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Occurrence of 2n pollen and *ps* gene frequencies in cultivated groups and their related wild species in tuber-bearing *Solanums* *

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Summary. The gene frequency for parallel spindles (*ps*) was estimated from the frequency of plants producing 2n pollen in three cultivated groups: 2x Phureja (phu), 2x Stenotomum (stn), and 4x Andigena (adg), as well as in four related wild taxa: 2x *Solanum brevicaule* (brc), 2x *S. sparsipilum* (spl), 4x *S. gourlayi* (grl) and 4x *S. gourlayi-S. infundibuliforme* hybrids (grl-ifd). Plants with more than 1% large pollen were considered as 2n pollen producers. Observations of meiosis in a sample of 2n pollen-producing plants indicated that parallel spindles is the mechanism of 2n pollen formation. The number of plants with 2n pollen among the total examined was 228 plants (15.5%) of 1,473 in 2x spl, 31 (26.7%) of 116 in 2x brc, 92 (17.4%) of 528 in 2x stn, 665 (22.1%) of 3,008 in 2x phu, 731 (51.4%) of 1,421 in 4x adg, 591 (41.2%) of 1,436 in 4x grl, and 36 (64.3%) out of 56 in 4x grl-ifd. The *ps* gene frequencies assuming Hardy-Weinberg equilibrium were: 0.393 for 2x spl, 0.462 for 2x brc, 0.417 for 2x stn, 0.470 for 2x phu, 0.847 for 4x adg, 0.801 for 4x grl, and 0.895 for 4x grl-ifd. Twenty-five adg clones were randomly selected from a large population and were crossed with 2x clone W5295.7, which produces 2n pollen by parallel spindles (*ps*). The 4x progenies from 4x × 2x crosses were used to determine the genotypes at the *ps* locus by screening 10–20 plants in each family for 2n pollen. Based on chromosome segregation at the *ps* locus, 9, 14, 1, and 1 clones were nulliplex, simplex, simplex or duplex, and duplex, respectively. The frequency of the *ps* gene in the adg population was estimated to be 0.825 and 0.815 for chromosome and chromatid segregation, respectively. The high frequencies of 2n pollen and the *ps* gene in cultivated 2x and 4x groups, and in wild taxa closely related to them, provide evidence for sexual polyploidization in the tuber-bearing *Solanums*.

Key words: Tuber-bearing *Solanums* – Sexual polyploidization – 2n pollen – Parallel spindles – Gene frequency

Introduction

2n gametes in the tuber-bearing *Solanums* have been reported in many species involving several taxonomic series and different ploidy levels (Camadro and Peloquin 1980; den Nijs and Peloquin 1977; Hermundstad 1986; Quinn et al. 1974). The cytological mechanisms of 2n gamete formation have been identified in microsporogenesis (Höglund 1970; Iwanaga 1984; Johnston et al. 1986; Mok and Peloquin 1975a, b; Matsubayashi 1981a; Okwuagwu 1981; Ramanna 1974, 1979, 1983; Souter et al. 1980) and in megasporogenesis (Iwanaga and Peloquin 1979; Jongedijk 1985; Parrot and Hanneman 1988; Stelly and Peloquin 1986; Werner and Peloquin 1987). The genetic control of 2n egg formation is not known, but that of 2n pollen has been well established. The three main mechanisms of 2n pollen formation are parallel spindles (*ps*), premature-cytokinesis (*pc*-1, *pc*-2), and synaptic mutants (*sy*-2, *sy*-3, *sy*-4). Those are simply inherited recessives, and are characterized by variability in penetrance and expressivity (Iwanaga 1984; Johnston et al. 1986; Mok and Peloquin 1975b; Okwuagwu 1981).

