trisomics in identifying the chromosomes involved in an interchange in *S. phureja*.

Chapter 1 describes the identification of mitotic chromosomes using conventional staining, Giemsa C-banding and *in situ* hybridization. The identification of the pachytene chromosomes of *S. phureja* and those involved in the interchange is reported in chapter 2. Meiosis in plants carrying the interchange and in some I_1 plants obtained from an interchange heterozygote is described in chapter 3.

Chapter 4 deals with the meiotic behaviour of 11 primary potato trisomics and its consequences for the female transmission of the extra chromosome.

The estimation of relative distances to the centromeres of three genes involved in resistance to the potato viruses X and Y and to nematodes, and of the gene *ym* by means of half-tetrad analysis is described in the chapters 5 and 6 respectively.

Finally, the chapters 7 and 8 report the successful localization of the recessive genes *ym* (yellow margin) and *tp* (topiary) respectively, using trisomic analysis.

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CHAPTER 1

Chromosome identification of potato trisomics ($2n=2x+1=25$) by conventional staining, Giemsa C-banding and non-radioactive *in situ* hybridization.

With : G.J.A. Rouwendal, G. Kuiper-Groenwold and H.P.J. de Vries-van Hulten

Summary

Identification of the potato chromosomes 1 (the longest one), 2 (the carrier of the nucleolar organizer, NOR) and 12 (the shortest one) is possible at mitosis using a combined Feulgen-haematoxylin staining or a Giemsa C-banding technique. In two aneuploids containing 27 chromosomes, three specimens of chromosome 1 were found through conventional staining. Variation of the length of the NORs and the size of the satellite of homologues or homoeologues of chromosome 2 was observed in various cytotypes. In what was identified by means of pachytene analysis as trisomics of chromosome 2 of interspecific origin, involving *S. tuberosum* L. ssp. *tuberosum* Hawkes and *S. phureja* Juz. et Buk., only two specimens of chromosome 2 could be identified using the Giemsa C-banding technique. However, in six different interspecific chromosome 2-trisomics, three chromosomes per cell were found to show a hybridization signal by non-radioactive *in situ* hybridization with heterologous rDNA from pea. The high polymorphism of chromosomes observed after Giemsa C-banding made it impossible to bring the identity of the mitotic chromosomes in accordance with the results from pachytene identification in cells containing one triplicate chromosome. The number of chromosomes responding to the sequences of the tuber protein patatin varied betwen 0 and 8, but was inconsistent when *in situ* hybridization was applied with a genomic DNA clone as large as 6.1 kilobases. Therefore, the results of this study suggest that more than one of the 12 basic potato chromosomes contain patatin genes.

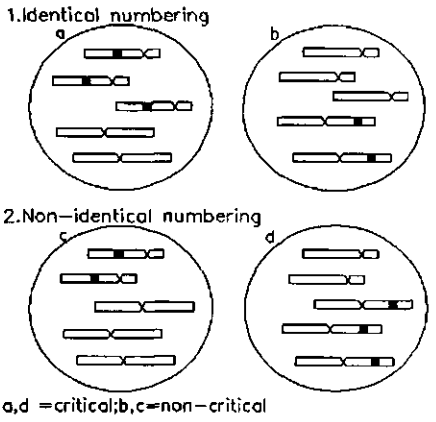
Key words: biotin/digoxigenin labelled probe, rRNA genes, NORs, chromosome polymorphism, potato trisomics.

Introduction

Identification of somatic chromosomes of the potato is difficult because of their small size and very similar morphology. Pre-treatment of plant tissues with chemicals such as 8-hydroxyquinoline or α-bromonaphthalene leads to mitotic metaphases with condensed chromosomes, which can be visualized by conventional staining as small blocks of heterochromatin. For research purposes, it is important to karyotype somatic cells, e.g. by banding techniques or via *in situ* hybridization. The urgent need for such techniques has been prompted by the finding of numerical and structural chromosome mutations in callus and suspension cultures as well as in plants regenerated from protoplasts (Creissen and Karp, 1985; Pijnacker *et al.* 1986a, 1986b; Pijnacker and Ferwerda, 1987; Pijnacker and Sree Ramulu, 1990). The finding of chromosome elimination in triploid cells of hybrids between *S. tuberosum* L. ssp. *tuberosum* Hawkes and *S. phureja* Juz. et Buk., which incidentally may give rise to *S. tuberosum* dihaploids with aneuploid cells still containing some DNA from *S. phureja* (Clulow *et al.* 1991) further emphasizes the importance of reliable karyotyping. *In situ* hybridization using species-specific DNA probes might have the potential to identify each chromosome by specific banding and offers the opportunity to study chromosome elimination. Mok *et al.* (1974) attempted to identify the somatic chromosomes of potato through a modified Giemsa staining technique. In their opinion the banding pattern resulted from natural condensation of heterochromatin after denaturation of DNA. They observed at prophase, one to five "bands" per chromosome and claimed to be able to identify all twelve basic chromosomes. Using the same technique, Lee and Hanneman (1976) identified in Giemsa-stained somatic cells from trisomics of *S. chacoense*, the extra chromosomes that were previously identified at pachytene. The technique of Mok *et al.* (1974), could not be reproduced in our laboratory. Another constraint of this technique is that the centromeres of prophase chromosomes are not visible. Later on Pijnacker and Ferwerda (1984) developed a Giemsa C-banding technique for the identification of somatic metaphase chromosomes.

In tomato, a species closely related to potato, chromosomes can be identified both in somatic cells and at pachytene stages. Based on the size, arm ratios, proportion of chromatic and achromatic parts, all 12 pairs of somatic chromosomes of the tomato were identified by Ramanna and Prakken (1967). Because of the highly similar morphology of tomato and potato chromosomes, the same traits were used for the characterization of the pachytene chromosomes of diploid ($2n=2x=24$) *S. tuberosum* L. ssp. *andigena* Hawkes (Yeh and Peloquin, 1965) and that of diploid *S. tuberosum* ssp. *tuberosum* (Ramanna and Wagenvoort, 1976) and

S. phureja (Wagenvoort, 1988). Successful pachytene analysis, however, requires skill, patience and experience, and is very time-consuming. The trisomics of the potato identified by Wagenvoort and Ramanna (1979), are expected to be ideal tools for establishing morphological concurrence between pachytene and somatic chromosomes identified by Giemsa C-banding or by *in situ* hybridization. However, the number of the chromosomes in pachytene and that resulting from the analysis of mitotic cells using banding methodologies may not be identical. If a comparison of the two systems of chromosome characterization is desired, i.e. the Giemsa C-banding technique or *in situ* hybridization on the one hand and chromosome identification at pachytene on the other, it is essential to use the same trisomics for both approaches. If the outcome of identification of chromosomes in a mitotic and a meiotic plate of the pachytene of a trisomic plant is identical, two situations are expected. Probing with a known chromosome-specific DNA clone or Giemsa C-banding, will reveal three chromosomes per cell with a hybridization signal or a specific C-banding pattern in the critical trisomic and two in the non-critical situation. If non-identical numbering of mitotic and meiotic chromosomes is occurring, only two hybridization signals will be observed and an other trisomic than expected on the basis of the pachytene analysis will show three chromosomes with a hybridization spot. The two situations are diagramatically visualized (see diagram).



The diagram shows two situations: (i) identical numbering of mitotic and meiotic chromosomes. In the critical situation (a) three chromosomes and in the non-critical situation (b) only two chromosomes will show a hybridization signal if a known chromosome-specific DNA clone is used. (ii) If non-identical numbering is occurring, an other trisomic than expected on the basis of the pachytene analysis will show three chromosomes with a hybridization spot.

Tandemly repeated DNA sequences such as the ribosomal genes (rDNA) have been successfully detected on the chromosomes through *in situ* hybridization in a large number of plant species including maize (Phillips *et al.* 1979; Mascia *et al.* 1981), cereals (Hutchinson *et al.* 1981; Mukai *et al.* 1990, 1991; Leitch and Heslop-Harrison, 1992), legumes (Ellis *et al.* 1988; Skorupska *et al.* 1989; Griffor *et al.* 1991), tomato (Zabel *et al.* 1986; Ganal *et al.* 1988; Lapitan *et al.* 1991), potato (Visser *et al.* 1988) and others. In plant cytogenetics, the use of biotin-labelled probes was introduced by Rayburn and Gill (1985). Unique sequences of human chromosomes as small as 1 kilobase (kb) are detectable with a non-isotopic *in situ* hybridization technique (Garson *et al.* 1987). In plants, the legumin gene of the pea, as large as 13.5 kb, is the smallest unique target sequence which until now has been detected on metaphase chromosomes by *in situ* hybridization using a non-radioactive labelled probe and the light microscope (Simpson *et al.* 1988).

It was attempted to detect moderately repetitive DNA sequences coding for the tuber protein patatin through non-radioactive *in situ* hybridization. Patatin in potato is encoded by approximately 10-15 genes per basic genome (Park *et al.* 1983; Mignery *et al.* 1988; Twell and Ooms, 1988).

In this paper we report the identification of the triplicate mitotic chromosomes in trisomics (already identified before by pachytene analysis) and other cytotypes, after conventional staining, Giemsa C-banding and *in situ* hybridization.

Material and methods

Plant material

The monohaploids ($2n=x=12$) used in this study to identify somatic chromosomes were induced using trisomics ($2n=2x+1=25$) of *S. tuberosum* ssp. *tuberosum* cv. Gineke by the *S. phureja* haploid inducer IVP35. The latter was kindly provided by Prof. Dr. J.G.Th. Hermsen, Agricultural University, Wageningen, the Netherlands. In addition, the following material was investigated: aneuploids ($2n=25,26,27$), triploids ($2n=36$) and several trisomics. The origins of trisomics were as follows: chromosome 3 and 12-trisomic from the cultivar Gineke; chromosome 2 and 5-trisomic derived from interspecific hybrids of *S. tuberosum* x *S. phureja* and the multiple *Solanum* species hybrids involving *tuberosum* - *chacoense* - *yungasense* (chromosome 5-trisomic) and *tuberosum* - *maglia* - *microdontum* - *stenotomum* (chromosome 10-trisomic). The trisomic for chromosome 10 was kindly provided by Dr. R.E. Hanneman Jr. University of Wisconsin, Madison, USA. The extra chromosomes of the trisomics were identified by

pachytene analysis or were expected to be identical by descent to the extra chromosomes in related identified trisomics.

Probes used for in situ hybridization

Two different clones were used for *in situ* hybridization. One of them was the pea ribosomal RNA gene fragment which consisted of a 4.0 kb *Eco* R_I fragment subcloned in pAcyc 184 which had been derived from a phage selected from an EMBL3 genomic library of *Pisum sativum* cv. Rondo (J.P. Nap, personal communication). The other clone, B106 is a 6.1 kb Bam H_I fragment containing an entire class II patatin gene; it originated from a genomic lambda clone designated λ2PAT1 in pUC18 (see Nap *et al.* 1992).

Conventional staining of somatic chromosomes

Root tips of young potato plants were pretreated with 0.002 M 8-hydroxyquinoline for 4-24 hours at 4°C and fixed in acetic acid alcohol (1:3,v/v). After hydrolysis in *N* HCl at 60°C for 6-8 minutes, the roots were stained with Feulgen (leuco-basic fuchsin). Squashing was carried out in a mixture of nine parts haematoxylin (2%) and one part iron alum (0.5%) both in 50 % propionic acid (Henderson & Lu, 1968), the haematoxylin solution being well ripened.

Giemsa C-banding

Root tips of young plants were pretreated in 0.002 M 8-hydroxyquinoline or in saturated α-bromonaphthalene for 4-6 h at 4°C and fixed in cold acetic acid alcohol (1:3,v/v). Maceration, preparation of slides (chromosome spreading and air drying) and C-banding were performed according to the method of Pijnacker and Ferwerda (1984). To achieve more consistent C-banding, slides were immersed in 0.2 M HCl for 1 h at room temperature before the barium hydroxide step (L.P. Pijnacker, Groningen University, personal communication). Chromosome spreads used for *in situ* hybridization were made as described for the Giemsa C-banding technique.

Labelling of plasmid DNA

Plasmid DNA was either labelled with biotin-11dUTP or biotin-21dUTP using a nick translation kit from Bethesda Research Laboratories (BRL), or it was labelled with digoxigenin-11 dUTP using a random primer labelling kit from Boehringer Mannheim. The extent of labelling of the probe was checked by spotting labelled plasmid DNA on nitrocellulose and visualizing the labelled plasmids according to the descriptions of the manufacturers.

In situ hybridization with chromosome spreads

Hybridization and detection of biotinylated plasmid DNA

In situ hybridization with biotinylated probes was performed following the protocol described by Garson *et al.* (1987) for human chromosomes, with slight modifications. The slides were treated with 100 µg ml^{-1} RNase A in 2xSSC (1xSSC is 0.15 M NaCl, 0.015 M sodium citrate) for 1 h at 37°C and sealed with rubber solution (Lero) during this treatment. Then they were dehydrated sequentially in an ethanol series of 70%, 96% and 100% (5 min. each step) and air dried. Thirty µl of hybridization mixture was added to each slide and covered with a 4.84 cm^2 cover slip and sealed with Lero. The hybridization mixture contained 50 % deionized formamide, 10 % dextran sulphate, 2xSSC, 0.1 mM EDTA, 0.05 mM Tris-HCl, at pH 7.5, 100 µg ml^{-1} herring sperm DNA, and 1 µg ml^{-1} biotinylated plasmid DNA. Denaturation of the probe and the chromosomal DNA was carried out simultaneously by incubating the slides on the metallic bottom of an incubator (Heraeus) for 5-10 min. at 80°C. Slides were then incubated overnight (about 16 h) in a humidified box (plastic container faced inside with filter paper) and placed in an incubator at 40-42°C. After hybridization the cover slips were discarded by incubating the slides in 2xSSC and washed consecutively in 2xSSC (30 min. at room temperature), 0.1 x SSC (30 min. at 42°C) and 2xSSC (15 min. at room temperature). The slides were then placed in TNM-A blocking reagent (0.1 M Tris-HCl, at pH 7.5, 0.1 M NaCl, 2 mM $MgCl_2$, 0.05% Triton X-100, 3% bovine serum albumin) for 15 min. at room temperature. Hybridized probe was detected by incubating the slides for 20 min. with 1 µg ml^{-1} streptavidin conjugated alkaline phosphatase (BRL) in TNM-A followed by 3 washes (5 min. each) in TNM (TNM-A without bovine serum albumin) and once in alkaline (pH 9.5) buffer (0.1 M Tris-HCl at pH 9.5, 0.1 M NaCl, 50 mM $MgCl_2$). For coloration, 30 µl substrate solution (330 µg ml^{-1} NBT and 165 µg ml^{-1} X-phosphate in alkaline buffer) was added to each slide. In contrast to the protocol of Garson *et al.* (1987), no levamisole was added for the inhibition of any residual endogenous alkaline phosphatase activity. Colour development was performed in the dark and terminated after 2-4 h by washing for 5 min. in 20 mM Tris-HCl, pH 7.5, 5 mM EDTA. Finally slides were mounted in Aquamount (Gurr, BDH) and metaphases viewed under phase contrast illumination.

Alternatively, the hybridized biotinylated probe was detected through streptavidin conjugated horseradish peroxidase (BRL). In this case the protocol followed the above mentioned description up to and including the blocking step. After blocking, 1 ml TNM-A containing 2 µl streptavidin horseradish peroxidase (0.02 mg streptavidin horseradish peroxidase per ml TNM-A)

was added to each slide for 20 min., followed by three washes (5 min. each) in TNM solution. The colour reaction was performed by incubating the slides for 2-10 min. in 5% solution of diaminobenzidine tetrahydrochloride (DAB from BRL) and 0.05% (v/v) H_2O_2 in pH 7.5 buffer to which 68 mg imidazole was added, preceded by a short rinse in pH 7.5 buffer (0.05 M Tris-HCl). The colour reaction was terminated by placing the slides in running tap water for 30 min. Finally, the slides were dehydrated successively in an ethanol series of 70%, 96% and 100%, 5 min. each step, and air dried. Slides without cover glasses were viewed with phase contrast illumination or more successfully using reflection contrast microscopy.

Hybridization and detection of digoxigenin labelled plasmid DNA

Digoxigenin labelled plasmid DNA was added to the hybridization mixture containing 5xSSC, 5% (w/v) blocking reagent from Boehringer, 0.1% (w/v) N-lauroylsarcosine, Na salt, 0.02% (w/v) SDS and 50% (v/v) de-ionized formamide. RNase treatment, denaturation , hybridization and post-hybridization washes were performed as described for the detection of the biotinylated probes. The slides were blocked in 5% blocking reagent in pH 7.5 buffer (100 mM Tris-HCl, 150 mM NaCl) and incubated in an anti-digoxigenin alkaline phosphatase conjugate (dilution 1:5000 in pH 7.5 buffer) for 30 min. at 37°C followed by three washes (10 min. each) in pH 7.5 buffer. A rinse of 5 min. in alkaline buffer (100 mM Tris HCl pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$) preceded the addition of 30 µl substrate solution having the same composition as that used for the biotinylated probes.

Results

Conventional staining of chromosomes

The chromosomes 1, 2 and 12 could be identified in the monohaploid unambiguously in the somatic cells, because chromosome 1 is the longest and chromosome 12 the shortest chromosome of the complement and chromosome 2 carries the weakly stained secondary constriction (nucleolar organizer, NOR) and a darkly stained satellite (Fig. 1). Table 1 presents the results of the identification of the chromosomes 1 and 2 in various cytotypes using the Feulgen-haematoxylin staining. These chromosomes are illustrated in the Figs. 2 and 4. Another landmark for the identification of chromosome 1 is the structure of this chromosome in cells with less condensed chromosomes. It exhibits large blocks of heterochromatin at both sides of the centromere and has a less condensed and therefore less stained euchromatic region at the distal end of the long arm. Attempts were made to identify the trisomic for chromosome 1,

Table 1. Identification of individual chromosomes in mitotic cells from various cytotypes of *S. tuberosum* ssp. *tuberosum* cv. Gineke stained by Feulgen-haematoxylin. In parentheses the number of identifiable specimens per chromosome type. Yes = chromosome identifiable. Yes (?) = identification based on chromosome size only and therefore uncertain. No = chromosome not identifiable.

Cytotype	Chromosome No.					
	1	2		3	4	12
		NOR(s)	Satellite(s)			
Monoploid from chromosome 12-trisomic	yes	yes	yes	yes (?)	yes (?)	yes
Monoploid from chromosome 9-trisomic	yes	yes	no	yes (?)	yes (?)	yes
Chromosome 12-trisomic	yes (2)	yes (2)	yes (2)	no	no	no
Double trisomic ⫩	yes (2)	yes (2)	yes (3)	no	no	no
Aneuploid with 27 chromosomes ⫩	yes (3)	yes (2)	yes (2)	no	no	no
Triploid	yes (3)	yes (3)	yes (3)	no	no	no

⫩ Not identified at pachytene

Table 2. Identification of individual chromosomes in mitotic cells from intraspecific and interspecific *Solanum* trisomics by Giemsa C-banding. In parentheses the number of identifiable specimens per chromosome type.

Trisomic type	Triplicate chromosome	Number of cells	Chromosome No.						
			1	2		3	4	10	12
				NORs	Satellites				
Intraspecific⫩	-	5	yes (2)	yes (2)	no	yes (2)	no	no	no
Intraspecific⫩	-	5	yes (2)	no	yes (2)	yes (2)	yes (2)	no	no
Interspecific	2	15	yes (2)	no	yes (2)	yes (1)	yes (1)	no	no
Interspecific	2	6	yes (2)	no	yes (2)	no	yes (1)	no	no
Interspecific	10	10	yes (1)	no	yes (1)	yes (1)	yes (1)	no	no

⫩ Not identified at pachytene

since this trisomic type is lacking in our primary trisomics series (Wagenvoort & Ramanna , 1979). Three specimens of chromosome 1 were identified in five cells from aneuploids, however containing 27 chromosomes (Fig. 3). One of the three specimens of chromosome 1 identified in these aneuploids with 27 chromosomes was nearly metacentric whereas the other specimens were sub-metacentric. This difference in centromere position is not easy to explain. In these cells variation of the length of the NORs and the size of the satellite were also observed. The size of the satellite varied even among homologues in the same cell (cf. the satellites of the chromosomes from three different cells shown in the Figs. 4a-c). A mitotic cell from a monohaploid, in which the satellite of chromosome 2 is absent, is shown in Fig. 5. The presence of chromosome 2 in triplicate could be unambiguously ascertained in cells of the double trisomic since three chromosomes per cell showed a darkly stained satellite (Fig. 4c).

Giemsa C-banding of chromosomes

Table 2 presents the results of the Giemsa C-banding of chromosomes from intra- and interspecific trisomics. The chromosomes of the two intraspecific trisomics have been arranged according to their length and specific banding pattern as described by Pijnacker and Ferwerda (1984). Because the banding pattern was unsatisfactory, a proper identification of all chromosomes was impossible. Chromosomes in the same numbering position are therefore not necessarily concurrent. According to our classification both trisomics contain three specimens of the chromosome designated with f (Figs. 6f and 7f). It is however not certain whether these chromosomes are identical to chromosome 6 described by Pijnacker and Ferwerda (1984). Two unsatellited chromosomes 2 are shown in Fig. 6b. Polymorphism for the band on the short arm of chromosome 4 was observed in all five cells analysed in the other trisomic. One of the two homologues of chromosome 4 showed a wide band, whereas the other had a very weak band (Fig. 7d). Polymorphism for the chromosomes 1, 2 and 4 was found. In the cell shown in Fig. 8, from a chromosome 2-trisomic one specimen of chromosome 1 (in the centre), probably from *S. tuberosum* ssp. *tuberosum*, had an interstitial band in the short arm and no telomeric bands. The homoeologous chromosome, probably from *S. phureja* also showed an interstitial band and weakly stained telomeric bands on the short arm. Only two instead of three specimens of chromosome 2, and one instead of two specimens of the chromosomes 3 and 4 could be identified in this C-banded cell. The remaining chromosomes could not be identified. In another chromosome 2-trisomic, also two instead of three chromosomes with banded satellites were observed (Fig. 9). In addition, polymorphism for the interstitial band in the short arm of chromosome 4 occurred in this trisomic. Chromosome 10 could not be

recognised in an interspecific chromosome 10-trisomic which was previously identified at pachytene.

In situ hybridization

Identification of chromosome 2 in trisomics using heterologous rDNA

Table 3 shows the results of the *in situ* hybridizations using ribosomal DNA and a patatin gene probe. The biotinylated and hybridized rDNA probe could unambiguously be detected on the NORs of two nucleolar chromosomes by the horseradish peroxidase enzyme reaction (Fig. 10). Similarly, the same probe detected the hybridization spot on the NOR at the distal end of each chromatid of the two specimens of chromosome 2, but in this case following the alkaline phosphatase enzyme reaction (Fig. 11). Visualization of the spots created by the horseradish peroxidase enzyme reaction was also possible by using reflection contrast microscopy (Fig. 12). With this visualization procedure, three spots were clearly detected in interphase nuclei from an interspecific trisomic for chromosome 2. These spots represent the NOR regions of the three specimens of chromosome 2 (Fig. 13). The nucleolar chromosome was also detected through the hybridization of the digoxigenin labelled rDNA probe and the enzyme linked immunoassay for detecting the hybridized rDNA (Fig. 14). Fig. 14 shows a cell in which two chromosomes show major hybridization spots representing the nucleolar chromosomes whereas two other chromosomes show minor spots. Using this method, two hybridization spots per cell were found in 24 out of 36 cells analysed but chromosomes with minor spots were found in only a few cells. No differences in sensitivity regarding the detection of the NORs were found, independent of ligand or visualization method used. In six different interspecific chromosome 2-trisomics (identity based on pachytene analysis) cells were found with three chromosomes showing a hybridization signal when the heterologous rDNA probe from pea was used (Fig. 15). This result emphasizes the strength of the *in situ* hybridization technique for chromosome identification since in one out of the six trisomics analysed the Giemsa C-banding technique failed to identify all three nucleolar chromosomes.

Detection of the number of chromosomes carrying patatin genes

Incubation in a soluble complex of alkaline phosphatase and mouse monoclonal anti-alkaline phosphatase antibody (APAAP, diluted 1:20 in pH 7.5 buffer) was included preceding the colour reaction in order to test the possibility of amplification of the hybridization signal at low copy sequences (small targets) such as those of patatin. Amplification of the signal was

Table 3. Identification of the number of chromosomes carrying rRNA or patatin genes in mitotic cells from intraspecific and interspecific trisomics of *Solanum* by *in situ* hybridization using two different haptens. $rDNA^{Biot}$= biotinylated ribosomal DNA; $rDNA^{Dig}$= idem but labelled with digoxigenin; $B106^{DIG}$= patatin probe labelled with digoxigenin. $Streptavidin^{HRP}$ and $streptavidin^{AP}$= streptavidin horseradish peroxidase and alkaline phosphatase conjugate respectively; $Anti\text{-}Dig^{AP}$= anti-digoxigenin alkaline phosphatase conjugate. APAAP= alkaline phosphatase anti-alkaline phosphatase antibody.

Trisomic type	Triplicate chromosome	Probe	Detection	No. of cells	No. of chromosomes/cel with spot
Intraspecific	3	$rDNA^{Biot}$	$Streptavidin^{HRP}$	10	0-2
Interspecific	5	$rDNA^{Biot}$	$Streptavidin^{AP}$	15	0-2
Interspecific	5	$rDNA^{Dig}$	$Anti\text{-}Dig^{AP}$	24	0-2 ⫩
Interspecific	2	$rDNA^{Biot}$	$Streptavidin^{AP}$	20	0-3
Interspecific	2	$B106^{Dig}$	$Anti\text{-}Dig^{AP}$+APAAP	9	0-8
Intraspecific	3	$B106^{Dig}$	$Anti\text{-}Dig^{AP}$	5	0-2
Interspecific	5	$B106^{Dig}$	$Anti\text{-}Dig^{AP}$	5	0-3
Interspecific	5	$rDNA^{Dig}$+$B106^{Dig}$	$Anti\text{-}Dig^{AP}$	5	0-8

⫩ In addition minor spots on two chromosomes in a few cells

doubtful when rDNA was labelled with digoxigenin and detection of the hybridized probe with an anti-digoxigenin alkaline phosphatase conjugate was carried out combined with an incubation in APAAP. Up to eight chromosomes per cell showed spots when B106 labelled with digoxigenin was used and the signal amplified by APAAP (Fig. 16). The same maximum number of spots per cell were found by simultaneous *in situ* hybridization with rDNA and B106, both labelled with digoxigenin. No amplification with APAAP was used in the simultaneous hybridization of which the results are shown in the Figs. 17 and 18. Two chromosomes from the prometaphase plate show more than one spot (Fig. 17). However, in 10 out of 13 cells from two other trisomics, maximally two and three chromosomes per cell were detected carrying a spot (Figs. 19 and 20).

Discussion

The results show that identification of the chromosomes 1,2 and 12 at mitosis is possible, though only in monohaploid cells and with Feulgen - haematoxylin staining, or with the Giemsa C-banding technique. Since chromosome 1 is the longest of the complement and its structure in somatic and meiotic cells is highly similar, it corresponds with the pachytene bivalent 1. A prerequisite for an accurate identification of this chromosome in somatic cells is that less condensed chromosomes are available. It is less certain that the shortest chromosome in somatic cells corresponds with the pachytene bivalent 12. In meiotic cells at pachytene this chromosome is not always the shortest of the complement. Variation for the length of the NORs of homologues or homoeologues of chromosome 2 as well as the presence of a satellite on this chromosome was found in various cytotypes in this study. The nucleolar organizer chromosomes from two clones, designated IVP35 and IVP48 from *S. phureja*, were not recognizable using the C-banding technique (Pijnacker and Ferwerda, 1984). The absence of a satellite on chromosome 2 (NOR was visible) in one of the Gineke monohaploids studied suggests that one of the four nucleolar chromosomes of Gineke is an unsatellited chromosome. However, in view of a report by Clulow *et al.* 1991, who found aneuploid cells in dihaploids of *S. tuberosum* ssp. *tuberosum*, the presence of an unsatellited chromosome from *S. phureja* in the monoploid plant from Gineke is another possibility since IVP35 was used for induction of monoploid plants from trisomics. Distinction between the two types of chromosome 2 by *in situ* hybridization using a species specific probe may elucidate the provenance of the chromosomes 2.

In trisomic interspecific hybrids including original trisomics from *S. tuberosum* ssp. *tuberosum*

and *S. phureja* (IVP35) only two specimens of chromosome 2 were found using the Giemsa C-banding technique. This result coincides with that reported by Pijnacker and Ferwerda (1984), who found unsatellited specimens of chromosome 2 in IVP35. These authors could reliably identify the chromosomes 1-4, 7, 11 and 12 using unbanded Giemsa stained chromosomes. The same authors identified directly all 12 metaphase chromosomes after C-banding but the chromosomes 3-12 do not necessarily coincide with the chromosomes as identified through pachytene analysis. Polymorphism for the C-band of chromosome 2 and differences in Giemsa C-banding pattern of chromosomes suspected to be the numbers 1, 3, 4, 5, 8, 9, 11 and 12 compared to that established for monohaploid Gineke has been found by Puite *et al.* (1986), Pijnacker *et al.* (1987), De Vries *et al.* (1987) and Jacobsen *et al.* (1989). The polymorphic chromosomes found in this study made it impossible to establish a correlationship between the morphology of somatic chromosomes and pachytene bivalents. Furthermore, from the present study the chromosomes 5-12 could not be reliably identified using the Giemsa C-banding technique.

Ribosomal RNA genes have been found to be highly variable both in copy number and in intergenic spacer length, even among somatic cells of individual plants (Rogers and Bendich, 1987). The difference in length of the NORs found among the trisomics in this study shows that the variation occurs in potato as well. The heterologous rDNA probe also hybridized to the NORs of triploid ($2n=3x=27$) sugar beet, cv. Monohil (Wagenvoort, unpublished results). Successful *in situ* hybridization of the heterologous rDNA probe from pea indicated that in potato and sugar beet the transcribed units of the rRNA genes are highly conserved. The 5S rRNA genes represent another highly conserved family of repeated sequences consisting of tandem copies of a repeating unit. However, the 5S rRNA genes are not closely linked to the genes coding for the large ribosomal RNA species. In *Vicia faba*, rye, wheat and *Triticum tauschii*, the 5S DNA loci and the rDNA loci are found on the same chromosomes (Knälmann and Burger, 1977; Appels *et al.* 1980; Dvořák *et al.* 1989; Mukai *et al.* 1990; Lagudah *et al.* 1991). In other species such as maize, pea and tomato 5S DNA occurs in chromosomes other than those carrying the NOR (Mascia *et al.* 1981; Ellis *et al.* 1988; Lapitan *et al.* 1991; Heslop-Harrison *et al.* 1992). The 5S rRNA genes of tomato were assigned to a region of chromosome 1 using RFLPs and a single hybridization signal was localized by *in situ* hybridization on the short arm of this chromosome close to the centromere (Lapitan *et al.* 1991). Comparative mapping studies between tomato and potato revealed that the RFLP maps of tomato and potato are very similar (Bonierbale *et al.* 1988, Gebhardt *et al.* 1991). If the 5S rRNA genes from the potato also reside on chromosome 1 an additional cytogenetic marker for this

chromosome would be available for chromosome identification.

The results obtained with the patatin probe were highly inconsistent regarding the number of chromosomes per cell showing a hybridization signal. This number varied from zero to eight using the genomic probe B106 and labelling with digoxigenin. This DNA clone contains an entire class II patatin gene and an intergenic region. All sequenced patatin genes show a high degree of homology (>90%) in their coding sequences (Ganal *et al.* 1991). However, with respect to their chromosomal position there is no consensus in the literature. Gebhardt *et al.* (1989) mapped the patatin genes to linkage group 2 (later on assigned to chromosome 2 by Gebhardt *et al.* 1991) and to linkage group 7 (later on assigned to chromosome 8 by Gebhardt *et al.* 1991) using the genomic clone pgT5 containing a patatin gene. Ganal *et al.* (1991), however, mapped all patatin genes, both the class I and class II genes, to the distal end of chromosome 8 using a full length cDNA clone designated pGMO1. In addition these authors found a single copy of the class I specific promoter region on chromosome 3 of potato. Later on Gebhardt *et al.* (1991) localized the patatin genes on the chromosomes 4 and 8 and could not reproduce their previous localization on chromosome 2. The results of the present study do not elucidate the question whether one or more chromosomes are carrying patatin genes since our results were highly inconsistent. It seems that hybridization with a genomic clone, whether *in situ* (this paper) or on blots (Gebhardt *et al.* 1989 and 1991) may detect more sites in the genome related to patatin than using a cDNA clone (Ganal *et al.* 1991). A number of patatin pseudogenes have been found and sequenced. Recently, it has been shown by Nap *et al.* (1992) that such pseudogenes contain regions with long direct repeats that in themselves are also highly repetitive. In addition, the remnants of previously active patatin genes also may account for the high number of chromosomes marked by *in situ* hybridization with the patatin probes in this study.

