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ORIGINAL PAPER

Meiotic behavior of pollen mother cells in relation to ploidy level of somatic hybrids between *Solanum tuberosum* and *S. chacoense*

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Abstract Potato somatic hybrids obtained by protoplast fusion between *Solanum tuberosum* (4x) and *Solanum chacoense* (2x) were investigated for genome stability and meiotic behavior associated with the pollen viability in order to elucidate the mechanism influencing the fertility of the somatic hybrids. The ploidy level detections conducted in 2004 and 2007 demonstrated that 68 out of 108 somatic hybrids had their ploidy level changed to be uniform and euploidy after successive in vitro subcultures, which mainly occurred in octaploids, aneuploids, and mixoploids, while 74% hexaploids were still stable in their genome dosage in 2007. Different types of abnormal meiotic behavior were observed during the development of pollen mother cells (PMCs) including the formation of univalents, multivalents, laggard chromosomes, and chromosomal bridges, as well as triads and polyads. A higher proportion of abnormal meiosis seemed to be accompanied with a genome dosage higher than the hexaploids expected in this study. A significant positive correlation between defective PMCs and the number of small pollen grains and negative correlation between number of small pollen grains and pollen viability strongly suggested that abnormal meiosis could be a causal factor influencing the fertility of the somatic hybrids. The hexaploids with stable genome dosage and a certain level of fertility will have great potential in a potato breeding program.

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Introduction

The cultivated potato (*Solanum tuberosum* L.), which is a tetraploid ($2n = 4x = 48$), is grown worldwide and plays an important role in the global economy. Improvement of potato varieties through sexual crossing was first reported in 1807 and has been widely applied to potato breeding programs since the latter half of the nineteenth century (Bradshaw and Mackay 1994). To broaden the genetic base of the cultivated potato, a desirable strategy is to introgress new genes from wild species which possess useful traits such as tuber quality, disease resistance, environmental adaptability, etc. Unfortunately, potato germplasm from only a few species has been incorporated into modern cultivars because of the limited gene pool and the incompatibility of inter- and intra-specific hybridization which results in a narrow genetic background of modern varieties (Hermsen 1994).

A useful alternative method for crossing many wild species is by means of protoplast fusion which can avoid the sexual barriers (Wolters et al. 1994). This technology, known as somatic hybridization, has been successfully utilized by many breeders in several plant species (Butenko and Kuchko 1980; Puite et al. 1986; Laferriere et al. 1999; Binsfeld et al. 2001; Guo and Deng 2001; Patra et al. 2001; Thieme et al. 2008). Somatic hybrids of potatoes have been reported, for example, Austin et al. (1988) obtained hexaploid somatic hybrids with high-level resistance to soft rot between the non-tuber-bearing diploid wild species *S. brevidens* and tetraploid potato PI203900. Almost all somatic hybrids obtained by Novy and Helgeson (1994) by

protoplast fusion from *S. etuberosum* + *S. tuberosum* × *S. berthaultii* were significantly more resistant to potato virus Y (PVY) than their fusion parents. Laferriere et al. (1999) produced six somatic hybrid plants by protoplast fusion between *S. tuberosum* and *S. commersonii*. Five of the somatic hybrids had a resistance level to bacterial wilt similar to the wild fusion parent, suggesting that the resistance genes were successfully transferred from the wild species *S. commersonii* into the cultivar. Gavrilenko et al. (2003) obtained interspecific somatic hybrids between a dihaploid clone of *S. tuberosum* and the diploid species *S. etuberosum* by protoplast fusion, and several of the fusion hybrids and BC1 progeny did not show PVY infection. More recently, Thieme et al. (2008) obtained 63 hexaploid somatic hybrids of *S. tuberosum* + *S. tarnii* by protoplast electrofusion, and some of these hybrids were highly resistant to PVY or late blight. These examples, and others, demonstrated a potential for somatic hybridization to create pre-breeding or intermediate breeding material with introgressed genes from highly divergent species.

Utilization of somatic hybrids depends on their cross-ability with *S. tuberosum* cultivars in order to transmit desirable genes and eliminate the undesirable characteristics of the wild parent from the derived varieties. However, only some somatic hybrids are fertile. The mechanisms that result in such a large proportion of sterile somatic hybrids remain unknown although the change in genome dosage represented by variation of ploidy levels (Szczerbakowa et al. 2003) and abnormal tapetal development and meiotic defects (Conicella et al. 1997) have been reported.

A diploid wild species *S. chacoense* has resistance to bacterial wilt caused by *Ralstonia solanacearum* (Tung et al. 1990). In the present study, we investigated the genome stability, meiotic behavior, and the microspore development of somatic hybrids of *S. tuberosum* + *S. chacoense* in relation to the potential use of the somatic hybrids.