Parallel spindles is present in diploid taxa in several taxonomic series (Camadro and Peloquin 1980; den Nijs and Peloquin 1977; Hermundstad 1986; Höglund 1970; Johnston et al. 1986; Ramanna 1979; Quinn et al. 1974), in haploids of group *Tuberosum* and haploid *Tuberosum-*

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wild species hybrids (Hermundstad 1986; Iwanaga 1984), and in 4 × cultivars (den Nijs and Peloquin 1977; Iwanaga and Peloquin 1982; Mok and Peloquin 1975 b; Souter et al. 1980). Thus, it appears that parallel spindles is the most common mechanism for 2n pollen formation in tuber-bearing *Solanums*. Premature cytokinesis occurs in several clones of *S. chacoense,* Phureja-haploid *Tuberosum* hybrids, Phureja and tetraploid progenies from crosses between 4x USA *Tuberosum* cultivars, and a 2x clone that is a Phureja-haploid *Tuberosum* hybrid (Iwanaga and Peloquin 1982; Matsubayashi 1981 a; Mok and Peloquin 1975 a, b; Ramanna 1974). Synaptic mutants, variation in meiosis which affects chromosome pairing and/or chiasma formation, have been identified in Phureja, haploids of *Tuberosum,* Phureja-haploid *Tuberosum* hybrids, haploid *Tuberosum*-wild species hybrids, and *S. commersonii* (Iwanaga 1984; Johnston et al. 1986; Okwuagwu 1981; Ramanna 1983).

The frequency of the *ps* gene has been reported in several diploid species and tetraploid cultivars (Camadro and Peloquin 1980; Hermundstad 1986; Iwanaga and Peloquin 1982). However, extensive information on the occurrence of 2n pollen formed by parallel spindles is not available for large populations of most taxa.

According to recent evolutionary hypotheses, the primitive cultivated tetraploid Andigena either arose from a hybrid between the diploid cultivated Stenotomum and the weedy diploid species, *Solanum sparsipilum* (Cribb and Hawkes 1986, Hawkes 1956), or evolved from a hybrid between the cultivated diploids Phureja and Stenotomum (Matsubayashi 1981 b; Swaminathan and Magoon 1961), or from hybrids between the diploid cultivated groups and a diploid wild species other than *S. sparsipilum* (Grun 1979).

The finding of 2n gametes in putative ancestral species of Andigena suggests that the mode of origin of polyploidy in Andigena is sexual polyploidization (den Nijs and Peloquin 1977). Iwanaga and Peloquin (1982) provided evidence of sexual polyploidization based on the high frequency of *ps* in USA 4x *Tuberosum* cultivars, which are most likely descendants of Andigena. Thus, if sexual polyploidization from diploids to tetraploids occurred through functioning of 2n pollen from parallel spindles, one should find 2n pollen and a relatively high frequency of *ps* in both diploid and tetraploid populations.

This investigation was undertaken to determine the occurrence and frequency of 2n pollen, and the *ps* gene frequencies in a large number of Plant Introductions of the cultivated groups, Andigena, Phureja, and Stenotomum, and the wild species, *S. brevicaule, S. gourlayi,* and *S. sparsipilum,* which are closely related to the cultivated groups in terms of taxonomy, genetics, and cytogenetics.

Materials and methods

The name of the taxa and numbers of Plant Introductions (PIs) and plants used for each taxon are listed in Table 1. Hereafter, the following abbreviations will be used for *S. brevicaule* (brc), *S. sparsipilum* (spl), *S. tuberosum* group Stenotomum (stn), group Phureja (phu), group Andigena (adg), *S. gourlayi* (grl) and *S. gourlayi-S. infundibuliforme* hybrid (grl-ifd), respectively.

Table 1. Number of Plant Introductions (PIs) with 2n pollen, frequency of plants with 2n pollen and cytology of samples from 2n pollen-producing plants among four 2x and 4x three taxa. ps, pc and sy: parallel spindles, premature cytokinesis and aberrant synapsis, respectively

Taxon	No. of PIs		No. of plants			Cytology[a]			
	Total	2n pollen	Total	Male fertile	2n pollen (%)	Total	ps	pc	sy
S. brevicaule (brc, 2x)	2	2	120	116	31 (26.7)	20	16	2	2
S. sparsipilum (spl, 2x)	51	44	1,505	1,473	228 (15.5)	46	46	0	0
S. tuberosum									
Group Phureja (phu, 2x)	114	107	3,092	3,008	665 (22.1)	123	122	1	0
Group Stenotomum (stn, 2x)	19	16	541	528	92 (17.4)	16	16	0	0
Group Andigena (adg, 4x)	76	75	1,506	1,421	731 (51.4)	85	84	1	0
S. gourlayi (grl, 4x)	63	59	1,454	1,436	591 (41.2)	65	65	0	0
S. gourlayi–S. infundibuliforme hybrid (grl-ifd, 4x)	5	4	56	56	36 (64.3)	4	4	0	0