Biotin and digoxigenin labelling was found to be a rapid, consistent and reliable technique to detect highly repeated sequences on the relative small chromosomes of potato. Its value for physical mapping of low copy or unique DNA sequences in these plant species has yet to be established. However, another approach may be to use pachytene chromosomes. As pachytene chromosomes are much less contracted than somatic chromosomes, mapping efficiency and accuracy may be expected to increase substantially using this type of chromosomes as shown already by Shen *et al.* (1987) and Albini and Schwarzacher (1992) for maize and rye pachytene chromosomes respectively. Whether this is also the case for potato remains to be investigated.

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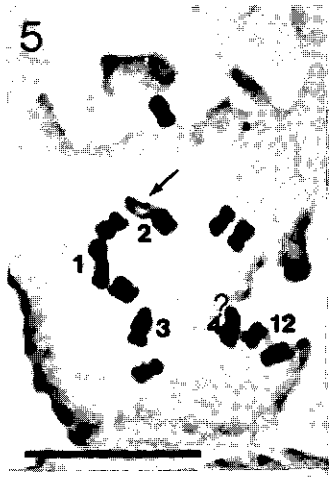
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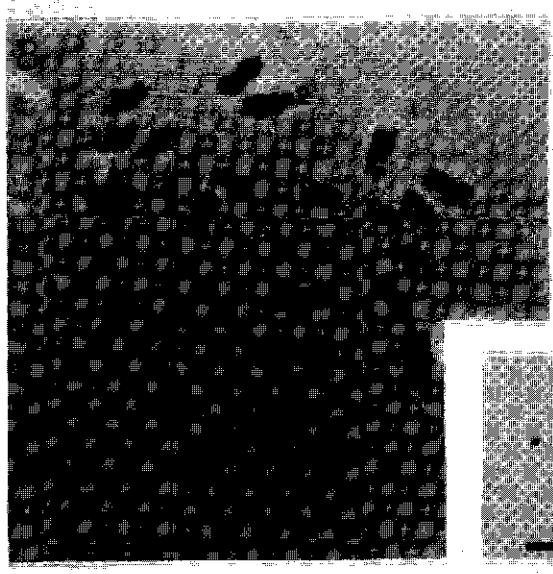
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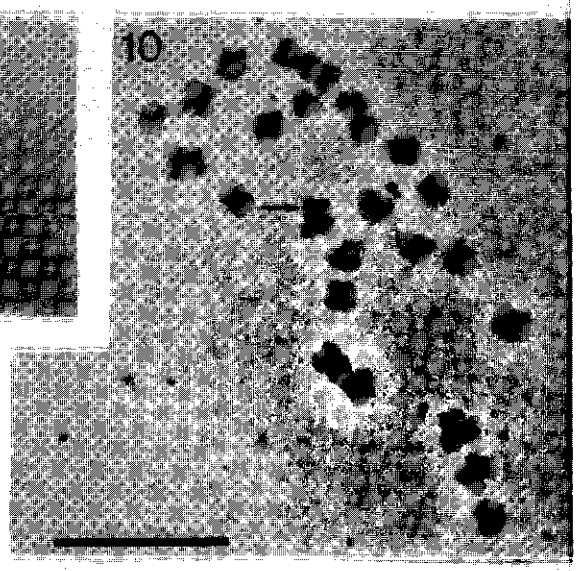
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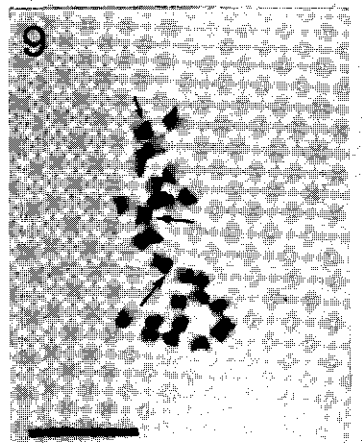
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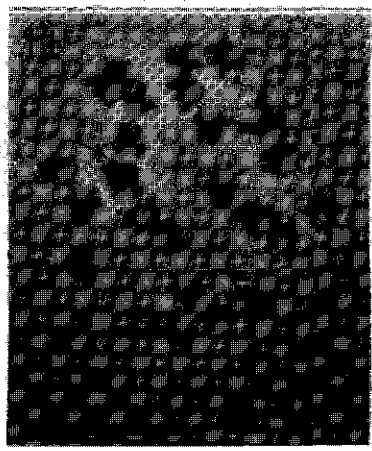


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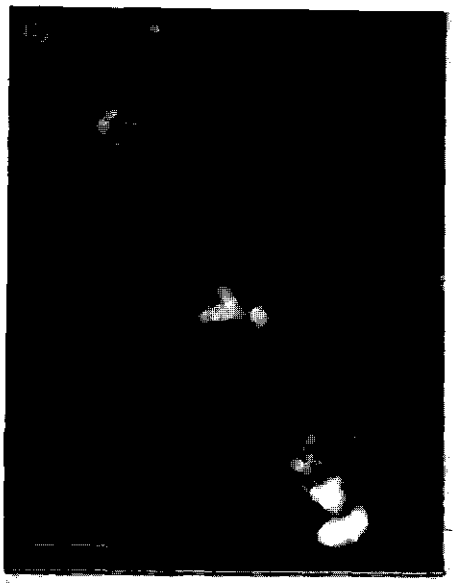
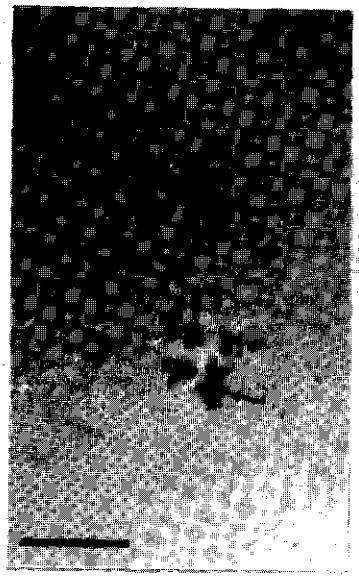
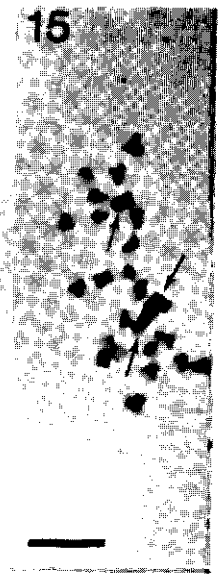
Figs. 1, 3, 5, (Feulgen-haematoxylin staining), 10 and 12. Cytotypes of *Solanum tuberosum* ssp. *tuberosum* cv. Gineke. Figs. 8 and 9. Interspecific trisomics including *S. tuberosum* ssp. *tuberosum* and *S. phureja*. Fig. 11. Interspecific trisomic including the species *S. tuberosum* ssp. *tuberosum*, *S. chacoense* and *S. yungasense*. Fig. 1. Metaphase from a monohaploid. The chromosomes 1, 2 and 12 are identifiable but the identity of the chromosomes 3 and 4 based on their size is not certain. Fig. 3. Metaphase from an aneuploid with 27 chromosomes. Three specimens of chromosome 1 and two of chromosome 2 are identifiable. Fig. 5. Metaphase of a monoploid with an unsatellited chromosome 2; NOR arrowed. Chromosome 3 shows a massive long arm. The identity of chromosome 4 is not certain. Fig. 8. C-banded chromosomes. Two specimens of each of the chromosomes 1 and 2 and one specimen of each of the chromosomes 3 and 4 are identifiable. Fig. 9. C-banded chromosomes. Two specimens of chromosome 2 arrowed. Note that one specimen of chromosome 4 (indicated by small arrow) has a wide band on the short arm. Fig. 10. Metaphase from intraspecific chromosome 3-trisomic. Two specimens of chromosome 2 show spots on the NORs. *In situ* hybridization (ISH) with biotinylated rDNA from pea and detection of the hybridized probe by the peroxidase-DAB reaction. Spots on each chromatid of one chromosome arrowed. Viewed with phase-contrast illumination. Fig. 11. Same probe as in Fig. 10, but interspecific chromosome 5-trisomic and detection with the alkaline-phosphatase reaction. Fig. 12. The same cell as shown in Fig. 10, but in mirror image and viewed with reflection contrast microscopy. Bar represents 10 µm.



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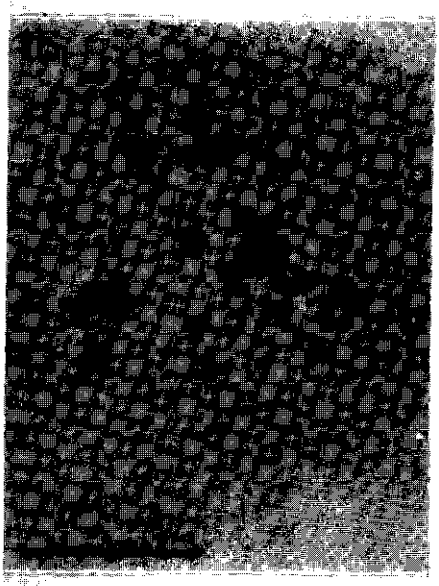
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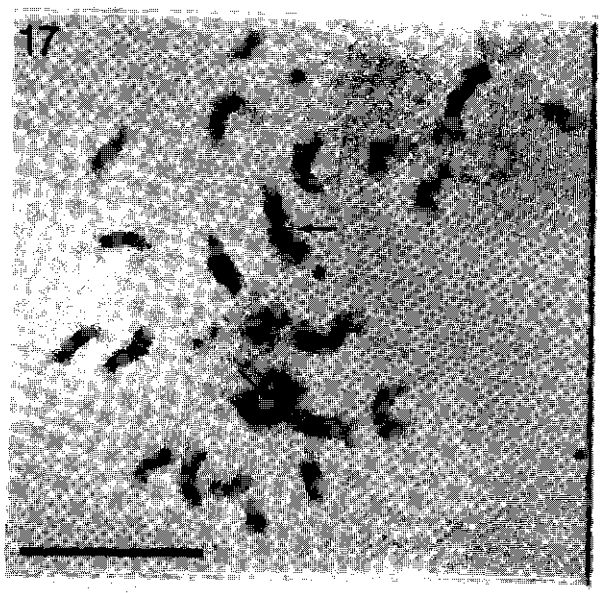
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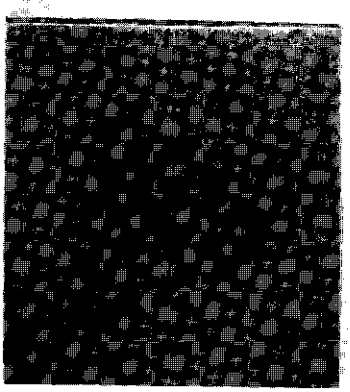

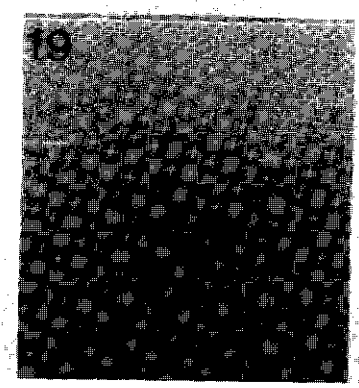
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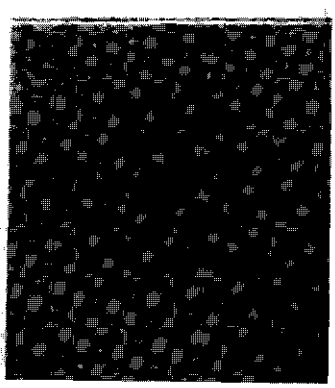
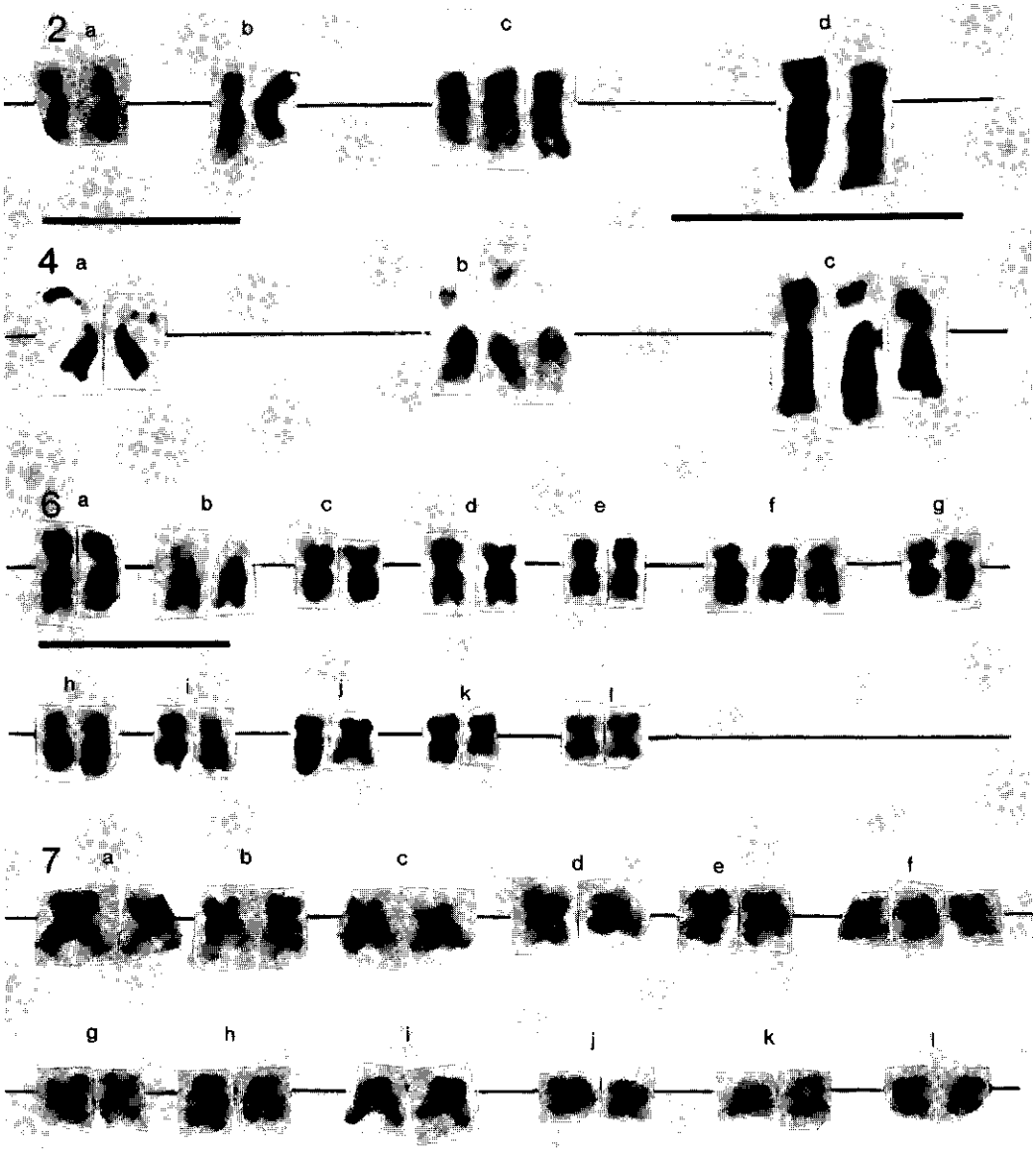


Figs. 13-19 and 20. Interspecific trisomics including *S. tuberosum* ssp. *tuberosum* and *S. phureja* and, intraspecific trisomic from cv. Gineke respectively. Fig. 13. Interphase nuclei from chromosome 2-trisomic. Three spots are visible, representing the NORs of the nucleolar chromosomes. ISH with biotinylated rDNA and detection by the peroxidase-DAB reaction and viewed with reflection contrast microscopy. Fig. 14. Metaphase from chromosome 5-trisomic. Two chromosomes with major spots on the NORs (large arrows) and two chromosomes with minor spots (small arrows). ISH with rDNA labelled with digoxigenin and detection by an anti-digoxigenin alkaline phosphatase conjugate. Fig. 15. Metaphase from chromosome 2-trisomic. Three specimens of chromosome 2 show a spot on the NORs. ISH with biotinylated rDNA and detection by an streptavidin-alkaline phosphatase conjugate. Fig. 16. Metaphase from chromosome 2-trisomic. Eight spots are visible. ISH with the patatin probe, designated B106, labelled with digoxigenin. Detection of the hybridized probe by an anti-digoxigenin alkaline phosphatase conjugate and amplification of the signal by APAAP. Figs. 17 and 18. Prometaphase and metaphase respectively from chromosome 5-trisomic. Simultaneous ISH with rDNA and B106 labelled with digoxigenin. Detection of the hybridized probe by an anti-digoxigenin alkaline phosphatase conjugate. Two chromosomes in Fig. 17 show more than one spot per chromosome (arrowed). Figs. 19 and 20. Metaphase from interspecific chromosome 5-trisomic and intraspecific chromosome 3-trisomic respectively. ISH with B106 labelled with digoxigenin. Detection of the hybridized probe by an anti-digoxigenin alkaline phosphatase conjugate. Three and two chromosomes show a hybridization spot respectively. Bar represents 10 μm.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Figs. 2 and 4 (Feulgen-haematoxylin staining), and 6 and 7 (Giemsa C-banding). Cytotypes of *S. tuberosum* ssp. *tuberosum* cv. Gineke. Fig. 2. Specimens of chromosome 1 in non-critical trisomic (a, b), in triploid (c), and in non-critical double trisomic (d). Fig. 4. Specimens of chromosome 2 in non-critical trisomic (a), note the double satellite of one of the chromosomes; in triploid (b), and in double trisomic critical for one of the chromosomes (c), note the shorter long arm of one of the three specimens. Figs. 6 and 7. Chromosomes of two not yet identified trisomics were arranged according to their length and their specific C-banding pattern. Because the banding pattern was unsatisfactory, chromosomes in the same position are therefore not necessarily identical. The chromosomes in Fig. 6 are overstained and cannot be properly identificated. It is, therefore, uncertain whether the extra chromosome in these trisomics designated f is identical to chromosome 6 according to the description by Pijnacker and Ferwerda (1984). Note the wide band in the short arm of one of the specimens of chromosome 4 (Fig. 7d). Bar represents 10 μm.

CHAPTER 2

Spontaneous structural rearrangements in *Solanum phureja* Juz. et Buk. 1. Chromosome identification at pachytene stage[1]

Summary

Meiosis was studied from pachytene onwards in two clones of *Solanum phureja* Juz. et Buk. At pachytene the bivalents of *Solanum phureja* appeared to be morphologically very similar to those of *Solanum tuberosum* L. ssp. *tuberosum* Hawkes cv. Gineke. Cross-shaped quadrivalent configurations at pachytene confirmed the presence of a heterozygous interchange. From the configurations at pachytene it was identified that the short arm of chromosome 3 and possibly one of the arms of chromosome 12 was involved in the interchange.

In addition, several abnormalities were observed: these included the occurrence of loops in euchromatic and heterochromatic parts, non-homologous pairing and centromere associations between different bivalents.

Key words: Solanum phureja Juz. et Buk., interchange, inversion loop, chromosome identification, trisomics, pachytene.

[1]Slightly revised version of the paper published in Euphytica 5: 159-167 (1988).

Introduction

In diploid potatoes chromosomal interchanges and inversions are rare, as it is evident from the experience of several decades in which a vast number of different interspecific hybrids have been produced and investigated cytologically. In the diploid hybrids, regular chromosome pairing at pachytene, diakinesis and MI were most commonly observed (Magoon *et al.* 1958a, 1958b; Dvořák 1983). Irregularities, such as multiple associations, univalents, delayed separation of bivalents, bridges with or without fragments, etc., have been seldom, suggesting that structural differences between chromosomes are not common (For a review, see Magoon *et al.* 1962).

The widely accepted opinion that tuberous *Solanum* species cannot be distinguished by gross structural differences has been discussed by several authors especially those who studied the pachytene stage of meiosis. Van Breukelen *et al.* (1976) studied meiotic chromosome pairing in monohaploids of *Solanum tuberosum* L. ssp. *tuberosum* Hawkes, cv. Gineke through the occurrence of bivalent, trivalent and quadrivalent-like structures. In dihaploids of cultivar Gineke looplike configurations could be observed in 8-20% of the bivalents (Ramanna & Wagenvoort 1976). Quadrivalents as well as univalents were rather common in almost all clones of dihaploids derived from the tetraploid cultivar Atzimba (*S. tuberosum* ssp. *tuberosum* with *S. demissum* Lindl. in its pedigree), (Sosa & Sosa 1971).

Comparison of pachytene complements revealed the existence of very clear differences in the fine structure of apparently homologous chromosomes of different *Solanum* species belonging to the section Petota (Gottschalk 1954; Gottschalk & Peters 1955, 1956; Peters 1954). Gottschalk also described the structure of nine heteromorphic bivalents of the interspecific hybrid *S. stenotomum* Juz. et Buk. x *S. ajuscoense* Buk., and observed very clear structural differences between the homologous chromosomes. These differences result in the formation of heteromorphic bivalents showing unpaired loops. Pachytene studies of Hermsen & Ramanna (1976) have revealed the existence of small structural differences between some of the chromosomes in the F_1 hybrids of *S. verrucosum* Schlechtd. x *S. bulbocastanum* Dun. The same authors found in an F_1 hybrid between two diploid Mexican species, viz. *S. pinnatisectum* Dun. x *S. bulbocastanum* a thick block of heterochromatin, adjacent to the centromere on the long arm of chromosome 11 of *S. bulbocastanum*. In F_1 hybrids between *S. verrucosum* and *S. commersonii* Dun. trivalents and quadrivalents were found at metaphase I and diakinesis of meiosis (Matsubayashi & Misoo 1979). These authors suggest the presence of a detectable structural differentiation between the chromosomes of the two species.

Two entirely different chromosome complements are brought together in the allotetraploid species *S. antipoviczii* Buk. (Gottschalk 1972). In one of the complements of this species very big heterochromatic blocks were observed which could not be found in the cultivated potato and its close relatives.

Marks (1968) showed that a hybrid between two diploid *Solanum* species, viz. *S. morelliforme* Bitt. et Muench and *S. clarum* Corr., was heterozygous for a chromosomal interchange and possibly for two inversions.

The cited literature suggest that structural differences between chromosomes of *Solanum* species are more common than generally is suspected. Although such structural differences can be detected during late prophase and metaphase I stages, they can be more convincingly demonstrated by pachytene analysis. Detection and identification of interchanges in diploid *Solanums* is especially important in view of localizing the position of centromeres in the linkage groups of this crop plant.

In the course of a research programme on unreduced gametes in *S. phureja*, multivalents at metaphase I of microsporogenesis were found in two siblings of this species. This paper reports part of the results of a cytological study (mainly pachytene) of these two clones and of some diploid and trisomic descendants. The remaining results (chromosome associations at diakinesis and later meiotic stages) will be published in a second paper (Wagenvoort, 1994).

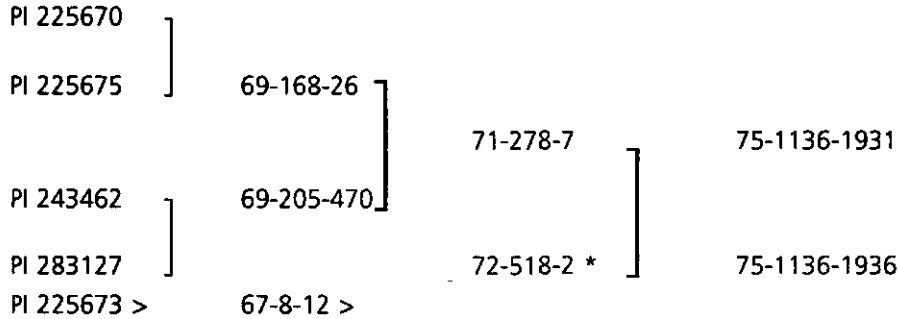
Material and methods

The diploid clones *S. phureja* 75-1136-1931 and *S. phureja* 75-1136-1936 (abbreviated 1931 and 1936) were selected by Dr. B. Maris, (former SVP, Wageningen, the Netherlands) in the course of a study concerning an adaptation programme with *S. phureja*. The pedigree of the two clones is shown in Fig. 1. The PI numbers mentioned in this figure refer to seed samples described in the Inventory of Tuber-bearing *Solanum* species, Bulletin 533, from the Inter-Regional Potato Introduction Project, IR-1, edited by R.E. Hanneman, Jr and J.B. Bamberg, Sturgeon Bay, WI, USA.

For meiotic studies young flower buds were fixed for 48 h or more in a freshly prepared 3:1 mixture of ethanol (96%) and propionic acid (99%) saturated with ferric acetate. The anthers were stained in alcoholic hydrochloric acid carmine for 16-24 h at 60°C according to the method of Snow (1963) and squashed in a drop of 45% acetic acid. The methods used to study mitotic chromosomes were the same as described by Wagenvoort & Lange (1975). Pollen grains were stained in a mixture of 100 ml lactophenol and 8 ml 1% acid fuchsin in water

(Sass 1964).

Fig. 1. Pedigree of the clones 1931 and 1936 of *S. phureja* used in this study.



* Clone 72-518-2 was selected in a spontaneous offspring of clone 67-8-12.

Results

Meiosis was studied from pachytene stage and onwards in the clones designated 1931 and 1936 and in some diploid ($2n = 2x = 24$) and trisomic ($2n = 2x + 1 = 25$) descendants. The occurrence of 10 bivalents + 1 quadrivalent in about 70% of the PMCs at MI pointed to the presence of a heterozygous interchange in both clones of *S. phureja* (Wagenvoort, 1994).

The clones 1931 and 1936 were male fertile and had a pollen stainability of 42% and 50% respectively. Although *S. phureja* is considered to be a self-incompatible diploid species, clone 1936 produced seeds after selfing. Three out of 475 plants of the first inbred generation were trisomics. No aneuploids were found among 79 full sibs of the cross 1936 x 1931.

The morphology of the pachytene chromosomes of *S. phureja* did not differ from that of the chromosomes of *S. tuberosum* ssp. *tuberosum*. The latter have been identified and described by Ramanna & Wagenvoort (1976) using dihaploid *S. tuberosum* ssp. *tuberosum* cv. Gineke. Features, such as size, positions of chromomeres and centromeres, size of telomeres, lengths of heterochromatic and euchromatic parts, used for chromosome identification in *S. tuberosum* ssp. *tuberosum* also holds good for the identification of individual pachytene bivalents in *S. phureja*.

Identification of the interchange chromosome

Fig. 2. shows individual pachytene bivalents of the chromosomes 1(a), 2(b), 9(c), 4(d), 7(e), 10(f), 5(g), 8(h), 11(i), probably 4 or 6 (j), a bivalent (k) which presumably consists of chromosome 12 plus the interchange chromosome. No bivalent for a normal chromosome 3 was found. On the basis of chromosome morphology it was concluded that the interchange chromosome had originated from the exchange of the short arm of chromosome 3 or a part of it, with possibly a part of one of the arms of chromosome 12. Evidence for the involvement of the short arm of chromosome 3 was acquired from some heteromorphic bivalents as shown in Fig. 3 (illustrations a and f) and from quadrivalent configurations as shown in the Figs. 4a and 4b. Although the bivalent shown in Fig. 2k is similar to a normal bivalent of chromosome 12, it is most likely that this configuration has originated through the association of a normal chromosome 12 and the interchange chromosome 12^3. The formation of a completely paired bivalent in this case could have resulted through non-homologous association.

The involvement of chromosome 12 in the interchange was difficult to prove by analysis of the bivalent and quadrivalent configurations observed at pachytene stage alone. However, by crossing a series of primary trisomics with the clones having the interchange chromosomes as pollen parents, F_1 trisomics including the interchange chromosomes were produced and analysed at metaphase I of meiosis. In such trisomic + interchange cytotypes the formation of a quinquevalent is expected if the critical trisomic is involved. Indeed, in F_1 plants from trisomics for the chromosomes 3 and 12, and 1931 or 1936, chain quinquevalents were observed at metaphase I (Wagenvoort, 1994).

The configuration shown in Fig. 3a represents the pairing between an interchange chromosome 12^3 and a normal chromosome 3. The interchange chromosome 12^3 probably equals a normal chromosome 12 in length. The very large loop in the euchromatic part of this configuration belongs to the long arm of chromosome 3. The bivalent illustrated in Fig. 3f clearly shows the three distinct chromomeres on the short arm of chromosome 3. The morphology of this bivalent is abnormal with respect to chromosome 3 of the karyotype of dihaploid *S. tuberosum* ssp. *tuberosum* cv. Gineke.

Some cross-shaped configurations were observed and analysed. The point of exchange of the four chromosome arms is clearly visible in the quadrivalent shown in Fig. 4a. Unfortunately a proper identification of the breakpoint of the chromosomes involved in this interchange was hampered by the fact that the positions of the centromeres remained unclear. For chromosome 3 the breakpoint must have been very close to the centromere or in the centromere itself. From this configuration it is obvious that the breakpoint in chromosome 12 also is situated in the heterochromatic part of the chromosome, near to the centromere. The quadrivalent shown in

Fig. 4b is difficult to interpret. It resembles the one shown in Fig. 4a with respect to the lengths of the four different chromosome arms. The big loop in the euchromatic part of the longest chromosome arm (that of chromosome 3) is rather the result of non-homologous association than inversion heterozygosity.

Abnormalities of chromosome association

The loop indicated by two small arrows in the bivalent of Fig. 3f and in one of the bivalents of Fig. 3c resulted from association of two chromosomes with different lengths. This type of abnormal chromosome pairing was observed in other bivalents (Fig. 3a, 3d) and in the multivalent configuration, shown in Fig. 3b, too. They resemble the configurations, described by McClintock (1933), which were the result from deficiencies involving internal segments of chromosomes. In this case it is not clear whether the loops result from deletion or duplication of internal segments.

Another abnormality is the occurrence of a small heterochromatic segment in the distal euchromatic part of the long arm of this bivalent (Fig. 3f). An interruption of the heterochromatic part of the long arm by a relatively large region of euchromatin (see Fig. 3e, between the small arrows) is another example of gross structural differences between a chromosome of this particular clone of *S. phureja* and its homeologous chromosome of the complement of dihaploid Gineke.

True loops were observed in the euchromatic parts of certain bivalents (Figs. 3g and 4e). The chromosomes shown in the Figs. 3g and 4e are morphologically similar. The loop is situated in the euchromatic part of the long arm of chromosome 4 or chromosome 6. The chromomere on the short arm of the bivalent shown in Fig. 3g points to chromosome 6, whereas the length of the short arm of this bivalent is more concurrent with the length of the short arm of chromosome 4 than with that of chromosome 6. However, the chromomeres found in both configurations, just behind the loop on the euchromatic part of the long arm, is a characteristic feature of chromosome 4 rather than of chromosome 6. The loops indicate to heterozygosity for an inversion, which can give rise to the occurrence of bridges at anaphase I of meiosis, with or without a fragment. In both clones 1931 and 1936, such irregularities at anaphase I have been observed indeed (Wagenvoort, 1994). A bivalent with a loop in the heterochromatic part of the long arm (Fig. 3h) could not be identified.

The quadrivalent configuration shown in Fig. 4d occurs frequently in other PMCs. This configuration does not represent an interchange multivalent but is rather the result of the occurrence of centromere associations at pachytene. Such configurations occur in normal

diploid plants in considerable frequencies (De Jong & Stam 1984).

Fig. 4c shows a phenomenon earlier reported in primary trisomics of diploid *S. tuberosum* ssp. *tuberosum* by Wagenvoort & Ramanna (1979). They revealed that in primary trisomics very frequently all three homologous chromosomes were found to be paired for a considerable part of their length. From Fig. 4c it is clear that a distal euchromatic part of a bivalent involving chromosomes with a median centromere position has been associated with an internal euchromatic part of a second bivalent involving chromosomes with submedian centromeres. It clearly demonstrates the possibility of full non-homologous pairing of four chromosomes without partner exchange.

Discussion

The cross-shaped configurations found at pachytene of the microsporogenesis in the two *Phureja* siblings, 1931 and 1936, clearly confirm the occurrence of a heterozygous interchange in these clones. These pachytene configurations are comparable with those described by Marks (1968) who observed similar figures in an F_1 hybrid between *S. morelliforme* and *S. clarum*.

