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Microsporogenesis in three tetraploid somatic hybrids of potato and their di(ha)ploid fusion partners

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Summary. The microsporogenesis of three somatic hybrids of potato, i.e. one tetraploid *Solanum tuberosum* (+) *S. phureja,* one tetraploid and one hypertetraploid *S. tuberosum* (+) desynaptic mutant, has been examined and compared with the microsporogenesis of the di(ha)ploid fusion partners. The somatic hybrids had a first meiotic division with uni-, bi-, and multivalents like that of tetraploid potatoes, illustrating introgression and dominance over desynapsis. Abnormal spindle orientations at second meiotic division, sporad types with reduced and unreduced cells and viable pollen occurred at various frequencies. Pollen fertility could not be predicted on the basis of pollen fertility of the fusion partners. Pollen sterility was partially due to abnormal chromosome numbers. Only the tetraploid *S. tuberosum* (+) desynaptic mutant produced normal amounts of viable seeds.

Key words: Fertility – Meiosis – Potato – Somatic hybrids – Unreduced gametes

Introduction

The aim of somatic hybridization between potato (*Solanum tuberosum*) clones and between potato and wild *Solanum* species has generally been to combine beneficial agronomic traits from both parents for crop improvement. The ideal genotype of potatoes, for example heterozygous tetraploidy, could be obtained by the fusion of different heterozygous di(ha)ploid protoplasts (Wenzel et al. 1979; Debnath and Wenzel 1987; Deimling et al. 1988). In general, somatic hybrids have a lowered fertility, which is a major problem of concern in sexual reproduction. Information about gametogenesis is thus indispensable though hardly available for potato somatic hybrids (Shepard et al. 1983; Austin et al. 1985; Ehlenfeldt and Helgeson 1987; Williams et al. 1990; Mattheij et al. 1992). In the paper presented here we describe the microsporogenesis of one hypertetraploid and two tetraploid somatic hybrids and of their diploid fusion partners.

Materials and methods

Plants

Three somatic hybrids obtained by Mattheij and Puite (1992) were cultured from tubers for three generations in a greenhouse under natural daylight (with supplementary light from high pressure iodine lamps, Philips HPI-T, 400 W, when the daylight intensity was lower than 100 W m^{-2}) with a 16-h photoperiod and 22°/18 °C day/night temperature. Their genotypes (for details see Mattheij and Puite 1992) are:

35-4-1a1 ($2n=4x=48$) = SVP11 (dihaploid *S. tuberosum*, $2n=24$) (+) SVP20 (dihaploid *S. phureja*, $2n=24$);
97-3A ($2n=4x=48$) = SVP104 (dihaploid *S. tuberosum*, $2n=24$) (+) SY7 (diploid hybrid from crosses *S. tuberosum* × *S. phureja*, $2n=24$);
RI-7A ($2n=4x=49$) = SVP103 (dihaploid *S. tuberosum*, $2n=24$) (+) SY7.

Cytology

For microsporogenesis, young flower buds were either pretreated with 0.2% α-bromonaphthalene for 5 h at 4 °C (to obtain better spreading of metaphase I configurations) or directly fixed in Carnoy (ethanol-glacial acetic acid, 3:1) at 4 °C for at least 16 h. Air-dried slides were made as follows: fixed anthers were rinsed in distilled water, macerated in 15% pectinase (v/v; Sigma P5146)/1.5% cellulase (w/v; Onozuka R-10) in citrate buffer pH 4.8 at 37 °C for 90 min and rinsed in distilled water. One anther was transferred to a clean slide, excess water was re-

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moved, and a drop of 60% acetic acid was added. The cells were suspended in the drop of acetic acid with the aid of fine (Wolfram) needles. The suspension was first surrounded with Carnoy and then two drops of Carnoy were put on top of the suspension. The slides were left to air-dry.

The slides were stained in 2% Giemsa in $1/15\ M$ Na_2HPO_4 for 15 min or C-banded as follows: the slides were incubated in (1) $0.2\ M$ HCl for 60 min at room temperature, then rinsed in distilled water, (2) 5% $Ba(OH)_2 \cdot 8\ H_2O$ for 15 min at room temperature, rinsed in running tap water, then distilled water and (3) $2 \times$ SSC, 30 min at 60 °C, then rinsed in distilled water. They were finally (4) stained in 2% Giemsa (Merck) in Sörensen's buffer pH 6.9 for 60 min, then rinsed in buffer and distilled-water. After air-drying, the slides were mounted, via xylol, in DePeX.