Materials and methods

Plant materials

One hundred and eight somatic hybrids were obtained from two different somatic fusion combinations between *Solanum tuberosum* and *S. chacoense*. The procedure for the production and identification of the somatic hybrids has been reported elsewhere (Cai et al. 2004). The materials used for fusion were *S. tuberosum* clones, $3^{\#}$ and $8^{\#}$ ($2n = 4x = 48$), from cv. *Zhongshu* 2 by pollination inducing gynogenesis and automatic chromosome doubling during in vitro subculture (kindly provided by Institute of Vegetables and Flowers of Chinese Academy of Agricultural Sciences) and C9701, a seeding-clone of *S. chacoense* obtained from its natural pollination seeds. The plants derived from $3^{\#}$ and $8^{\#}$ + C9701 were numbered 3C*i–j* and 8C*i–j*, respectively (*i* represents the *i*th callus, *j* represents the *j*th plant regenerated from callus *i*). For example, 3C1-1 is the first plant of the first callus from $3^{\#}$ + C9701, and 8C3-2 is the second plant of the third callus from $8^{\#}$ + C9701.

Ploidy analysis

The ploidy levels of the subcultures of the somatic hybrids and the fusion parents maintained in vitro for 3 years were measured to investigate the variation in genome dosage. Ploidy determination was conducted in a Partec flow cytometry (D248161 Münster Germany). Approximately 0.5 cm^2 of leaves from in vitro grown plantlets were chopped in a plastic Petri dish containing 0.4 ml Partec HR-A buffer (Partec high resolution nuclei extraction solution). After being filtered, the samples were stained for 3 min with 1.6 ml of HR-B buffer (Partec high-resolution DAPI staining solution), and the relative fluorescence of total DNA was measured. Each histogram was generated by analyzing at least 5,000 nuclei.

Meiotic analysis

Flower buds of somatic hybrids were collected and fixed in Carnoy (ethanol:glacial acetic acid 3:1 v/v) for 24–36 h at room temperature, followed by hydration in 90 and 80% ethanol separately for 10 min each, and finally stored in 70% ethanol at 4°C. Anthers were isolated from the flower buds and rinsed in distilled water for 5 min, incubated in 10% HCl for 10 min at 60°C, and in distilled water again for 5 min, and then stained with Carbol fuchsin. All phases of meiosis were investigated using Olympus light microscope, and photographs were taken with a computer-assisted cooled (CCD) camera.

Pollen viability analysis

The number of pollen grains per anther was determined using five anthers collected from at least three flowers. The anthers were crushed separately and mixed with 3.0 ml of distilled water, and counts made of at least five hematocytometer fields. Pollen viability of the somatic hybrids and their respective parents ($3^{\#}$, $8^{\#}$, and C9701) was determined by staining the pollen grains using acid magenta and in vitro pollen germination. Pollen grains from five anthers collected from different flowers were divided into two parts: one part was suspended in a drop (20 µl) of staining solution and distributed to three slides. After 5 min, the

viable pollens were counted. The other pollen grains were divided into three replicates and were cultured on the medium (20% sucrose + 5 mg/100 ml boric acid) for 4–8 h at 25°C in the dark before determining the germination.

Results

Changes in ploidy level of 108 somatic hybrids

The control diploid plant C9701 was set at about channel 50 (2x) of the flow cytometer. Compared with the diploid control, the peak channels of the detected materials showed their ploidy level. To check the genome variations, the ploidy level of the 108 somatic hybrids was determined in 2004 and of the in vitro propagated subcultures in 2007. The two showed a wide range of ploidy levels, from 4x to 8x, and over 60% of the somatic hybrids changed their ploidy levels in 2007 compared with the results in 2004 (Table 1).

There were 54 hexaploids obtained in 2004 which were more stable in ploidy level than other somatic hybrids since 40 of them were still hexaploids in 2007. Of the 14 hybrids that the genome dosage changed, one was detected as octaploid and 13 were mixoploids showing two peaks at 4x,6x, 4x,8x, 5x,6x, or 6x,8x (Table 1).

In contrast, the ploidy level of all ten octaploids detected in 2004 changed in 2007 to two hexaploids and eight mixoploids. It is noticeable that all of the mixoploids derived from octaploids showed two peaks between 3x and 7x except for 8C57-2 that had peaks at 6x and 8x (Table 1). The results showed that somatic hybrids with a higher ploidy level than expected hexaploid (derived from a 4x–2x fusion) showed reduced ploidy levels during successive in vitro subcultures.