There was a striking similarity between the morphology of the chromosomes of *S. phureja* and that of dihaploid *S. tuberosum* ssp. *tuberosum* cv. Gineke. The abnormalities, such as non-homologous association of certain chromosome regions, the occurrence of loops, and the pairing of more than two chromosomes without partner exchange, as observed in the meiosis of dihaploids of cv. Gineke, also were seen in the *phureja* clones studied. Although these irregularities, especially the occurrence of quadrivalent-like configurations, hampered an accurate identification of the chromosomes, a careful analysis of some multivalents at pachytene could be performed. The true quadrivalents shown in the Figs. 4a and 4b were not subject to misinterpretation because of the certainty with which the exchange points (indicated by small arrows) of the four chromosomes could be detected. The interference of the phenomenon of non-homologous association of chromosomes with the identification necessitates the analysis of a large number of configurations at pachytene stage, as stated by Ramanna & Wagenvoort (1976).

The loops, found in the clones 1931 and 1936 of *S. phureja* differed with respect to the position in the chromosomes from those observed in diploid *S. tuberosum* ssp. *tuberosum*. In the latter, loops in the euchromatic parts of the chromosomes rarely occurred (Ramanna & Wagenvoort 1976).

Gill *et al.* (1980) showed that breakages in tomato chromosomes that gave rise to

interchange chromosomes occurred more frequently in centromeric and heterochromatic regions than in euchromatic parts of the chromosomes. Also in maize there are more interchanges reported with breakages in the centromeres compared with the euchromatic regions of the chromosomes (Jancey & Walden 1972). The breakages in the heterochromatic regions of both chromosome 3 and 12, involved in the interchange in *S. phureja* seem to be in agreement with the non-random distribution of breakage points in the chromosomes of tomato and maize.

The phenomenon of the occurrence of centromere associations at pachytene and diplotene has been reported for *Beta* by De Jong & Stam (1984). From meiotic studies in *B. vulgaris* and in several monosomic additions of a chromosome of *B. patellaris* or *B. procumbens* to the diploid genome of *B. vulgaris*, these authors concluded that the centromere associations do not disturb chromosome pairing and meiotic transmission. The observations in *S. phureja* concerning a regular chromosome pairing are in accordance with the conclusion of De Jong & Stam.

This report is the first in describing an intraspecific interchange in a tuberous diploid *Solanum* species, complete with the identification of the chromosomes involved. Such interchanges could be most useful for the localization of genes to specific arms as well as for the localization of centromeres on the linkage maps.

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Fig. 2a-h. Eleven pachytene bivalents of *S. phureja*. Chromosome 1(a), 2(b), 9(c), 4(d), 7(e), 10(f), 5(g), 8(h), 11(i), probably 4 or 6 (j), a bivalent (k) which presumably consists of chromosome 12 plus the interchange chromosome. The centromeres are indicated by large *arrows*. Important chromomeres of the chromosomes 4(d) and 5(g) which are helpful for identification are indicated by *small arrows*.

Magnification: Illustrations a and c-k, about x 3240. Illustration b, about x 2025. For description of individual bivalents, see Ramanna & Wagenvoort (1976).

a
c
d
e
f
g
k
h
j

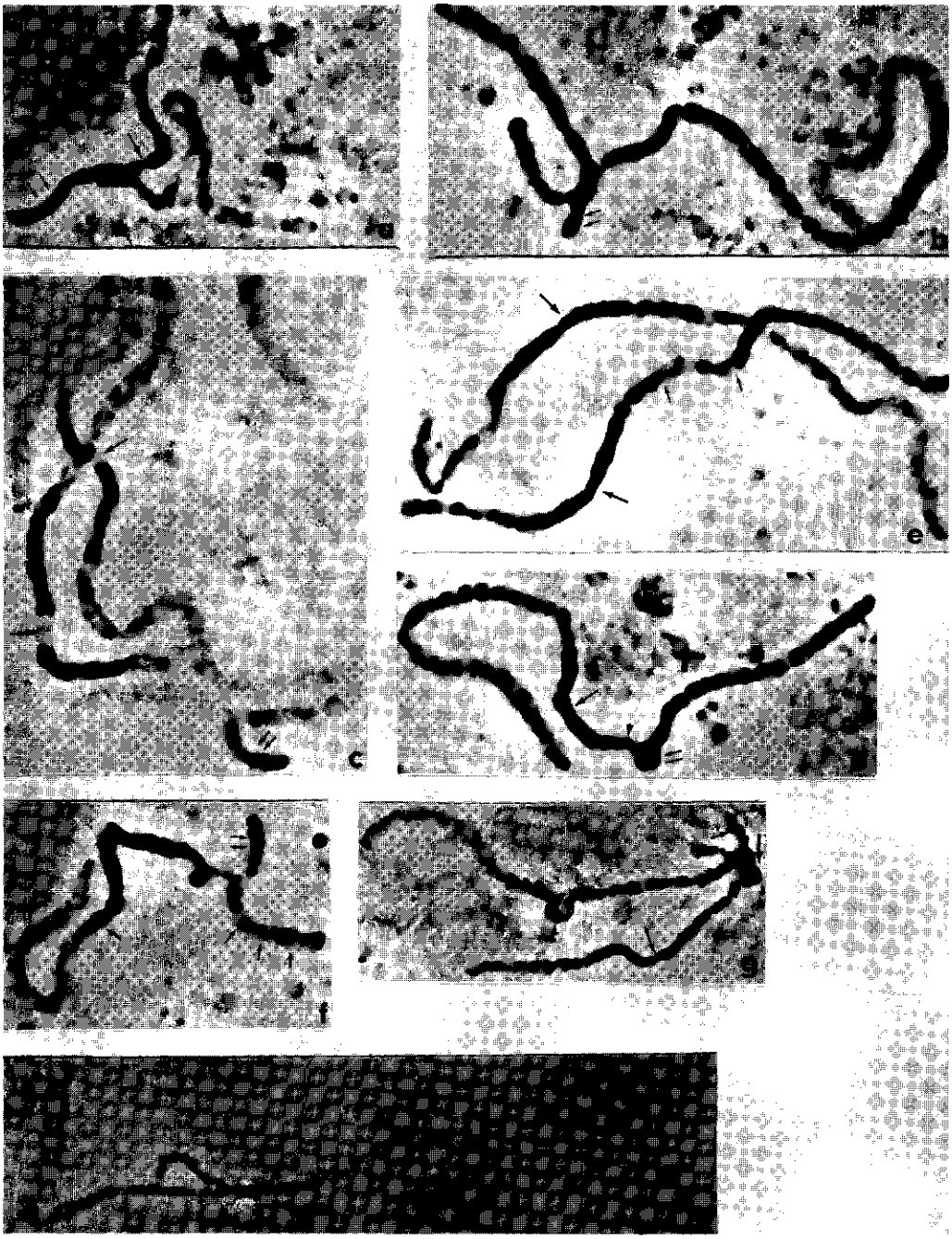


Fig. 3. Showing bivalents (a en c-g), a multivalent (b) of *S. phureja* and a bivalent (h) of a diploid hybrid between *S. tuberosum* ssp. *tuberosum* and *S. phureja*.

Illustration a. This configuration is assumed to be the result of pairing between an interchange chromosome 12^3 and a normal chromosome 3. Note the three distinct chromomeres on the short arm of chromosome 3 (*small arrows*) which also are shown in illustration f.

Illustration b. A three-armed multivalent showing 'fold-back pairing' of a heterochromatic part (indicated by two *small arrows*) of a chromosome. This type of non-homologous association also is shown in the illustration a and d.

Illustration c. In one of the bivalents a loop-like structure (two *small arrows*) is shown.

Illustration d. Note the thick knob of heterochromatin, due to non-homologous pairing and the short interruption of the heterochromatic part (arrow-head) of this bivalent.

Illustration e. One of the bivalents shows an interruption of the heterochromatin by an euchromatic part (between the *small arrows*) of the bivalent.

Illustration f. A bivalent configuration of chromosome 3. Note the three distinct chromomeres (*small arrows*), the non-homologous association of heterochromatin (two *small arrows*) and the small heterochromatic part (*small arrow*) in the euchromatic region of the long arm of this chromosome.

Illustration g. The bivalent configuration showing the loop in the euchromatic region (*small arrow*) probably represents chromosome 4 or 6. For further explanation see text.

Illustration h. This bivalent shows a loop in the heterochromatic part of the long arm.

Magnification: Illustrations a-f, about x 3240. Illustration g and h, about x 2025.

a
b
c
d
e

Fig. 4. Illustration a. A quadrivalent configuration is shown including two normal chromosomes and two interchange chromosomes. Note the exchange point indicated by a *small arrow.*
Illustration b. This configuration represents a real quadrivalent. Note the loop-like structure in the euchromatic part of the long arm.
Illustration c. A distal euchromatic part of a bivalent has been associated with an internal euchromatic part of a second bivalent, resulting in a three-armed configuration.
Illustration d. This configuration resulted from the non-homologous association of centromeres of two bivalents. It is assumed to be a pseudo-quadrivalent.
Illustration e. A bivalent of probably chromosome 4 or 6 showing a loop in the euchromatic part of the long arm.
Magnification: Illustrations a-e, about x 3240.

CHAPTER 3

Spontaneous structural rearrangements in *Solanum phureja* Juz. et Buk. 2. Meiotic behaviour and identification of interchange chromosomes using primary trisomics.

Summary

Meiosis was studied in two diploid ($2n = 2x = 24$) siblings of *Solanum phureja*, Juz. et Buk., and in eleven disomic and two trisomic descendants. The diploid siblings carry the same heterozygous interchange and either one or two inversions. The frequency of quadrivalents at diakinesis/metaphase I in these clones was 0.56 and 0.62 per pollen mother cell. In two plants from the first inbred generation (I_1) this frequency was about the same, but in some other I_1 plants and a full sib the frequency was substantially lower and varied from 0.00-0.16. Most quadrivalents, 78-83%, were rings. A variety of quadrivalent configurations at diakinesis and metaphase I was observed giving rise to balanced and unbalanced gametes. The absence of ring quadrivalents in trisomic descendants of one of the siblings implied that tertiary trisomics or primaries being homozygous for the interchange were present in the I_1 generation.

Regular chromosome distribution (12-12) at anaphase I occurred in 46.5 and 73.2 % of the pollen mother cells studied in the two original clones. Irregularities, such as 11-13 distribution, lagging chromosomes, and a bridge and fragment were detected on average in 2.7, 3.3 and 32.5% respectively of the anaphase I cells analysed.

In hybrids from crosses between six primary trisomics as females with the interchange heterozygote, the involvement in the interchange of the chromosomes 3 and 12 was clearly demonstrated.

Key words: *Solanum phureja*, interchange heterozygote, paracentric inversion, chromosome identification, compensating trisomic, meiosis.

Introduction

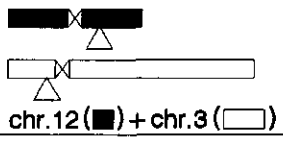
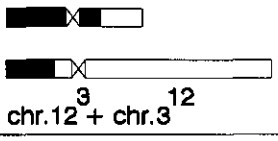
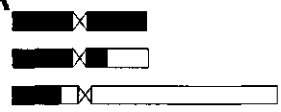
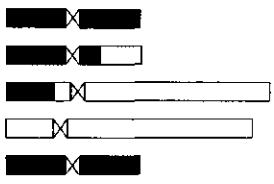
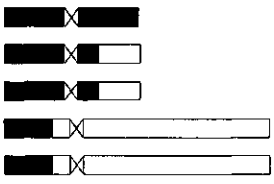
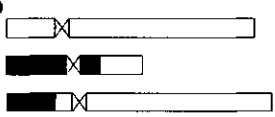
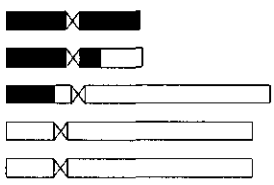
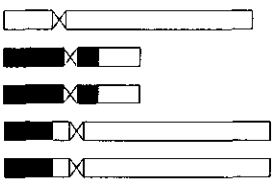
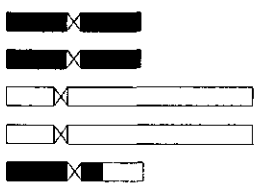
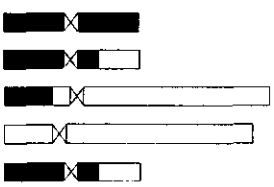
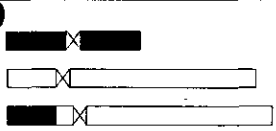
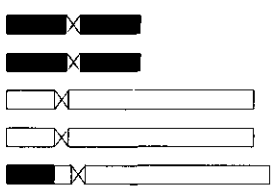
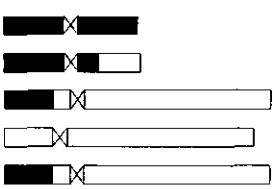
In the first paper in this series (Wagenvoort, 1988) the results of pachytene analysis in two siblings of *Solanum phureja* Juz. et Buk., being heterozygous for the same interchange, were reported, showing the involvement in the interchange of the short arm of chromosome 3 and possibly one nearly full arm of chromosome 12. The regions between the breakpoints and the centromere were assumed to be too small to allow chiasma formation, although the occurrence of chiasmata in these regions cannot be ruled out. Chiasma formation in all homologous chromosome arms will result in a ring quadrivalent. However, if in one out of the four pairs of chromosome arms of a quadrivalent no chiasma is formed, a chain quadrivalent will result. In addition, chromosome loops were found at pachytene and the preliminary observation of the incidence of a bridge and a fragment at anaphase I (AI) in both clones indicated heterozygosity for a paracentric inversion in chromosome 4 and/or chromosome 6.

Trisomics were found in the first inbred generation (I_1) of one of the siblings, whereas the I_1 segregated aberrantly for the morphological marker *ym* (yellow margin). The incidence of segregation of *ym*, previously localised on chromosome 12 (Wagenvoort, 1982), together with the presence of an interchange involving presumably one arm of chromosome 12, stimulated further cytogenetical analysis of this material.

Background considerations

An interchange heterozygote producing an asymmetrical quadrivalent at metaphase I (MI) of meiosis can give rise to a 3:1 disjunction of the chromosomes at AI. As a result, four types of $n+1$ gametes can be formed, two of which contain an interchange chromosome in addition to the normal complement (C + D in Fig. 1), whereas the other two carry the interchange complement with a normal chromosome extra (A and B in Fig. 1). Figure 1 (modified from Khush, 1973) includes the eight different trisomics expected in selfed progeny of an interchange heterozygote, assuming (1) that only $n+1$ female gametes are incorporated, (2) that only euploid male gametes are viable, carrying either two normal or two interchange chromosomes, (3) that progeny arising from fusion of $n+1$ female with functional male gametes is viable.

Fig. 1. Functional n (pollen) and $n+1$ (eggs) gametes produced by an interchange heterozygote (the putative chromosomes being involved in the interchange are the chromosomes 3 and 12) and the $2n+1$ zygotes expected in its selfed progeny (Modified from Kush, 1973). →

Viable n+1 female gametes from 3:1 disjunction	Functional male gametes	
	 chr.12 (■) + chr.3 (□)	 chr.12^{3} + chr.3^{12}
A  n + chr. 12	1  2n + chr. 12	2  2n + chr. 12
B  n + chr. 3	3  2n + chr. 3	4  2n + chr. 3
C  n + chr. 12^{3}	5  2n + chr. 12^{3}	6  2n + chr. 12^{3}
D  n + chr. 3^{12}	7  2n + chr. 3^{12}	8  2n + chr. 3^{12}

These eight trisomics are expected to consist of two types each of - primary trisomic interchange heterozygote (Fig. 1; 1 and 3) - primary trisomic interchange homozygote (Fig. 1; 2 and 4) - tertiary trisomic (Fig. 1; 5 and 7) - tertiary trisomic interchange heterozygote (Fig. 1; 6 and 8). A tertiary trisomic in which the extra chromosome consists of one complete arm of one chromosome and one complete arm of the other chromosome can be used for determining arm location and approximate distance from the centromere of genetic markers as the genetic ratios are modified only for genes located in one chromosome arm. In such a tertiary trisomic test, the recessive gene to be located, has been previously associated with a specific chromosome by the primary trisomic test (Wagenvoort, 1982).

The involvement of chromosome 12 in the interchange in *S.phureja* could not be detected unambiguously by direct chromosome identification at pachytene. Therefore, a series of primary trisomics as females were crossed with the interchange heterozygote. Two types of each primary trisomic and compensating trisomic (identical with the trisomics shown in Fig. 1; 1 and 3) are expected in the critical (extra chromosome involved in the interchange) situation of the F_1 progeny, assuming that only balanced male gametes are functional (Table 1). Compensating trisomics (individuals in which one chromosome is missing but is compensated for by two other modified chromosomes, Khush, 1973), also referred to as translocation trisomics by Sybenga (1975), will result from the fusion of balanced male gametes containing two interchange chromosomes and $n+1$ female gametes. In the non-critical situation two types of each, primary trisomic and primary trisomic interchange heterozygote, are expected when only balanced gametes are functional. Unbalanced gametes are expected to be hardly or not at all functional.

To distinguish the different types of F_1 trisomics and to discriminate between the critical and non-critical situation, it is necessary to study chromosome association at MI. Table 1 presents the possible chromosome configurations and their combinations per pollen mother cell (PMC) occurring in the different types of trisomics. In case of the critical situation, the compensating trisomics are able to form a chain of five chromosomes at MI of meiosis. Thus the occurrence of a quinquevalent at MI identifies the chromosome involved in the interchange, being similar to the extra chromosome of the primary trisomic used in the cross. Only the compensating trisomic might have a ring quadrivalent + a univalent at MI instead of the quinquevalent.

For the morphological marker *ym*, 34.16-36.08% crossing-over between the locus and the centromere was estimated with the aid of a half-tetrad analysis by Jongedijk *et al.* (1991). The relatively large distance of *ym* to the centromere together with the fact that the breakpoint of the interchange was situated in the heterochromatic part of chromosome 12, near to the centromere (Wagenvoort, 1988), led to the view that there is no tight linkage between *ym* and

Table 1. Possible multivalent configurations at MI in primary trisomics, primary trisomics interchange heterozygotes and compensating trisomics derived from crosses between a series of primary trisomics as females, with an interchange heterozygote. V = quinquevalent; IV = quadrivalent; III = trivalent; I = univalent; R = ring; Ch = chain; A = non-critical trisomic; B = critical trisomic.

	V	IV		III	Combination/Cell
	Ch	R	Ch		
A_1 Primary trisomic	-	-	-	++	-
A_2 Primary trisomic interchange heterozygote	-	++	+	++	IVR+III or IVCh+III or 2 III+I
B_1 Primary trisomic	-	-	-	+	-
B_2 Compensating trisomic	+	+	+	+	VCh or IVR+I or IVCh+I or III+II

the breakpoint of the interchange. In a testcross population from Tt *Ymym* x tt *ymym* (Tt = interchange heterozygote and tt = plant without interchange) the ratio of plants forming bivalents only vs plants forming one quadrivalent is expected to be 1:1, no matter whether *ym* or *Ym* is on the interchanged chromosome arm. However, within the group of phenotypically normal plants (*Ymym*) the ratio will approach 1:2 (*Ym* on the interchanged chromosome arm) or 2:1 (*ym* on the interchanged chromosome arm), based on the crossing-over percentages mentioned above.

Aim of research

The experiments described in this paper aimed at answering the following questions:

(i) Is chromosome 12 indeed involved in the interchange?

(ii) If so, is the gene *ym* located on the interchanged or the non-interchanged arm of chromosome 12?

(iii) Which of the two alleles is on the interchanged chromosome arm?

(iv) Are the trisomics found in the I_1 of one of the siblings primary or tertiary trisomics?

In order to answer these questions, meiosis was studied in the two siblings and in several of their descendants. In addition, the chromosomal identification of the interchange in *Solanum phureja* using a series of primary trisomics of mainly *S.tuberosum* L. ssp. *tuberosum* Hawkes, and the various types of quadrivalents involving the interchange are reported and discussed.

Materials and methods

The siblings *S.phureja* 75-1136-1931 and *S.phureja* 75-1136-1936, for the sake of brevity designated 1931 and 1936, were used in this study. Their pedigree has previously been described by Wagenvoort (1988). Both diploid ($2n=2x=24$) clones were heterozygous for an interchange between chromosome 3 and possibly chromosome 12, for the morphological marker *ym* and presumably for one or two inversions (Wagenvoort, 1988). Selfing 1936 and crossing 1936 x 1931 yielded the I_1 and the F_1 generation respectively. In order to determine whether chromosome 12 was involved in the interchange, the diploid interchange heterozygotes, 1936 and I_1 plant 73 were crossed as males to the primary trisomics for the chromosomes 3, 4, 7, 9, 10 (Wagenvoort and Ramanna, 1979) and 12 (Hermsen *et al.*, 1973). Use was also made of the *S. phureja* clone Ym 76-1-15, a recessive mutant for yellow margin (Wagenvoort, 1982). F_1 trisomics were selected by chromosome counting in the root tips of seedlings. Chromosome association at diakinesis/MI was studied in twelve F_1 trisomics.

The siblings 1931 and 1936 were crossed to the mutant yellow margin, and their progenies assessed for yellow margin and for interchange heterozygotes.

The methods used for the study of mitotic and meiotic chromosomes were the same as described by Wagenvoort & Lange (1975) and Wagenvoort (1988) respectively. Meiosis was studied from diakinesis onwards in 1931 and 1936, in ten disomic and two trisomic descendants selected in the first inbred generation (I_1) of 1936, and in one F_1 plant of the cross 1936 x 1931.

Results

Average seed set in five berries from 1936 selfed was 114 seeds, whereas 183 seeds were found in one berry from crossing 1936 with 1931. The average seed set in 53 different crosses within *S. phureja* was 237 ± 131.3 (M. Wagenvoort, unpublished results).

Chromosome association at diakinesis/MI

The results of meiotic analysis at diakinesis and MI in the interchange are presented in Table 2 and Fig. 2. Chromosome association generally was regular. A rather high frequency of quadrivalents per PMC was found in the diploid clones 1931 and 1936, viz. 0.62 and 0.56, respectively. Quadrivalents were also observed in four out of ten I_1 plants from 1936 and in the F_1 plant from the cross 1936 x 1931. The frequency of quadrivalents in two I_1 plants was of the same order of magnitude as that found in 1931 and 1936. In the I_1 plants 134 and 138 and in the F_1 plant, this frequency was substantially lower.

At diakinesis six different quadrivalent configurations were observed (Figs. 2a-2f). Adjacent and alternate ring quadrivalents (Figs. 2i and 2j), alternate chain quadrivalents (Fig. 2k) in addition to cells with twelve bivalents (Fig. 2l) were observed at MI. About 83 and 79% ring quadrivalents were found in 18 PMCs from 1931 and 59 PMCs from 1936 respectively. Therefore, ring quadrivalents tend to occur more frequently than chain quadrivalents. However, an accurate quantitative estimation of the frequency of adjacent and alternate types could not be made, mostly due to stickiness. Some other rarely occurring configurations were found such as an U-shaped quadrivalent (Fig. 2m), a ring quadrivalent with possibly one extra chiasma (Fig. 2n) and an adjacent type of orientation with either co-orientation of all four chromosomes to one pole or co-orientation of two centromeres to opposite poles (the ones positioned nearest to the poles) and the other two not co-orientated (Fig. 2o).

The non-interchanged chromosomes of 1931 and 1936 were regularly associated,

Table 2. Chromosome association at diakinesis/MI of meiosis in two *S. phureja* siblings (1931 and 1936) heterozygous for an interchange between the putative chromosomes 3 and 12, in an F_1 hybrid plant, and in ten disomic and two trisomic I_1 descendants. I_1 = selfed progeny from 1936. F_1 = 1936 x 1931. V = quinquevalent; IV = quadrivalent; III = trivalent; II = bivalent; I = univalent.

Plant No.	Number of cells	Configurations/cell (range)				
		V	IV	III	II	I
1931	50	-	0.62	-	10.72 (9-12)	0.08 (0- 2)
1936	179	-	0.56	-	10.85 (9-12)	0.07 (0- 2)
Disomic I_1 plants from 1936						
55	8	-	0.62	-	10.50 (8-12)	0.50 (0- 4)
73	22	-	0.50	-	11.00 (10-12)	-
79	21	-	-	-	12.00 -	-
134	36	-	0.11	-	11.78 (10-12)	-
138	25	-	0.16	-	11.68 (10-12)	-
162	13	-	-	-	12.00 -	-
258	50	-	-	-	12.00 -	-
268	168	-	-	-	12.00 -	-
323	21	-	-	-	12.00 -	-
396	31	-	-	-	12.00 -	-
Trisomic I_1 plants from 1936						
82	25	0.08	0.08	0.28	11.24 (9-12)	0.96 (0- 3)
237	17	0.17	0.05	0.11	10.23 (10-12)	3.05 (0-25)
Disomic F_1 plant from 1936 x 1931						
680	22	-	0.14	-	11.73 (10-12)	-

Table 3. Chromosome distribution at AI of meiosis in two interchange heterozygotes (1931 and 1936) of *S. phureja*. L=lagging chromosome; b=bridge; f=chromosome fragment; II ds=bivalent separating with delay.

Plant No.	Number of cells	Distribution (%)					
		12-12	11-13	11-12+L/ 11-11+2L	11-11+b	11-11+b+f	10-12/11-11 + II ds
1931	143	46.2	2.8	3.5	25.2	20.3	2.0
1936	224	73.2	2.7	3.1	10.7	8.5	1.8

forming mostly bivalents. In some cells two univalents occurred, which in view of their position on the metaphase plate, resulted from precocious separation of a rod bivalent.

I_1 plant 55 had a univalent frequency of 0.50 and a maximum of four univalents per cell. Six out of the ten diploid I_1 plants had twelve bivalents in all the cells studied (Table 2). The nature of these plants could not be elucidated cytologically since both interchange homozygotes and plants carrying only standard chromosomes may form twelve bivalents. Fig. 2g shows twelve bivalents at diakinesis. A heteromorphic bivalent could be recognized in an incidental case (Fig. 2h). Such bivalent may originate from association of a normal and an interchange chromosome.

In the trisomic descendants of 1936 besides quadrivalents, bivalents and univalents also quinquevalents and trivalents (Figs. 3a and 3b) were observed. The frequency of quadrivalents in the trisomic I_1 plants 82 and 237 was 0.08 and 0.05, respectively (Table 2). This frequency of quadrivalents was low compared to that found in 1936. All quadrivalents found in both I_1 trisomics were chains, pointing to tertiary trisomy or to primaries being homozygous for the interchange. The trisomic I_1 plant 237 had in one cell up to 25 univalents and an average of 3.05 univalents per cell (Table 2).

Chromosome distribution at AI and some later stages

The distribution of chromosomes at AI is presented in Table 3 and in Fig. 3 c-h. The high frequency at AI of a bridge or a bridge and fragment (Fig. 3g and 3h) was notable. These abnormalities were found in 1931 and in 1936 in 45.5 and 19.2% respectively of the AI cells analysed (Table 3). In one PMC in 1936; a ring bivalent was still present at second prophase (Fig. 4a). In a single cell a chromatid bridge was observed at AII/TII (Fig. 4b). A double bridge and fragments were observed in one AI cell in 1931 (Fig. 4c). In the trisomic I_1 plant 82, a regular 12-13 distribution (Fig. 4d) was observed in five AI cells. Fig. 4e shows a 11-12 distribution and two scattered chromosomes at telophase I in the same trisomic. Both in the I_1 plants 82 and 237 a bridge and fragment were observed in some AI cells.

Chromosome identification of the interchange

Results from meiotic analysis (diakinesis/AI) in trisomic F_1 plants from the cross of six primary trisomics (3, 4, 7, 9, 10, 12) x interchange heterozygote are presented in Table 4. In trisomic F_1 plants from chromosome 3 and chromosome 12-trisomics chain quinquevalents (Figs 5a-c) were found. This result strongly indicates the involvement of the chromosomes 3 and 12 in the interchange as expected from the results of pachytene analysis (Wagenvoort, 1988).

Table 4. Chromosome association at diakinesis/MI of meiosis in six types of F_1 trisomics from the cross primary trisomic x interchange heterozygote.

Trisomic for chromosome	Number of plants	Number of cells	Configurations/cell (range)				
			V	IV	III	II	I
3	1	29	0.31	0.07	0.14	10.52 (8-12)	1.72 (0- 5)
4	1	6	-	0.33	0.17	11.00 (9-12)	1.17 (0- 3)
7	1	12	-	0.25	0.08	3.92 (0-12)	15.92 (0-25)
9	2	57	-	0.68	0.61	10.00 (9-12)	0.42 (0- 3)
10	1	4	-	-	0.50	11.25 (11-12)	1.00 (0- 3)
12	4	38	-	-	0.16	11.42 (9-12)	1.68 (0- 7)
12	2	119	0.07	0.05	0.26	10.04 (0-12)	3.60 (0-25)

Table 5. Chromosome association at MI in meiosis of fifteen normal and five yellow margin plants selected from the progenies of the testcross *Ymym* Tt (both 1931 and 1936) ♀ x *ymym* tt (Ym 76-1-15). Tt=interchange heterozygote; II=bivalent; IV=quadrivalent.

Genotype for yellow margin	Number of plants with		χ^2 for the ratios		
	12II	10II + 1IV	1:2	2:1	1:1
Normal	6	9	0.3	4.8*	-
Mutant	1	4			
Total	7	13	-	-	1.8

* = Significant at $P < 0.05$.

An asymmetrical Y-shaped trivalent was found at MI in a trisomic F_1 plant from the chromosome 3-trisomic (Fig. 5d). Two trisomic F_1 plants, one from the chromosome 7-trisomic and one from the chromosome 12-trisomic (Fig. 5e) had a high number of univalents in some cells. A ring quadrivalent together with a trivalent was observed at MI in PMCs from two trisomic F_1 plants from the chromosome 9-trisomic (Fig. 5f) and in PMCs from one trisomic F_1 plant from the chromosome 4-trisomic. A trivalent was the highest chromosome association observed in four trisomic F_1 plants from the chromosome 12-trisomic and in one trisomic F_1 plant from the chromosome 10-trisomic. No ring quadrivalents were observed in 119 PMCs from two other trisomic F_1 plants from the chromosome 12-trisomic and in 29 PMCs from a trisomic F_1 plant from the chromosome 3-trisomic.

Verification of chromosome arm position of the gene ym

The number of normal and mutant plants in both F_1s from the cross *Tt Ymym* x *tt ymym* fitted the expected ratio 1:1 (χ^2=1.03 and 1.21 respectively and P≈ 0.30). Table 5 presents the results of a study of meiosis in twenty F_1 plants. Fifteen normal plants segregated 6:9, six plants producing 12 bivalents only, and nine plants forming a quadrivalent at MI, which indicates their heterozygosity for the interchange. The five mutants analysed segregated 1:4 (Table 5). The observed ratio 6:9 fits the expected ratio 1:2 (χ^2=0.3, P= 0.70-0.50) and deviates significantly (χ^2=4.8, P=0.05-0.02) from the ratio 2:1. Therefore, it corroborates the view that there is no tight linkage between *ym* and the breakpoint of the interchange. Furthermore, it allows the conclusion that the dominant allele *Ym* is located on the interchanged arm of chromosome 12.

Discussion

The relatively high frequency of quadrivalents, viz. 0.62 and 0.56 per cell, estimated for 1931 and 1936 in this study, was similar to that found in the diploid interspecific hybrids between *S. morelliforme* and *S. clarum* (Marks, 1968) and between *S. verrucosum* and *S. commersonii* (Matsubayashi & Misoo, 1979). Such frequencies demonstrate the occurrence of structural differentiation within a diploid species and in diploid interspecific hybrids of *Solanum*.

Orientation of multiple chromosome associations

According to Rickards (1983) three orientation types can in general be distinguished in chromosome multiples: (i) Alternate orientation, (ii) Adjacent orientation; (iii) Amphitelic

orientation, in which sister chromatid centromeres of at least one chromosome in an otherwise alternate or adjacent orientation are oriented to opposite poles rather than syntelically oriented to the same pole. Sybenga (1984) stated that within a population of configurations with alternate orientations up to six types may be distinguished, but that this has no biological significance.

In this study a full quantitative estimation of adjacent and alternate orientation of quadrivalents could not be made but some generally occurring types of orientation could be interpreted qualitatively. The nature of the U-shaped quadrivalent (Fig. 2m) observed in the interchange of *S. phureja* might be explained as an adjacent orientation of a chain quadrivalent (1/2/1 configuration) or a flattened three dimensional ring quadrivalent. The observed spectacle-shaped configuration at diakinesis is difficult to interpret. It is suggested that the two-armed frying pan quadrivalent (Fig. 2e) has four chiasmata, but one of the four is an interstitial chiasma and two non-interchanged arms are unbound. Asymmetry of some ring quadrivalents was observed, e.g. in the configurations shown in the Figs. 2i & 2j. Chromosome morphology of these quadrivalents is similar, and in both configurations a small and a large region of heterochromatin is directed to one pole, representing two non-homologous centromeres. Thus the quadrivalent shown in Fig. 2i represents an adjacent-1 orientation because this is the only possible type of adjacent orientation with two non-homologous centromeres parallel across the spindle. The asymmetrical quadrivalents in *S. phureja* resulted from association of unequal sized chromosomes, originating from an interchange between chromosome 12, the shortest of the complement with median centromere, and chromosome 3, one of the four longest chromosomes with submedian centromere.

