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The occurrence and frequency of 2n pollen in 2x, 4x, and 6x wild, tuber-bearing *Solanum* species from Mexico, and Central and South America *

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Summary. The occurrence of 2n pollen-producing plants was investigated in 187 plant introductions (PIs) of 38 wild species of tuber-bearing *Solanum.* These 2x, 4x, and 6x species are from Mexico, and Central and South America. The determination of 2n pollen-producing plants was conducted using acetocarmine glycerol. Plants with more than 1% large-size pollen were regarded as 2n pollen-producing plants. 2n pollen-producing plants were identified in the following species: 10 out of 12 Mexican 2x species, seven of nine South American 2x species, seven of seven Mexican and Central American 4x species, five of five South American 4x species, and five of five Mexican 6x species. The frequency of 2n pollen-producing plants varied among species at the same ploidy level, but the range of frequency, generally between 2 and 10% among species, was similar over different ploidy levels. The general occurrence of 2n pollen in both 2x and polyploid species, which are evolutionarily related, is evidence that the mode of polyploidization in tuber-bearing Solanums is sexual polyploidization. Furthermore, the frequencies of 2n pollen-producing plants in autogamous disomic polyploid species were not markably different from those of their related diploid species. It is thought that the frequent occurrence of 2n gametes with autogamy tends to disturb the fertility and consequently reduce fitness of polyploids. Thus, we propose that the breeding behavior of polyploids and the occurrence of 2n gametes may be genetically balanced in order to conserve high fitness in polyploid species in tuber-bearing *Solanum.*

Key words: 2n pollen – Tuber-bearing *Solanum* – Sexual polyploidization – Breeding behavior – Disomic polyploid

Introduction

The tuber-bearing Solanums are distributed over the American continents from the southern part of Nebraska to southern Chile. There are two fairly distinct centers of diversity. One of these is in the central to southern part of Mexico; the other is in the Andes of Peru, Bolivia, and northwestern Argentina (Hawkes 1979a).

The general occurrence of 2n pollen in many accessions has been reported in more than 15 diploid wild species and at least one tetraploid species from South America (den Nijs and Peloquin 1977; Camadro and Peloquin 1980; Hermundstad 1986). The occurrence of 2n eggs has also been reported in several diploid, wild species (den Nijs and Peloquin 1977). These species are in the taxonomic series *Commersoniana, Cuneolata, Megistacroloba,* and *Tuberosa,* in which polyploid species also occur.

The occurrence of 2n gametes in tuber-bearing Solanums was surveyed by den Nijs and Peloquin (1977). Although they suggested that 2n gametes may occur generally in tuber-bearing Solanums, more than 50% of *Solanum* species, especially Mexican species and species in the series *Circaeifolia, Conicibaccata, Ingaefolia, Olmosiana,* and *Piurana* from South America, have not been investigated. Thus, extensive information on the occurrence of 2n gametes in the tuber-bearing Solanums is still lacking in many taxa.

A relationship between 2n gametes and polyploidization in cultivated taxa and closely related wild species has

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Table 1. Number of plant introductions with 2n pollen and frequency of plants with 2n pollen among 12 Mexican 2x taxa

Taxon	No. of PIs examined	PIs with 2n pollen	Total plants	Male-fertile plants	2n pollen plants (%)
S. bulbocastanum (blb[a], BUL[b])	10	5	138	136	8 (5.9)
S. clarum (clr, BUL)	2	0	25	25	0 (0.0)
S. morelliforme (mrl, MOR)	2	1	37	37	3 (8.1)
S. brachistotrichum (bst, PIN)	3	1	8	8	2 (25.0)
S. cardiophyllum (cph, PIN)	10	7	153	153	12 (7.8)
S. jamesii (jam, PIN)	7	4	77	77	6 (7.8)
S. pinnatisectum (pnt, PIN)	5	5	54	54	8 (14.8)
S. trifidium (trf, PIN)	5	0	42	41	0 (0.0)
S. bulbocastanum × *S. cardiophyllum* hybrid (blb-cph)	1	1	11	11	1 (9.1)
S. *lesteri* (les, POL)	1	1	17	16	1 (6.3)
S. polyadenium (pld, POL)	10	5	119	119	8 (6.7)
S. verrucosum (ver, TUB)	6	3	74	74	3 (4.1)

[a] Abbreviation for species name
[b] Abbreviation for taxonomic series, *Bulbocastana*, *Morelliformia*, *Pinnatisecta*, *Polyadenia*, and *Tuberosa*, respectively

been proposed, and sexual polyploidization is probably the mode of origin of polyploid taxa of tuber-bearing *Solanum* (den Nijs and Peloquin 1977; Iwanaga and Peloquin 1982; Watanabe and Peloquin 1989). However, the origin of mode of polyploidization in wild polyploid species is not known. Sexual polyploidization might be involved in the origin of these polyploid species, since some putative parental 2x species have been observed to produce 2n gametes (den Nijs and Peloquin 1977). However, there is not clear indication of the occurrence of 2n gametes in many 2x progenitor species and their descendent polyploid species.