[a] Microsporogenesis observed in sample plants from 2n pollen-producing plants

Table 2. Expected segregation ratios for 2n pollen-producing plants in the progeny of 4x × 2x I (*ps/ps*) crosses, based on either chromosome or chromatid segregation in the 4x clones

Genotype of tetraploid	Chromosome segregation		Chromatid segregation	
	Normal:2n producer		Normal:2n producer	
pspspsps	0	:1	0	:1
Pspspsps	1	:1	0.87	:1
PsPspsps	5	:1	3.7	:1
PsPsPsps	1	:0	27	:0
PsPsPsPs	1	:0	1	:0

Seeds of the taxa were obtained from the Inter-Regional Potato Introduction Project (IR-1), at Sturgeon Bay, Wisconsin. Those materials were grown at Rhinelander and Hancock Research Stations in 1986 and 1987, and in the greenhouse at Madison in 1987.

The determination of 2n pollen frequency was done according to Iwanaga and Peloquin (1982), using acetocarmine glycerol for pollen staining. The plants with more than 1% large-size pollen were regarded as 2n pollen-producing plants (hereafter, 2n pollen plants) and those with less than 5% pollen stainability were considered male sterile. The number of plants examined for 2n pollen per PI varied from 20–36.

A diploid W5295.7 (I), that is a Phureja-haploid *Tuberosum* hybrid and produces 2n pollen by parallel spindles (Mok and Peloquin 1975a, b), was used as a tester in estimating the frequency of 2n pollen plants in the 4x progenies from tetraploid × I crosses. Twenty-nine plants of Andigena and two of 4x grl were randomly selected and were pollinated with this tester. The hybrid seedlings were grown in the greenhouse at Madison or in the field at Rhinelander in 1987. Occurrence of 2n pollen in those hybrids was determined from pollen samples by acetocarmine glycerol staining.

The frequency of 2n pollen plants in each family was used to estimate the genotype of each tetraploid parent as to the *ps* gene using the procedures of Iwanaga and Peloquin (1982), under an assumption that all 2n pollen was produced by parallel spindles. For example, if a tetraploid is nulliplex (*pspspsps*), all 4x hybrid progeny will produce 2n pollen, if simplex (*Pspspsps*) 50% of the progeny are expected to produce 2n pollen based on random chromosome segregation. Table 2 indicates the expected segregation ratios for 2n pollen production in the progeny of the tetraploid × I crosses. Since the location of the *ps* locus in relation to the centromere is not known, the segregation ratios were tested against both chromosome and chromatid segregations.

Results

The results of screening 2n pollen plants are summarized in Table 1. Chi-square tests for homogeneity of the frequency of 2n pollen plants indicated no significant difference in the same PI between years and between locations (Watanabe 1988). Thus, the data from different years and locations was pooled in Table 1.

The frequency of 2n pollen plants was very high for both diploid and tetraploid taxa, ranging from 15.5% in 2x spl (lowest) to 64.3% in 4x grl-ifd (highest). It should be noted that the frequency of 2n pollen plants in tetraploids was about twice as high as that in diploids.

The percentage of 2n pollen in all 2n pollen plants was determined. In both diploids and tetraploids, most 2n pollen plants had from 5% to 30% 2n pollen. Plants having more than 50% 2n pollen were rare. The occurrence of functional 2n pollen was confirmed by seed set following 4x × 2x crosses (Watanabe 1988).