Pollen stainability, seed set and orientation of multiples

Pollen grains with unbalanced chromosome complements usually abort. Pollen stainability in the *S. phureja* genotypes 1931 and 1936, was 42 and 50% respectively (Wagenvoort, 1988), suggesting abortion of the products of adjacent orientation. Also cytogenetically unbalanced embryo sacs in plants usually produce abortive seed; hence the percentage seed set in these plants relative to that in normal plants provides an indirect measure of the frequency of alternate orientation in female meiosis (Rana 1965; Soriano, 1957). The low seed set observed in 1936 suggests that alternate and adjacent orientation occur with approximately the same frequency. However, this conclusion must be interpreted cautiously with respect to pollen abortion in view of the incidence of a paracentric inversion in 1931 and 1936 as well. Inversions will give rise to pollen abortion due to duplication and deficiency in crossing-over

chromatids whereas embryo sac abortion will be low or absent (Burnham, 1962).

Characterization of I_1 and F_1 trisomics

Tertiary and primary trisomics from selfed progenies of interchange heterozygotes have been obtained in several species. For a literature review may be referred to Khush (1973) and Schulz-Schaeffer (1980). Tertiary trisomics of the tomato were used in determining position of centromere and arm location of markers (Khush & Rick, 1967) and those in barley were important for use in hybrid seed production (Ramage & Tuleen 1964; Ramage, 1965). More recently tertiary trisomics were also produced in *Pennisetum americanum* L. by Singh et al. (1982), and in rye by De Vries (1983). Based on chromosome association at MI it was concluded that the trisomics found in the progeny upon selfing of 1936 are tertiaries or primaries homozygous for the interchange.

The incidence of a ring quadrivalent together with a trivalent at MI in PMCs of certain trisomic F_1 plants obviously characterizes these trisomics as primary trisomic interchange heterozygotes. The absence of ring quadrivalents in trisomic F_1 plants from the chromosome 3-trisomics and chromosome 12-trisomics suggests the incidence of tertiary trisomics. However, the low frequency of quadrivalents made it impossible to elucidate the nature of these trisomics unambiguously. F_1 trisomics in which a trivalent was observed as the highest chromosome association, were identified as primary trisomics. Thus, among the trisomic F_1 plants, derived from the cross primary trisomic x interchange heterozygote, compensating trisomics, primary trisomics and primary trisomics interchange heterozygotes were identified in this study. The frequency of three trisomics among 475 I_1 plants (0.63%) found by Wagenvoort (1988) was significantly ($\chi^2 = 8.90$, $P < 0.01$) lower than expected on the basis of chromosome distribution at AI, six of the 224 AI cells (2.68%) in 1936 having an 11-13 distribution (Table 3). Since at the male side only n gametes are assumed to be functional, this points to non-viability of some n+1 female gametes.

The incidence of bridges and fragments at AI was expected from the analysis of the pachytene stage (Wagenvoort, 1988) where true loops were seen in chromosome 4 and/or chromosome 6 indicating heterozygosity for a paracentric inversion. A bridge and a fragment at AI imply a chiasma in the inversion segment of the bivalent. When one of the two chromatids involved in an interstitial chiasma is also involved in the loop chiasma, the bridge is transformed into a loop at AI which turns into a chromatid bridge at second anaphase (Sybenga, 1975). Such a transformation may account for the bridge observed at AII in this

study. Two chiasmata in the inversion and one in the proximal segment may lead to the presence of a double bridge and fragments at AI (Sybenga, 1975). This configuration was observed in one AI cell from 1931. However, the incidence of a paracentric inversion in two non-homologous chromosomes would also adequately explain the double bridge and fragments and therefore should not be precluded.

The interchange heterozygote found in *S. phureja*. was not associated with any morphological aberration.

This report is the first in describing the use of a series of primary trisomics to identify the chromosomes involved in structural deviations in potato chromosomes.

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c
f
g
h
i
j
k
l
m
n
o

Fig. 2a-h. Chromosome association at diakinesis in PMCs of the diploids 1931 (c, f, g) and 1936 (a, b, d, e, h) both heterozygous for an interchange between the putative chromosomes 3 and 12. (a) Asymmetrical ring quadrivalent (IV), (b) Figure - eight IV, (c) Spectacle shaped IV, (d) Frying pan IV, (e) Two-armed frying pan IV, (f) Linear chain IV, (g) Twelve bivalents (II), (h) heteromorphic II (*arrow-head*). Figure 2i-o. Chromosome association at MI in PMCs of 1936. Quadrivalents indicated by *arrows*. (i) Adjacent ring IV, (j) Alternate ring IV, (k) Alternate chain IV, (l) 12 II, (m) U-shaped IV, (n) ring IV with possibly one extra chiasma, and (o) adjacent type of orientation of ring IV (explanation in text).

Bars represent 10 µm. Scale on Fig. 2g applies also to Figs. 2a, 2b, 2d and 2e; scale on Fig. 2i applies also to Figs. 2c and 2f.

c
d
e
f
g
h

Fig. 3. Chromosome association at diakinesis in PMCs of trisomic I_1 plant 82 (a, b) and AI chromosome distribution in PMCs of 1936 (c-f and h) and 1931 (g).

(a) 10 II + chain quinquevalent (V, *arrow*), (b) 11 II + one III (*arrow*), (c) 12-12 distribution, (d) 11-13 distribution, (e) One laggard (*arrow-head*) + II-like structure (with delayed separation (*arrow*)) in cell with 23 chromosomes, (f) Delayed separating II (*arrow*), (g) Bridge (*arrow*), (h) Bridge (*arrow*) + fragment (*small arrow*).

Bars represent 10 μm.

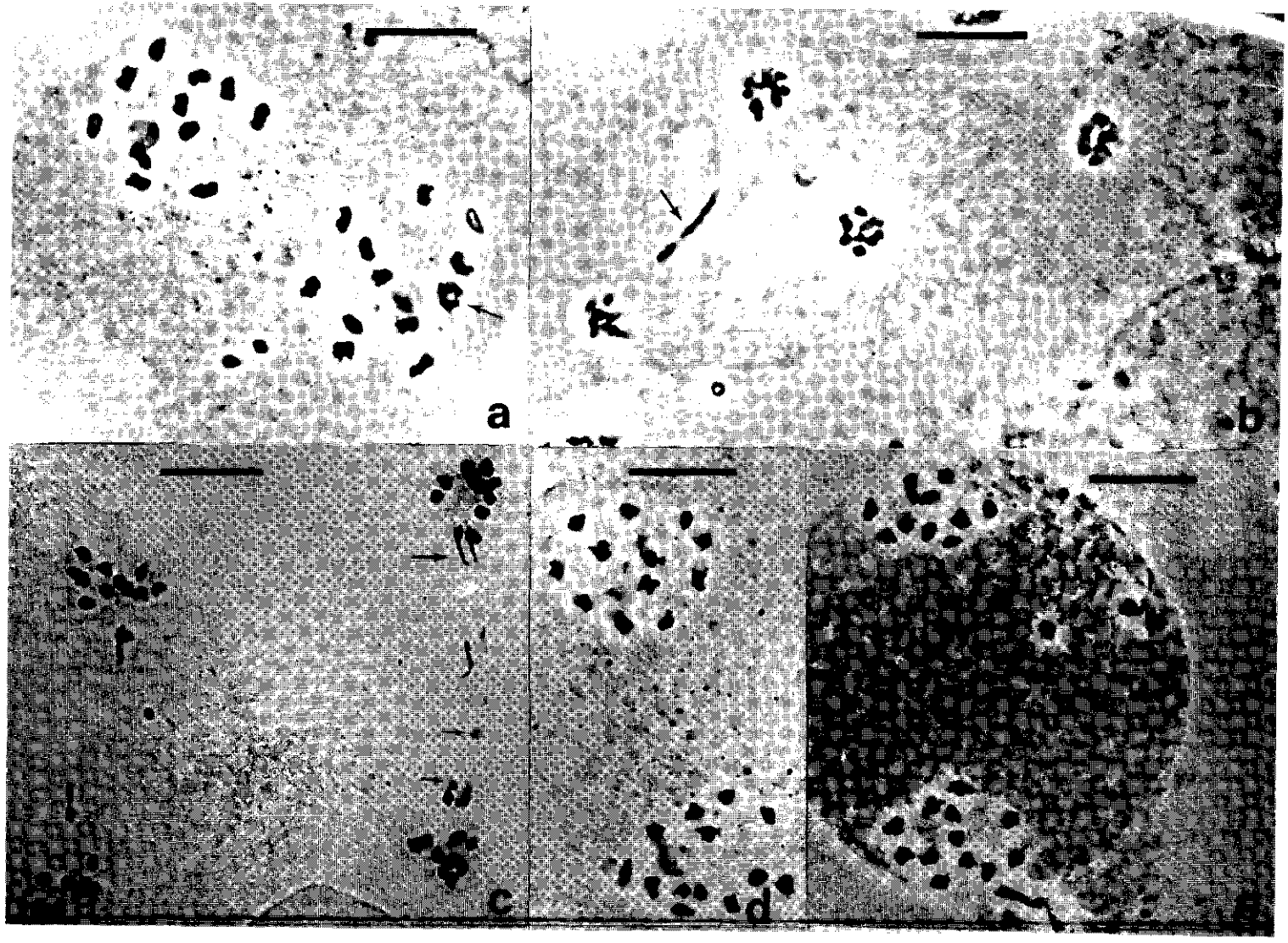
a
b
c
d

Fig. 4. Chromosome distribution at second prophase and A II/T II in PMCs of 1936 (a, b), at A I in 1931 (c), and at second prophase in trisomic I_1 plant 82 (d, e).

(a) Ring II at second prophase (*arrow*), (b) Chromatin bridge (*arrow*), (c) Double bridge (*arrow*) + fragments (*small arrows*), (d) 12-13 distribution, (e) 11-12 distribution + two scattered chromosomes.

Bars represent 10 µm.

d
c
b
a
f
e

Fig. 5. Chromosome association at MI in PMCs of trisomic F_1 plants from the cross primary trisomic x interchange heterozygote (a-f).
(a, b) One V (*arrow*) in a trisomic F_1 plant from the chromosome 3-trisomic, (c) As a and b, but from the chromosome 12-trisomic, (d) Asymmetric Y-shaped III (*arrow*) in a trisomic F_1 plant from the chromosome 3-trisomic, (e) 9 II + 7 I in a trisomic F_1 plant from the chromosome 12-trisomic (f) ring IV (*arrow*) + one III (*small arrow*) in a trisomic F_1 plant from the chromosome 9-trisomic.
Bars represent 10 μm.

CHAPTER 4

Meiotic behaviour of 11 primary potato trisomics (2*n*=2*x*+1=25) and its consequences for the transmission of the extra chromosome

Abstract

Meiosis was studied in 11 primary trisomics (2*n*=2*x*+1=25) from diploid *Solanum tuberosum* L. ssp. *tuberosum* Hawkes (2*n*=2*x*=24) and from interspecific *Solanum* hybrids. The three homologous chromosomes were associated in a trivalent in 90% of more than 175 pollen mother cells analysed at pachytene. Trivalents showing a two by two pairing and partner exchanges at pachytene along with incomplete triple synapsis were frequently observed. Fold-back pairing, predominantly observed in the heterochromatic parts of the chromosomes, occurred in 28.3% of the trivalents analysed. Non-homologous association of chromosome segments was observed in 29.1% of the trivalents. Up to six telomeres were associated homologously in 46.2% of the trivalents. Genotypic differences with respect to trivalent formation at metaphase I occurred in almost all the trisomic types, and was significant for the trisomics for the chromosomes 4, 7, and 9. The coefficient of realization of a trivalent (CRT) at metaphase I in the 11 primary trisomics varied from 0.20 to 0.80, and was positively correlated with the absolute length of the extra chromosome (r = 0.61, P $\leq$ 0.05) and with the absolute as well as the relative length of the euchromatic segments of the extra chromosome (r = 0.70, P $\leq$ 0.05). There was no apparent relationship between the CRT and the distribution pattern at anaphase I or anaphase II. The rate of female transmission of the extra chromosome varied from 10.0 to 45.0% among different trisomics, and differed significantly also within the trisomics for the chromosomes 4, 7, and 9.

Key words: primary trisomics, meiosis, non-homologous chromosome association, telomere pairing, triple synapsis, female transmission, *Solanum*.

Introduction

Trisomics of the diploid potato have previously been produced at a high frequency, using triploids of *Solanum tuberosum* L. ssp. *tuberosum* Hawkes (Wagenvoort and Lange 1975). All trisomics identified at pachytene by Wagenvoort and Ramanna (1979) proved to be primaries, containing one complete chromosome in triplicate. Einset (1943) pointed out, that in maize the probability of chiasma formation and hence the frequency of trivalents at metaphase I (MI) increases with increasing length of the triplicate chromosomes. To check this statement for the primary trisomics of the potato the total length and the length of the euchromatic segments were measured of each of the chromosomes from a complete pollen mother cell at pachytene of which all chromosomes could be reliably identified. From these data were calculated the relative length of each chromosome (absolute chromosome length divided by absolute genome length) as well as the relative lengths of the euchromatic segments of each chromosome (absolute length of the euchromatic segments of the chromosome divided by the absolute length of the euchromatic segments of the genome). As the complete data from a single cell were available, both the absolute and the relative lengths of the potato chromosomes were compared to those of the tomato chromosomes, as determined by Barton (1950) from 10 camera lucida drawings. Furthermore, chromosome association at MI was analysed in previously identified trisomics in order to study the possible relationship between the coefficient of realization of a trivalent (CRT) at MI and the absolute chromosome length. In tomato, the chiasma frequency is expected to be higher in the euchromatic segments than in the heterochromatic segments of the chromosomes (Khush and Rick, 1968). As the euchromatic and heterochromatic segments of the potato chromosomes can be distinguished at pachytene, the CRT at MI was also related to the relative euchromatin lengths of the chromosomes.

Wagenvoort and Lange (1980) found no relationship between chromosome length at pachytene and the rate of female transmission of the extra chromosome. A genetic control of the transmission rate was suggested. The relationship could however have been disturbed by the action of lethal genes causing seedling death. As new data regarding the rate of female transmission of the extra chromosome in additional genotypes (F_1 and backcross trisomics) became available, the relationship between chromosome length at pachytene and the rate of female transmission of the extra chromosome could be re-examined in genetically more diverse genotypes.

In this paper the meiotic behaviour of 11 different primary trisomics and new data regarding the rate of female transmission of the extra chromosome are reported. The possible

relationships between the absolute length as well as the relative length of euchromatin of each chromosome and the CRT at MI are discussed. Finally, the relation between chromosome length at pachytene and rate of female transmission of the extra chromosome is re-examined and compared with data from literature.

Materials and methods

Plant material

Trisomics for the chromosomes 2, 3, 4, 5, 6, 7, 8, 9, 11 and 12 were derived from triploid *S.tuberosum* ssp. *tuberosum* (cf Wagenvoort and Lange, 1980) whereas the trisomic for chromosome 10, coded V1063.8, had a complex hybrid origin, involving the species *S.maglia*, *S.microdontum*, *S.stenotomum*, *S.phureja* and *S.tuberosum* ssp. *tuberosum*. The latter trisomic was obtained from Dr. R. E. Hanneman Jr., Madison, USA. The rate of female transmission of the extra chromosomes 2, 3, 4, 5, 7, 8, 9, 11 and 12 was determined using the original trisomics as well as trisomic F_1 hybrids between seven original trisomics (4, 5, 6, 7, 9, 11 and 12) and *S. phureja*. In addition, the rate of female transmission of the extra chromosomes 3, 4, 6, 7 and 11 was also determined using trisomic F_1 hybrids from crosses between the corresponding original trisomics and *S.infundibuliforme*, whereas for the extra chromosomes 4, 9, and 10 trisomic F_1 plants were used from crosses of the corresponding original trisomics with the PVY immune diploid clone H76-76-5 (obtained from Dr. G. Wenzel, Grünbach, Germany).

Cytology

The method for studying the chromosomes in meiosis was the same as described by Wagenvoort and Ramanna (1979). Chromosome length measurements were made from photomicrographs, using a divider and ruler. The coefficient of realization of a trivalent (CRT) in a trisomic was defined as the mean frequency of trivalents at MI.

Statistics

Significance of correlation coefficients (r) was tested by calculating $F = [(n\text{-}2)r^2]/(1\text{-}r^2)$, and using the F-distribution on 1 and (n-2) degrees of freedom, where n is the sample size (Mead and Curnow 1983). Significant differences between two proportions with respect to the occurrence

of trivalents were tested by calculating $\chi^2_{(1)}$:

$N(ad-bc)^2/(a+b)(a+c)(b+d)(c+d)$ using a one tailed test, where in a sample of $(a+b)$ items, a contains a trivalent and b does not, and in a second sample of (c+d) items, c contains a trivalent and d does not. $N=a+b+c+d$. When entries were smaller than five, *Yates' correction* (see Clarke 1982) which reduces the value of $(ad-bc)$ numerically by 1/2 N before squaring, was applied.

Results

Pachytene

In a part of the pollen mother cells (PMCs) the extra chromosome could not be analysed as these cells showed entangled chromosomes. In about 90% of the PMCs observed the extra chromosome was bound in a trivalent. Out of 159 PMCs analysed, representing 11 of the 12 possible trisomic types, 158 showed the association of the three homologous chromosomes in a trivalent. Only a single PMC from the trisomic for chromosome 12 had three univalents. Fig. 1 shows 11 bivalents and the trivalent configuration for chromosome 7 in a complete cell at pachytene in which all bivalents could be identified. Trivalents were frequently observed to show a two by two pairing along with partner exchanges (Fig. 1, chromosome 7). In addition, many trivalents showed triple synapsis, i.e. association of three homologous chromosomes over a considerable part of their length (Fig. 2). Fold-back pairing of the univalent part of the trivalent configuration was predominantly observed in the heterochromatic parts of the chromosomes and occurred in 28.3% of the trivalents analysed. Non- homologous association of chromosome segments was observed in 29.1% of the trivalents analysed. This phenomenon occurred in the heterochromatic and euchromatic parts of the chromosomes in 24.7 and 4.4%, respectively, of the trivalents. The duplicate chromosomes were nearly always associated completely as bivalents.

The frequencies of homologous association of the telomeres and the centromeres of the trivalents were established. In 8.9% of the 158 trivalents analysed, four telomeres of the trivalent configuration were associated, whereas in the remaining trivalents association of five or six telomeres occurred. The association of three centromeres was observed in 11.4% of the trivalents, whereas in 88.6 % of the trivalents only two centromeres were associated.

Polymorphism for the completely heterochromatic short arm of the nucleolar chromosome (chromosome 2), was observed in one trisomic derived from *S. tuberosum* ssp. *tuberosum* cv.

Gineke, and in the trisomic for chromosome 10. Fig. 3 shows a heteromorphic bivalent of chromosome 2 in a trisomic for chromosome 3, derived from the Gineke-material. The difference in length between the two homologous chromosome arms in this trisomic was small. However, in the trisomic for chromosome 10, genotype V1063.8, the difference in length between the two short arms was much greater than in the Gineke trisomic (Fig. 4). The occurrence of a heteromorphic bivalent for chromosome 2 in V1063.8 is probably due to the hybrid origin of this trisomic. In a trisomic for chromosome 2, also *S.tuberosum* ssp. *tuberosum*, derived from an other genotype than cv. Gineke, no polymorphism for the short arm of chromosome 2 could be observed (cf. Figs. 2 and 3).

Table 1 presents the results of the chromosome measurements of a single PMC at pachytene of a trisomic for chromosome 7 of the potato and, for comparison, data on tomato chromosomes collected by Barton (1950). Chromosome identification was based on landmarks such as the position of specific chromomeres, arm lengths, the lengths of euchromatic and heterochromatic segments, and secondary constrictions. Chromosome 6 was longer than chromosome 5, and chromosome 10 was the shortest chromosome of the complement, instead of chromosome 12. Since the chromosomes were initially numbered according to their absolute lengths, the deviations from the normal complement are probably due to differences in condensation between chromosomes in a single cell. The genome length on the basis of the lengths of the chromosomes of the single cell was 355.4 µm of which 70% consisted of euchromatin. As the data from a single cell were available, the relative lengths of the potato chromosomes were compared to those of the tomato (*Lycopersicon esculentum*). The correlation coefficient *r* for the absolute lengths of the potato (Table 1) and tomato (data not shown) chromosomes was 0.94 ($P \leq 0.001$), whereas the correlation coefficient *r* for the relative lengths of the euchromatic segments of the potato and tomato chromosomes was 0.97 ($P \leq 0.001$).

Chromosome association at MI

At MI the extra chromosome was included in a trivalent or remained separate as a univalent. The mean number of trivalents (III), bivalents (II) and univalents (I) per PMC in the eleven primary trisomics are presented in Table 2. Most of the PMCs had 11 II + 1 III, 12 II + 1 I or 11 II + 3 I (Figs. 5-7). The trivalent configurations were either Y shaped (Fig. 8) or of the chain-type, e.g. V shaped (Fig. 9) or linear (not shown). Trichiasmate "frying pan" trivalents (Fig. 5) occurred only rarely. Significant differences in trivalent frequency occurred between several trisomic types. In the trisomics for the chromosomes 4, 7, and 9 significant differences in

Table 1. Absolute and relative lengths of chromosomes and euchromatin (in microns and percentage respectively) of potato chromosomes at pachytene, based on one complete cell and data of tomato chromosomes

	Chromosomes												
	1	2	3	4	5	6	7	8	9	10	11	12	Total
Chromosome length (μm)	46.3	36.4	34.9	31.8	25.7	30.6	32.2	25.8	25.0	21.5	23.1	22.1	355.4
Euchromatin length (μm)	31.4	30.2	25.0	25.4	17.4	22.5	18.2	18.6	18.0	13.8	15.1	12.4	248.0
Relative chromosome length* (%)	13.0	10.2	9.8	8.9	7.2	8.6	9.1	7.3	7.0	6.0	6.5	6.2	-
Relative euchromatin length* (%)	12.7	12.2	10.1	10.2	7.0	9.1	7.3	7.5	7.3	5.6	6.1	5.0	-
Relative euchromatin length* (%) of tomato‡	14.9	12.3	12.0	9.5	6.8	9.6	6.7	7.2	6.2	4.9	5.8	4.1	-

*Relative length = length per chromosome divided by total length of or in the entire genome.
‡Based on data from Barton (1950).

trivalent frequency were found between the genotypes of the same trisomic. The CRT of the eleven primary trisomics varied from 0.20 (trisomic for chromosome 10) to 0.80 (trisomic for chromosome 2). The mean CRT estimated from 1634 PMCs over all trisomic types was 0.48. Regular bivalent pairing of the duplicate chromosomes was common in almost all trisomics.

In some trisomics a higher number of univalents was found indicating some desynapsis. Distinction between ring bivalents and rod bivalents was possible only in some cases, and was dependent on the contraction of the chromosomes. A relatively high frequency of ring bivalents was observed in certain genotypes of the trisomics for the chromosomes 3 and 4 (Table 2). However, the variation within a trisomic type was as large as between different trisomics. Consequently, a possible relationship between the frequency of ring bivalents and a particular triplicate chromosome could not be established.

The CRT at MI, estimated for each trisomic type, was positively correlated with both the absolute length of the extra chromosome ($r = 0.61$, $P \leq 0.05$) and the absolute and relative length of the euchromatic segments of the extra chromosome ($r = 0.70$, $P \leq 0.05$).

Chromosome distribution at AI and AII

In 369 out of 407 AI cells the extra chromosome moved to one of the poles, resulting in a 12 : 13 distribution (Table 3 and Fig. 10). In the remaining 10% of the cells an 11 : 14 distribution (Fig. 11), or delayed separation of bivalents (Fig. 12) or lagging of chromosomes (Fig. 13) was observed. A maximum of five lagging chromosomes was observed in certain genotypes of the trisomics for the chromosomes 8 (Fig. 14) and 12. Occasionally lagging chromosomes separated precociously. There was no apparent relationship between the CRT and the distribution pattern at AI.

In most trisomics, a regular distribution of the chromosomes at AII was observed, e.g. 33 PMCs from the trisomic for chromosome 10 showed a 12-12-13-13 distribution (Fig. 15). The only aberration observed in the trisomics for the chromosomes 3 and 9, was the occurrence of fused spindles at MII (Fig. 16), leading to a 25-25 distribution at AII in 20 of 27 cells analysed. In three AII cells a 24-26 distribution was found.

Female transmission of the extra chromosome

New data regarding the rate of female transmission of the extra chromosome were gathered with 36 genotypes representing the trisomics for the chromosomes 2-12. They are presented in Table 4 together with earlier data from Wagenvoort and Lange (1980). The average rate of female transmission of the extra chromosome was 22.2%, but varied considerably (10-45%)

Table 2. Mean number of univalents (I), bivalents (II), and trivalents (III) per PMC, and the coefficient of realization of a trivalent at MI (CRT) in different genotypes of original (from 3*x*-2*x* crosses) or F_1 primary trisomics described in "Plant material".

Trisomic chromosomes	Codes	No. of PMCs*	Mean No. of					
			I	II			III‡	CRT
				Ring	Rod	Unspecified		
2	80-1-121	(49)	0.41	2.37	8.74	-	0.80	0.80
3	72-5-31	119(51)	0.50	1.37	9.94	11.52	0.55 a	0.54
	77-61-6	(68)	0.52	6.65	4.82	-	0.52 a	
4	72-4-10	(153)	0.60	2.61	8.73	-	0.57 a,b	
	72-4-21	19	0.53	-	-	11.53	0.47 a	
	77-62-56	92(28)	0.66	5.00	6.36	11.38	0.53 a	0.62
	73-16-27	78	0.26	-	-	11.10	0.85 c	
	75-10-2	73	1.43	-	-	10.74	0.70 b	
5	75-31-26	115(50)	0.61	0.72	10.80	11.40	0.50	0.50
6	72-5-18	31	0.55	-	-	11.26	0.65 a	0.72
	75-33-2	(14)	0.50	1.00	10.07	-	0.79 a	
7	72-5-32	111	0.60	-	-	11.41	0.52 a	0.35
	77-61-25	33	1.30	-	-	11.58	0.18 b	
8	72-5-23	(55)	2.42	1.09	9.76	-	0.29 a	0.31
	73-23-48	138	1.37	-	-	11.33	0.33 a	
9	72-3-167	53	0.77	-	-	11.55	0.38 a,b	
	72-5-7	(26)	0.92	1.92	9.89	-	0.15 a	0.33
	72-5-61	77	0.69	-	-	11.46	0.47 b	
10	1063.8	71	1.13	-	-	11.66	0.18 a	0.20
	79-8-25	116(90)	0.93	0.08	11.82	11.00	0.22 a	
11	72-4-22	9	0.33	-	-	11.33	0.67 a	0.59
	73-17-14	(70)	0.91	1.70	9.57	-	0.51 a	
12	74-17-28	(64)	0.95	2.86	8.48	-	0.45	0.45

*In parentheses number of PMCs in which specification of ring and rod bivalents was possible while the bivalents in the remaining PMCs are unspecified.

‡Different letters refer to significant differences at the 5% level between genotypes of one type of trisomic.

Table 3. Chromosome distribution at AI of meiosis in the 11 original primary trisomics described in "Plant Material". Data from different genotypes per trisomic are pooled.

Trisomic chromosomes	No. of		No. of cells showing distribution of chromosomes		No. of cells showing number of lagging chromosomes/cell				Mean no. of lagging chromosomes/cell
	genotypes	cells	12 - 13	11 - 14	1	2	4	5	
2	1	9	8	1	0	0	0	0	0.00
3	3	44	41	1	1	1	0	0	0.07
4	3	44	35	1	2	3	3	0	0.45
5	1	46	44	0	0	2	0	0	0.09
6	1	5	5	0	0	0	0	0	0.00
7	2	19	17	2	0	0	0	0	0.00
8	2	28	21	1	3	1	1	1	0.50
9	3	92	85	3	2	2	0	0	0.07
10	2	107	102	2	3	0	0	0	0.03
11	2	8	8	0	0	0	0	0	0.00
12	2	5	3	0	0	1	0	1	1.40

Table 4. Rates of female transmission of the extra chromosome in progenies from trisomic-disomic crosses. Original trisomics derived from *Solanum tuberosum* ssp. *tuberosum* and F_1 and backcross trisomics with *S. phureja* and *S. infundibuliforme* were used as females.

Trisomic chromosomes	No. of genotypes	No. of plants pooled per trisomic type		Transmission rate (range) of extra chromosome (%)*	χ^2(homogeneity)
		trisomic	disomic		
2	2	67	230	22.6 (16.7 - 27.1)	0.20
3	2	45	72	38.5 (31.4 - 48.9)	3.64
4	7	200	832	19.4 (1.3 - 44.1)	98.10***
5	3	109	385	22.1 (11.8 - 24.3)	4.63
6	2	15	135	10.0 (9.5 - 12.5)	0.20
7	7	278	846	24.7 (3.6 - 41.0)	30.63***
8	1	28	88	24.1 -	-
9	5	138	638	17.8 (6.6 - 32.1)	24.28***
10	1	54	67	44.6 -	-
11	3	81	257	24.0 (20.9 - 34.1)	5.41
12	3	68	241	22.0 (14.6 - 40.9)	5.94
Total	36	1083	3791	22.2 (1.3 - 48.9)	248.3***

*Part of data from Wagenvoort and Lange (1980).
***Significant at $P \leq 0.001$.

among different trisomics. Also the variation between genotypes within trisomics was generally large; P (χ^2 homogeneity) < 0.001 for the trisomics for the chromosomes 4, 7 and 9. The average rate of female transmission of the original trisomics for chromosome 4 differed significantly from that of the trisomic F_1 hybrids. This higher rate of female transmission in the trisomic F_1 hybrids from chromosome 4 was not found for trisomic F_1 hybrids of other trisomic types.

Discussion

In a primary trisomic, assuming two major points of pairing initiation (one at either end of each chromosome), random chromosome association will lead to a trivalent frequency at pachytene of 67%. The occurrence of higher frequencies may be due to : (i) pairing initiation at more than two major points, or (ii) preferential pairing between specific chromosomes at one end and the other two at the other end (Sybenga 1975). Although the pachytene analysis led to biased frequencies, the high trivalent frequency (90%) observed in the present study, indicates that one or both statements made by Sybenga (1975) may hold true for the potato trisomics. Furthermore, the triple synapsis of homologous chromosomes observed over long segments of the chromosomes also contributed to the high trivalent frequency at pachytene in certain trisomics. The association of five or six telomeres per configuration, may be attributed to this type of synapsis occurring in about 90 % of the trivalents. The association of three centromeres of the trivalent was observed in only 11 % of the PMCs analysed and therefore, it is not known whether the centromere plays a role in the increase of the trivalent frequency. The formation of trivalents in diplotene and MI of autotriploids of the potato (Lange and Wagenvoort 1973) showed that crossing over can occur between all three homologues. Previous observations on meiosis of other triploid species have suggested that if three homologues are present, in general they will occur at pachytene as a pair and a single univalent (Newton and Darlington 1929; Darlington and Mather 1932; McClintock 1933; Sybenga 1975). These authors concluded that "only two homologous chromosomes can pair at one time at any one site" and that pairing partner exchanges between homologues allow the formation of trivalents in triploid and trisomic individuals. In the early thirties, however, triple synapsis at pachytene had already been observed with a light microscope in triploid *Hyacinthus orientalis* (Belling 1931), in diploid and triploid *Gossypium* species (Skovsted 1933), and in *Nicotiana tabacum* (Olmo 1934), and more recently in trisomics of diploid *Solanum* by

Wagenvoort and Ramanna (1979). Initial electron microscopic studies in lily confirmed the view of the two by two pairing of chromosomes, in that the synaptonemal complex of trivalents is formed by only two lateral elements (Moens 1968). However, in a later study in lily, the same author reported that the association between three homologues at pachytene can be as intimate as that between any two homologues, and the phenomenon was referred to as "partner fusion" (Moens 1969). Evidence for triple synapsis by the formation of a double synaptonemal complex consisting of three lateral elements and two central elements in trivalents of triploid chicken was first published by Comings and Okada (1971). The occurrence of triple and even quadruple synapsis in tetraploid *Solanum tuberosum* observed by Stack (1982) using electron microscopy confirmed the observations made in trisomics by Wagenvoort and Ramanna (1979). In the primary trisomics of the potato such associations were often found in the euchromatic parts of the chromosomes. Triple synapsis may be considered as a more general feature of chromosome association in trisomic and autotriploid plants.