All of the 27 mixoploids, i.e. hybrids without clear-cut peak(s) in the cytometry detection of the genome dosage, which composed the second large group of somatic hybrids in 2004 changed their ploidy levels in 2007. Fourteen of them changed to euploids including nine hexaploids, three heptaploids, one tetraploid, and one pentaploid, and the percentage of euploidy accounted for 51.9% of all the 27 somatic hybrids, and the rest 13 were with two clear peaks, except 3C5-2 which showed three peaks (4x,6x,8x) (Table 1). Table 1 also showed that 12 of 17 aneuploids, which had a peak not reflecting a certain euploid in 2004, became euploids in 2007 including four pentaploids, seven hexaploids, and one heptaploid. But five of the aneuploids were observed as mixoploids having two peaks during the genome dosage detection in 2007. The results demonstrated a trend of uniformization or euploidization of the hybrids with complex genome dosages in continuous in vitro propagations.

Meiotic behaviors of somatic hybrids

Among the 108 somatic hybrids, 63 (58.3%) produced flower buds, but only 40 hybrids could flower normally including 27 hexaploids, 2 pentaploids, 3 heptaploids, and 8 mixoploids. Some hybrids with different ploidy levels were chosen for meiotic analysis and pollen viability test.

The meiosis of the potato pollen mother cell (PMC) occurred in buds from 1.0 to 2.5 mm in length. Those smaller than 2.0 mm had pre-dyad phases, and those longer than 2.5 mm had post-tetrad phases. Division of cytoplasm did not happen during the first meiosis, and the tetrad had a tetrahedron conformation. Compared with the fusion parents, especially the wild diploid clone C9701, the meiotic behavior was complex in the PMCs of the somatic hybrids, and abnormal chromosomal behavior was found in each stage of meiosis.

The ubiquity of chromosomal abnormalities and the difficulty in identifying homoeologous pairing clearly have focused our attention on univalent or multivalent configurations at diakinesis and laggard chromosomes and chromosomal bridges in other meiotic phases. There were some different multivalent configurations at diakinesis, such as “8” type (Fig. 1a), “X” type (Fig. 1b), and ring and line type (Fig. 1c). All of these abnormalities revealed that these chromosomes were partial homoeologous. At the same stage, there were also many univalents that could not pair with any chromosome (Fig. 1a–c). Laggard chromosomes were observed during the phases from metaphase I to telophase II (Fig. 1d, f, i, k, n, o, q). Chromosome bridges were also observed in anaphase I, and telophase I and II (Fig. 1g, j, s). The micronuclei in the tetrads (Fig. 1u) were formed by the concentration of the laggard chromosomes. The formation of dyads (Fig. 1w) was related to the absence of the second meiotic division in microsporocytes. Some other meiotic aberrations were observed at different meiotic phases. For example, homologous chromosomes did not separate entirely (Fig. 1e); chromosomal behavior of both poles was not at the same phase, one pole at anaphase I, the other pole at telophase I (Fig. 1h), or one pole at prophase II, the other pole at metaphase II (Fig. 1l); PMCs in the same anther could be at different phases (Fig. 1v); three micronuclei at telophase II were not of the same size (Fig. 1r); chromosome number was less than that expected (Fig. 1m); and the arrangement of tetrads could be at right angles at anaphase II (Fig. 1p) or in a line (Fig. 1t).

Cytological analysis of the cultivated parent $8^{\#}$, especially the wild species C9701, revealed only a few abnormalities. The percentage of abnormal PMCs in parent $3^{\#}$ was 20.44% which was higher than that of $8^{\#}$ (12.12%) and C9701 (1.29%). At diakinesis, most of the paring homologous chromosomes of the diploid fusion parent C9701

Table 1 Ploidy level of potato somatic hybrids and the respective parent genotypes $3^{\#}$, $8^{\#}$, and *S. chacoense* (C9701) detected by the flow cytometry in 2004 and 2007