The frequency of 2n pollen plants from 4x × I (2x, *ps/ps*) crosses is shown in Table 3. Ten families grown in both Madison and Rhinelander indicated no significant difference in the frequency of 2n pollen plants for the two locations, as determined by the Chi-square test for homogeneity (Watanabe 1988). Thus, the data from the two locations were combined. Some plants might have produced 2n pollen by premature cytokinesis, because I is heterozygous for *pc*-1 and *pc*-2. However, the meiotic observation of 2n pollen plants indicated that 43 plants out of 44 had parallel spindles, and only one plant showed premature cytokinesis. This fact indicates that the frequency of *ps* gene is little overestimated when it is determined via the frequency of 2n pollen plants.

The genotypes of adg and 4x grl clones at the *ps* locus based on chromosome segregation and chromatid segregation hypothesis were estimated by using the Chi-square test for fitness for the ratios expected at the 5% probability level (Tables 2 and 4). Families 2, 10, 17, and 22 gave segregation ratios which did not fit for any expected ratio. This might be due to lack of penetrance of *ps* in some plants in these families or to the effect of a major modifying locus. Based on the chromosome segregation hypothesis, 9, 14, 1, and 1 plants of adg were assumed to be nulliplex, simplex, simplex or duplex, and duplex, respectively, and two clones of 4x grl were simplex. According to the chromatid segregation hypothesis, 9, 12, 3, and 1 plants of adg were nulliplex, simplex, simplex or duplex, and duplex, respectively, and 2 plants of 4x grl were simplex. The assigned genotypes of 25 Andigena clones were used to estimate the frequency of the *ps* gene after Iwanaga and Peloquin (1982) (Table 5).

Most observations of meiosis in a sample of 2n pollen producing plants indicated that *ps* is the mechanism of 2n pollen formation. Thus, the gene and genotype frequencies at the *ps* locus were estimated using the 2n pollen frequency data given in Table 1. Assuming Hardy-Weinberg equilibrium, the *ps* gene frequencies in diploid and tetraploid were estimated (Table 6). In regard to brc, there were 2 plants with premature cytokinesis and 2 synaptic mutant-like plants among 20 2n pollen producing plants, but the remaining 16 plants (80%) had parallel spindles. Hence, 2n pollen frequency of 0.214 (=0.8 × 0.267) was used to estimate the *ps* gene frequency for brc.

The estimated gene frequencies for both diploid and tetraploid taxa were very high, ranging from 0.393 for spl

Table 3. Frequency of 2n pollen plants in progenies from crosses between 4x adg (codes 1–29) or 4x grl (codes 30–31) clones and 2x clone I (*ps*/*ps*)

Code no.	Cross	No. of plants				Cytology[a] ps/total
		Total	Male fertile	n pollen	2n pollen	
1	PI 338623-1 × I	38	32	14	18	2/2
2	PI 338623-2 × I	40	37	3	34	2/2
3	WRF 1744-22 × I	28	27	11	16	2/2
4	WRF 1744-30 × I	34	30	11	19	3/3
5	WRF 1605-12 × I	27	18	0	18	2/3
6	WRF 1605-30 × I	42	24	0	24	4/4
7	PI 473299-8 × I	21	20	0	20	2/2
8	PI 473299-22 × I	21	20	9	11	0/0
9	PI 473301-2 × I	19	19	0	19	2/2
10	PI 473301-10 × I	21	21	4	17	3/3
11	WRF 1767-21 × I	12	10	6	4	1/1
12	PI 245931-9 × I	12	11	6	5	1/1
13	PI 280995k-17 × I	12	12	0	12	1/1
14	PI 280995-19 × I	12	12	5	7	1/1
15	PI 280995-22 × I	12	12	4	8	1/1
16	PI 243435-1 × I	12	12	3	9	1/1
17	PI 243435-1 × I	12	12	2	10	1/1
18	PI 230470-1 × I	12	11	5	6	2/2
19	PI 230470-14 × I	12	9	0	9	2/2
20	PI 230470-23 × I	12	8	0	8	3/3
21	PI 246979-11 × I	8	6	3	3	1/1
22	PI 246979-21 × I	12	11	1	10	0/0
23	PI 246979-26 × I	11	10	0	10	0/0
24	PI 246979-27 × I	12	11	0	11	2/2
25	PI 473248-1 × I	12	12	4	8	0/0
26	PI 473249-1 × I	12	11	8	3	1/1
27	PI 473270-6 × I	12	12	10	2	1/1
28	PI 473270-9 × I	12	10	5	5	1/1
29	PI 473270-29 × I	12	10	4	6	1/1
30	PI 473010-1 × I	12	12	3	9	1/1
31	PI 473014-1 × I	12	12	2	10	1/1