Pollen fertility was tested according to Alexander (1969). The buds and pollen were collected randomly over the year. Seasonal influences were not found, and quantitative data were thus pooled.

Results

Fusion partners

The meiotic divisions in the microsporocytes of the dihaploids SVP11, SVP103 and SVP104 of *S. tuberosum* and of the diploid *S. phureja* SVP20 conform to earlier descriptions of di(ha)ploid genotypes (Yeh et al. 1964; Höglund 1970; Mok and Peloquin 1975; Ramanna 1979; Veilleux et al. 1982; and in reviews by Swaminathan and Howard 1953; Magoon et al. 1962; Gottschalk 1976). Chromosome behaviour was normal, indicating the absence of structural heterozygosity for reciprocal translocations and paracentric inversions. During second meiotic division the two spindles, which are oriented normally at various angles, could be parallel, fused or tripolar (i.e. converged at one pole). Through normal and parallel spindles tetrads with four haploid cells were formed, dyads with two unreduced, diploid cells were formed through fused spindles and through tripolar spindles triads with one diploid and two haploid cells were formed. The frequencies of spindle and sporad types differed between the genotypes (Table 1). The origin of triads in SVP20 and of dyads in SVP11 escaped detection.

The meiosis of the interspecific hybrid SY7 was influenced by the homozygous presence of a gene for desynapsis (*dsds*) that has been described for other desynaptic mutants (Mok and Peloquin 1975; Ramanna 1983; Iwanaga 1984; Jongedijk and Ramanna 1988; Sadanandam 1991). First metaphases showed bivalents and univalents at various frequencies, and first anaphases showed a precocious movement to the poles, and the lagging and non-disjunction of chromosomes (Fig. 1a). Of the first telophases 68% ($n = 82$) obtained polar groups having different numbers of chromosomes with combinations of 11–13 till 5–19. After first telophase dyads with "unreduced" nuclei were formed. Some triads and tetrads likely originated from multipolar first divisions (Table 1).

Some pollen sterility was evident in all genotypes except SVP104 (Table 1). Large-sized fertile pollen, likely with an unreduced number of chromosomes, occurred in all five genotypes at frequencies lower than might be expected from the percentages of unreduced sporad cells.

Somatic hybrids

The somatic hybrids followed a meiosis pattern characteristic of one for tetraploid potatoes (cf. Swaminathan and Howard 1953; Magoon et al. 1962; Gottschalk 1976). Uni-, bi- and multivalents were observed from pachytene till first metaphase (Fig. 1b, c). Their frequencies at first metaphase show that, on average, at least half of the chromosomes were organized as bivalents and about a quarter as quadrivalents (Table 2). Penta-, hexa- and heptavalents were observed in RI-7A only (Fig. 1c), and all of the chromosomes of RI-7A could undergo pairing, meaning that the additional chromosome was involved. Heteromorphic quadrivalents and bivalents of the nucleolar chromosomes were regularly observed in all three somatic hybrids (Fig. 1b, c). In general the heteromorphic bivalents consisted of a nucleolar chromosome of SVP20 or SY7 characterized by a C-banded arm with a distinct satellite and one with a smaller arm and satellite from the fusion partner. It is notable that desynapsis was not observed in 97-3A and RI-7A and that 97-3A demonstrated stickiness at metaphase I, which was also observed in SVP104.

Anaphase bridges were rare ($< 1\%$) during both meiotic divisions. Through non-disjunction and, rarely, elimination of one or more chromosomes 30% of the first telophase nuclei in 35-4-1a1 ($n = 50$) did not reach the expected number of chromosomes; in 97-3A this was 14% and in RI-7A, 22%. The combinations with the maximum deviations were 22–26, 23–25, and 22–27, respectively. Thus, in RI-7A 39% of the telophase nuclei contained 24 chromosomes (Fig. 1d). The frequency at which non-disjunction occurred at second meiosis could not be established, and elimination occurred rarely ($< 1\%$). The percentages of second meiotic divisions with parallel, fused or tripolar spindles differed from those in the fusion parents (Table 1). 35-4-1a1 showed hardly any abnormal spindle orientation, and the other two somatic hybrids demonstrated a higher frequency of parallel spindles. The percentages of dyads and triads were higher than expected from the frequencies of fused and tripolar spindles. In RI-7A we observed first telophase sporocytes that differentiated into sporads and likely caused the high percentage of dyads. The meiosis of 35-4-1a1 and 97-3A resulted in a high production of tetrads and fertile reduced pollen (Table 1). RI-7A appeared to be