The results obtained in the present study show the frequent occurrence of fold-back pairing of heterochromatic segments in the univalent parts of trivalent configurations. This type of pairing is similar to that in autotriploid *Allium*, where the third chromosome of each homologous group, which is precluded from homologous pairing, forms extensive fold-backs (Loidl and Jones 1986). Whether the fold-backs in the primary trisomics of the potato reflect the occurrence of small duplications or are the result of real non-homologous association is unknown. Non-homologous association in the heterochromatic parts of the chromosomes was described earlier by Ramanna and Wagenvoort (1976) and Wagenvoort (1988).

The results obtained in the present study show a significant correlation between the relative chromosome or euchromatin length and the CRT of the primary trisomics of the potato. These data are at variance with those of Lee and Rowe (1975), who concluded that the frequency of trivalent formation was not affected by the length of the extra chromosome of secondary trisomics of *S.chacoense*. In tomato, the degree of trivalent pairing in late meiotic prophase was found to be correlated with pachytene chromosome length (Rick and Barton, 1954). Sree Ramulu *et al.* (1977) found a positive correlation between metaphase chromosome length and the frequency of trivalent formation in two trisomics from *Lycopersicon peruvianum*. Thus, it seems that chromosome length and CRT at MI are correlated both in potato and tomato.

The occurrence of fused spindles at MII, leading to a 25-25 chromosome distribution at AII is genetically equivalent to first division restitution, whereas the 24-26 chromosme distribution is genetically equivalent to second division restitution. The latter is caused by the omission of the second division along with an aberrant cytokinesis.

No relationship could be established between female transmission and the length of the extra chromosome in the primary trisomics of the potato (Wagenvoort and Lange 1980, this study). This was in agreement with the findings reported by Lee and Rowe (1975), and Lam and Erickson (1971) for trisomics in *S.chacoense* and in other plant species, such as *Lycopersicon esculentum*, (Rick and Barton 1954), *Lolium perenne* (Meiger and Ahloowalia 1982), and *Oryza sativa* (Khush et al. 1984). Thus, although the observation by Einset (1943) that in maize trisomics transmission of the extra chromosome is a function of chromosome length, while also Chen and Grant (1968) found such a relation for trisomics in *Lotus pedunculatus*, this could not be confirmed for the primary trisomics of the potato in spite of the significant correlation between chromosome length and the CRT at MI. Therefore, the low transmission of the extra chromosome in the primary trisomics of the potato must have been caused by other factors. Such factors may be differential seed germination, seed weight, seedling viability, as was recently suggested by Premachandran and Sarkar (1991) for transmission of the female gametes of triploid maize. If, indeed, differential seedling viability influences female transmission, it is desirable that care should be taken to determine female transmission in the total progeny of each trisomic under investigation, or at least in a representative sample of the progeny.

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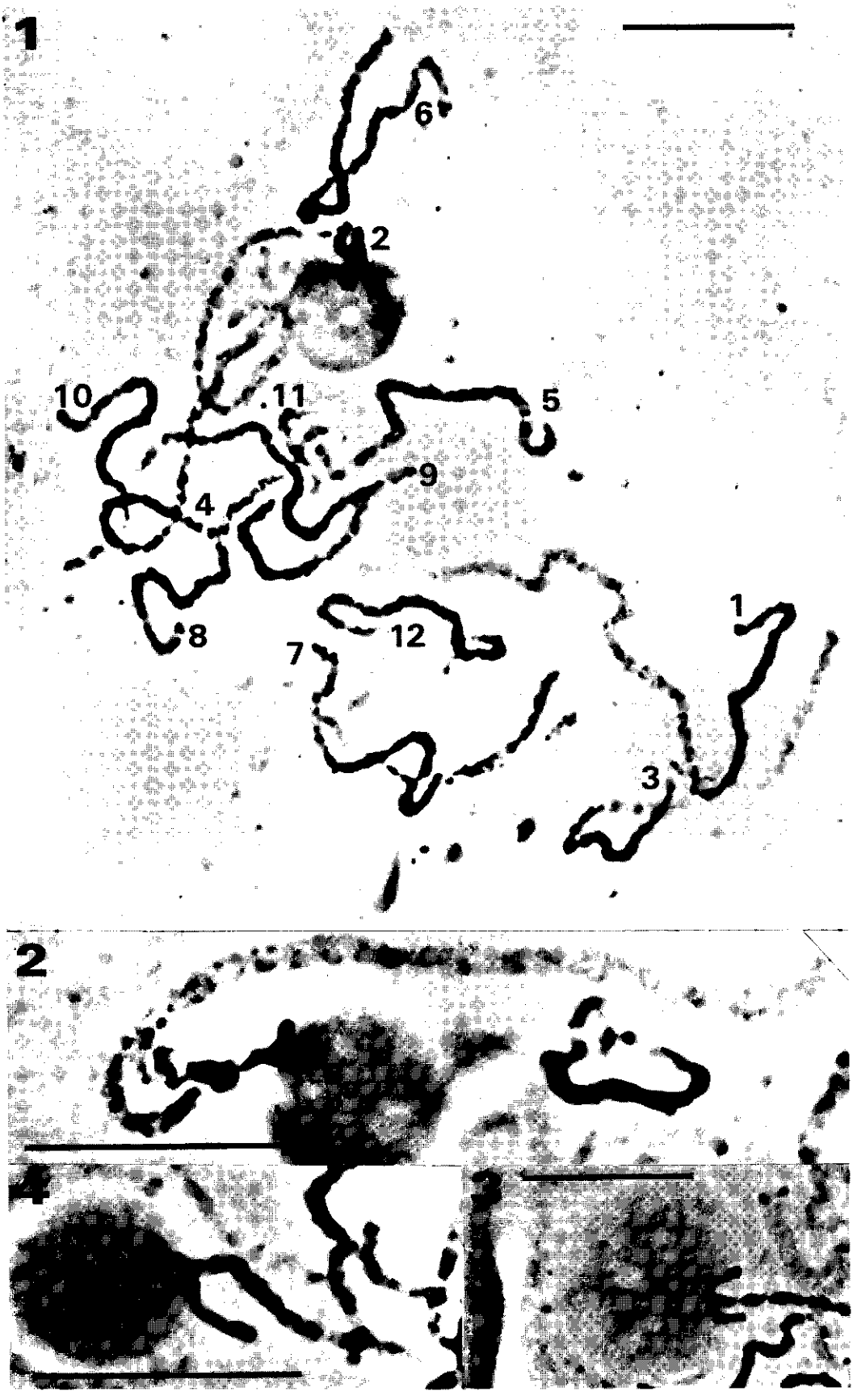
1
6
2
10
11
5
9
4
8
7
12
1
3
2
4

Fig. 1. A complete cell at pachytene of a trisomic for chromosome 7 showing one trivalent and 11 bivalents. The numbers 1-12 refer to the chromosome numbers.

Fig. 2. A trivalent of a trisomic for chromosome 2 showing triple synapsis over nearly the entire length of the long arm.

Fig. 3. A part of the nucleolar chromosome of a trisomic for chromosome 3 showing little difference in length of the satellites.

Fig. 4. A heteromorphic bivalent of a trisomic for chromosome 10 showing size differences of the satellites.

Bars represent 10 μm.

5
6
6
7

Fig. 5. Metaphase I of a trisomic for chromosome 4, showing one frying pan trivalent, seven ring bivalents and four rod bivalents.

Fig. 6. Metaphase I of a trisomic for chromosome 3, showing nine ring bivalents, three rod bivalents and one univalent.

Fig. 7. Metaphase I of a trisomic for chromosome 4, showing 11 bivalents and three univalents.

Fig. 8. Metaphase I of an F_1 trisomic from a trisomic for chromosome 6, showing one Y-shaped trivalent and 11 rod bivalents.

Fig. 9. Metaphase I of a trisomic for chromosome 12, showing one V-shaped trivalent, five ring bivalents, five rod bivalents and two univalents.

Bars represent 10 μm

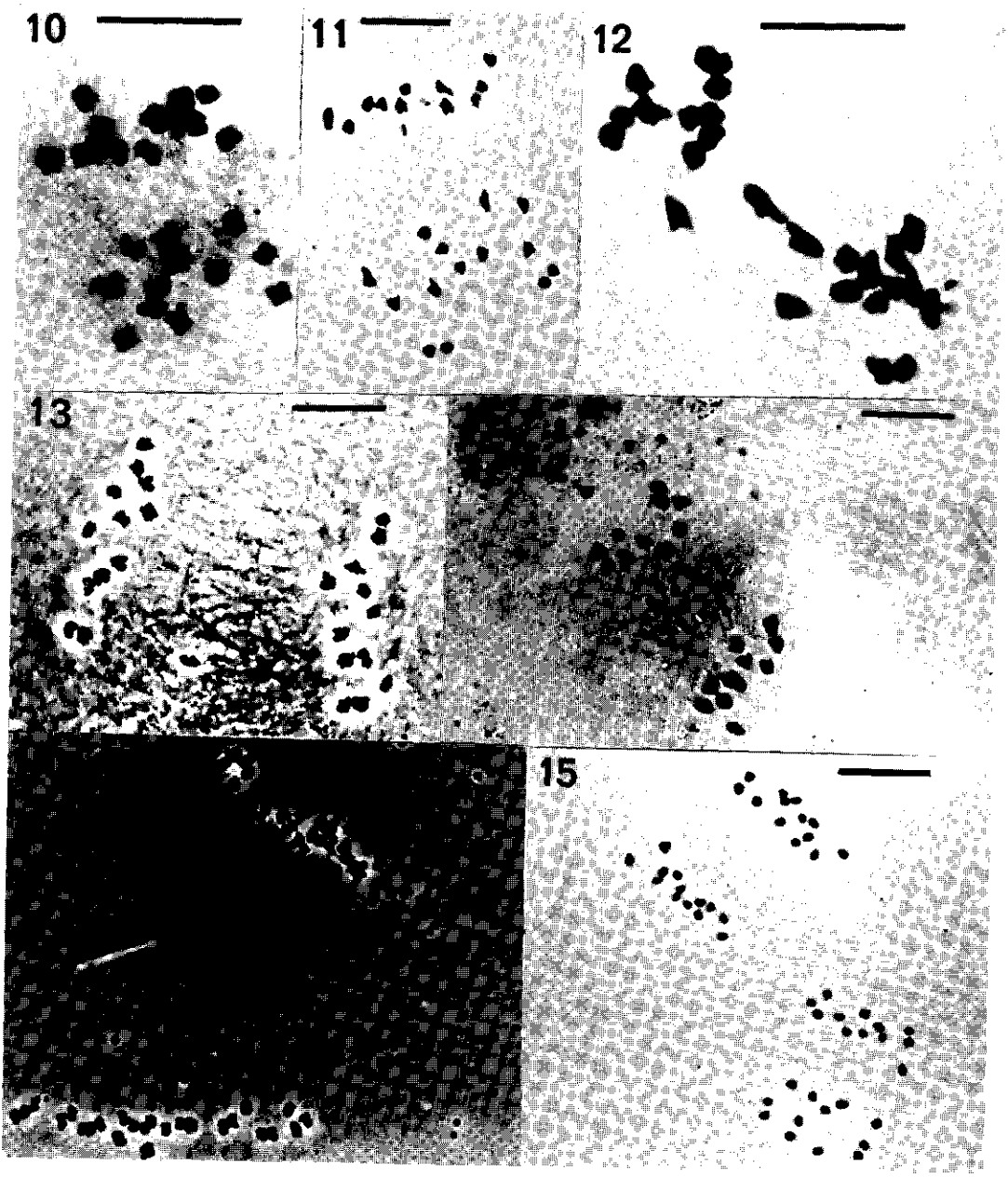
10
11
12
13
15

Fig. 10. Anaphase I of a trisomic for chromosome 12, showing a 12-13 distribution.

Fig. 11. Anaphase I of an F_1 trisomic from a trisomic for chromosome 10, showing an 11-14 distribution.

Fig. 12. Anaphase I of an F_1 trisomic from an unidentified trisomic, showing delayed separation of a bivalent.

Fig. 13. Anaphase I of a trisomic for chromosome 4, showing a 12-12 distribution and one lagging chromosome.

Fig. 14. Anaphase I of a trisomic for chromosome 8, showing a 10-11 distribution and four lagging chromosomes, three of them dividing precociously.

Fig. 15. Anaphase II of an F_1 trisomic from a trisomic for chromosome 10, showing a 12-12-13-13 distribution.

Fig. 16. Metaphase II of an F_1 trisomic from a trisomic for chromosome 6, showing two PMCs with 25 chromosomes due to fused spindles formation.

Bars represent 10 µm.

CHAPTER 5

Gene-centromere mapping in potato by half-tetrad analysis: map distances of H_1, *Rx* and *Ry* and their possible use for ascertaining the mode of 2*n*-pollen formation[1]

With: Ewa Zimnoch-Guzowska

Abstract

Diploids from the tetraploid potato varieties 'Alcmaria' and 'Pansta' and from the tetraploid CPRO-DLO genotypes Y66-13-610 and Y66-13-636 were used in half-tetrad analyses to estimate the gene-centromere map distances of the genes *Rx*, *Ry* and H_1. Employing tetraploid progeny from 2*x* (second division restitution)-4*x* testcrosses the gene-centromere map distance of H_1, conferring resistance to pathotype Ro_1 of *Globodera rostochiensis* was estimated to be 16.3 centimorgans (cM). For *Rx*, conferring extreme resistance to potato virus X (PVX), a map distance of 33.9 cM was estimated. The gene *Ry* conferring extreme resistance to potato virus Y (PVY), was estimated to be located 14.2 cM from the centromere. Using the estimated map distance for *Rx*, it was attempted to determine the mode of 2*n*-pollen formation in four diploid interspecific hybrids, including the species *Solanum tuberosum*, *Solanum chacoense*, *Solanum yungasense*, and *Solanum phureja*, by half-tetrad analysis in tetraploid progeny from 4*x*-2*x* testcrosses. The mean frequency of 8.7% nulliplex plants for *Rx* was outside the range of the 95% confidence intervals, for both first division restitution and second division restitution 2*n* pollen.

Key words: nematode resistance, potato virus X resistance, potato virus Y resistance, 2*n* eggs, gene-centromere mapping, *Solanum*.

[1]Slightly revised version of the paper, published in Genome (1992) 35: 1-7

Introduction

The concept of breeding potatoes at the diploid level ($2n$=$2x$=24) and the use of $2n$ gametes to restore the tetraploid chromosome number was originally described by Chase 1963 and has since been worked out by, for instance, Peloquin (1982) and Hermsen (1984a, 1984b).

In diploid potatoes, several meiotic restitution mechanisms resulting in $2n$ gametes have been found. The two basic types are described as first division restitution (FDR) and second division restitution (SDR). If crossing-over does not occur, FDR gametes retain the parental genotypes and can preserve intralocus and interlocus interactions present in the diploid genotype. Furthermore, the transfer of more or less intact chromosome sets to the tetraploid progeny should maximize heterotic potential. In contrast, SDR breaks up the parental genotype and may lead to homozygosity (Mendiburu *et al.* 1974; Peloquin 1982; Hermsen 1984a, 1984b). Ramanna (1979) stated that it is not always possible to predict on the basis of a certain meiotic abnormality during microsporogenesis alone that either FDR or SDR will occur.

Taylor (1978) concluded that tetraploid parthenogenetic progeny of *Solanum tuberosum* ssp. *andigena* obtained from $4x$-$2x$ crosses resulted from SDR $2n$ female gametes.

On the basis of a sequential study of the embryo-sac development, typical for the female gametophyte in *Solanum*, Jongedijk (1985) suggested that under normal synaptic conditions, SDR $2n$ egg cells should prevail and the occurrence of FDR $2n$ eggs be an exception. In certain diploids SDR $2n$ eggs occur exclusively (Stelly and Peloquin 1986a, 1986b; Douches and Quiros 1988). Because of the occurrence of a single restitution mechanism in the diploid parent, the $2x$-$4x$ cross is advantageous in gene-centromere mapping studies. In potato, a number of loci has been mapped by establishing the map distance to the centromere through $4x$-$2x$, $2x$-$4x$ and $2x$-$2x$ crosses. Table 1 presents a summary of data from the literature. We were especially interested in the genes *Rx*, *Ry* and H_1, conferring extreme resistance to the potato viruses X (PVX) and Y (PVY) and to pathotype Ro_1 of *Globodera rostochiensis*, respectively. *Rx* and H_1, both from *S. tuberosum* ssp. *andigena*, are on different chromosomes (H.T. Wiersema, unpublished). No relationship has been established so far between *Rx* and *Ry* and between *Ry* and H_1. If these genes are located close to the centromere, they should provide a means for discriminating between FDR and SDR $2n$ pollen, because the half-tetrad analyses (HTAs) will provide nonoverlapping confidence intervals (CIs) for the frequencies of nulliplex plants in the case of FDR or SDR (Mendiburu and Peloquin 1979). This paper reports on the estimation of the gene-centromere map distances of the genes *Rx*, *Ry* and H_1 by means of HTA. The possibility to use *Rx* for genetic identification of FDR and SDR $2n$-pollen formation is tested.

Table 1. Data from the literature concerning gene-centromere map distances of 22 genes estimated via HTA in 4*x*-2*x*, 2*x*-4*x* and 2*x*-2*x* potato progeny.

Gene Symbol*	Average map distance (cM)†	Range (cM)	Reference(s)
P	13.0*a*	-	Mendiburu and Peloquin 1979
Y	16.7*a,b,c*	13.0-20.9	Mok *et al.* 1976; Veilleux and Lauer 1981; Stelly and Peloquin 1986*a*; Douches and Quiros 1987; Jongedijk *et al.* 1991
ym	33.3*a,b*	31.7-34.9	Jongedijk *et al.* 1991; M. Wagenvoort, in preparation
Np	22.5*a*	-	Mok 1981 (original not seen)
Ea	21.7*a*	-	Mok 1981 (original not seen)
Eb	8.8*a*	-	Mok 1981 (original not seen)
Ro	12.2*a*	-	Masson 1985
EM	13.4*a*	-	Masson 1985
D	47.6*a*	-	Masson 1985
Got-1	2.7*a,c*	0.9 - 4.4	Douches and Quiros 1987; Jongedijk *et al.* 1991
Got-2	5.3*c*	-	Jongedijk *et al.* 1991
Pgm-2	2.0*a*	-	Douches and Quiros 1987
Sdh-1	8.3*a*	-	Douches and Quiros 1987
Aps-1	13.5*a*	-	Douches and Quiros 1987
Prx-3	18.0*a*	-	Douches and Quiros 1987
Idh-1	18.4*a*	-	Douches and Quiros 1987
Adh-1	15.8*a*	-	Douches and Quiros 1987
Pgi-1	26.0	-	Douches and Quiros 1987
6Pgdh-3	30.1*a*	-	Douches and Quiros 1987
Mdh-1	33.5	-	Douches and Quiros 1987
Tpi-1	25.0*b*	-	Douches and Quiros 1988
ds-1	23.7	-	Jongedijk *et al.* 1991

*P, purple colour in various organs and tissues of the potato; *Y*, yellow tuber flesh; *ym*, yellow margin; *Ro*, round tubers; *EM*, closely linked genes responsible for red tuber colour and restriction of pigmentation, respectively; *D*, a basic gene for brownish and red colour in stems and inflorescences; *Np*, *Ea* and *Eb*, protein markers; *ds-1*, desynapsis; *Got-1*, *Got-2*, *Pgm-2*, *Sdh-1*, *Aps-1*, *Prx-3*, *Idh-1*, *Adh-1*, *Pgi-1*, *6Pgdh-3*, *Mdh-1* and *Tpi-1*, various isozymes. For a detailed description of the isozymes see Quiros and McHale (1985) and Jongedijk *et al.* (1991).
† *a*, *b*, and *c* following values represent 4*x*-2*x*, 2*x*-4*x*, and 2*x*-2*x* potato progeny, respectively.

Material and methods

Plant material

Table 2 lists the origin of the diploid clones used in the HTAs and their ability to produce 2*n* gametes. In all diploid clones the extreme resistance to PVX traces back to *S. tuberosum* ssp. *andigena* and that to PVY to *Solanum stoloniferum*. In the diploids, derived from the cv. Alcmaria and the CPRO-DLO genotypes Y66-13-610 and Y66-13-636, the resistance to pathotype Ro_1 of *Globodera rostochiensis* is conferred by the dominant gene H_1 from *S. tuberosum* ssp. *andigena*. In the gene symbols the origin of the genes has been omitted, thus $Rx=Rx_{and}$, etc. Both parents of the cv. Pansta are resistant to pathotype Ro_1. In the female parent the resistance is caused by H_1, whereas in the male parent polygenes from *Solanum vernei* are involved. The 4*x* progeny from 2*x*-4*x* crosses of 2*x*-Pansta pl 3 were found to be either completely susceptible or resistant. Each genotype was tested in duplicate through a so-called pot test. Because no intermediate types of resistance were found, we have assumed that only a major gene (presumably H_1) is present in 2*x*-Pansta pl 3. All diploid clones used in the HTAs were heterozygous for the resistance genes, whereas all tetraploids susceptible to PVX, PVY, or nematodes had the nulliplex condition.

Screening for resistance to PVX

From each genotype, two plants of the first clonal generation were inoculated mechanically with strain X_5 of PVX, provided by the Research Institute for Plant Protection (IPO-DLO), Wageningen. From the second clonal generation two plants per genotype, derived from the two inoculated clones of the former generation, were selected visually for resistance to PVX. In addition, an ELISA test was performed on tubers from 50 genotypes of the third clonal generation, which genotypes had been identified as resistant to PVX based on the two previous cycles of selection. The aim of this test was to elucidate whether tolerant plants were present among the group of plants assumed to be resistant. In the ELISA test two plants per genotype were tested, of which one plant was reinoculated to evaluate the presence of escapes after the first inoculation. Seedlings from 4*x*-2*x* crosses were treated as follows. Young seedlings were twice inoculated mechanically with PVX (isolate from cv. Osa) with a 2-day interval. Screening for resistance was done three to six times, in a combination of a visual and a serological test. Subsequently, the first and second clonal generation were screened in the same manner. Infected tobacco plants were used as a positive control. A third test was performed using plants identified as resistant in the second clonal generation. Infected scions of tobacco were

Table 2. Origin of diploid clones in HTAs and their ability to produce 2*n* gametes and genotypes of tetraploid clones for the marker loci H_1, *Rx*, and *Ry*

Code	Origin	Marker(s)/ genotype	2*n* pollen %	2*n* pollen Range	2*n* egg cells[b] *N*	2*n* egg cells[b] Range
DG 79-986[c]	T x (CY x T)[d]	Rx_{and}	5.0	2.7 - 8.4		
DG 81-68[c]	(T x CT) x {T x (CY x T)}	Rx_{and}	16.5	8.1 - 29.0		
DG 82-23[c]	{T x (CY x T)} x TC	Rx_{and}	7.6	0.6 - 17.0		
DG 82-201[c]	P x {T x (CY x T) }	Rx_{and}	18.1	0.0 - 41.6		
2*x*-Alcmaria pl 8[e]	Diploid of cv. Alcmaria	Rx_{and} H_{1and}			3.8	0- 8
2*x*-Alcmaria pl 17[e]	Diploid of cv. Alcmaria	Rx_{and}			7.5	2-13
2*x*-Pansta pl 3[e]	Diploid of cv. Pansta	Rx_{and} H_{1and}			27.6	8-58
2*x*-Pansta pl 8[e]	Diploid of cv. Pansta	Rx_{and}			8.6	4-17
2*x*-Y66-13-610 pl 4[e]	Diploid of 4*x* clone Y66-13-610	Rx_{and} H_{1and}			2.1	0- 5
2*x*-Y66-13-636 pl 6[e]	Diploid of 4*x* clone Y66-13-636	Rx_{and} Ry_{sto}			10.6	1- 8
AT 81-1843-326	4*x-Andigena-tuberosum* hybrid	$(h_1)^4$, $(rx)^4$	(Personal communication, Dr.ir. B. Maris, former SVP)			
And 77-1347-276	4*x*-Advanced *andigena* clone	$(rx)^4$				do
And 83-2242-887	4*x*-Advanced *andigena* clone	$(h_1)^4$, $(rx)^4$, $(ry)^4$				do
DH 81-7-1461	4*x-Tuberosum* clone	$(h_1)^4$, $(rx)^4$				
Estima	4*x*-Dutch variety	$(h_1)^4$, $(rx)^4$, $(ry)^4$				
Bryza	4*x*-Polish variety	$(rx)^4$				
Certa	4*x*-Polish variety	$(rx)^4$				
Wilga	4*x*-Polish variety	$(rx)^4$				

[a]Percent 2*n* pollen was estimated by pollen diameter measurements. Pollen grains with a diameter > 22.5 µm were assumed to be 2*n*.
[b]*N*, number of seeds per berry in 2*x*-4*x* crosses.
[c]Diploid clones used in 4*x*-2*x* crosses to determine the mode of 2*n*-pollen formation.
[d]T, *S.tuberosum*; C, *S.chacoense*; Y, *S.yungasense*; P, *S.phureja*; CT, *S.chacoense* x *S.tuberosum*.
[e]Diploid clones used in 2*x*-4*x* mapping.

grafted onto potato rootstocks (three plants per genotype). Two nongrafted plants per genotype were used as a negative control. The presence or absence of PVX in the potato plants was detected serologically in two samples. The serological detection of PVX was additionally done on tuber progeny from graft inoculated plants.

Screening for resistance to PVY

From each genotype, two plants of the first clonal generation were inoculated mechanically with strain PVY°, provided by the IPO-DLO, Wageningen. Screening for resistance was done by visual selection. From the second clonal generation again two plants per genotype, derived from the inoculated two clones of the former generation, were selected visually for resistance to PVY.

Screening for resistance to nematodes

For testing nematode resistance plants were grown in pots filled with loam and inoculated with a volume unit of about 30 cysts per pot (pathotype Ro_1). About 8 weeks after inoculation the number of cysts on the root ball of each plant was determined. Plants containing roots without cysts were assumed to be resistant.

Cytological methods and method of estimating gene-contromere map distances

Tetraploid testcross progeny were selected by establishing the mean number of chloroplasts in stomata or by counting the number of chromosomes in root-tip meristems (Wagenvoort and Lange 1975). Chromosome behaviour in meiosis was studied as described by Wagenvoort and Ramanna (1979). For estimating gene-centromere map distances we used the 2*x*-4*x* cross instead of the reciprocal cross, as was advocated by Mendiburu and Peloquin (1979). These authors assumed single-exchange tetrads (SETs) only, and consequently in that case the proportion of SETs is a linear function of the map distance between the locus in question and the centromere. The heterozygous diploids used in this study were assumed to have SDR 2*n* egg cells, because they had normal synapsis at meiosis. FDR at the female side predominantly occurs under desynaptic conditions because of the successive type of cell wall formation at megasporogenesis (Jongedijk 1985). If double or higher order crossovers do not occur, the gene-centromere map distance in the case of SDR can be calculated with the formula:
(0.50 - frequency of nulliplex progeny) x 100 cM (Mendiburu and Peloquin, 1979). In the case of FDR the gene-centromere map distance = 2 (frequency of nulliplex progeny) x 100 cM. Once the gene-centromere map distance is known, it can be used to deduce the mode of 2*n*-gamete

formation in 2*n* pollen producing diploids. Binomial confidence intervals were calculated using the expression:

$$\hat{p}-2\sqrt{\frac{\hat{p}\hat{q}}{N}}<p<\hat{p}+2\sqrt{\frac{\hat{p}\hat{q}}{N}}$$

giving approximate 95% limits to p, where $\hat{p}$ and $\hat{q}$ are the frequencies of susceptible and resistant plants, respectively, and N is the total number of plants (Clarke 1982).

Heterozygosity at the *Rx* locus in diploid pollen parents and seed parents employed in half-tetrad analysis was checked by 2*x*-2*x* testcrosses. Segregation of the genes *Ry* and H_1 was not evaluated at the diploid level because from the present genotypes no suitable offspring was available. However, tetraploid testcross progeny segregated for these genes, indicating heterozygosity of the diploid parents. In the case the diploids were homozygous, the tetraploid testcross progeny would not segregate irrespective of the incidence of SDR or FDR.

Results

Evidence for the formation of 2*n* eggs by SDR in one diploid was obtained from segregation of the *P* locus in the progeny from the cross (2*x*-Pansta pl 3) x (And 83-2242-887). The *P* locus is responsible for the purple colour in various organs and tissues of the potato. From progeny evaluated for sprout colour, it could be deduced that 2*x*-Pansta pl 3 was heterozygous for *P*, whereas And 83-2242-887 had the simplex condition. Thus the cross was *Pp* x *Pppp*. Assuming chromosome assortment in the tetraploid and using the map distance of *P*, viz., 13 cM, estimated by Mendiburu and Peloquin (1979), the predicted frequency of nulliplex plants is 18.5% in the case of SDR 2*n* eggs. The ratio 73:25 found for purple sprouts to red sprouts did not deviate significantly from the expected ratio 80:18 at P=0.05. With respect to *Rx*, all diploid populations segregated in accordance with the expected 1:1 ratio of resistant to susceptible except the offspring of 2*x*-Alcmaria pl 8 (Table 3). The deviating ratio found in this population could be due to lethal or sublethal factors occurring in 2*x*-Alcmaria pl 8, because 29.5% of the seeds sown did not germinate.

Table 3. Segregation of extreme resistance to PVX in diploid progeny derived from 2*x*-2*x* (resistant x susceptible) testcrosses

Resistant parent (code)	No. of seeds sown	No. of plants tested			$\chi^2_{1:1}$
		Total	Resistant	Susceptible	
DG 79-986		125	63	62	0.00
DG 81-68		7	2	5	1.28
DG 82-23		169	72	97	3.70
DG 82-201		301	137	164	2.42
2*x*-Alcmaria pl 8	1000	705	459	246	64.35*
2*x*-Alcmaria pl 17	28	23	8	15	2.13
2*x*-Pansta pl 3	176	164	85	79	0.22

* Significant at P = 0.001.

About 300 plants from 2*x*-4*x* progeny appeared to be tetraploids (2*n*=4*x*=48). No triploids were found, pointing to a strong triploid block operating in the 2*x*-4*x* crosses. Some triploids were found in the offspring of 4*x*-2*x* crosses.

Estimation of the gene-centromere map distance of the gene H_1

Table 4 presents the pooled numbers of resistant and susceptible clones of four families derived from 2*x*-4*x* crosses. Three heterozygous diploids and four male parents, nulliplex for H_1, were used for establishing these families. In total 65 plants (33.7%) out of 193 were susceptible. To test the homogeneity for segregation of H_1 across the four families their ratios for resistant to susceptible were compared with the ratio 128:65 of the pooled data (Table 4). All were homogeneous: P(χ^2 homogeneity)> 0.05. The calculated gene centromere map distance of H_1, based on the mean frequency of nulliplex plants and calculated from the pooled data, was 16.3 cM with a 95% CI of 9.5-23.1 cM. This CI was derived from the CI for the percentage of nulliplex plants.

Estimation of the gene-centromere map distance of the genes Rx and Ry

Table 4 presents the pooled numbers of resistant and susceptible clones of seven tetraploid families segregating for *Rx* and two tetraploid families segregating for *Ry*. Four diploids heterozygous at the *Rx* locus and one diploid heterozygous at the *Ry* locus were used for establishing these families. Homogeneity tests revealed no significant differences between seven populations: P (χ^2 homogeneity) > 0.05 for segregation of both *Rx* and *Ry*. About 16% of the 273 clones investigated for resistance to PVX were susceptible. The calculated gene-centromere map distance of *Rx*, based on the mean frequency of nulliplex plants and calculated from the pooled data was 33.9 cM with a 95% CI of 29.5-38.3 cM. Compared to H_1, the gene *Rx* is sited more distally on the chromosome.

In total 53 tetraploid clones were screened for resistance to PVY. The percentage of nulliplex progeny was 35.8%, which estimates the gene-centromere map distance for *Ry* at 14.2 cM with a 95% CI of 1.0-27.4 cM.