Several polyploid species are known to be autogamous, disomic polyploids (Hawkes 1978). Tetraploid and hexaploid *S. acaule,* 4x *S. stoloniferum,* and 6x *S. demissum* have disomic inheritance as regards some disease resistances (Mckee 1962; Cockerham 1970). Microsporogenesis in these species is characterized by a high frequency of bivalents but no multivalents (survey by Dvořák 1983). The disomic genetic behavior of polyploids is similar to diploids in terms of inheritance. The advantage of disomic inheritance in autogamous polyploids is the built-in heterosis between homoeologous loci (MacKey 1970). 2n pollen has only occasionally been found in these disomic polyploid species, although only a small number of plants were screened (den Nijs and Peloquin 1977; Brown 1988). However, the frequencies of plants with 2n pollen in allogamous, tetrasomic tetraploids, Andigena, and 4x *S. gourlayi,* were very high (Watanabe 1988). Thus, there may be some relationship between occurrence of 2n pollen and autogamous disomic polyploids.

This investigation was undertaken to determine whether 2n pollen occur generally in 2x, 4x, and 6x species from Mexico, Central America, and South America. An attempt was also made to relate the occurrence of 2n pollen in these species and the mode of polyploidization.

Materials and methods

One hundred and ninety-two plant introductions (PIs) of 38 species, which consist of diploids (Tables 1 and 2), tetraploids (Tables 3 and 4), and hexaploids (Table 5), were screened for the occurrence and frequency of 2n pollen. Taxonomy is based on Hawkes (1978). The abbreviations of names of species and of series were based on Huamán and Ross (1985) and on Simmonds (1963).

Table 2. Number of plant introductions with 2n pollen and frequency of plants with 2n pollen among nine South America 2x species

Species	No. of PIs examined	PIs with 2n pollen	Total plants	Male-fertile plants	2n pollen plants (%)
S. commersonii (cmm[a], COM[b])	5	3	126	125	6 (4.8)
S. capsicibaccatum (cap, CIR)	4	0	41	41	0 (0.0)
S. circaeifolium (crc, CIR)	4	2	73	71	4 (5.6)
S. alborinozii (abz, CON)	1	0	12	12	0 (0.0)
S. chomatophilum (chm, CON)	4	2	94	93	4 (4.3)
S. laxissimum (lxs, CON)	2	0	26	26	0 (0.0)
S. violaceimarmoratum (vio, CON)	6	2	72	70	2 (2.8)
S. acroglossum (acg, PIU)	2	1	28	28	1 (3.6)
S. piurae (pur, PIU)	2	1	29	29	1 (3.5)

[a] Abbreviation for species name
[b] Abbreviations for taxonomic series, *Commersoniana*, *Circaeifolia*, *Conicibaccata*, and *Piurana*, respectively

Table 3. Number of plant introductions with 2n pollen and frequency of plants with 2n pollen among seven Mexican 4x species

Species	No. of PIs examined	PIs with 2n pollen	Total plants	Male-fertile plants	2n pollen plants (%)
S. agrimonifolium (agf[a], CON[b])	6	3	102	102	14 (13.7)
S. oxycarpum (oxc, CON)	6	3	87	87	8 (9.2)
S. fendleri (fen, LON)	13	8	127	127	18 (14.2)
S. hjirtingii (hjt, LON)	7	5	89	88	8 (9.1)
S. papita (pta, LON)	10	4	98	96	5 (5.2)
S. polytrichon (plt, LON)	9	4	179	178	15 (8.4)
S. stoloniferum (sto, LON)	9	3	134	134	9 (6.8)

[a] Abbreviation for species name
[b] Abbreviation for taxonomic series, *Conicibaccata* and *Longipedicellata*, respectively

Seeds were obtained from the Inter-Regional Potato Introduction Project (IR-1), at Sturgeon Bay/WI. Plants were grown in the greenhouse in the spring of 1987 and 1988. Flower samples for 2n pollen screening were also collected in the fields of IR-1 through the courtesy of Dr. R. E. Hanneman, Jr., project leader of IR-1.

2n pollen frequency was determined as described in Watanabe and Peloquin (1989). Pollen was stained using acetocarmine glycerol. Plants with more than 1% large-size pollen were regarded as 2n pollen-producing plants and plants with less than 5% stainable pollen were considered male sterile. The number of plants observed per PI varied from 10 to 20.