[a] ps and total represent parallel spindles and 2n pollen plants observed for microsporogenesis, respectively

to 0.895 for grl-ifd (Table 6). The gene frequency of tetraploids is about twice as high as that of diploids. The gene frequency estimated from the adg × I crosses (0.825 and 0.815) did not differ significantly from the estimated gene frequency of adg (0.847) under Hardy-Weinberg equilibrium. The estimated genotype frequencies for the *ps* gene indicate high frequencies of *ps* homozygotes and heterozygotes in diploids, and very high frequencies of nulliplex and simplex genotypes in tetraploids. About 50% of plants were heterozygotes in diploids, and 80%–90% of the tetraploids were nulliplex or simplex.

Discussion

Recent results on haploid *Tuberosum* × 2x wild species hybrids have demonstrated that the *ps* locus is homologous among series *Commersoniana, Cuneoalata, Megistacroloba,* and *Tuberosa* (Hermundstad 1986). This indicates general occurrence of parallel spindles in South American taxa of the tuber-bearing *Solanums*. Formation of parallel spindles in adg and 4x × 2x (*ps*/*ps*) crosses gave the segregation ratios which fit single-gene inheritance (Table 4). The results also indicated that the *ps* gene is in common among adg, 4x grl, phu, and *Tuberosum*, as the diploid tester 'I' is a Phureja-haploid *Tuberosum* hybrid.

The Plant Introductions containing 2n pollen plants were collected from many different locations in South America. Further cytological observation indicated the frequent production of 2n pollen via parallel spindles formation. Apparently, there is neither geographical nor ecological specificity in the distribution of the *ps* gene in the taxa observed. Thus, the *ps* gene is probably common among tuber-bearing *Solanums* in South America.

The high frequency of *ps* in both 2x and 4x taxa emphasizes the importance of meiotic mutants for 2n pollen formation. This feature would be disadvantagous

Table 4. Proposed genotypes of 29 adg (1–29) and 2 grl (30 and 31) clones at the *ps* locus for chromosome and chromatid segregations

Code no.	Genotype	
	Chromosome segregation	Chromatid segregation
1	*Pspspsps*	*Pspspsps*
2	Unassigned	Unassigned
3	*Pspspsps*	*Pspspsps*
4	*Pspspsps*	*Pspspsps*
5	*pspspsps*	*pspspsps*
6	*pspspsps*	*pspspsps*
7	*pspspsps*	*pspspsps*
8	*Pspspsps*	*Pspspsps*
9	*pspspsps*	*pspspsps*
10	Unassigned	Unassigned
11	*Pspspsps*	*Pspspsps* or *PsPspsps*
12	*Pspspsps*	*Pspspsps*
13	*pspspsps*	*pspspsps*
14	*Pspspsps*	*Pspspsps*
15	*Pspspsps*	*Pspspsps*
16	*Pspspsps*	*Pspspsps*
17	Unassigned	Unassigned
18	*Pspspsps*	*Pspspsps*
19	*pspspsps*	*pspspsps*
20	*pspspsps*	*pspspsps*
21	*Pspspsps*	*Pspspsps* or *PsPspsps*
22	Unassigned	Unassigned
23	*pspspsps*	*pspspsps*
24	*pspspsps*	*pspspsps*
25	*Pspspsps*	*Pspspsps*
26	*Pspspsps* or *PsPspsps*	*Pspspsps or PsPspsps*
27	*PsPspsps*	*PsPspsps*
28	*Pspspsps*	*Pspspsps*
29	*Pspspsps*	*Pspspsps*
30	*Pspspsps*	*Pspspsps*
31	*Pspspsps*	*Pspspsps*

[a] Unassigned – did not fit any expected ratio

Table 5. Frequency of assigned genotypes at the *ps* locus for 25 Andigena clones and gene frequencies estimated based on two hypotheses