The mode of 2n-pollen formation in four diploid interspecific hybrids

From the map distance of *Rx* estimated by the 2*x*-4*x* cross (33.9 cM), the frequency of susceptible plants in progeny of reciprocal (4*x*-2*x*; see Table 2) crosses may be predicted. In the

Table 4. Gene-centromere map distances for H_1, *Rx* and *Ry* in female meiosis of normal synaptic diploid potato clones as estimated by HTA mapping

Marker	2*x* x 4*x* crosses ♀	♂	Mapping feature	No. of tetraploid progeny Total	Resistant	Susceptible	Gene-centromere map distance (cM)	95% CI (cM)	No. of families[a]
H_1	H_1h_1 x	$h_1h_1h_1h_1$	♀ SDR	193	128	65	16.3	[9.5; 23.1]	4
Rx	*Rxrx* x	*rxrxrxrx*	♀ SDR	273	229	44	33.9	[29.5; 38.3]	7
Ry	*Ryry* x	*ryryryry*	♀ SDR	53	34	19	14.2	[1.0; 27.4]	2

[a]All families within a mapping group are homogeneous, P(χ^2 homogeneity) > 0.05.

Table 5. Segregation of extreme resistance to PVX in 4*x* offspring of 4*x*-2*x* crosses, conferred by the genes *Rx*

4*x* x 2*x* crosses (♀ x ♂)	No. of tetraploid progeny Total	Resistant	Susceptible	% susceptible	95% CI	No. of families[a]
rxrxrxrx x *Rxrx*	781	713	68	8.7	[6.7; 10.7]	8

Note: Pooled data are shown involving eight families derived from four heterozygous diploids and three susceptible tetraploid varieties.
[a]All families are homogeneous, P(χ^2 homogeneity) > 0.05.

case of FDR and SDR 2*n* pollen the 95% CIs of the percentages of susceptible plants under the assumption of SETs only would be 14.8-19.2 and 11.7-20.5%, respectively. These CIs were calculated on the basis of the observed 16.1% susceptible plants in the 4*x* families, derived from the 2*x*-4*x* crosses, and using the formulas of Mendiburu and Peloquin (1979). Because these CIs largely overlap, a HTA aimed at discriminating between FDR and SDR 2*n*-pollen formation is useless. To ascertain whether the assumption of SETs is correct, 4*x* progeny from 4*x*-2*x* crosses were evaluated. Table 5 presents the segregation of *Rx* in 4*x* progeny from 4*x*-2*x* crosses involving diploid interspecific hybrids. The 4*x*-2*x* crosses resulted in a total of 781 plants of which 68 (8.7%) plants were susceptible to PVX. The frequency of nulliplex plants falls outside the range of the predicted 95% CIs of both FDR and SDR 2*n* pollen. In addition, the results (not shown) of cytological analyses of the four diploids involved in the 4*x*-2*x* crosses could not fully elicit the mode of 2*n*-pollen formation in the four hybrids.

Discussion

The purpose of the study was to assess the gene-centromere map distances of the genes *Rx*, *Ry* and *H*, and their possible use in discriminating between FDR and SDR gametes. The position of both *H*, and *Ry* was estimated to be relatively close to the centromere. If the model of Mendiburu and Peloquin (1979), assuming SETs only, is accepted, *H*, and *Ry* should be valuable markers to discriminate between FDR and SDR via a HTA. Unfortunately, for these genes, progeny of 4*x*-2*x* (Table 2) crosses were not available. The position of *Rx*, relative to the centromere, is unfavourable because with a map distance of 33.9 cM the expected frequency of nulliplex plants in progeny of 2*x*-4*x* or reciprocal crosses is the same, irrespective of the occurrence of FDR or SDR.

Ross and Langton (1974) found 13.3% susceptible plants in a HTA with the gene *Rx* from *Solanum acaule* using a 2*x*-4*x* cross. These authors considered it unlikely that all resistant progeny had resulted from crossing over between the centromere and *Rx* and, therefore, concluded that the 2*n* eggs were produced via FDR. However, FDR in normal synaptic eggs is less likely. The putative FDR restitution mechanisms in synaptic clones reported by Conicella *et al.* (1991) and Werner and Peloquin (1991), in our view, hardly have any significance.

If only SDR and SETs occurred in the diploid clones used by Ross and Langton (1974), the calculated map distance would have been 36.7 cM which is within the 95% CI for SDR estimated in this study. This might suggest that the genes for extreme resistance to PVX in *S. andigena* and *S. acaule* are allelic. However, recently Ritter *et al.* (1991) reported that the genes

for extreme resistance to PVX in *S. andigena* and *S. acaule* mapped to the distal end of chromosome 12 and an intermediate site on chromosome 5, respectively. For this reason, these genes are not allelic.

The frequency of 8.7% nulliplex plants found in the 4*x* progeny of the 4*x*-2*x* crosses was neither within the predicted range of 14.8-19.2% for FDR nor within the predicted range of 11.7-20.5% for SDR. Several hypotheses can be put forward to explain this discrepancy.

Double or higher order crossovers between the *Rx* locus and the centromere might occur. In the absence of chiasma interference, which implies a Poisson distribution of chiasmata, the proportion of nulliplex plants no longer would be a linear function of the map distance (Jongedijk *et al.* 1991). In addition, map distances exceeding the theoretical limit of 33.3 cM would rarely be detected. However, for one gene (*D*, Table 1) a map distance beyond 33.3 cM was reported, possibly as a result of positive chiasma interference favouring the formation of single exchanges. If random multiple exchange tetrads (METs) occur and 2*n* gametes are produced by SDR only, the expected frequency of nulliplex plants would range between 16.7 and 50% (Jongedijk *et al.* 1991). The 8.7% nulliplex plants found in this study was not within this range, so the hypothesis of a gametic pool originating from random METs and SDR 2*n* gametes only was rejected.

In the case of random METs and 2*n* gametes, produced by FDR only, the mean number of chiasmata formed between *Rx* and its centromere (*x*) can be calculated with the formula f^{FDR} (nulliplex) = $\Sigma_n\ e^{-x}.x^n/n!.1/6.[1-(-1/2)^n]$ (Jongedijk *et al.* 1991). By substituting the observed frequency of nulliplex plants (8.7%) in the given formula, it was estimated that *x* is about four, suggesting that the *Rx* locus is not actually linked to its centromere. However, it seems unlikely that the assumption of random METs and FDR only holds true. Firstly, random METs in combination with SDR could not be responsible for the 16.1% nulliplex plants found in the offspring of the 2*x*-4*x* cross. Secondly, a difference in chiasma formation in male and female meiosis is unlikely, as Jongedijk and Ramanna (1989) and Jongedijk *et al.* (1991) found consistent absence of sex differences in overall chiasma frequencies and chiasma distribution among chromosomes in potato. There is also cytological evidence from a study of Stack and Anderson (1986) that double and higher order crossovers do not occur frequently in another *Solanaceae* viz., the tomato (*Lycopersicon esculentum*), where they found only one to three recombination nodules per synaptonemal complex (SC) during pachytene. Because of the striking structural resemblance of chromosome arm lengths and distribution of heterochromatin between tomato and potato chromosomes at pachytene (Gottschalk and Peters 1955), the low frequency of recombinations (0.5-1.5 per chromosome arm) found in tomato might also occur

in potato. Recently, M.W. Bonierbale (personal communication) found that double and higher order crossovers are rare in a hybrid population segregating for restriction fragment length polymorphisms. Therefore the hypothesis of a gametic pool originating from random METs and FDR 2*n* gametes only was rejected.

The chiasma frequency could be lower in the diploid hybrids used in the 4*x*-2*x* crosses (Table 2) than in the diploids used in the 2*x*-4*x* crosses. FDR 2*n* pollen is assumed to occur in three diploid hybrids used in the 4*x*-2*x* crosses since fused spindles and (or) parallel spindles were observed at second metaphase of meiosis. Although the frequency of these spindle orientation types did not correspond well with the frequency of dyads in two of three clones (data not shown), the incidence of predominantly FDR 2*n* pollen was expected since no other cytological aberrations were found. If no crossovers have occurred between *Rx* and the centromere, all FDR 2*n* gametes are expected to be *Rxrx*. However, in the case of a single crossover between the locus in question and the centromere, the frequency of heterozygous gametes is reduced to 50%, and the frequency *RxRx* and of *rxrx* gametes would be 25%. Thus, it can be calculated that a frequency of SETS of 34,8%, being 33% lower than in the diploids used in 2*x*-4*x* mapping, would be sufficient to explain the 8.7% nulliplex plants found in the offspring of the 4*x*-2*x* cross. Douches and Quiros (1988) also suggested that genomic differentiation between *S. tuberosum* and *S. chacoense* could account for the reduced recombination levels found in the species hybrids.

Using 2*x*-4*x* crosses gene-centromere map distances have been estimated for three marker loci. Since the diploid seed parents are likely to form 2*n* eggs by SDR only, these estimates are probably reasonable in case of *Ry* and H_1. As to *Rx*, the 33.9-cM map distance indicates that this locus segregates independently from its centromere. Another conclusion might be that reduced recombination rates due to genomic differentiation may seriously defeat attempts to determine the mode of 2*n*-pollen formation in diploid clones genetically, even if marker loci that are located close to their centromeres are used. HTAs using the 4*x*-2*x* cross are less valuable to predict the frequency of susceptible plants as well as to estimate the gene-centromere map distance because both FDR and SDR restitution mechanisms might simultaneously occur in the same diploid clone (Ramanna 1979). The 4*x*-2*x* cross is more useful in HTAs if desynaptic diploid clones are involved, as was demonstrated by Jongedijk *et al.* (1991).

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CHAPTER 6

Spontaneous structural rearrangements in *Solanum phureja* Juz. et Buk. 3. Gene-centromere mapping of *ym* (yellow margin) by half-tetrad analysis and variable expression of I^{sp} (spectacle).

Summary

A diploid ($2n = 2x = 24$) interchange heterozygote of *Solanum phureja* Juz. et Buk. produced $2n$ pollen by fused, 'tripolar' and/or parallel spindles at second metaphase of meiosis, giving rise to predominantly first division restitution $2n$ pollen. A relative map distance of 31.7 centimorgan was estimated by a half-tetrad analysis (HTA) for the distance between *ym* (yellow margin) and the centromere which was similar to the distance estimated for the non-interchange situation. In diploid progenies from diploid testcrosses, segregation for spectacled tubers fitted the hypothesis of control by heterozygosity (genotype $I^{sp}i$) at the *I*-locus and the presence of the basic pigmentation genes *R* or *P*. A remarkable shortage of spectacles was found in $4x$ progenies from both $2x$ x $2x$ and $4x$ x $2x$ crosses probably due to non-expression of the genes involved. This made HTA-mapping of the gene I^{sp} impossible.

Key words: *Solanum phureja* Juz. et Buk., interchange heterozygote, $2n$ pollen, gene-centromere mapping, gene expression

Introduction

In diploid ($2n=2x=24$) potatoes, several meiotic restitution mechanisms which can give rise to $2n$ gametes have been found. Considering the genetic consequences, two distinct modes of $2n$ gamete-formation are distinguished: first division restitution (FDR) and second division restitution (SDR). Basically FDR $2n$ gametes include non-sister chromatids, whereas SDR $2n$ gametes comprise sister chromatids. Once the mode of $2n$-gamete formation has been determined cytologically, the so-called half-tetrad analysis (HTA), which takes advantage of $2n$-gamete formation in the diploid parent in $4x$-$2x$ or $2x$-$2x$ matings, can be used to map single genes with respect to the centromere (Mendiburu *et al.*, 1974). Ramanna (1979) stated that it is not always possible to predict on the basis of a certain meiotic abnormality during microsporogenesis alone whether FDR or SDR will occur. In potato, a number of loci have been mapped by establishing the map distance to the centromere through $4x$-$2x$, $2x$-$4x$, and $2x$-$2x$ crosses. A summary of relative map distances of morphological characters and isozymes reported in the literature has been composed by Wagenvoort & Zimnoch-Guzowska (1992). The morphological marker *ym* (yellow margin) was mapped 34.16 centimorgans (cM) relative to the centromere via $4x$-$2x$ crosses, and 36.08 cM via $2x$-$4x$ crosses, (Jongedijk *et al.*, 1991). The diploid parents used in these HTAs were synaptic and produced $2n$ pollen by FDR or $2n$ eggs by SDR.

The diploid clones of *Solanum phureja* Juz. et Buk. designated 1931 and 1936 are heterozygous for an interchange between chromosome 3 and possibly chromosome 12, for one or two paracentric inversions, and also for the marker *ym* (Wagenvoort, 1988). In these clones the dominant allele *Ym* was assigned to the interchanged arm of chromosome 12. Clone 1936 produced seeds after selfing. Trisomics ($2n=2x+1=25$) were found in the first inbred generation of 1936 and were identified as tertiary trisomics or primaries being homozygous for the interchange (Wagenvoort, 1994).

In order to establish the genetic constitution of both the normal chromosomes 3 and 12 and the interchanged chromosomes 3^{12} and 12^{3} concerning the loci *S* (gametophytic incompatibility) and *ym* and with respect to the breakpoints (T) of the interchange, it was necessary to ascertain the recombination rate of *ym* in the interchange heterozygote. If this recombination value could be compared with the estimate made by Jongedijk *et al.*, 1991, who used a different approach, inferences regarding the degree of interference, if any between *ym* and the centromere, could be drawn. In that case the relative map distance of *ym* to the centromere estimated in the interchanged chromosome can be used in further genetic analysis

of progenies of 1931 and 1936. Thus, 4*x* x 2*x* testcrosses were performed for mapping the monogenic recessive gene *ym* by HTA using the genotypes 1931 and 1936.

Dodds & Paxman (1962) showed that in cultivated diploid potatoes the spectacle pattern (failure of pigmentation around the eyes of an otherwise pigmented potato tuber) is controlled by heterozygosity at the *I*-locus, expressed only in the presence of the basic pigmentation genes *R* or *P*. The *I*-locus is genetically independent of the loci *R* and *P* (Dodds & Long, 1956). Together with *R* or *P* only combinations of the alleles *I* and I^{sp} are spectacle; the other combinations are either self-coloured (uniformly coloured) (*II*, *Ii*) or white (*ii*, ii^{sp}, $I^{sp}I^{sp}$)(Dodds and Paxman, 1962). The distance between the *I* locus and the centromere is not known. The genetics of spectacle in diploid potatoes and somatic segregation of the spectacle pattern in tetraploid *S. tuberosum* L. ssp. *andigena* Juz. et Buk. has later been studied by Simmonds (1969, 1973). This author suggested that *I* is a compound locus such that *I=Isp*, I^{sp}=*iSp* and *i=isp*. Simmonds could not detect the double dominant *ISp* but found one heterozygote (*Isp isp*) with self-coloured tubers and a rather consistent shortage of spectacles in several diploid families suggesting that such heterozygotes may be fairly frequent. Furthermore, Howard (1967) concluded that variability of spectacle expression depends upon the presence of the gene *M*, sofar found only in the tetraploid potatoes.

In this paper the relative distance of *ym* to the centromere in the interchange situation is estimated and the occurrence of chiasma interference and the mode of 2*n*-gamete formation. In addition, it was attempted to map I^{sp} with respect to the centromere since in our study normal segregation for spectacle was observed in diploid populations.

Materials and methods

Plant material

In Table 1 the origin is listed of the diploid and tetraploid clones used in test-crosses and in HTA-mapping. Also included in Table 1 are their respective tentative genotypes, based on phenotype and the presence or absence of segregation in the diploid and tetraploid progenies (Table 3) for the traits yellow margin and tuber pigmentation. The diploid mutant for yellow margin, viz. Ym76-1-15 was selected from *S. phureja* (Wagenvoort, 1982). From this clone a tetraploid (2*n*=4*x*=48) was produced through tissue culture using the method by Roest & Bokelmann (1976). The two diploid desynaptic clones designated M6 and SY7, produce FDR 2*n* egg cells, and were kindly supplied by Dr. M. Masson, Evry, France. These clones were previously selected by the group of Dr. S.J. Peloquin (University of Wisconsin, Madison, USA),

Table 1. Origin of diploid and tetraploid potato clones used in half-tetrad analysis and their genotypes as reported in the references

Code	Origin	Assumed ⫩ genotype	Reference
75-1136-1931[a]	2*x*-*S. phureja* Juz. et Buk.	*Ymym*	Wagenvoort 1988
75-1136-1936[a]	2*x*-*S. phureja* Juz. et Buk.	*Ymym*	do
4*x* from Ym 76-1-15[d]	2*x*-*S. phureja* Juz. et Buk.	*ymymymym*	Wagenvoort 1982
I_1258[b]	2*x*-Inbred clone from 1936	*II rr*	M. Wagenvoort, unpublished
I_1268[b]	2*x*-Inbred clone from 1936	Ii^{sp} *Rr*	do
F_1640[b]	2*x*-F_1 plant from 1936x1931	ii^{sp} *Rr*	do
M6[c]	2*x*-*tuberosum*-*phureja* hybrid	unknown	Masson 1985
SY7[c]	2*x*-*tuberosum*-*phureja* hybrid	unknown	do
Bintje[d]	4*x*-Dutch variety	*iiii PPpp (R?)*	
Civa[d]	4*x*-Dutch variety	*iiii pppp (R?)*	

⫩ *ym*, yellow margin, monogenic recessive marker; *I*, a dominant gene involved in the tuber-specific expression of the basic pigmentation genes *R* (red) and *P* (purple). The combination of *I* with the recessive allele i^{sp} suppresses the action of *R* or *P* only around the eyes resulting into white patches (spectacle).

[a] Diploid clones with the ability to produce FDR 2*n* pollen and used in 4*x*-2*x* mapping.

[b] Diploid clones used in 2*x*-2*x* or 4*x*-2*x* testcrosses for the trait spectacle.

[c] Diploid clones with the ability to produce FDR 2*n* eggs and used in 2*x*-2*x* testcrosses.

[d] Tetraploid clones used in 4*x*-2*x* testcrosses.

from the progeny of the cross W5295.7 x W5337.3, both parents being hybrids between *S. phureja* (2*n*=2*x*=24) and *S. tuberosum* dihaploids (2*n*=2*x*=24).

Crosses were made on plants grafted onto tomato root stocks and on plants grown on bricks in a temperature-conditioned glasshouse.

Heterozygosity at the *ym*-locus in the diploids 1931 and 1936 was checked in the first inbred generation of 1936 and in an F_1 progeny of both clones. Segregation for spectacle (I^{sp}) was checked in diploid offspring of white x spectacle and reciprocal crosses within *S. phureja*. Testcrosses (2*x* x 2*x* and 4*x* x 2*x*) for i^{sp} were carried out using two tetraploid cultivars of *S. tuberosum* L. ssp. *tuberosum* Hawkes and two diploid hybrids between *S. phureja* and 2*x* *S. tuberosum* ssp. *tuberosum* as female and $I_1$268 as male parent. The *I*-locus in the diploid species is equivalent to the *D*-locus in tetraploid *S. tuberosum* ssp. *tuberosum* and ssp. *andigena* (Howard, 1970). The dominant gene *D* causes a brown-reddish colour in the stem, the leaf petioles and the inflorescences, and is along with another dominant gene *R* (anthocyanin production) responsible for deeply red colouring of the phelloderm of the tubers (Howard, 1970). The *P*-locus is responsible for the biosynthesis of purple pigments in various plant parts. The inheritance of anthocyanin pigmentation in the cultivated potato has recently been reviewed by De Jong (1991). According to Dodds & Long (1956), the effect of *I* is limited to the tuber whereas, in the tetraploid scheme, *D* is considered to have an effect throughout the whole plant (De Jong, 1991). In this paper the notation of Dodds & Long (1956) is used for the gene which is responsible for the tuber-specific expression of the genes *R* and *P* in diploid and tetraploid potatoes. A *iiii-R* genotype has white skinned tubers. The two cultivars, Bintje and Civa, are expected to be nulliplex at the *I*-locus as both have white tubers and purple and red pigments in the sprouts respectively. Progeny of testcrosses of Bintje x *S. phureja* (recessive for the *P*-locus) segregated for purple hypocotyl colour in ratios (data not shown) pointing to Bintje being duplex *P* at the *P*-locus. Seedlings of testcrosses of Civa had uncoloured hypocotyls, this cultivar was considered to be nulliplex at the *P*-locus. If there is no crossing-over between i^{sp} and the centromere and only FDR 2*n* pollen is functional, all pigmented tubers in the 4*x* progenies (the crosses being *ii* x Ii^{sp} and *iiii* x Ii^{sp} respectively) are expected to be spectacled. If there is always one cross-over, the ratio of self-coloured:spectacle: white in the 4*x* mapping population will approach 1:2:1.

Cytological methods and method of estimating gene-centromere map distance

Ploidy level of 4*x*-2*x* progenies and of plants from tissue culture was determined by counting the number of chloroplasts in the stomatal guard cells after staining with iodine-potassium

iodine. Whenever in doubt the number of chromosomes was counted in root meristems according to the method described by Wagenvoort & Lange (1975). Study of various stages of meiosis in the diploids were carried out as described by Wagenvoort & Ramanna (1979) in order to ascertain the mode of male and female 2*n*-gamete formation.

For estimating the map distance of *ym* to the centromere in the interchange situation the 4*x*-2*x* cross was used since 1931 and 1936 produced 2*n* gametes only at the male side.

Mendiburu & Peloquin (1979) assumed only single-exchanges to occur in potato. Under that assumption the proportion of single-exchange tetrads is a linear function of the map distance between the locus in question and the centromere. In case of FDR the gene-centromere map distance = 2 (frequency of nulliplex progeny) x 100 cM (Mendiburu & Peloquin 1979). The binomial confidence interval was calculated using the expression

$$\hat{p}-2\sqrt{\frac{\hat{p}\hat{q}}{N}}<p<\hat{p}+2\sqrt{\frac{\hat{p}\hat{q}}{N}}$$

giving approximately 95% limits to p, where $\hat{p}$ and $\hat{q}$ are the frequencies of plants with and without the trait, respectively, and N is the total number of plants (Clarke 1982).

Results

Half-tetrad analysis with ym

The diploid clones 1931, 1936 and I$_1$268 were studied at MII and later stages of meiosis in order to trace the mode of 2*n*-pollen formation. In Table 2 the frequencies of fused spindles and parallel spindles at MII are presented along with the distribution of the chromosomes at AII/TII and the dyad, triad and tetrad frequencies at the sporad stage. The relative contribution of fused spindles to the pool of big pollen grains in the clones 1931, 1936 and I$_1$268 was 52.9, 66.7 and 85.0 % respectively, whereas the dyad frequency exceeded the frequency of fused spindles. Triads were found in 3-5 % of the PMCs analysed at the sporad stage. They will give rise to SDR or FDR 2*n* pollen depending on whether either one of the equational walls has failed to form or the reductional wall is partly formed respectively (Ramanna, 1974). In I$_1$268, where parallel spindles were nearly lacking, there was a close correspondence between the frequencies of fused spindles at MII and dyads at the sporad stage (Table 2; Figs. 1 and 2).

Table 2. Frequencies of parallel spindles (PS) and fused spindles (FS) at MII, chromosome distribution at AII/TII and the dyad, triad and tetrad frequency at the sporad stage in the diploid clones 1931, 1936 and $I_1$268 from *S. phureja* Juz. et Buk. - = no observations

Plant no	Number of cells	MII (%)			Number of cells	AII/TII (%)			Number of cells	Sporad stage (%)		
		Normal	PS	FS		12-12-12-12	12-12-24	24-24		tetrad	triad	dyad
1931	305	61	21	18	548	71	4	25	836	61	5	34
1936	979	70	20	10	1400	70	9	21	1491	82	3	15
$I_1$268	425	47	2	51	-	-	-	-	2007	37	3	60

In Fig. 2 a region of an anther is shown in which only dyads occur. Variation in the frequency of dyads occurred within and between anthers of the same plant. Therefore, it was reasonable to conclude that fused spindles predominantly contributed to the pool of 2*n* pollen of I_1268 and consequently gave rise to FDR 2*n* pollen. For 1931 and 1936 fused as well as parallel spindles probably contributed to the pool of 2*n* pollen, also giving rise to FDR 2*n* pollen.

The 4*x* mutant for yellow margin was crossed to 1931 and 1936, the crosses being *ymymymym* x *Ymym*. The triploid offspring of these 4*x*-2*x* crosses segregated for *ym* in 20 normal versus 26 mutant, which was consistent with the expected ratio 1:1 ($\chi^2 = 0.78$, $P > 0.30$). In the tetraploid offspring the ratio 249 normal to 47 mutant was found. In the case of FDR 2*n* pollen all mutants originate from crossing-over between *ym* and the centromere. Using the formula Mendiburu & Peloquin (1979) a map distance of 31.7 cM was calculated with a 95% binomial confidence interval of 27.5-35.9 cM.

Test-crosses with F^0.

In microsporogenesis of M6 and SY7 normal chromosome pairing at pachytene (Fig. 3) and predominantly univalents at diakinesis (Fig. 4) and MI (Fig. 5) were found. After MI the chromosomes orientated and divided mitotically (Fig. 6) forming restitution nuclei. This chromosome behaviour in microsporogenesis of M6 and SY7, indicated pseudo-homotypic division by Gustafson (1935), confirmed that these clones were desynaptic and had the ability to produce viable FDR 2*n* pollen. SDR 2*n* gametes would be unviable owing to genetical unbalance. The 2*n* eggs produced by these clones are also expected to be of the FDR type because the recessive gene responsible for desynapsis in these clones equally affects microsporogenesis and megasporogenesis (Jongedijk & Ramanna, 1988). Some Telophase I cells were found with a 12-12 chromosome distribution. Not a single PMC was found in both clones showing chromosome orientation as in a regular second Metaphase. Cells with four groups of $n = 12$ chromosomes along with cells containing two groups of $2n = 24$ chromosomes were found (Fig. 7).

Tubers from I_1268 were spectacled red and those from I_1258, F_1640, M6, SY7, Bintje and Civa were entirely unpigmented. In the progenies from the diploid testcrosses, I_1258 x I_1268 and I_1268 x F_1640 the ratios found for white:self-coloured:spectacle fitted the expected ratios, calculated on the basis of the assumed genotypes (Table 3). In 4*x* progenies from the crosses between M6, SY7, Bintje and Civa, used as female parents and I_1268 as male parent, the frequency of spectacles among the plants with pigmented tubers is expected to vary from

Table 3. Results of testcrosses with *ii*^{sp} (spt=spectacle); $I_1$268 produced FDR 2*n* pollen whereas M6 and SY7 produced FDR 2*n* eggs. The following genotypes are assumed: $I_1$258=*ii rr pp*; $I_1$268=*ii*^{sp} *Rr pp*; $F_1$640=*ii*^{sp} *Rr pp*; Bintje=*iiii PPpp*, Civa=*iiii pppp*. The genotypes for M6 and SY7 are unknown.

Cross combination			Phenotype parents		Progeny				Expected ratio	χ^2	P
♀		♂	♀	♂	Ploidy	White	Self-Coloured	Spec-tacle			
$I_1$258	x	$I_1$268	white	spt	2x	18	11	5	2:1:1	2.24	0.50-0.30
$I_1$268	x	$F_1$640	spt	white	2x	31	13	14	10:3:3	1.88	0.50-0.30
SY7	x	$I_1$268	white	spt	4x	9	20	1			
M6	x	$I_1$268	white	spt	4x	17	49	9			
Bintje	x	$I_1$268	white	spt	4x	13	55	0			
Civa	x	$I_1$268	white	spt	4x	14	49	2			

66.6-100%, depending on the rate of crossing-over of f^{sp}. However, a remarkable shortage of spectacles was found in these 4*x* progenies (Table 3). In the offspring of the cross between Bintje and $I_1$268, spectacles were not at all observed among the 55 plants with self-coloured tubers. Out of these 55 plants, 12 had red tubers and 43 plants had purple tuber skin varying from reddish-purple to flecked light purple. In addition, all plants of the 4*x* progenies had round tubers suggesting that $I_1$268 is homozygous for a single dominant gene controlling round tuber-shape, since Bintje and Civa themselves produce tubers with oval and long-oval shape respectively.

Discussion

Parallel orientation of MII spindles may, but need not necessarily result in 2*n*-pollen formation (cf. Mok & Peloquin, 1975; Ramanna, 1979; Wagenvoort, 1986). Parallel spindles found in 1931 and 1936 in this study may have partly contributed to the pool of 2*n*-pollen, indicated by the strong correlation between the occurrence of fused/parallel spindles, and dyads (Table 2).

The relative distance of *ym* (31.7 cM) to the centromere as found in this study was similar to that (viz. 34.16 cM) reported by Jongedijk *et al.* (1991) who in the 4*x*-2*x* testcross used the same 4*x* mutant and a synaptic diploid clone not carrying an interchange. The similarity of the map distances of *ym* assessed in both studies suggests that crossing over in 1931 and 1936 was not influenced by the presence of the interchange. This outcome also demonstrates that the position of the locus relative to the centromere has not been changed much owing to the interchange. Therefore, this map distance can be used in further genetic analysis of progenies from 1931 and 1936.

The cytological observations in M6 and SY7, made in this study, partly correspond to those by Douches and Quiros (1988). With respect to the desynaptic condition of clone M6, no difference was found with the results by Douches and Quiros (1988). However, fused spindles orientation at Telophase II was reported by these authors but not observed in the present study. Meiotic stages of the second division were hardly seen and when PMCs in these stages were recognized as such, they showed non-reduction, i.e. the formation of (un)-balanced tetrads. Therefore, it seems more likely that the FDR 2*n* pollen in these clones is caused by desynapsis combined with pseudo-homotypic division than with fused spindles.

The shortage of spectacles in the 4*x* progenies of the 2*x* x 2*x* and 4*x* x 2*x* crosses might support Howard's (1967) opinion that expression of spectacle in cultivated tetraploid potatoes depends upon action of the pigment-restricting gene *M*. In the varieties Bintje and Civa this gene might be recessive. In cultivated diploid potatoes patterns that resemble the effect of *M* do occur but are rare according to Simmonds (1969). The same author concluded that expression of spectacle is largely dependent upon unidentified internal physiological factors and much less upon external environment (Simmonds, 1973). On the other hand, spectacle in the cultivated diploids is due to an allele at the *I*-locus and not to a gene similar to *M* (Dodds & Paxman, 1962). The possibility that the shortage of spectacles in the 4*x* progenies is due to differential expression of the gene I^{sp} at the 2*x* and 4*x* level, rather than to action of gene *M*, would however, fit a genetical effect rather than an effect of internal physiological factors which somehow affects the pigment-producing enzyme system. In the present study the low and variable expression of spectacle made it impossible to determine the gene-centromere map distance of I^{sp}. A similar case of apparent differential expression at the 2*x* and 4*x* level was found for the gene *Me* (metribuzin tolerance) by H. de Jong, Agriculture, Canada (Personal Communication). It therefore appears that for certain genes there may be differential expression at different ploidy levels. This, in turn, would have implications for a breeding and selection programme on the diploid level. Many breeders today develop superior diploid parents which will eventually contribute to superior tetraploid progeny. However, the underlying assumption of this procedure is that genes are expressed at various ploidy levels. Although this may be true for many characteristics (Keijzer-van der Stoel *et al.*, 1991), the results discussed here indicate that it may not be true for all cases.

The relative map distance of *ym* was successfully estimated by half-tetrad analysis and the mode of 2*n*-pollen formation by cytological analysis. The interchange apparently had no effect on the distance to the centromere. This map distance can be used for the placement of the gene on a gene map in potato.