Results

The number of plant introductions (PI) with 2n pollen and the frequency of plants with 2n pollen among 12

Table 4. Number of plant introductions with 2n pollen and frequency of plants with 2n pollen among five South American 4x species

Species	No. of PIs examined	PIs with 2n pollen	Total plants	Male-fertile plants	2n pollen plants (%)
S. acaule (acl[a], ACA[b])	4	1	76	76	1 (1.3)
S. colombianum (col, CON)	5	2	48	48	2 (4.2)
S. paucijugum (pcj, CON)	1	1	3	3	2 (66.7)
S. tuguerrense (tuq, PIU)	2	1	40	40	4 (10.0)
S. sucrense (scr, TUB)	5	2	104	104	5 (4.8)

[a] Abbreviation for species name
[b] Abbreviation for taxonomic series, *Acaulia*, *Conicibaccata*, *Piurana*, and *Tuberosa*, respectively

Table 5. Number of plant introductions with 2n pollen and frequency of plants with 2n pollen among five Mexican 6x species

Species	No. of PIs examined	PIs with 2n pollen	Total plants	Male-fertile plants	2n pollen plants (%)
S. brachycarpum (bcp[a], DEM[b])	5	3	89	86	8 (9.3)
S. demissum (dms, DEM)	5	2	91	86	2 (2.3)
S. querreroense (grr, DEM)	2	2	2	2	2 (100)
S. hougasii (hou, DEM)	2	2	8	8	3 (37.5)
S. iopetulum (iop, DEM)	2	2	16	14	4 (28.6)

[a] Abbreviation for species name
[b] Abbreviation for taxonomic series *Demissa*

Mexican 2x species are listed in Table 1. Ten of 12 species have plants with 2n pollen, whereas the number of PIs examined per taxon varied from one to ten.

The number of PIs with 2n pollen and the frequency of plants with 2n pollen among nine South American 2x species are listed in Table 2. Six of nine species have plants with 2n pollen, although the number of PIs screened per species is small.

The number of PIs with 2n pollen and the frequency of plants with 2n pollen in polyploid taxa are listed in Tables 3, 4, and 5. 2n pollen-producing plants were observed in all seven Mexican 4x species (Table 3), in all five South American 4x species (Table 4), and in all five Mexican 6x species (Table 5). However, the percentage of 2n pollen in 2n pollen-producing plants was low and was generally less than 10%.

Discussion

General occurrence of 2n pollen

2n pollen was identified in most of 2x, and in all of 4x and 6x species in this investigation. The frequency of 2n pollen-producing plants in a species is low, generally ranging from 2 to 10% among species over all ploidy levels. It should be pointed out that the number of PIs and the number of plants observed were relatively small. Thus, it is possible that a larger sample, as to the numbers of accessions and of plants, would have led to detection of 2n pollen-producing plants in species in which 2n pollen was not identified in this investigation, as well as to different frequencies of 2n pollen-producing plants. In spite of this, it is evident that 2n pollen occurs in most of the species investigated.

As regards geographical distribution, species with 2n pollen-producing plants were found all over Mexico, and Central and South America. It is notable that 2n pollen was identified in this investigation in most Mexican and Central American species that had not been previously surveyed for 2n pollen.

The frequent occurrence of 2n pollen-producing plants has been reported in other 2x wild species, haploid *Tuberosum* × 2x wild species hybrids, and cultivated taxa from South America (den Nijs and Peloquin 1977; Camadro and Peloquin 1980; Iwanaga and Peloquin 1982; Hermundstad 1986; Watanabe and Peloquin 1989). Thus, it is well documented that 2n pollen occurs generally among tuber-bearing Solanums.

Genetic control of 2n pollen formation

The inheritance of 2n pollen formation by meiotic mutant genes such as parallel spindles (*ps*) and premature-cytokinesis (*pc*-1, *pc*-2) has not been established in this investigation. However, the interpretation of several recent reports provides evidence for the genetic control of 2n pollen formation in these species. Hermundstad (1986) selected 2n pollen-producing plants from haploid *Tuberosum* – 2x *S. verrucosum* (ver) hybrids, where both haploid and ver were selected for 2n pollen. This would indicate that the mechanism of 2n pollen formation is heritable and under the same genetic control both in haploids of *Tuberosum* and 2x ver. Similarly, there are several reports on the occurrence of 2n pollen in hybrids in which either of the parental species such as *S. bulbocastanum*, *S. jamesii*, *S. pinnatisectum*, and *S. stoloniferum* produced 2n pollen (Masutani 1962; Irikura 1976; Matsubayashi and Misoo 1977). Thus, the mechanism(s) of 2n pollen formation would be under genetic control and probably would be similar, since most 2n pollen-producing plants had either parallel spindles or premature-cytokinesis (Watanabe 1988).