Genotype	No. of *ps* genes	Chromosome segregation	Chromatid segregation
pspspsps	4	9	9
Pspspsps	3	14	12
Pspspsps or *PsPspsps*	2.5	1	3
PsPspsps	2	1	1
PsPsPsps	1	0	0
PsPsPsPs	0	0	0
Gene frequency		0.825	0.815

Table 6. Gene and genotype frequencies at the *ps* locus in 2x and 4x taxa assuming Hardy-Weinberg equilibrium

Taxon	*ps* gene frequency	Genotype frequency				
		p^2 (*Ps*/*Ps*)	2pq (*Ps*/*ps*)		q^2 (*ps*/*ps*)	
2x						
brc	0.462	0.289	0.497		0.214	
spl	0.393	0.368	0.477		0.155	
phu	0.470	0.281	0.498		0.221	
stn	0.417	0.340	0.486		0.174	
4x		p^4 Q[a]	$4p^3q$ T[a]	$6p^2q^2$ D[a]	$4pq^3$ S[a]	q^4 N[a]
adg	0.847	0.0005	0.0121	0.1008	0.3719	0.5147
grl	0.801	0.0016	0.0252	0.1524	0.4091	0.4117
grl-ifd	0.895	0.0001	0.0042	0.0530	0.3011	0.6416

[a] Q, T, D, S and N: quadriplex (*PsPsPsPs*), triplex (*PsPsPsps*), duplex (*PsPspsps*), simplex (*Pspspsps*) and nulliplex (*pspspsps*), respectively

for conservation of the diploid level, but it provides good opportunity for sexual polyploidization, which gives higher fitness and genetic flexibility for the 4x progenies.

The high frequency of the *ps* gene in the 2x taxa can be explained by the following factors: (1) heterozygosity is favored, (2) modifier(s) controls the expressivity of *ps*, (3) segregation distortion in favor of *ps* allele more than *Ps* allele, (4) pollen competition, (5) close linkage of *ps* gene with genes affecting vigor, and (6) presence of certain feedback mechanisms between tetraploid and diploid populations (De Wet 1980). Yerk and Peloquin (1988) compared n pollen plants and 2n pollen plants in diploids, and found that 2n pollen plants were significantly better in terms of flowering and vigor, and significantly later in maturity than the n pollen plants. The possible existence of modifier(s) for *ps* was suggested by the recurrent selection for 2n pollen in red clover (Parrot and Smith 1986) and in diploid potato species (McHale 1983). The percentage of 2n pollen was mostly between 5% and 30% among 2n pollen plants, which rarely showed higher percentages than 50% (present results). This seems to indicate the presence of a mechanism that allows production of 2n pollen in low percentages, holding high percentages of n pollen. Owing to this mechanism, the diploid level could have been maintained at a certain frequency by crossing with other diploids, while allowing polyploidization.

Another question raised is why octoploids are not found in natural populations, although many tetraploids can produce a modest amount of 2n pollen. The possible explanations for lack of octoploids could be: (1) the frequency of 2n eggs is very low; (2) the chance of 2n pollen of a tetraploid uniting with a 2n egg of another 4x is rare, since both male and female parents can produce n gametes at high frequencies; (3) octoploids may be weak in vigor because of their high chromosome number

Table 7. Effects of asexual and sexual polyploidization on the genotypic and gametic frequencies of the *ps* gene in diploid populations, and genotypic frequencies in newly arisen tetraploid populations, assuming 2n eggs are formed by second division restitution (SDR) in sexual polyploidization

Diploid population		Genotype			
Genotypic frequency					
p^2		*PsPs*			
2pq		*Psps*			
q^2		*psps*			
Mode of polyploidization					
Somatic doubling		Sexual polyploidization; all 2n pollen is *ps/ps*			
Each genotype has equal chance of doubling		a. SDR 2n, *ps* locus adjacent to centromere		b. SDR 2n egg, *ps* locus far from centromere	
Parent		Female gamete		Female gamete	
Frequency	Genotype	Fequency	Genotype	Frequency	Genotype
p^2	*PsPs*	p^2+pq	*PsPs*	p^2	*PsPs*
2pq	*Psps*			2pq	*Psps*
q^2	*psps*	q^2+pq	*psps*	q^2	*psps*
Tetraploid population Somatic doubling		Sexual polyploidization			
Zygote		a. Zygote		b. Zygote	
Frequency	Genotype	Frequency	Genotype	Frequency	Genotype
p^2	*PsPsPsPs*	p^2+pq	*PsPspsps*	p^2	*PsPspsps*
2pq	*PsPspsps*			2pq	*Pspspsps*
q^2	*pspspsps*	q^2+pq	*pspspsps*	q^2	*pspspsps*