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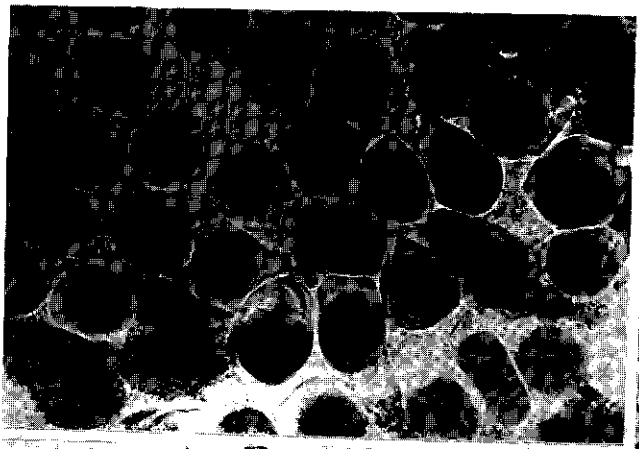

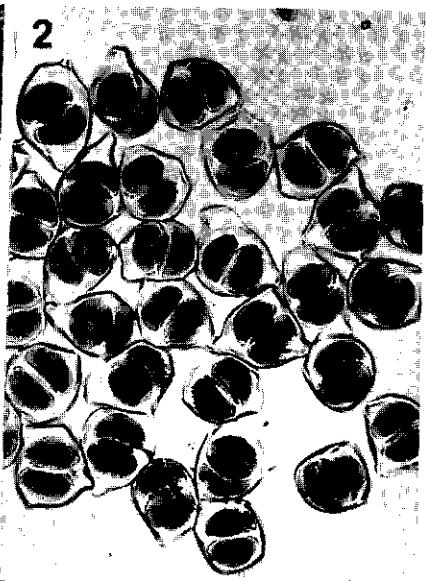
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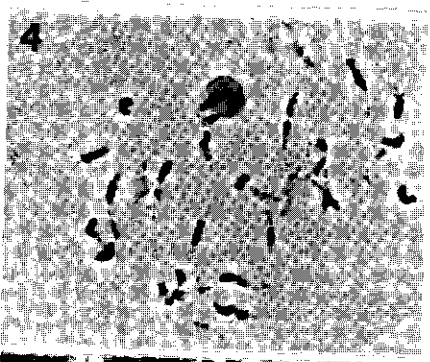
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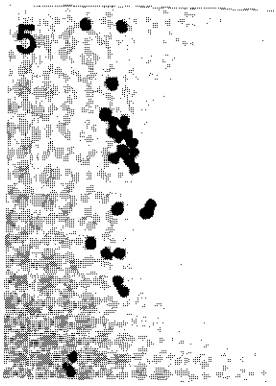
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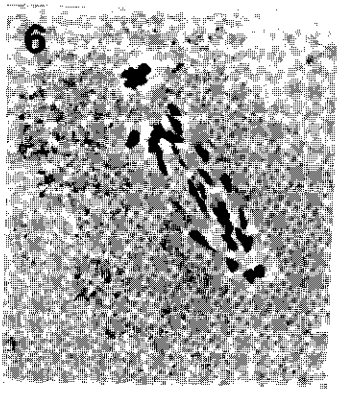
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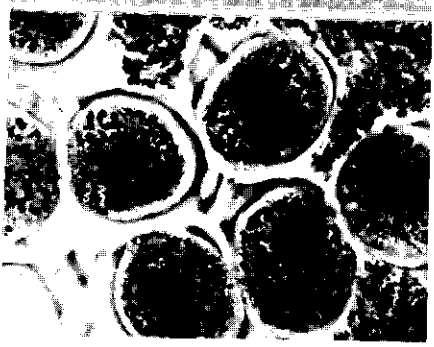

Fig. 1. PMCs from $I_1$268 showing fused spindles at MII.

Fig. 2. Dyads at the tetrad stage in $I_1$268.

Fig. 3. Two PMCs from SY7 showing complete synapsis at pachytene.

Fig. 4. PMC from M6 showing desynapsis at diakinesis.

Fig. 5. PMC from M6 showing 24 univalents at MI.

Fig. 6. PMC from M6 showing mitotic division of chromosomes after orientation at MI.

Fig. 7 PMC from SY7 showing cells with 4 groups of chromosomes and one cell with 2 groups of chromosomes.

CHAPTER 7

Location of the recessive gene *ym* (yellow margin) on chromosome 12 of diploid *Solanum tuberosum* by means of trisomic analysis[1]

Summary

Ten out of twelve primary trisomics of diploid *S. tuberosum* were crossed as females with a recessive mutant for yellow margin (*ym ym*) obtained from *S. phureja*. All primary trisomics used proved to be homozygous dominant. Trisomic plants from all ten F_1's were backcrossed with the mutant and trisomics from eight F_1's were crossed also with a heterozygous F_1 plant from the chromosome 10-trisomic.

In both BC_1 and half sib progeny of each trisomic type the mutant plants were easily identified because of their typical small roundish leaflets with yellow or reddish margins. The observed segregation ratios for normal to mutant were tested against the expected non-critical ratios and against various expected critical ratios.

From the results of these tests it is concluded that the gene *ym* is most probably located on chromosome 12 of the potato. A hypothesis of linkage between *ym* and a gene l_x for lethality is put forward. It is concluded that l_x is not identical with a previously detected recessive gene l_2 which is responsible for yellow cotyledons and lethality.

Key words: *Solanum tuberosum* - trisomics - gene location - yellow margin - lethality

[1]Slightly revised version of the paper published in Theor. Appl.Genet. 61:239-243 (1982).

Introduction

In the genus *Solanum* only a few cases of gene location by trisomic analysis have been reported. A gene *a* for albinism was located on the long arm of chromosome 12 of *S. chacoense* by Lam and Erickson (1971), who used a di-isotrisomic of that species. Hermsen *et al.* (1973) associated gene *v* for chlorophyl deficiency with chromosome 12 of *S. tuberosum*. The latter chromosome 12 was numbered according to the identification of Yeh and Peloquin (1965) and is different from the chromosome 12 of *S. chacoense* reported by Lam and Erickson (1971). These authors used their own numbering of pachytene chromosomes of *S. chacoense* (Lam and Erickson 1968). Lee and Rowe (1975) reported the association of the genes *P* and *Ac* with unknown iso-chromosomes of *S. chacoense*.

The *P* locus controls the production of delphinidin in both flowers and tubers. *Ac* is concerned with the acylation of anthocyanins with p-coumaric acid (Harborne 1960). Lee and Rowe also located one of the two genes Gl_1, and Gl_2 on the long arm of chromosome 9.

The genes Gl_1 and Gl_2 were found to control the glucosylation of rutin (Harborne 1962). Gl_1 is linked with *Ac*. The gene *df* (deformed flower), which in sensitive cytoplasm df^s is expressed as the character 'short anther' (Grun 1970; Grun *et al.*, 1962) was associated with trisomic V 1682.3 by Lee and Ruhde (1976). The extra chromosome of this trisomic was not identified. As soon as 11 of the 12 possible types of primary trisomics were available (cf. Wagenvoort and Ramanna 1979), crosses between these trisomics and plants which carried several marker genes were made.

In this paper the location of a recessive gene *ym* (yellow margin) is reported and the possible linkage with a gene l_x for lethality is discussed.

Materials and Methods

Pedigrees of all *S. tuberosum* material (trisomics, dihaploids and inbred clones) used in this study have been described earlier (Wagenvoort and Ramanna 1979; Wagenvoort and Lange 1980). The chromosome 10-trisomic which had an interspecific hybrid origin, was obtained from Dr. R.E. Hanneman Jr., Madison, Wisconsin, USA. The mutant for yellow margin was selected from the diploid species *S. phureja*. Seeds of this species were kindly supplied by Dr. B. Maris (former SVP, Wageningen). A crossing scheme for the production of the BC_1's of crosses between F_1 trisomics (*Ym ym* or *Ym Ym ym*) and the mutant parent, and for the production of the half sibs of crosses between F_1 trisomics and a heterozygous (*Ym ym*) F_1 plant

from the chromosome 10-trisomic, is presented in Fig. 1. Trisomic F_1 plants of crosses between trisomics and the mutant were tentatively selected morphologically and their possible trisomy was checked in root tip cells.

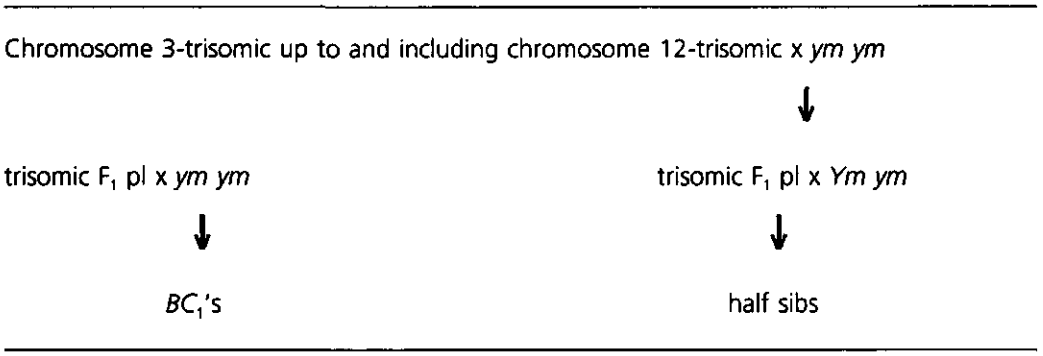

Fig. 1. Crossing scheme for the production of BC_1's and half sibs of crosses between F_1 trisomics (*Ym ym* or *Ym Ym ym*) and the mutant parent and a heterozygous (*Ym ym*) F_1 plant from the chromosome 10-trisomic respectively.

Both the BC_1 and half sib progenies were assessed for the proportion of mutant plants and the observed ratios were tested for goodness of fit to the expected critical and non-critical ratios. In general random chromosome association was assumed.

The methods for studying the chromosomes in mitosis and meiosis were the same as described by Wagenvoort and Lange (1975) and Wagenvoort and Ramanna (1979). Male fertility was estimated by staining the pollen with lactophenol - acid fuchsin (Sass 1964).

Results and Discussion

The feature of yellow margin is generally characterized by small roundish leaflets with yellow or reddish margins. In some populations, however, the mutants showed variation with respect to the size of the leaflets. Fig. 2 shows three leaves: two are of the mutant phenotype, but only the leaf at the right shows the typical small roundish leaflets in combination with the yellow leaf margin. Originally the mutant was found in two families of crosses between normal plants of *S. phureja*, whose families segregated 65:28 and 78:21 for normal to mutant. The observed ratios fit the expected ratio 3:1, indicating that for both families the parental plants were heterozygous for the *ym* locus. A homozygous recessive plant was selected from one of these populations. This plant had a pollen stainability of 80-90%. Meiosis appeared to be

regular and no 2*n* gametes were observed in second metaphase or anaphase.

F_1 plants from trisomics x mutant never showed the mutant character, indicating that the original trisomics are homozygous for the dominant allele *Ym*. The segregation ratios of normal versus mutant plants in the BC_1 generation are summarized in Table 1. The observed ratios were tested against the non-critical ratio 1:1 and against the critical ratios 2:1 (if f=0) and 3:1 (if f=0.25), where f is the female transmission of the extra chromosome. For many trisomics a female transmission of 25% is a good estimate (cf. Wagenvoort and Lange 1980). The first test revealed that the observed ratios fitted the non-critical ratio 1:1 except in the chromosome 11-trisomic x *ym ym* (Table 1). But in this case the deviation from 1:1 was an excess of mutants, which does not point to trisomic inheritance at all.

Table 1. Segregation of normal versus mutant (*ym ym*) plants in ten BC_1 progenies of crosses between F_1 trisomics (*Ym ym* or *Ym Ym ym*) and the mutant parent (*ym ym*), as well as tests for goodness of fit to 1:1 (expected non-critical ratio), to 2:1 (expected critical ratio, if f=0.0) and to 3:1 (expected critical ratio if f=0.25), where f is the female transmission of the extra chromosome

Trisomic chromosomes	Normal	Mutant	$\chi^2_{1:1}$	$\chi^2_{2:1}$	$\chi^2_{3:1}$
3	39	36	0.12	7.26*	21.16*
4	24	32	1.14	14.29*	30.86*
5	46	44	0.04	9.80*	27.39*
6	9	8	0.06	1.44	4.41*
7	72	75	0.06	20.69*	53.08*
8	35	28	0.78	3.50	12.70*
9	47	46	0.01	10.89*	29.68*
10	24	35	2.05	17.93*	37.07*
11	4	13	4.76*	14.24*	24.02*
12	22	20	0.09	3.86*	11.46*

* Significant at a probability level of P= 0.05

The second test is the most severe because with f=0 the difference between critical and non-critical ratio is the smallest. Populations of at least 131 plants are needed for a reliable distinction between 1:1 and 2:1. This number was reached only for the chromosome 7-trisomic. It is expected that any significant deviation in this test will also be significant in tests to ratios that are based on higher f-values. The observed ratios in the F_1's involving the trisomics for the chromosomes 6 and 8 fitted both 1:1 and 2:1 but deviated significantly from 3:1, whereas all other F_1's fitted neither 2:1 nor 3:1. So with the assumption of f=25% none of the observed ratios were critical.

Table 2. Segregation of normal versus mutant (*ym ym*) plants in nine half sib progenies of crosses between eight F_1 trisomics *Ym ym* or *Ym Ym ym*) and a male fertile F_1 plant from the chromosome 10-trisomic (supposed genotype *Ym ym*), as well as tests for goodness of fit to 3:1 (expected non-critical ratio), to 5:1 (expected critical ratio, if f = 0.0) and to 7:1 (expected critical ratio if f=0.25), where f is the female transmission of the extra chromosome

Trisomic chromosomes	Normal	Mutant	$\chi^2_{3:1}$	$\chi^2_{5:1}$	$\chi^2_{7:1}$
3	107	28	1.31	1.61	8.38*
4	6	7	5.77*	12.94*	20.32*
5	21	8	0.10	2.49	6.03*
6	12	15	13.44*	29.40*	45.76*
7	200	64	0.08	10.91*	33.28*
9	13	8	1.92	6.94*	12.58*
11	8	4	0.44	2.40	4.76*
12	31	9	0.13	0.98	3.66
12	115	15	12.56*	2.67	0.07

* Significant at a probability level of P = 0.05

Table 2 presents the ratios observed in the half sib progenies and the chi-squares calculated on the basis of 3:1, 5:1 and 7:1. The ratios observed in the progenies involving trisomics for the chromomsomes 4 and 6, as well as that in one population of the chromosome 12-trisomic

deviated significantly from the expected non-critical ratio 3:1. For the chromosome 4 and chromosome-6 trisomics, however, the deviation observed was an excess of mutants, as was the case in the BC_1 from the chromosome 11-trisomic (Table 1). These deviations cannot have been brought about by trisomic inheritance, for in that case a large surplus of normal plants would be expected. Therefore, only the significance for the chromosome 12-trisomic may point to trisomic inheritance. This was corroborated by the results of testing against the two critical ratios. Only the two populations from the chromosome 12-trisomic showed non-significance both with f=0 and f=0.25. This result led to the tentative conclusion that the gene *ym* might be located on chromosome 12.

Additional evidence for this conclusion was obtained by counting the number of chromosomes of some normal as well as mutant plants from both the BC_1 and half sib progenies. The results of this analysis are presented in Table 3.

Table 3. Results of cytological analysis of parts of the groups of normal and mutant plants of both BC_1 and half sib progenies of crosses between F_1 trisomics (*Ym ym* or *Ym Ym ym*) and the mutant parent (*ym ym*) or a male fertile F_1 trisomic of the chromosome 10-trisomic (supposed genotype *Ym ym*), respectively

Trisomic chromosomes	BC_1				Half sib			
	Normal		Mutant		Normal		Mutant	
	Disomic	Trisomic	Disomic	Trisomic	Disomic	Trisomic	Disomic	Trisomic
3	2	3	8	3				
4			5	0				
5	3	1	10	8				
6	3	0	3	2				
7	1	0	0	3	129	51	44	17
8	1	2	15	4				
9	29	17	34	10	12	1	4	1
10			5	2				
11	1	2	8	2			1	1
12	16	6	19	0	(29)[a]	2	9	0
12					81	31	10	0

[a]This number is based on morphological selection only

The group of mutants will reveal the most relevant information: if the critical trisomic is involved and random chromosome assortment is assumed, all plants of this group will be disomic and consequently all trisomics in BC_1 as well as half sib progeny will show the normal phenotype; if, however, random complete chromatid assortment is assumed, one out of 15 trisomics will be a mutant (Hermsen 1970). In the case of disomic inheritance both groups of normal and mutant plants will show about equal proportions of trisomics, the size being dependent on the f-value. Table 3 shows that the chromosome 12-trisomic and perhaps the

chromosome 4-trisomic, fulfil the criteria of trisomic inheritance: only these trisomics revealed a complete absence of trisomics in the groups of mutants.

Although only five mutants were investigated cytologically from the chromosome 4-trisomic and these five appeared to be disomic, it seems rather unlikely that this trisomic is critical for the *ym* locus because it was already rejected on the basis of segregation ratios in BC_1 and half sib progeny. Therefore, it can be concluded that the gene *ym* is most probably located on chromosome 12 of the potato. This chromosome is equal to the one on which Hermsen *et al.* (1973) located the gene *v* for virescens. The results in the BC_1 of the chromosome 12-trisomic need further discussion because two observed ratios were not in agreement with the above mentioned conclusion. First, the ratio 22:20 for normal to mutant (Table 1) deviated significantly (χ^2=7.46) from the expected critical ratio 2.5:1, calculated on the basis of the actual value of f=0.15. Second, the observed ratio 16 normal to 19 mutant in the group of disomics (Table 3) deviated significantly from the expected critical ratio 2:1(χ^2=6.21). To explain this phenomenon it can be hypothesized that in some populations the ratios were disturbed because of the activity of lethality genes. Dodds and Paxman (1962) suggested that the gene *ym* is linked to a recessive lethal in the repulsion phase.

In the progeny of a cross between two normal plants of *S. phureja* these authors found a segregation ratio for yellow margin which deviated significantly from the expected ratio 3:1. Hermsen *et al.* (1978) described three lethal genes viz l_1(seed non-emerging from the soil), l_2 (yellow cotyledons) and l_3 (tiny dwarf) in a dihaploid plant (G254) of cultivar 'Gineke'. These genes affect the germination rate of the seeds and may be present and segregating in the trisomics used in this study, as G254 was the male parent in the original 3*x* x 2*x* crosses, except for the chromosome 10-trisomic, which has another origin.

Indeed, in seven BC_1 populations, as well as six half sib progenies, mutants for l_2 occurred and were readily observable by their yellow cotyledons, segregation for l_3 was not observed in any of the populations, and the occurrence of l_1 in the same populations was indistinct. The three populations of the chromosome 12-trisomic did not segregate for l_2, but nevertheless in BC_1 only 42 plants out of 126 germinated seeds could be reliably assessed for the yellow margin character. This loss of seedlings was not due to the action of l_2. Consequently it may be hypothesized that an unknown recessive gene for lethality (l_2) is involved that is linked with *ym*. In the original population of *S. phureja*, from which the homozygous recessive *ym ym* clone was selected, no seedlings died. Therefore, it can be presumed that one of the parents of this cross was heterozygous for l_x, the cross being

$$\frac{Lx\ Ym}{Lx\ ym} X \frac{Lx\ Ym}{lx\ ym}.$$

The genotype of the homozygous yellow margin clone, which was used to incorporate the gene *ym* into the original trisomics as well as to produce the BC_1 could have been

$$\frac{Lx\ \ ym}{lx\ \ ym}.$$

If it is further assumed that the original chromosome 12-trisomic was duplex for L_x the genotype of the trisomic of the F_1 progeny of the chromosome 12-trisomic could have been

$$\frac{\frac{Lx\ Ym}{lx\ Ym}}{Lx\ ym}.$$

The cross for the production of the BC_1 then can be reproduced as

$$\frac{\frac{Lx\ Ym}{lx\ Ym}}{Lx\ ym} X \frac{Lx\ ym}{lx\ ym}.$$

This situation will lead to segregation of l_x in the BC_1 and consequently will disturb the segregation ratio of *ym*. The ratio in the group of trisomics however will not be influenced and thus remains 1:0 for normal to mutant because the F_1 trisomic is assumed to be duplex for L_x, which means that all the gametes with an extra chromosome contain at least one dominant allele.

With this hypothesis the deviating ratios in the BC_1 of the chromosome 12-trisomic and the group of mutants can be explained satisfactorily. Since the size of the groups of plants was limited and the stage at which the seedlings died was not clearly established, the possible relationship between *ym* and l_x should be studied more extensively.

Conclusions

(i) The recessive gene *ym* is most probably located on chromosome 12 of the potato and presumably linked to a recessive lethal gene l_x.
(ii) The gene l_x is not identical with l_2.

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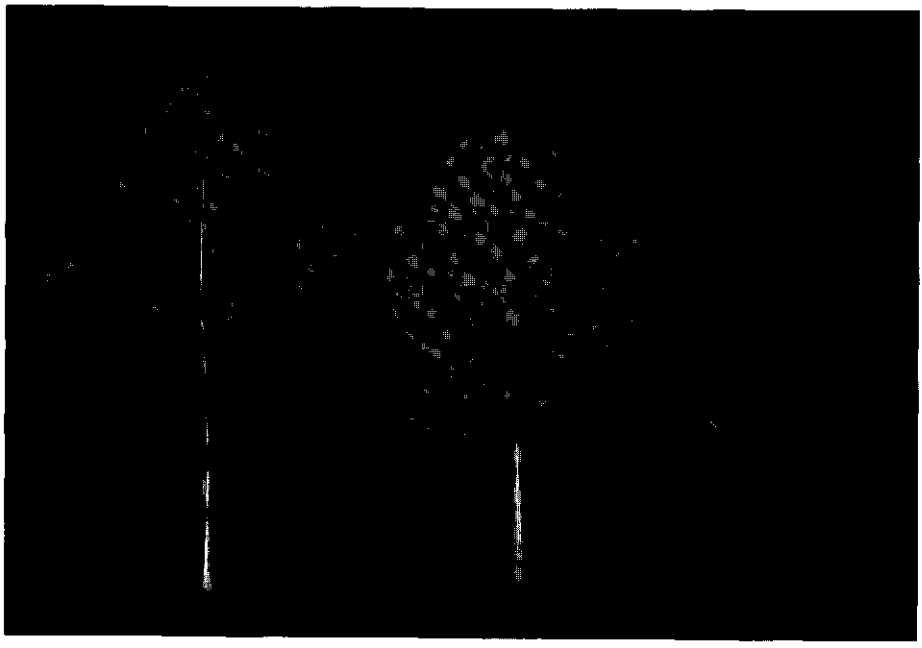

Fig. 2. Three leaves of BC_1 plants: normal phenotype for the yellow margin character (left), mutant type (middle) and mutant type showing the yellow leaf margin in combination with the typical small roundish leaflets.

CHAPTER 8

Chromosomal localisation of a recessive gene *tp* controlling the pleiotropic character topiary in *Solanum*[1]

Summary

Seven out of twelve possible types of primary trisomics of dihaploid *Solanum tuberosum* L. were crossed as females with a disomic recessive mutant for topiary (*tp tp*) identified in *S. infundibuliforme* Phil. All primary trisomics used proved to be homozygous dominant. Trisomic plants from the seven F_1's were crossed with a disomic heterozygous F_1 plant (supposed genotype *Tp tp*). In the half sib progeny of each trisomic type the mutant plants could be easily identified by the presence of typical lateral shoots, particularly at the cotyledonary nodes. The observed segregation ratios for normal to mutant were tested against the expected non-critical ratio 3:1 and against various critical ratios. It is concluded that the gene *tp* is most probably located on chromosome 3 of the potato.

Key words: *Solanum tuberosum* L. - potato - trisomics - gene location - topiary

[1]Revised version of the paper presented in Theor Appl Genet (1988) 75: 712-716

Introduction

There has been increasing interest and activity in the field of somatic cell genetics and tissue culture of the economically important potato crop. Recently, significant progress has been made in somatic hybridization, including both tuber-bearing and non-tuber-bearing *Solanum* species (Gressel *et al.* 1984; Austin *et al.* 1985; Helgeson *et al.* 1986; Puite *et al.* 1986; De Vries *et al.* 1987). For a demonstration of the hybrid character of fusion products, specific markers for either parent are of significant value. However, the number of available morphological markers, i.e. genes mapped on chromosomes of the potato, is restricted to only a few cases. Furthermore, genetic transformation of the potato with the aid of *Agrobacterium tumefaciens* (Ooms and Lenton 1985; Burrell *et al.* 1985) and *Agrobacterium rhizogenes* (Ooms *et al.* 1985, 1986) has been successful. Therefore, the construction of a genetic map of the potato is a necessity in regard to gene transfer in the near future.

A gene for albinism (*a*) was located on the long arm of chromosome 12 of *Solanum chacoense* Bitt. by Lam and Erickson (1971), who used a di-isotrisomic clone of that species. Hermsen *et al.* (1973) associated gene *v* for chlorophyll deficiency with chromosome 12 of *S. tuberosum* L.. The latter chromosome 12 was numbered according to the pachytene identification of Yeh and Peloquin (1965) and is different from chromosome 12 of *S. chacoense* reported by Lam and Erickson (1971). These authors used their own numbering of pachytene chromosomes of *S. chacoense* (Lam and Erickson 1968).

Genes Gl_1 and Gl_2 control the glucosylation of rutin (Harborne 1962). Gl_1 is linked with the gene *Ac*, which is involved in the acylation of anthocyanins with p-coumaric acid (Harborne 1960). Lee and Rowe (1975) located either Gl_1 or Gl_2 on the long arm of chromosome 9. For gene localisation studies in potato, trisomic analysis appeared to be useful.

Wagenvoort and Ramanna (1979) established a nearly complete series of primary trisomics in diploid *S. tuberosum*. Eleven of the twelve possible types of primary trisomics are available and crosses between these trisomics and plants with some marker genes were made. In a previous paper, the location of a recessive gene *ym*, responsible for yellow margin, on chromosome 12 of diploid *S. tuberosum* was described (Wagenvoort 1982). In this paper the location of a recessive gene *tp* (topiary) is reported.

Materials and methods

Pedigrees of the *S. tuberosum* material (trisomics for the chromosomes 2 through 11, except

for chromosome 10) used in this study have been described earlier (Wagenvoort and Ramanna 1979; Wagenvoort and Lange, 1980). The chromosome 10-trisomic, which has an interspecific hybrid origin, was obtained from Dr. R.E. Hanneman Jr., Madison, Wisconsin, USA. The mutant for topiary was identified in the wild diploid species *S. infundibuliforme* Phil. by Den Nijs *et al.* (1980). It is a pleiotropic character, which can easily be recognized in the seedling stage by profuse branching at the cotyledonary nodes. Seeds of this species were kindly supplied by Dr. A.P.M. den Nijs and originally came from the laboratory of Prof. S.J. Peloquin, Madison, Wisconsin, USA. Crosses were made between the 11 primary trisomics and the disomic mutants for topiary. Trisomic F_1 plants of crosses between trisomics and the mutant were tentatively selected morphologically and their possible trisomy was checked in root tip cells. These F_1 trisomics (*Tp tp* or *Tp Tp tp*) were crossed with a heterozygous (*Tp tp*) disomic F_1 plant from the chromosome 10-trisomic as male parent. Seven progenies from these crosses were checked for the character involved and in both groups of normal and mutant plants samples were taken and used for counting the number of chromosomes.

Topiary seedlings were distinguished by the presence of excessive lateral branching, particularly at the cotyledonary nodes. Seedlings were assessed weekly for this character over a period of several weeks, starting when the plants were four weeks old. The observed ratios for normal to mutant plants were tested for goodness of fit to the expected critical and non-critical ratios. In general, random chromosome association was assumed. For a reliable distinction between disomic and trisomic inheritance the size of the population was calculated with the formula:

$$n = \left[\frac{1 + (\mu\lambda)^{1/2}}{\mu^{1/2} - \lambda^{1/2}}\right]^2 \cdot \chi^2_{\alpha,1\ d.f.}$$

where

n = the total number of plants

μ = the expected ratio dominant to recessive in the case of trisomic inheritance

λ = the expected ratio dominant to recessive in the case of disomic inheritance

$\chi^2_{\alpha,1\ d.f.}$ = Chi-square for P = 0.05 and one degree of freedom. (see Romagosa 1982).

The method used to study the chromosomes in mitosis was the same as described by Wagenvoort and Lange (1975).

Results and discussion

The mutant for topiary found in the wild tuber-bearing diploid species *S. infundibuliforme* develops lateral branches at nearly every node and shows a globular shape as it produces a dense growth of numerous slender stems. Stolons are absent or very short and the tubers are located in a tight cluster around the base of the stem. Fig. 1 shows three seedlings: the one on the left and the one in the middle have mutant phenotypes with numerous stems originating from nearly every node, whereas the seedling on the right shows only one stem as found in normal plants. In addition to these characters, earlier tuberization in the field and the appearance of knobby tubers were described by Den Nijs *et al.* (1980). These authors suggested that the topiary character could be the result of an altered cytokinin activity. This study focused on the first character only, viz. the presence of lateral branches.

Although some older F_1 plants from trisomics x mutant developed some lateral branches, they never showed the typical dense growth and globular shape of the mutant. Therefore, it was concluded that the original trisomics were homozygous for the dominant allele *Tp*.

Table 1 shows the segregation ratios of normal versus mutant plants in seven half sib progenies of crosses between F_1 trisomics and a male fertile F_1 disomic heterozygous for topiary.

The observed ratios were tested against the non-critical ratio 3:1 and against the critical ratios 5.67:1 (if f = 0.10) and 9.91:1 (if f = 0.45), where f is the female transmission of the extra chromosome. The test against the non-critical ratio revealed that the ratios for the trisomics for the chromosomes 4, 6, 7, 9 and 10 fitted the expected value. For the chromosome 3-trisomic and the chromosome11-trisomic there was a significantly deviating ratio (Table 1). Both trisomics had an excess of normal plants, pointing to trisomic inheritance. However, a reliable distinction between disomic and trisomic inheritance can only be made if the population is sufficiently large. With f = 0.10 or f = 0.45, populations of at least 240 and 80 plants, respectively, are needed for a reliable distinction. (See "Materials and methods".)

Table 1. Segregation of normal (*Tp*) vs mutant (*tp tp*) plants in half sib progenies of crosses between F_1 trisomics (*Tp tp* or *Tp Tp tp*), as well as tests for goodness of fit to 3:1 (expected non-critical ratio), 5.67:1 (expected critical ratio if f = 0.10) and 9.91:1 (expected critical ratio if f = 0.45) where f is the female transmission of the extra chromosome.

Trisomic chromosomes	Normal	Mutant	$\chi^2_{3:1}$	$\chi^2_{5.67:1}$	$\chi^2_{9.91:1}$
3	408	31	75.70*	21.84*	2.34
4	220	55	3.79	5.62*	39.60*
6	31	4	3.73	0.23	0.36
7	41	15	0.09	7.14*	21.96*
9	274	83	0.53	18.35*	83.48*
10	257	65	3.74	7.07*	45.03*
11	50	7	4.63*	0.52	0.88

* Significant at a probability level of P = 0.05

The population sizes of the trisomics for the chromosomes 3, 4, 9 and 10 fulfilled these criterions. In the progenies of the trisomics for the chromosomes 9 and 10 the segregations for the topiary gene were in accordance with disomic inheritance. For both a low female transmission (f = 0.10) and a high female transmission (f = 0.45), the observed ratios deviated significantly from the expected critical ratios (Table 1). For this reason no chromosome counts were made in these progenies. The observed ratios for the chromosome 3-trisomic deviated significantly from 5.67:1 and fitted the expected value 9.91:1. In the chromosome 11-trisomic the observed ratios were in accordance with both expected ratios, but the population size was insufficient for a reliable test. Therefore, it was necessary to split up the populations into trisomics and disomics in order to test for normal versus mutant ratios within these two groups. In the case of trisomic inheritance and if random chromosome segregation is assumed, all mutants will be disomic and consequently all trisomics will show the normal phenotype. With random complete chromatid segregation, however, one out of 15 trisomics will be mutant (Hermsen 1970).

The results of chromosome counts are presented in Table 2. For the chromosome 3, 6, 7 and

11-trisomics no trisomics were found among the mutants (Table 2). All trisomics under investigation had trisomic plants among the normal phenotypes. Fifteen out of 40 normal plants were trisomic for the chromosome 11-trisomic, in addition to no trisomics among the mutants. For this trisomic type a female transmission of 34% was estimated in this study. In the case of disomic inheritance, this transmission rate would lead to at least one of the four mutants being trisomic. However, possibly because of the small number of mutants investigated for the chromosome 11-trisomic, not a single trisomic was found among the mutants. The same probably holds true for the chromosome 6 and 7-trisomics. Therefore, it seems unlikely that the topiary gene is located on one of these three chromosomes. However, in the chromosome 3-trisomic among 45 normal plants 22 trisomics were found and no trisomics were found among 25 mutants (Table 2). In the last group nearly eight trisomics would be expected based on disomic inheritance and the actual female transmission of 31%. Hence, it was concluded that the gene *tp* for topiary is most probably located on chromosome 3 of the potato.

Table 2. Results of cytological analysis of parts of the groups of normal and mutant plants of five progenies of crosses between F_1 trisomics (*Tp tp* or *Tp Tp tp*) and a male fertile F_1 disomic of the chromosome 10-trisomic (supposed genotype *Tp tp*).

Trisomic chromosome	Normal		Mutant	
	Disomic	Trisomic	Disomic	Trisomic
3	23	22	25	0
4	19	16	19	14
6	19	3	2	0
7	20	7	10	0
11	25	15	4	0

The F_1 trisomics from the chromosome 3-trisomic used for the production of the half sib progenies were derived from the trisomic coded GNA77-61-6. Pachytene analysis of this trisomic clearly revealed the presence of a complete chromosome 3 as the extra chromosome.

Fig. 2 shows a trisomic configuration of chromosome 3 in GNA77-61-6. This chromosome has three distinct chromomeres in the achromatic part of the short arm. Meiotic studies in some F_1 trisomics revealed the presence of the short arm of chromosome 3, indicating that no univalent shift had taken place during transmission of the chromosome at meiosis. In the progenies studied at mitosis there were no indications of the occurrence of telos. For this reason, it seems justified to conclude that the extra chromosome in the F_1 plants of the chromosome 3-trisomic is indeed a complete chromosome 3, which carries the recessive gene for topiary.