Somatic hybrids/parents	2004	2007	Somatic hybrids/parents	2004	2007	Somatic hybrids/parents	2004	2007
Parents			8C38-1	6x	6x	8C48-2	8x	6x
C9701	2x	2x	8C42-1	6x	6x	8C65-1	8x	6x
$3^{\#}$	4x	4x	8C42-2	6x	6x	3C10-1	8x	5x,6x
$8^{\#}$	4x	4x	8C42-3	6x	6x	3C25-1	8x	3x,6x
Somatic hybrids			8C55-1	6x	6x	3C25-3	8x	4x,6x
3C1-1	6x	6x	8C62-1	6x	6x	8C13-1	8x	5x,7x
3C1-2	6x	6x	8C75-1	6x	6x	8C44-1	8x	5x,6x
3C1-3	6x	6x	8C29-3	6x	8x	8C44-3	8x	5x,6x
3C1-5	6x	6x	3C3-5	6x	4x,6x	8C57-2	8x	6x,8x
3C2-1	6x	6x	8C6-4	6x	4x,6x	8C60-1	8x	6x,7x
3C2-3	6x	6x	8C31-1	6x	4x,6x	8C1-2	Mixoploid (mainly 6x)	4x
3C3-2	6x	6x	8C68-1	6x	4x,8x	8C66-1	Mixoploid	5x
3C3-3	6x	6x	3C35-3	6x	5x,6x	3C12-1	Mixoploid	6x
3C3-6	6x	6x	3C1-4	6x	5x,6x	3C12-2	Mixoploid	6x
3C14-1	6x	6x	3C3-8	6x	5x,6x	3C15-1	Mixoploid	6x
3C18-1	6x	6x	8C7-1	6x	5x,6x	8C9-1	Mixoploid	6x
3C19-1	6x	6x	8C17-1	6x	5x,6x	8C15-1	Mixoploid	6x
3C27-1	6x	6x	3C35-2	6x	5x,6x	8C20-1	Mixoploid (mainly 8x)	6x
3C30-1	6x	6x	8C19-1	6x	5x,6x	8C26-1	Mixoploid	6x
3C30-2	6x	6x	8C55-3	6x	5x,6x	8C28-1	Mixoploid	6x
3C33-2	6x	6x	3C7-1	6x	6x,8x	8C43-1	Mixoploid	6x
3C35-1	6x	6x	3C8-1	Aneuploid (4x–6x)	5x	3C10-2	Mixoploid (mainly 4x)	7x
3C37-1	6x	6x	3C8-4	Aneuploid (4x–6x)	5x	8C17-3	Mixoploid (mainly 6x)	7x
8C6-5	6x	6x	8C53-1	Aneuploid 4x–6x	5x	8C22-1	Mixoploid	7x
8C8-1	6x	6x	3C3-7	Aneuploid (4x–6x)	6x	8C33-2	Mixoploid	4x,5x
8C10-1	6x	6x	3C8-2	Aneuploid 4x–6x	6x	3C34-1	Mixoploid	4x,6x
8C10-2	6x	6x	3C8-3	Aneuploid (4x–6x)	6x	8C35-1	Mixoploid	4x,6x
8C11-1	6x	6x	3C29-2	Aneuploid (4x–6x)	6x	3C36-1	Mixoploid	5x,6x
8C11-2	6x	6x	8C1-1	Aneuploid (4x–6x)	6x	8C48-1	Mixoploid (mainly 6x)	5x,6x
8C11-3	6x	6x	8C71-1	Aneuploid (4x–6x)	6x	8C54-1	Mixoploid	5x,6x
8C14-1	6x	6x	3C29-1	Aneuploid (4x–6x)	4x,6x	8C2-2	Mixoploid	5x,7x
8C16-1	6x	6x	3C33-1	Aneuploid (4x–6x)	4x,6x	8C27-1	Mixoploid	5x,7x
8C17-2	6x	6x	8C67-1	Aneuploid (6x–8x)	5x	8C56-1	Mixoploid	5x,7x
8C19-2	6x	6x	8C46-1	Aneuploid (6x–8x)	6x	8C57-1	Mixoploid (mainly 6x)	6x,7x
8C25-2	6x	6x	8C50-2	Aneuploid (6x–8x)	7x	8C23-1	Mixoploid (mainly 8x)	6x,8x
8C25-3	6x	6x	3C21-1	Aneuploid (6x–8x)	5x,7x	8C36-2	Mixoploid (mainly 8x)	6x,8x
8C30-1	6x	6x	8C43-2	Aneuploid (6x–8x)	6x,7x	3C5-2	Mixoploid	4x,6x,8x
8C31-2	6x	6x	8C50-1	Aneuploid (6x–8x)	6x,7x			

could form normal bivalents, and little meiotic abnormality revealed its diploid nature ($2n = 2x = 24$). In tetraploid fusion parents, the chromosome configuration at diakinesis was not regular, and bivalent, univalent, or multivalent could be observed. In the somatic hybrids, it was difficult to analyze the chromosome pairing configuration because of the more numerous chromosomes and their complex chromosome pairing. The general characteristics of the meiotic behavior of selected somatic hybrids are summarized in Table 2. The total abnormal PMCs of all the observed somatic hybrids were higher than their fusing parents. The proportion of abnormal PMCs had a significant linear correlation with abnormal meiosis occured at each meiotic phase, except for the triads formed at telophase II. The significance could be ordered by the values of the determination coefficients which arranged as polyads