Mode of polyploidization

The occurrence of 2n gametes in both 2x and polyploid species, which are probably their descendants, would be an indication that sexual polyploidization is the mode of polyploidization in the polyploid species. Tetraploid *S. paucijugum* is related to the diploids, *S. violaceimarmoratum* (vio) and *S. chomatophilum* (chm). 2n pollen was found in vio and chm (Lopez 1979). Mexican polyploid species such as 4x *S. polytricon* and *S. stoloniferum*, and 6x *S. demissum* could be descendants from certain diploid species in series *Pinnatisecta* and *Bulbocastana* (Hawkes 1978, 1979a, b; Marks 1955, 1965). 2n pollen was found both in those diploid and tetraploid species in this investigation. 2n eggs occur in the 2x species *S. jamesii* (den Nijs and Peloquin 1977). Thus, it is plausible that sexual polyploidization is the mode of polyploidization in 4x and 6x species from diploids via 2n gametes.

Relationships among polyploidy, 2n gametes, and breeding behavior

Wild 4x and 6x species behave like diploids in terms of chromosome pairing during microsporogenesis (Dvořák 1983; Watanabe 1988). Also, there is some genetic evidence that 4x *S. stoloniferum* and 6x *S. demissum* have disomic inheritance (Cockerham 1970; McKee 1962). Thus, the 4x and 6x species investigated could be regarded as stable disomic polyploids in which heterogenetic chromosome pairing is suppressed by genetic mechanisms. Furthermore, these disomic polyploid species could be self-fertilizing (Hawkes 1978).

There is a distinct difference in the frequency of 2n pollen-producing plants between diploids and outcrossing tetrasomic tetraploids, which are cultivated taxa and their related species (Watanabe and Peloquin 1989). Frequencies of 2n pollen-producing plants were about 20% in 2x and 50% in 4x. In contrast, it could be indicated from this investigation that there is not a significant difference in the frequencies of 2n pollen-producing plants between diploids and their related disomic polyploids which are autogamous, although the number of accessions and the number of plants used in this investigation are relatively small. This could be explained by the following: (1) poor penetrance of meiotic mutant genes which control production of 2n pollen in disomic polyploids, (2) existence of homoeologous loci for *ps* in disomic polyploids, and (3) suppression of expression of meiotic mutant genes by modifier(s), in order to maintain high fitness by autogamy of *n* gametes.

Autogamous disomic polyploids could have homoeologous loci for the *ps* gene. Each homoeologous locus is homozygous for either the *ps* or *Ps* allele, and one locus would have epistatic interaction onto another locus. As for disomic tetraploids, if there are plants with the *PsPs* genotype for one locus and *psps* for another homoeologous locus, there is no expression of 2n pollen production; however, the *ps* allele is maintained at a certain frequency in the population. If a rare intercrossing event occurs between disomic tetraploids or among disomic polyploids and diploids with 2n gametes, 2n pollen-producing genotype could still be possible. For example, assuming that one parent has *PsPs* for one locus (tentatively locus *A*) and *psps* for another (tentatively locus *B*), and that the other parent has *psps* for locus *A* and *PsPs* for locus *B*, the resulting progeny would be heterozygous for two homoeologous loci. One-sixteenth of selfed progeny from the hybrids could be *psps* for locus *A* and *psps* for locus *B*; thus, 2n pollen production would be possible and could maintain 2n pollen-producing genotypes.

These explanations are related to breeding systems in polyploid species. Self-fertilization of disomic tetraploid species requires an identical endosperm balance number (EBN) in both egg and pollen. EBN is under genetic control, and success in seed development requires a 2:1 ratio of maternal and paternal EBNs (Johnston and Hanneman 1980, 1982; Johnston et al. 1980; Ehlenfeldt and Hanneman 1988). Deviations from the 2:1 ratio of maternal:paternal EBNs results in breakdown of endosperm development. Thus, a high percentage of 2n pollen in a plant and high frequencies of meiotic mutant genes in disomic tetraploids would not be an advantage. Self-fertilization with 2n pollen in these disomic tetraploids (2EBN) results in an imbalance of EBNs between normal polar nuclei (2EBN) and a 2n sperm (2EBN). This results in 1:1 ratio of EBN in endosperm, unless both 2n egg and 2n pollen are produced and unite to result in an octoploid. As octoploids are not found in nature, the frequency of 2n eggs could be low, and the chances of 2n eggs uniting with 2n pollen would be small. Therefore, the frequency of 2n pollen produced by meiotic mutant genes should be suppressed to avoid gametic waste and maintain high fitness, even when meiotic mutant genes occur at modest frequency. The same speculation could be used to explain the low frequencies of 2n pollen plants and consequently the low frequency of meiotic mutant genes in disomic hexaploid species.

All of the sexual reproductive features mentioned here – occurrence of 2n gametes, disomic behavior of poylploids with autogamy, and EBN – could be controlled by a genetic system such as closely linked loci, in order to maintain high fitness in disomic polyploids.

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