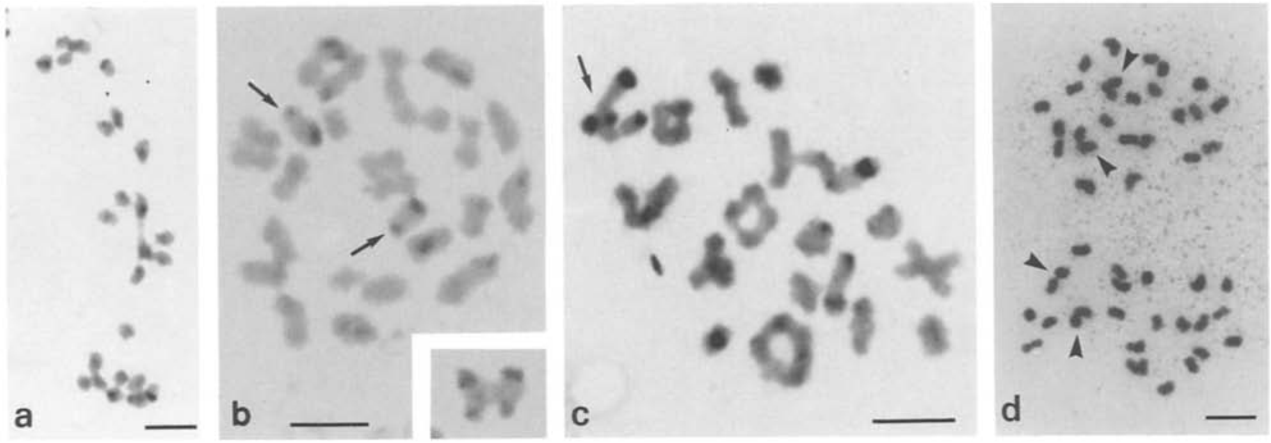

Fig. 1a–d. Giemsa-stained first microsporocytes of potato. **a** C-banded meta-anaphase I of desynaptic mutant SY7; **b** C-banded metaphase I with 2 I + 15 II + 4 IV, nucleolar chromosomes as heteromorphic bivalents (*arrows*) and as quadrivalent (*inset*) (97-3A); **c** C-banded metaphase I with 9 II + 2 III + 5 IV (*arrow* indicates nucleolar chromosomes) + 1 V (RI-7A); **d** Anaphase I with 25 (*top*) and 24 chromosomes, *arrowheads* indicate interconnected chromosomes from multivalents (RI-7A). *Bar*: 5 μm

Table 1. Spindle orientation at second division of microsporogenesis, sporad types, and pollen fertility of five genotypes and three somatic hybrids of potato

Genotype	Spindle orientation ($n>100$)				Sporad types ($n>200$)			Pollen ($n>500$)	
	% normal	% parallel	% fused	% tripolar	% dyad	% triad	% tetrad	% fertile	% fertile large-sized
SVP20	54	28	18	0	40	2	58	63	15
SVP11	85	0	0	15	10	31	59	10	19
SVP104	90	8	0	2	0	1	99	99	0.01
SY7	Absent				94	5	1	52	85
SVP103	88	6	2	4	9	11	80	14	49
35-4-1a1	97	3	0	0	2	11	87	70	1
97-3A	81	17	0	2	5	14	81	81	1
RI-7A	77	12	6	5	46	18	36	2	60

Table 2. Chromosome associations at metaphase I in microsporocytes of three genotypically different somatic hybrids of potato

Genotype	Number of metaphases	Range and mean frequency per metaphase of									
		Univalents		Bivalents		Trivalents		Quadrivalents		Penta-, Hexa-, Heptavalents	
35-4-1a1	39	0–10	1.00	9–22	17.82	0–2	0.23	0–6	2.67		
97-3A	30	0– 3	0.77	7–22	15.53	0–2	0.63	1–8	3.57		
RI-7A	30	0– 6	1.07	5–21	12.97	0–2	0.53	0–8	4.33	0–2	0.57

almost pollen sterile through degeneration after wall development.

To test whether RI-7A was also female sterile, it was crossed with 35-4-1a1 as the male parent (20 pollinations). Some fertility was demonstrated by the production of two berries with 4 and 6 viable seeds, respectively. Somatic hybrid 35-4-1a1 was self sterile and produced berries after crossing (two tests of 20 pollinations with different tetraploid genotypes) that fell off prematurely. After selfing, 97-3A produced four berries with about 200 viable seeds.