(Hawkes 1979). There are several reports on irregular microsporogenesis in cholchicine-induced octoploids on *S. acaule* (Lamm 1945; Swaminathan 1954), *S. stoloniferum* (Lamm 1953; Masutani 1962; Swaminathan 1954), and *S. punae* (Lamm 1953). However, there are no reports on octoploids obtained via sexual polyploidization.

It is worthwhile to compare the effects of somatic doubling versus sexual polyploidization in regard to the genetic constitution of tetraploid populations of adg and 4x grl (Tables 7 and 8). If tetraploids originate from somatic doubling, the *ps* gene frequency of the 4x population is expected to be similar to that of the putative ancestral 2x population. Alternatively, if they originate from sexual polyploidization via 2n pollen, a higher *ps* gene frequency can be expected in the tetraploids than in the diploids, because 2n pollen grains carry only the *ps* gene, namely, its frequency in 2n pollen is 1.0 (Iwanaga and Peloquin 1982). Assuming that all 2n eggs are formed by second division restitution (SDR) (Werner and Peloquin 1987), the frequency of the *ps* gene in 2n eggs is independent of whether the *ps* locus is adjacent to or far apart from centromere. Thus, the *ps* gene frequency in newly arisen tetraploid populations is subsequently increased from that in the original diploid population.

Table 8. Frequency of the *ps* gene in tetraploid populations calculated from the genotype frequencies in Table 7

Somatic doubling

$pq+q^2=q(p+q)=q$

Sexual polyploidization

a. SDR 2n egg, *ps* locus adjacent to centromere

$1/2(p^2+pq)+q^2+pq=1/2p^2+3/2pq+q^2$
$=1/2\{(p^2+2pq+q^2)+pq+q^2\}=1/2\{(p\times q)^2+q(p+q)\}$
$=1/2(1+q)$

b. SDR 2n egg, *ps* locus far from centromere

$1/2p^2+(3/4x2pq)+q^2=1/2p^2+3/2pq+q^2$
$=1/2\{(p^2+2pq+q^2)+pq+q^2\}=1/2\{(p+q)^2+q(p+q)\}$
$=1/2(1+q)$

The gene frequency of *ps* in adg is 0.847, whereas the gene frequencies of the ancestral diploids, spl, stn, and phu, are 0.393, 0.417, and 0.470, respectively. Further-

more, the frequency of *ps* in 56 USA cultivars is reported to be 0.69 (Iwanaga and Peloquin 1982), that is, significantly higher than those of the related diploid taxa. The difference in *ps* gene frequency between adg and USA cultivars (*Tuberosum*) could be due to either sampling error or to the narrow genetic background of USA cultivars, or to further introgression of *ps* to adg from diploid taxa via 2n pollen. According to Camadro and Peloquin (1980), the *ps* gene frequencies in 2x grl and ifd, which are closely related to 4x grl (Hawkes 1978), were 0.46 and 0.37, respectively. The *ps* frequency of 4x grl was 0.801 (present results). Thus, the frequencies of *ps* gene in tetraploid taxa are almost twice as high as those of ancestral diploids. This clearly indicates that the tetraploid taxa originated from sexual polyploidization.

Group adg has enormous variation in its morphology mainly due to introgression from diploid wild species (Hawkes 1978). Tetraploid grl also shows introgression from 2x ifd and 2x grl (Hawkes 1978; Okada 1974). The high frequency of *ps* in both 2x and 4x taxa investigated here also suggested the continuous introgression of this gene from diploid species to tetraploids via sexual polyploidization with 2n pollen.

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