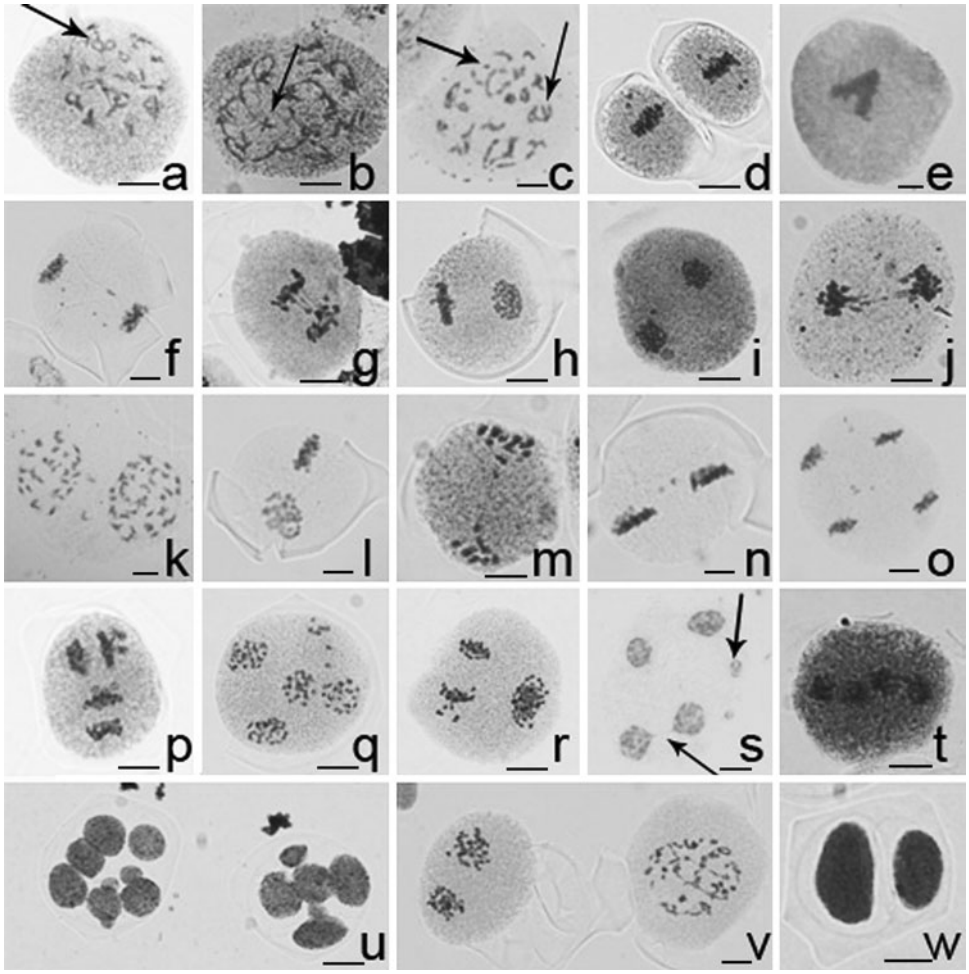

Fig. 1 Photomicrographs of microsporogenesis of the somatic hybrids ($3^{\#}$ + C9701, $8^{\#}$ + C9701). **a–c** Chromosome pairing at diakinesis showing "8" type, "X" type, line or ring multivalent and univalent chromosomes (*arrows*), **d** laggard chromosome at metaphase I, **e** partial chromosome did not separate, **f** laggard chromosome at anaphase I, **g** chromosome bridge at anaphase I, **h** chromosomal behavior of each pole was not at the same phase at anaphase I, **i** laggard chromosome at telophase I, **j** chromosome bridge at telophase I, **k** laggard chromosome at prophase II, **l** chromosomal behavior of each pole was not at the same phase at prophase II, **m** few chromosomes at both poles, **n** laggard chromosome at metaphase II, **o** laggard chromosome at anaphase II, **p** vertical spindle at anaphase II, **q** laggard chromosome at telophase II, **r** triad, **s** tetrad with chromosome bridge and micronucleus (*arrows*), **t** tetrad arrange in line, **u** different number micronucleus, **v** PMCs at different phases in a same anther, and **w** dyad result in cytoplasm division after the first meiosis. *Bars* 10 μm

formed at telophase II > abnormal meiosis from anaphase II to telophase II > laggard chromosomes at metaphase I > abnormal meiosis from anaphase I to metaphase II. However, there was no much difference in percentage of abnormal PMCs among the selected somatic hybrids.