Discussion

Microsporogenesis of the three somatic hybrids illustrated the characteristics of tetraploid potato meiosis. *S. tuberosum* is considered an autotetrapoid. At metaphase I uni-, bi- and multivalents are present at various frequencies, with the bivalent frequency being generally close to 20 (Gill et al. 1987; reviews Swaminathan and Howard 1953, Magoon et al. 1962). The frequencies in tetraploid hybrids of *S. tuberosum* × *S. phureja* have been found to be rather similar (Prakken and Swaminathan 1952). The somatic hybrids of this study may all be considered to be interspecific hybrids of *S. tuberosum* and *S. phureja* because SVP20 supplied two *phureja* genomes and SY7 only parts of these genomes. Comparing the frequencies of the first metaphase chromosome associations of the three somatic hybrids (Table 2) with the frequencies of tetraploid potatoes and their tetraploid hybrids with *phureja*, it becomes evident that those of the fusion of *S. tuberosum* (SVP11) (+) *S. phureja* (SVP20) do not deviate and that also those of *S. tuberosum* (SVP104) (+) *S. tuberosum* × *S. phureja* hybrid (SY7), though with fewer bivalents, have been established before. An effect of the somatic hybrid nature itself on pairing, for instance through cytoplasmic factors of either parents, is apparently not present. RI-7A has 49 chromosomes, and the extra chromosome, likely added during cell culture, may well have influenced pairing behaviour, resulting in the production of more multivalents (Sybenga 1975). The formation of associations higher than quadrivalents is indicative of the presence of heterozygosity for a translocation. This translocation between a chromosome of SVP103 and SY7 may have taken place during the cell culture period because structural rearrangements occur regularly at this time (Karp 1990; Pijnacker and Sree Ramulu 1990). Gill et al. (1987) observed similar variant multivalent formation in protoclones of the 'Russet Burbank' potato.

The formation of bivalents and multivalents of chromosomes of both fusion partners points to introgression, which may be expected because of the homeologous genotypes (Ramanna and Hermsen 1979). That incorporation of the DNA of one potato clone into the gene pool of another indeed occurs in somatic hybrids has been shown by RFLP analysis of the sexual progeny of somatic hybrids of *S. tuberosum* and *S. brevidens* (Williams et al. 1990; Helgeson and Williams 1991). When SY7, homozygous for the recessive gene desynapsis (*ds*), was fused with SVP104 or SVP103, desynapsis no longer occurred in the somatic hybrid because of the dominance of (one or) two *DS* genes of the *tuberosum* genotypes, like the situation in sexual hybrids (Jongedijk and Ramanna 1988). Whether the high incidence of dyad formation in RI-7A was related to some expression of *ds* genes is not known.

Abnormal spindle orientation is a common feature in potato, though the genetics thereof has not yet been solved fully because of variable expression (Mok and Peloquin 1975; Ramanna 1979; Watanabe and Peloquin 1991). Each somatic hybrid demonstrated abnormal spindle orientation of the type(s) also observed in its fusion partners. The frequencies, however, were different and not influenced significantly by the fusion partners. The frequencies of sporads with unreduced nuclei were higher than expected, which cannot be explained. In RI-7A the high incidence of dyad formation was likely due to first telophases entering sporad formation. Some variation may be somaclonal, for Gill et al. (1987) showed that cell culture itself can affect meiotic restitution events.

The degree of pollen sterility of SY7 may be caused by unbalanced chromosome numbers, but the lower fertility of SVP20, SVP11 and SVP103 remains unexplained. Partial fertility is common among di(ha)ploid potatoes. The somatic hybrids 35-4-1a1 and 97-3A had a high pollen fertility as compared with the fusion partners. Unbalanced chromosome numbers that arose at first anaphase may account for most of the lower fertility. In contrast to 97-3A, 35-4-1a1 did not produce mature berries, which seemed to be a character of the reproducing plant. RI-7A appeared to be almost male and female sterile, though it differed in one fusion partner from 97-3A. Besides unbalanced genomes through aneuploidy and through deletions and duplications as a consequence of pairing between translocated chromosomes, pollen sterility must have been provoked by another factor. Pollen fertility of the three somatic hybrids can not, therefore, be predicted on the basis of pollen fertility of the fusion partners.

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