The results in the half sib progenies need further discussion because the progeny of the chromosome 3-trisomic segregates 408 normal:31 mutant which does not fit the ratio 389:50 based on the actual female transmission of 31% ($\chi^2_{7.7\text{-}1}$=8.47 P<0.001). A shortage of mutants was also observed in five of the six half sib progenies, which were derived from the non-critical trisomics (χ^2 heterogeneity = 5.04, P = 0.50-0.30). In other progeny from the chromosome 3-trisomic, f-values were estimated ranging from 31.4-48.9% (see chapter 4). Because some bias could have taken place at the estimation of f in this study, a higher f-value of the chromosome 3-trisomic seems to be more realistic. If f is 0.45, the expected ratio for normal to mutant is 399:40 fitting the observed ratio 408:31 (Table 1). Hence, with the assumption of a higher f-value the results in the half sib progeny of the chromosome 3-trisomic can satisfactorily be explained. The shortage of mutants in the non-critical situations is more difficult to explain. In the six progenies analysed the percentage of non-viable seeds or seedlings non-emerging from the soil varied from 10.7 - 41.0% (data not shown). Only in the progeny from the chromosome 10-trisomic mutants for the lethal gene l_2 (yellow cotyledons) occurred. A model of two recessive genes (one linked to the topiary gene and the other independent) causing non-viability of the seeds or the young seedlings would adequately explain the shortage of mutants. However, loss of mutants was not found to occur in the BC_1 from the cross (*S. chacoense* x topiary) x topiary by Den Nijs *et al.* (1980). Therefore, the hypothesis put forward has to be tested further before it can be accepted for explaining the shortage of mutants.

Also from this study it can be concluded that a series of primary trisomics in potato is a suitable tool for the localisation of recessive genes. Dominant genes also can be assigned to chromosomes with the aid of a series of primary trisomics. Such studies, however, take much more labour and time, compared with the location of a recessive gene. For the location of a dominant gene the backcross populations have to be larger to allow for a reliable distinction between disomic and trisomic inheritance. A second approach would be to properly estimate

the female transmission in each backcross progeny and to test the observed ratios based on the actual f-value. Only when the f-value is low can the ratios in the total populations be used for a reliable detection of the critical trisomics. With increasing rates of female transmission, the critical ratios shift towards the non-critical ratios (see Hermsen, 1970), and for higher f-values a reliable distinction between disomic and trisomic inheritance is only practicable within the groups of disomics and trisomics separately.

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Fig. 1. Three seedlings of the half sib progenies of crosses between F_1 trisomics *(Tp tp)* or *(Tp Tp tp)* and a male fertile F_1 disomic (supposed genotype *(Tp tp)*. Two mutants (*left* and *middle*) for topiary clearly show the numerous slender stems originating from the nodes in the leaf axes. The normal plant on the *right* has one stem only and already shows the development of long stolons which can be seen extending across the pot.

Fig. 2. A complete PMC of GNA 77-61-6 (chromosome 3-trisomic) at mid-pachytene stage of meiosis. The PMC contains one trivalent and 11 bivalents, as expected for a trisomic with $2n = 24 + 1 = 25$. The trisomic configuration represents chromosome 3 and shows three chromomeres (*arrows*) on the achromatic part of the short arm. Some other bivalents, viz. chromosomes 2, 4, 5, 7 and 12 could also be identified with certainty. The centromeres are indicated by *arrow heads*.


Mutant 6p
Normal Tp.


7
2
3
12
5
4

GENERAL DISCUSSION

The cultivated potato *Solanum tuberosum* L. ssp. *tuberosum* Hawkes is considered by some researchers to be a segmental allotetraploid rather than an autotetraploid. Tetrasomic inheritance, a high degree of heterozygosity and the small and morphologically similar chromosomes seriously hamper genetic and cytogenetic studies in this important food crop. The development of methods to produce dihaploids and monohaploids not only enhanced the opportunities for analytic breeding but also created better tools for basic research.

Several methods to identify chromosomes at mitosis have been proposed. For two techniques, viz. a modified Giemsa technique (Mok *et al.*, 1974) and a Giemsa C-banding technique (Pijnacker and Ferwerda, 1984), the authors claimed that it is possible to identify the twelve basic chromosomes. Although both techniques were used in this study, we were not able to identify all 12 basic chromosomes. Polymorphism of the Giemsa C-banding pattern seems to be the main cause preventing the Giemsa C-banding technique to be generally applicable for chromosome characterization in somatic cells of the potato.

An accurate knowledge of the meiotic behaviour in parents of crosses that were made to create a mapping population for trisomic or RFLP analysis, is essential for detecting causes of distorted genetic ratios in segregating populations. In addition, such knowledge is necessary for determining the female transmission of the extra chromosome in trisomics which in its turn must be known for calculating the expected genetic ratios.

Half-tetrad analysis is a means to determine the gene-centromere map distance and takes advantage of the occurrence of numerically unreduced (2*n*) gametes in diploid potato species or species hybrids. Genes or markers with known distances to the centromere may be used as reference markers when creating a genetic map of the potato.

The research described in this thesis focuses on the perspectives for identification of meiotic and mitotic chromosomes using several cytological techniques and cytotypes, and deals with gene mapping by trisomic and half-tetrad analysis. Interchanges are rare in potato, but when available they can be very useful for chromosome arm location of genes. The available primary trisomics of *S. tuberosum* ssp. *tuberosum* were used for the identification of the chromosomes involved in the interchange found in *S. phureja* Juz. et Buk..

Several methods to identify somatic chromosomes were attempted (Chapter 1). In contrast to results reported in the literature, this study enabled the unambiguous identification of only three chromosomes (1, 2 and 12) in mitotic cells using conventional staining, and four (1, 2,

3 and 4) in case of Giemsa C-banding. It should be noted that by applying these techniques to certain cytotypes not all specimens of a specific chromosome were always identifiable. Polymorphism for the Giemsa C-banding pattern determined in various cytotypes in this study was the main cause preventing the detection of homology between pachytene and somatic chromosomes. The C-banding pattern varied between preparations, and this variation occurred within plants of the same species as well as in interspecific hybrids. It seems that induction of distinct C-banding is not feasible in the highly condensed and small chromosomes of the potato. Moreover, the preservation of the chromosomes throughout the entire procedure of C-banding was not always sufficient. Denaturation by the barium hydroxide step and successive re-association in a 2 x SSC (standard saline citrate concentration) buffer caused loss of DNA and/or chromosomal protein in some cells resulting in swollen chromosomes with poor morphology and weak banding. Both with conventional staining and Giemsa C-banding the mitotic chromosomes 1 and 2 could unambiguously be identified as being homologous to the pachytene chromosomes 1 and 2. A third method the so-called "*in situ* hybridization" was tried to identify somatic chromosomes and to determine the number of chromosomes carrying genes for the tuber protein patatin. This method combines both classical methods used in cytogenetics with powerful molecular biological techniques. By using *in situ* hybridization it was found in this study that one basic chromosome of the potato contains rRNA genes. Although the short arm of chromosome 2 is C-banding positive, except for the NOR (Pijnacker and Ferwerda, 1984), it was impossible to identify with Giemsa C-banding the three homologues of the nucleolar chromosome in mitotic cells from the trisomic for chromosome 2. In trisomics which were previously identified as trisomic for the nucleolar chromosome, the presence of three chromosomes with a signal of the hybridized rDNA probe was clearly demonstrated. The heterologous rDNA probe from pea also hybridized to the NORs of triploid sugar beet showing that the rRNA genes in potato, sugar beet and pea are highly conserved.

The development of high resolution *in situ* hybridization will become increasingly important for the physical mapping of DNA sequences, including single genes, along chromosome arms. In this context *in situ* hybridization using more than one probe simultaneously is of interest, because in that case the hybridization spot of a localized probe marks the chromosome arm and the other probe(s) will localize the gene(s) of interest.

Biotin and digoxigenin labelling was found to be a rapid, consistent and reliable technique to detect highly repeated sequences on the relatively small chromosomes of potato and sugar beet. Its value for physical mapping of low copy or unique sequences in these plant species has yet to be established. Success in localizing a low copy or single copy gene in chromosomal DNA

by *in situ* hybridization depends on several factors including the accessibility of the target DNA for interaction with the probe DNA, the specific activity and the amount of probe DNA, the absence of background hybridization and in particular the preservation of DNA throughout the entire procedure.

In plants it appears that low mitotic indices and the presence of cell-wall material in chromosome preparations hamper hybridization of low copy number sequences to the chromosome and their detection. In addition the degree of coiling and condensation of plant chromosomes in mitosis varies from species to species and between preparations. The presence of cell wall material could be avoided in potato by using wall-degrading enzymes such as cellulase and pectinase. However, low mitotic indices in root tips were a serious problem in the quantitative analysis of the Giemsa C-banding pattern or after *in situ* hybridization. By using aphidicolin or hydroxyureum, both of which inhibit the activity of the enzyme DNA polymerase α giving rise to accumulation of cells at the transition phase between G1 and S of the cell cycle, mitotic indices could not be increased in root tips of the potato (M. Wagenvoort, unpublished data).

Accurate chromosome identification in potato can be achieved by pachytene analysis (Chapter 2). At pachytene the bivalents of *S. phureja* appeared to be morphologically very similar to those of *S. tuberosum* ssp. *tuberosum* cv. Gineke. This result coincides with that of Matsubayashi (1991), who found a close similarity of pachytene morphology in *S. phureja* and *S. tuberosum* L. ssp. *andigena* Juz. et Buk.. In addition, there are only trivial differences in the fine structure of the pachytene chromosomes of *S. tuberosum* L. and *S. stenotomum* Juz. et Buk. (Gottschalk, 1972). In the literature considerable controversy exists with respect to interpretation of morphology of pachytene chromosomes in potato and related tuber-bearing species of *Solanum*. The use of different features in identifying pachytene chromosomes mainly contributed to this discrepancy along with misinterpretation of the observed configurations. Comparison of the studies of pachytene chromosomes of *S. canasense* Hawkes by Haynes (1964) and by Gottschalk and Peters (1956a) reveals very little similarity for most of the chromosomes. There is some agreement in the satellite (SAT) chromosome {chromosome 12 according to Haynes (1964) and chromosome 1 according to Gottschalk and Peters (1956a)} with respect to heterochromatic regions but the location of the NOR and the total chromosome length are very different. *S. canasense* belongs to the taxonomic series *Tuberosa*. However the morphology of its pachytene chromosomes differs greatly from that in other species of this series (Gottschalk and Peters, 1955). Also lack of agreement was established for the SAT chromosome when comparing the results of Haynes (1964) with the karyogram for

S. vernei Bitt. et Wittm. presented by Fiedler and Schreiter (1959). Furthermore, Lam and Erickson (1968) numbering the chromosomes of *S. chacoense* Bitt. according to their total length, identified the third chromosome as the SAT chromosome. This chromosome was morphologically similar to chromosome 2 of both *S. tuberosum* ssp. *andigena* and of *S. tuberosum* ssp. *tuberosum* described by Yeh and Peloquin (1965) and Ramanna and Wagenvoort (1976) respectively. Comparison of the pachytene chromosomes of *S. clarum* Corr. with those of *S.tuberosum* ssp. *andigena* revealed that chromosome 2 is the SAT chromosome in both species and the chromosomes 11 and 12 of *S. clarum* are similar to their counterparts in *S. tuberosum* ssp. *andigena* (Marks, 1969). The remaining chromosomes were highly different for the two species. There are three outstanding features in the pachytene karyotype of *S. clarum*: (i) the almost completely chromatic short arm of chromosome 9 (ii) the large interstitial chromatic chromomeres in the long arms of the chromosomes 2 and 6 (iii) the characteristically large telomeres of the chromosomes 5 and 10. These features are unique for *S. clarum* except for the chromomere in chromosome 2. Also Gottschalk and Peters (1956b) found very clear structural differences between the homologous chromosomes of a close relative of the diploid species *S. stenotomum* and the amphidiploid *S. ajuscoense* Buk.. In the species hybrid considerable differences of the total chromosome lengths were observed but these differences were restricted to the heterochromatic regions. The differences in chromosome length resulted in the formation of heteromorphic bivalents showing unpaired loops of different size within the heterochromatic zones comparable to loop formation due to deficiencies in the heterozygous condition. The loops found in pachytene chromosomes of *S. phureja* in this study were present in both the heterochromatic and the euchromatic regions of specific chromosomes. Those formed in the euchromatic regions pointed to the presence of a heterozygous inversion which was clarified by the incidence of a bridge and fragment at anaphase I. Finally, in the allotetraploid species *S. antipoviczii* Buk. one chromosome complement shows clear relations to *S. tuberosum* as far as chromosome structure is concerned and a second complement contains chromosomes with a very divergent structure. The formation of only bivalents at metaphase I confirms the allotetraploid nature of this species (Gottschalk, 1972). Both species, *S. ajuscoense* and *S. antipoviczii* are considered to be nearly identical to *S. stoloniferum* Schlechtd. et Bché., which belongs to the taxonomic series Longipedicellata (Correll, 1962). All these examples show the variation in the fine structure of the pachytene chromosomes in different species of the section Petota of the genus *Solanum*. This may have an evolutionary meaning. It can be concluded, that although in potato the pachytene is very difficult to analyse pachytene analysis will deliver more reliable results than

mitotic karyotype analyses. It will also give a more detailed insight into the degree of homology of the chromosomes compared to metaphase I analyses.

The structural differences in heterochromatin between homologous chromosomes, which are clearly discernable and analysable at pachytene may be responsible for the variation observed in the Giemsa C-banding pattern as it is generally thought that the C-bands are induced in the constitutive heterochromatin of the chromosomes. Therefore the value of the Giemsa C-banding technique for chromosome identification in potato should not be overestimated. The main disadvantage of the pachytene stage is the difficulty to get well spread chromosomes amenable to analysis and this is due to the very low degree of chromosomal spiralisation.

Interphase nuclei and pachytene chromosomes are thought to have advantages also in *in situ* hybridization since closely linked probe hybridization sites may be further apart than at metaphase where the chromatin is more condensed. A more efficient and accurate mapping should be obtainable by the combination of pachytene chromosomes and *in situ* hybridization and the use of an electron microscope. In this context a report by Lehfer *et al.* (1991) deserves some attention as these authors began to explore the potential of various *in situ* hybridization and probe labelling techniques on barley chromosomes at the detection level of both the light microscope and the electron microscope. Visualization of a 1.8 kilo-base pairs (kb) single copy probe on barley chromosome 7 was realized in interphase nuclei using streptavidin-gold complexes (diameter 15 nm) along with biotin labelled probes and high-resolution field emission scanning electron microscopy. It is worth noting that with this technique distinction between hybridized and non-hybridized signals is generally possible. The authors concluded that it is technically feasible to map single-copy DNA sequences on plant chromosomes. A novel "insert amplification/sandwich" technique for signal detection enabled them to localize successfully single-copy DNA fragments of 200 bp, and the authors expect that even smaller fragments might be detected at the ultrastructural level.

The advantages of the production of physical maps of chromosomes is that also unlinked loci can be mapped and an estimate of the size of an alien insert can be obtained.

In chapter 2 an intraspecific interchange in *S. phureja* is described and the involvement of the chromosomes 3 and 12 in this interchange could clearly be demonstrated by studying morphology of pachytene chromosomes and chromosome pairing behaviour during meiosis in trisomic F_1 hybrids. Such interchanges could be most useful for assigning genes to specific chromosome arms as well as for the localization of centromeres on the linkage maps. In the two siblings of *S. phureja* carrying the same interchange a variety of quadrivalent configurations at diakinesis and metaphase I was found to occur giving rise to balanced and

unbalanced gametes. Trisomic progeny resulted from irregularities such as 11-13 distribution and lagging chromosomes at anaphase I. A bridge and fragment detected in some anaphase I cells indicated the presence of one or two heterozygous inversions. The occurrence of such abnormalities in fertile diploid clones strengthen the necessity to study meiosis when using such clones in genetic research.

Trisomic descendants selected in the first selfed generation of the interchange heterozygote were primary trisomic and homozygous for the interchange, or tertiary trisomic, based on their phenotypes for yellow margin and the absence of ring quadrivalents at metaphase I of meiosis. Further distinction between the two trisomic types was impossible because the self-compatibility of the interchange heterozygote could not be satisfactorily explained.

The meiotic behaviour of 11 primary trisomics was studied and the transmission of the extra chromosome through the female gamete determined (Chapter 4). Triple synapsis of pachytene chromosomes was often found in the euchromatic parts of the chromosomes. This phenomenon may be considered as a more general feature of chromosome pairing in trisomic and autotriploid plants. Consequently it may influence recombination events.

The occurrence of deleterious recessives in diploid populations of *S. tuberosum* and in diploid relatives may seriously hamper genetic analysis of the potato. However, by using markers with co-dominant expression, such as RFLPs or isozymes, this handicap can be circumvented as the occurrence of skew ratios in the mapping population does not hinder the localization of the gene under investigation because of co-segregation with a molecular marker.

In classical genetic and cytogenetic studies the establishment of a complete series of primary trisomics is essential to assign genes or linkage groups to specific chromosomes. In potato, however, an exceptional situation exists as it is claimed that the genetic content of potato chromosomes is nearly identical to that of tomato chromosomes, i.e. there is no evidence of chromosomal translocations differentiating the two species. This statement is somewhat conflicting in view of the above mentioned variation in pachytene morphology observed among species of the series *Tuberosa* to which also *S. sparsipilum* (Bitt.) Juz. et Buk. belongs, one of the putative parents of *S. tuberosum*. Nevertheless, the molecular map established for tomato could also be used to assign linkage groups to specific potato chromosomes. Yet, five inversions of marker order within the chromosomes 5, 9, 10, 11 and 12 are reported in the literature. These inversions appear to be paracentric and involve entire chromosome arms. Moreover, only one breakpoint can be identified per chromosome and occurs in regions of the genetic map at or near the centromere. The outcome of the research described in this thesis, where the breakpoints of the interchange in *S. phureja* also were localized close to the

centromere, coincides with the above mentioned results which were obtained by RFLP analysis. If the heterochromatin distal to the breakpoint was lost or converted to euchromatin upon transposition to the telomeric end of the chromosome, as suggested by some researchers, a nearly entire euchromatic chromosome arm would result. However, this is not in agreement with the observation that all potato chromosomes show heterochromatic regions on both arms as shown in this thesis and elsewhere.

The average ratio kb/cM deviates greatly from estimates of physical distance. As the potato genome has fewer map units than tomato, the effective density of markers in potato is suggested by some researchers to be actually higher than in tomato (on average one marker per 1.2 and 0.7 cM in tomato and potato, respectively). However, the haploid DNA content of potato is approximately 1750 Mega-base pairs (Mbp) and that of tomato 1000 Mbp (Arumuganathan and Earle, 1991). This means that on average 1 cM $\approx$ 2500 kb in potato and 750 kb in tomato. Therefore, it seems that the average physical map distance between two consecutive loci in potato is about twice as large as in tomato. From results reported in the literature it appeared that: (i) reduction in crossing over affects all potato chromosomes since each of the potato chromosomes has a reduced genetic length compared with the corresponding tomato chromosome. Some chromosomes (e.g. chromosome 2) are affected more than others (ii) the total map length decreases when different species are involved in a cross. In the latter case restricted recombination may be involved, but also recombinant gametes or zygotes may be preferentially eliminated by deleterious recessives.

In this thesis the gene Rx_{and} was mapped 42 cM from the centromere by means of half-tetrad analysis. This estimate coincides with the mapping of the gene *Rx1* to the distal end of chromosome 12. For this reason it is most likely that the gene Rx_{and} is concurrent with the gene *Rx1*. In this case no large difference exists between the two estimates carried out by RFLP and half-tetrad analysis. It is striking, that the *R1* locus conferring hypersensitivity to all *Phytophthora infestans* races except race 1, maps precisely to the same genomic region on chromosome 5 of the potato (Leonards-Schippers *et al.*, 1992) as the locus for extreme PVX resistance (*Rx2*) from *S. acaule* Bitt. (Ritter *et al.*, 1991) although different genetic stocks were used in the two mapping studies. On the other arm of the same chromosome Gebhardt *et al.*, (1993) and Pineda *et al.*, (1993) mapped the gene H_1 for resistance to pathotype Ro1 of *Globodera rostochiensis*. Both research groups placed the gene H_1 on the distal end of the same arm of chromosome 5. This outcome of research is not in line with the map distance of 17 cM which was estimated by half-tetrad analysis in this thesis. This discrepancy might show another example of restricted recombination when different species are involved in a cross. No

correlation has been reported between the presence of *R*-genes, single or in combinations (*R1*, *R2*, *R3*, and *R4* were involved) and the level of field resistance to late blight. The observed linkage between *R1* and a locus for extreme PVX resistance is not in line with the outcome of a previous genetic study (Świeżyński *et al.*, 1974), where no correlation was found between the gene X^i from *S. acaule*, conferring extreme resistance to PVX, and the *R*-genes.

It is noteworthy that a hypothesis has been reported (Leonards-Schippers *et al.*, 1992) suggesting that allelism as well as tightly linked resistance gene-complexes might apply not only to different genes for race-specific resistance to the same pathogen, but also to genes for resistance to completely different pathogens, such as the fungus *Phytophthora infestans*, the virus X or the nematode *G. rostochiensis*. Moreover, it is remarkable that the potato gene *Gro1* for resistance to pathotype Ro1 of *G. rostochiensis* and a gene for resistance to *Fusarium oxysporum* f.sp. *lycopersici* race 1 both were mapped to the same RFLP marker (TG20) on chromosome 7 of potato and tomato, respectively (Sarfatti *et al.*, 1991).

From the research described in this thesis it can be concluded that primary trisomics of the potato are a useful tool for gene localization and are very helpful in identifying the chromosomes involved in an interchange.

Finally it is concluded that *in situ* hybridization is a very suitable technique in detecting the nucleolar chromosomes in trisomic and other cytotypes. This technique has good prospects for physical mapping of genes, although much work has to be done before single copy genes can be localized routinely on the pachytene chromosomes of potato.

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SUMMARY

The potato is an important arable crop in the Netherlands and other countries with a temperate climate. Potatoes are grown in the Netherlands for sale to the consumer, for the potato processing industry and for seed production, the larger proportion of which is being exported. Breeding of this important food crop is a long-term activity because varieties are highly heterozygous tetraploid clones, and in addition contain very small and morphologically similar somatic chromosomes. Therefore, it is not surprising that the potato is a subject not only for practical breeding, but also for fundamental research. The research described in this thesis deals with chromosome identification and gene mapping.

Several methods to identify somatic chromosomes were attempted (Chapter 1). In contrast to results from literature, in this study only three chromosomes (1, 2 and 12) could unambiguously be identified in mitotic cells using conventional staining, and four (1, 2, 3 and 4) in case of Giemsa C-banding. Both with conventional staining and Giemsa C-banding the chromosomes 1 and 2 could unambiguously be identified and are homologous to the chromosomes 1 and 2 as identified by pachytene analysis.

It was found in this study by using *in situ* hybridization that one basic chromosome of the potato contains rRNA genes. In trisomics previously identified as trisomic for the nucleolar chromosome, the presence of three chromosomes with a signal of the hybridized rDNA probe was clearly demonstrated. In contrast to a report in the literature about detection of one chromosome with gene(s) for patatin using a cDNA clone, hybridization with a genomic DNA clone used in this study detected more than one basic chromosome carrying genes related to patatin.

Biotin and digoxigenin labelling was found to be a rapid, consistent and reliable technique to detect highly repeated sequences on the relatively small chromosomes of potato and sugar beet.

Reliable chromosome identification in potato can be achieved by pachytene analysis (Chapter 2). At pachytene the bivalents in *S. phureja* Juz. et Buk. were morphologically very similar to those of *S. tuberosum* L. ssp. *tuberosum* Hawkes cv. Gineke. In the chapters 2 and 3 an interchange in *S. phureja* is described and the involvement of the chromosomes 3 and 12 in this interchange could clearly be demonstrated by pachytene analysis (Chapter 2) and the meiotic behaviour in trisomic F_1 hybrids (Chapter 3). In the two siblings of *S. phureja* carrying the same interchange a variety of quadrivalent configurations at diakinesis and metaphase I

was found to occur, giving rise to balanced and unbalanced gametes. Trisomic progeny resulted from irregularities such as 11-13 distribution and lagging chromosomes at anaphase I. A bridge and fragment observed in some anaphase I cells indicated the presence of one or two heterozygous inversions (Chapter 2). Trisomic descendants selected in the first selfed generation of the interchange heterozygote were primary trisomic being homozygous for the interchange or tertiary trisomic.

Meiotic behaviour in 11 primary trisomics was investigated and female transmission of the extra chromosome determined (Chapter 4). Triple synapsis of pachytene chromosomes was often found in the euchromatic parts of the chromosomes. In this study a significant correlation between the relative chromosome or euchromatin length and the coefficient of realization of a trivalent at metaphase I was found in the primary trisomics of the potato. In spite of this result no relationship could be established between female transmission and the length of the extra chromosome. Therefore, care should be taken to determine female transmission in the total progeny of each trisomic under investigation, or at least in a representative sample of the progeny.

By means of half-tetrad analysis the map distance relative to the centromere could be estimated of each of three dominant genes involved in resistance to potato viruses X and Y and to pathotype Ro1 from *Globodera rostochiensis*, and of the recessive gene for yellow leaf-margin (Chapters 5 and 6). The gene for yellow margin was localized on chromosome 12 (Chapter 7) and that for topiary on chromosome 3 (Chapter 8) by means of trisomic analysis.

SAMENVATTING

De aardappel is een belangrijk akkerbouwgewas in Nederland en in andere landen met een gematigd klimaat. Aardappelen worden geteeld voor de consumptieverkoop, voor de verwerkende industrie en voor de produktie van pootgoed, waarvan het grootste deel wordt geëxporteerd. Veredeling van dit belangrijke voedselgewas is een lange-termijnactiviteit, omdat de rassen sterk heterozygote tetraploide klonen zijn en bovendien zéér kleine en morfologisch sterk op elkaar lijkende somatische chromosomen bevatten. Het is daarom niet verwonderlijk, dat niet alleen in de praktische aardappelveredeling, maar ook in fundamenteel onderzoek van de aardappel veel wordt geïnvesteerd. Het onderzoek, dat is beschreven in dit proefschrift, omvat chromosoomidentificatie en genkartering.

Verscheidene methoden voor identificatie van somatische chromosomen werden beproefd (Hoofdstuk 1). In afwijking van resultaten vermeld in de literatuur, konden in ons onderzoek bij toepassing van conventionele kleuringen slechts drie chromosomen (1, 2 en 12) ondubbelzinnig worden geïdentificeerd in mitotische cellen en vier chromosomen (1, 2, 3 en 4) bij toepassing van Giemsa C-bandering. De chromosomen 1 en 2 konden zowel met conventionele kleuring als met Giemsa C-bandering ondubbelzinnig worden geïdentificeerd en hun homologie met de pachyteenchromosomen 1 en 2 worden aangetoond.

Er werd in dit onderzoek via *in situ* hybridisatie gevonden dat één basischromosoom van de aardappel rRNA genen bevat. In trisomen met het nucleolus-chromosoom in drievoud kon de aanwezigheid van drie chromosomen met een signaal van de gehybridiseerde rDNA probe duidelijk worden gedemonstreerd. In ons onderzoek kon door hybridisatie met een genomische DNA kloon meer dan één basischromosoom worden gedetecteerd als drager van genen gerelateerd aan patatineproduktie. In de literatuur wordt echter melding gemaakt van hybridisatie met een cDNA-kloon, waarmee slechts één basischromosoom als drager van gen(en) voor patatineproduktie kon worden aangetoond.

Biotine en digoxigenine labelling bleek een snelle, consistente en betrouwbare techniek te zijn om sterk repetitieve sequenties op de relatief kleine chromosomen van aardappel en suikerbiet zichtbaar te maken.

Chromosoomidentificatie in aardappel via pachyteenanalyse is een betrouwbare methode (Hoofdstuk 2). In het pachyteen bleken de bivalenten van *S. phureja* Juz. et Buk. morfologisch in hoge mate gelijk te zijn aan die van het ras Gineke van *S. tuberosum* L. ssp. *tuberosum* Hawkes. In de hoofdstukken 2 en 3 wordt een chromosoomtranslocatie in *S. phureja*

beschreven. De betrokkenheid van de chromosomen 3 en 12 bij deze translocatie kon duidelijk worden aangetoond door pachyteenanalyse (Hoofdstuk 2) en het meiotisch gedrag in trisome F_1-hybriden uit de kruising van primaire trisomen x translocatieheterozygoot (Hoofdstuk 3). In de twee zusterplanten met dezelfde translocatie van *S. phureja* werden verscheidene quadrivalente configuraties waargenomen in de diakinese en de eerste metafase die leidden tot gebalanceerde en ongebalanceerde gameten. Onregelmatigheden, zoals een 11:13 verdeling en achterblijvende chromosomen in de eerste anafase, resulteerden in trisome nakomelingen. Het voorkomen van een brug en een fragment in sommige anafase 1 cellen, duidde op de aanwezigheid van één of twee heterozygote inversies (Hoofdstuk 2). Trisome nakomelingen die werden gevonden in de eerste inteeltgeneratie van de heterozygote translocatie waren primaire trisomen en homozygoot voor de translocatie of tertiaire trisomen.

Het meiotisch gedrag van 11 primaire trisomen werd onderzocht en de transmissie van het extra chromosoom via de vrouwelijke gameten bepaald (Hoofdstuk 4). Drievoudige paring van pachyteenchromosomen werd dikwijls gevonden in de euchromatische delen van de chromosomen. Verder werd een significante correlatie aangetoond tussen de relatieve chromosoomlengte of de lengte van het euchromatine enerzijds en de coëfficient van realisatie van een trivalent in de eerste metafase anderzijds. Ondanks dit resultaat kon geen verband worden vastgesteld tussen de vrouwelijke transmissie en de lengte van het extra chromosoom. De vrouwelijke transmissie dient daarom te worden bepaald in een voldoende grote en repesentatieve nakomelingschap van trisoom x diploid voor elke trisoom, die in onderzoek is.

Van elk van drie dominante genen voor resistentie tegen de aardappelvirussen X en Y en tegen het pathotype Ro1 van *Globodera rostochiensis* en van het recessieve gen voor "yellow margin" (gele bladrand) kon de relatieve genetische afstand tot het centromeer worden bepaald via half-tetradenanalyse (Hoofdstukken 5 en 6). Het gen voor "yellow margin" werd gelocaliseerd op chromosoom 12 (Hoofdstuk 7) en dat voor "topiary" op chromosoom 3 (Hoofdstuk 8) via trisomenanalyse.

CURRICULUM VITAE

Marinus Wagenvoort werd geboren te Vierakker (gemeente Warnsveld, thans gemeente Vorden) op 26 juli 1939. In 1958 behaalde hij het diploma HBS-B met 5j.c. aan het Baudartius Lyceum te Zutphen en in 1960 het einddiploma van de Christelijke Hogere Landbouwschool (CHLS) te Ede (thans Agrarische Hogeschool te Dronten). Eveneens aan de CHLS te Ede verwierf hij de akte L1 (akte van bekwaamheid voor het geven van lager landbouwonderwijs). In 1973 werd het diploma van de cursus onderzoektechniek (Ministerie van Landbouw en Visserij) behaald. In augustus 1984 werd een begin gemaakt met de doctoraalopleiding biologie (in deeltijd), aan de Rijksuniversiteit te Utrecht, die in 1990 met succes werd afgesloten met "genoomevolutie" en "plantenveredeling" in het doctoraalvakkenpakket. Na het vervullen van de militaire dienstplicht werkte hij van 1 februari 1963-1 april 1964 als leraar aan de Lagere landbouwschool te Winsum (Gn). In 1964 begon hij zijn carrière in het onderzoek bij de toenmalige Stichting voor Plantenveredeling (SVP), aanvankelijk als assistent in het veredelingsonderzoek aan aardappelen en op het gebied van de cytogenetica. Vanaf 1984 was hij werkzaam als wetenschappelijk onderzoeker en sinds 1992 als senior-wetenschappelijk onderzoeker op de afdeling Akkerbouw- en Voedergewassen van het Centrum voor Plantenveredelings- en Reproduktieonderzoek (CPRO-DLO), waar hij onderzoek verricht op het gebied van sexuele polyploïdisatie van Engels raaigras en lelie. In de periode van 1994-1996 zal hij participeren in een internationaal, door de EG gesubsidiëerd project, betreffende de manipulatie van apomixie in tropische grassen.