Pollen characters

The diameter of normal size pollen grains was about 20 μm. Most of the normal pollen grains were round and plump, and they could be stained by aceto-carmine (Fig. 2a–c). Small and wizened pollen grains were the direct result of the abnormal meiosis and could not be stained or germinated (Fig. 2d).

There was a significant positive correlation in pollen viability between the two methods of testing, staining (S), and germination (G) ($S = 21.02 + 1.507G$, $r^2 = 0.519$, $n = 17$, $P < 0.01$). Therefore, only the data for staining is tabulated in Table 3. The pollen stainability of the two cultivated parents was 47.0% ($3^{\#}$) and 49.9% ($8^{\#}$), lower than the wild species *S. chacoense* (99.5%). The pollen stainability among the somatic hybrids tested presented a wide range; 8C50-1(mixoploid) had the lowest pollen viability (0.7%), and 3C33-2 (hexaploid) had the highest (79.7%).

The percentage of small pollen grains ($d \approx 10$ μm) from each somatic hybrid was analyzed and shown in Table 4. The mixoploids had more small pollen grains than

Table 2 Abnormal meiotic behavior of part somatic hybrids and the respective parent genotypes 3#, 8#, and *S. chacoense* (C9701)

Genotypes	Ploidy level	LCM I (%)	APA I–M II (%)	APA II–T II (%)	Triad-T II (%)	Polyad-T II (%)	Total Abnormal PMCs (%)
C9701	2x	3.54	0.73	0.00	0.00	0.00	1.29
3#	4x	31.82	15.19	19.86	0.00	0.00	20.44
8#	4x	22.46	17.31	4.25	0.00	0.00	12.12
3C3-3	6x	45.10	13.30	4.75	2.67	34.42	28.52
3C33-2	6x	30.33	69.23	9.02	4.10	9.84	30.86
8C8-1	6x	19.57	39.10	13.14	0.00	2.29	24.86
8C20-1	6x	56.86	40.22	61.54	0.00	92.31	55.13
8C30-1	6x	41.86	20.62	15.56	0.89	0.89	21.10
8C50-2	7x	46.06	11.28	0.00	6.73	16.35	23.92
3C35-3	5x,6x	11.36	39.13	1.67	0.21	34.10	28.35
8C50-1	6x,7x	48.00	36.73	22.71	0.97	14.01	39.62
Correlation to the total abnormal PMCs (y) as $y = a + bx$	a	6.57	14.13	17.59	25.39	18.61	–
	b	0.60	0.43	0.61	0.44	0.40	–
	r^2	0.52**	0.36*	0.60**	0.01	0.63**	–

LCMI laggard chromosome at metaphase I; *APA I–M II* abnormal PMCs at anaphase I to metaphase II resulted from the abnormal meiosis as indicated in Fig. 1; *APA II–T II* abnormal PMCs at anaphase II to telophase II resulted from the abnormal meiosis as indicated in Fig. 1; *Triad-T II* triads formed at telophase II; *Polyad-T II* polyads formed at telophase II; *Total abnormal PMCs* sum of the abnormal PMCs observed at whole meiotic process; the percentage of it is calculated as total number of abnormal PMCs/total number of PMCs investigated × 100/100

* Significant at $P < 0.05$

** Significant at $P < 0.01$

the hexaploids or pentaploids and their fusion parents except for 8C50-2. There was a strong positive correlation between the proportion of small pollen grains (P_s) and the percentage of abnormal PMCs (P_a) ($P_s = -1.087 + 0.667P_a$, $r^2 = 0.719$, $n = 11$, $P < 0.01$). A significant negative correlation between the pollen stainability (P_{stain}) and the percentage of small pollen grains (P_s) was observed ($P_{stain} = 71.618 - 2.000P_s$, $r^2 = 0.412$, $n = 9$, $P < 0.05$). As expected, a negative linear regression between the proportion of abnormal PMCs and the pollen stainability existed as $P_{stain} = 81.253 - 1.559P_a$ ($r^2 = 0.462$, $n = 9$, $P < 0.05$).

There were 35 hybrids which produced pollen grains. The number of grains per anther varied between 8.5×10^4 and 5.7×10^5/anther (partial data are shown in Table 4 as examples). The amount of pollen produced by most hybrids was less than that of the parents.

Discussion

Because of the variable and instable genomes of the interspecific potato somatic hybrids, the ploidy level tested by the flow cytometry could give primary information on genome size (Szczerbakowa et al. 2003). The present research demonstrated that successive subcultures in vitro could lead to remarkable change in ploidy level of the potato somatic hybrids, but it differed among hybrids with different original ploidy levels. Our results showed that most of the expected hexaploids (from a 4x–2x fusion) were stable, whereas the ploidy level changed in 100% of mixoploids, aneuploids, and octaploids with a trend to a uniform or stable ploidy level (Table 1). Similar results were reported by Menke et al. (1996). A higher proportion of ploid instability in mixoploids and those with larger genome dosage might result from the loss of fragments following genomic rearrangements or whole chromosome block elimination. The changes might happen more frequently in these somatic hybrids. Aversano et al. (2009) also reported that the loss of ISSR fragments was the most frequent event in regenerated plants of different *Solanum* genotypes with varied somatic chromosome numbers, and the genotype had obvious effects on the integrity of the genome under in vitro conditions.

Complexity in ploidy level and its uncertain changes in somatic hybrids may result in diverse meiotic behaviors, especially when the fusion parents were remotely related. The abnormal chromosome behavior in the PMCs of somatic hybrids at different meiosis phases, such as univalent, multivalent, laggard chromosome, and different number of micronuclei, were more complex than in their fusing parents (Fig. 1). Different proportions of abnormal meiosis were observed among somatic hybrids with varied sets of chromosomes. Similar results have been reported in other potato somatic hybrids, such as *S. tuberosum* + *S. phureja* (Pijnacker et al. 1992) and *S. commersonii* +

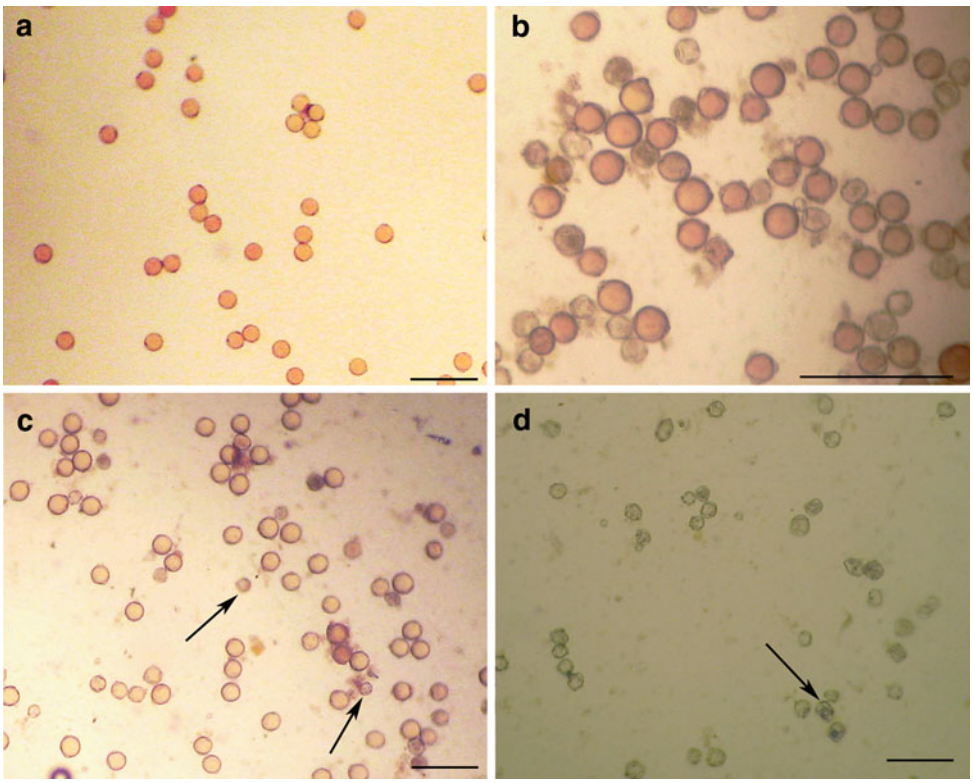

Fig. 2 Pollen characters of the fusion parents and two somatic hybrids: **a** C9701, **b** 3#, **c** 3C33-2 small pollen grains (*arrows*), and **d** 8C50-1 wizened and sterile pollen grains (*arrow*). *Bars* 80 μm

Table 3 Comparison of percentage viability of pollen grains determined by acid magenta staining for selected potato somatic hybrids and the fusion parents 3#, 8#, and *S. chacoense* (C9701) (the significance was analyzed by *t* test)

	C9701	3#	8#	3C3-3	3C33-2	8C20-1	8C50-2	3C35-3	8C50-1
C9701	99.5	52.5**	49.6**	80.5**	19.8**	95.1**	96.0**	46.5**	98.8**
3#		47.0	2.9	27.9**	32.7**	42.5**	43.5**	6.0	46.3**
8#			49.9	30.9**	29.8**	45.4**	46.4**	3.1	49.2**
3C3-3				19.0	60.7**	14.6**	15.5**	34.0**	18.3**
3C33-2					79.7	75.3**	76.2**	26.7*	79.0**
8C20-1						4.5	1.0	48.5**	3.7*
8C50-2							3.5	49.5**	2.8*
3C35-3								53.0	52.3**
8C50-1									0.7
Pollens/anther	4.4×10^5	4.4×10^5	/	4.2×10^5	/	1.6×10^5	2.9×10^5	1.6×10^5	3.0×10^5
Ploidy level	2x	4x	4x	6x	6x	6x	7x	Mix	Mix

The cross met by the same genotype is percentage of pollen viability of that genotype and that met by two different genotypes is the difference of the pollen viabilities between them

* Significant at $P < 0.05$

** Significant at $P < 0.01$

S. tuberosum (Conicella et al. 1997). Meiotic aberrations were found also in the pollen mother cells of somatic hybrids of *Brassica* + *Sinapis* (Gaikwad et al. 1996), passion fruit (Barbosa and Vieira 1997), potato + tomato (Garriga-Calderé et al. 1998; Chetelat and Meglic 2000), Citrus (Bosco et al. 1999; Chen et al. 2004), sunflower (Binsfeld et al. 2001), and tomato (Gavrilenko et al. 2001). There was no obvious difference of meiotic aberrations among different ploidy level hybrids. This implied that there were other factors that could influence the development of microsporocytes, such as the homology of the chromosomes in somatic hybrids, the environment and so on. The appearance of different numbers of univalents suggested the lack of homology of different chromosomes, or this could result from an early separation of bivalents. Different kinds of bivalents (open rod and end–end)

Table 4 The percentage of small pollen grains of selected potato somatic hybrids and their respective parent genotypes $3^{\#}$, $8^{\#}$, and *S. chacoense* (C9701)

Genotypes	Ploidy	Pollens counted	Small pollens	Percentage of small pollens
C9701	2x	1,390	13	0.9
$3^{\#}$	4x	3,820	209	5.5
$8^{\#}$	4x	2,357	244	10.4
3C3-3	6x	1,275	237	18.6
3C33-2	6x	5,914	1,084	18.3
8C8-1	6x	8,543	2,264	26.5
8C20-1	6x	3,785	1,230	32.5
8C30-1	6x	1,381	118	8.5
8C50-2	7x	3,199	294	9.2
3C35-3	Mix	7,270	1,044	14.4
8C50-1	Mix	4,406	1,504	34.1

showed partial homology between the paired or parental chromosomes (Jan 1997). The formation of multivalents may favor gene exchange between fusion parents and could suggest intergenomic homology (Barbosa and Vieira 1997), or another effect on the fertility of the hybrids. These phenomena might result in chromosome elimination which was probably ascribed to different cell cycle of the remote parents, mutations during fused cell growth and regeneration after protoplast fusion and interaction between cytoplasmic and nuclear genomes in somatic hybrids (Wang et al. 2007). The highest proportion of polyads was found in the hexaploid 8C20-1 (Table 2), and this phenomenon might result from the presence of multivalents or laggards, which could contribute to the formation of small pollen grains (32.5%; Table 4).

Meiotic abnormalities in somatic hybrids were the results of genome instability or chromosomal elimination, which contributed to partial fertility or complete sterility. Pollen viability was related to the abnormal meiosis caused by deficient pairing, non-separation, or asymmetrical chromosome distribution (Singh 1992). These suggestions are supported by our findings of high frequency of abnormal chromosome behaviors throughout the whole meiotic process (Fig. 1; Table 2). Direct relationships observed in present research between defected PMCs and the formation of small pollen grains and pollen viability provide persuasive evidences that abnormal meiosis occurred in potato somatic hybrids, especially those having higher genome dosage than the sum of the fusion parents, is a causal factor influencing the fertility of the hybrids. Although the mechanism of hexaploids to maintain a relative higher genome stability needs further investigation, its potential used in potato breeding program to introgress genes from distant species to *S. tuberosum* has been highlighted elsewhere (Gavrilenko et al. 2003; Thieme et al. 2008).

Present results allow us to conclude that successive in vitro subcultures can lead to the change in ploidy level of potato somatic hybrids toward uniform and euploidy. Abnormal meiotic behaviors are associated with complexity in chromosome recombination in the somatic hybrids. The expected hexaploids derived from a 4x–2x protoplast fusion have a higher stability in ploidy level than others with higher genome dosages. With a possible cross compatibility in backcrossing, these hybrids may have great potential in potato breeding programs.

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