

## The Occurrence and Frequency of 2n Pollen in Three Diploid Solanums from Northwest Argentina

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**Summary.** A group of wild, tuber-bearing species from Northwest Argentina, belonging to the series Tuberosa, *Solanum spgazzini* Bitt. (spg,  $2n = 2x = 24$ ), *S. gourlayi* Hawkes (grl,  $2n = 2x = 24$  and  $2n = 4x = 48$ ) and *S. oplocense* Hawkes (opl,  $2n = 6x = 72$ ), and Cuneolata, *S. infundibuliforme* Phil (ifd,  $2n = 2x = 24$ ), is being used to investigate the mode of origin of polyploids in the genus *Solanum*. 2n gametes have been detected in the diploid species ifd and spg and in a diploid race of grl, using cytological and breeding approaches. Twenty-two introductions of spg, 8 of grl and 26 of ifd have been tested for 2n pollen; 59%, 63% and 54% of them, respectively, had at least one 2n pollen producing plant. These introductions comprised 238, 76 and 235 plant respectively, of which 20, 16, and 32 plant produced 5% or more 2n pollen. The mechanism of 2n pollen formation was determined in several plant of 2x spg, 2x grl and 2x ifd. All of them were found to form diplandroids via parallel spindles. This mechanism, which gives meiotic products genetically equivalent to first division restitution gametes, is under control of the Mendelian recessive *ps*. The results suggest that the allele *ps* is widely distributed in natural populations of the three diploids, and that its frequency is very high. These species are seen as valuable material for population genetic studies, and for the eventual incorporation into a breeding scheme involving sexual polyploidization via 2n gametes.

**Key words:** Diploid pollen – Evolution – Sexual polyploidization – *Solanum* – Tuberosa – Diplandroids

### Introduction

The genus *Solanum*, to which both wild and cultivated potatoes belong, is characterized by extensive polyploidization. A few tuber-bearing series appear to be entirely

diploid, others exhibit a range of ploidy levels from 2x to 6x, and some contain only polyploid species. Moreover, some species are mixtures of ploidy races.

2n gametes (gametes or gametophytes with the sporophytic chromosome number) have been detected in diploid, triploid and tetraploid species (Quinn, Mok and Peloquin 1974; den Nijs and Peloquin 1977). Mok and Peloquin (1975a, b) discovered three mechanisms of 2n pollen formation in diploid potatoes, parallel spindles (*ps*), premature cytokinesis-1 (*pc-1*) and premature cytokinesis-2 (*pc-2*), and found that they were simple inherited. Parallel spindles result in 2n pollen genetically equivalent to first division restitution (FDR) gametes, whereas premature cytokinesis-1 and premature cytokinesis-2 give meiotic products genetically equivalent to second division restitution (SDR) gametes.

FDR gametes were first recognized as a powerful tool in breeding by Mendiburu (1971), since 75 to 80% of the parental genotype is transmitted intact to the progeny. This means that not only the heterozygosity can be transmitted from parents to offspring but most of the inter-locus interactions as well. 2n gametes (2n eggs and 2n pollen) also provide the opportunity for unilateral and bilateral sexual polyploidization, in both breeding and evolution, and introgression between different ploidy levels (Mendiburu and Peloquin 1976; den Nijs and Peloquin 1977). The genetic determination of 2n gamete formation and the consequences of sexual polyploidization led den Nijs and Peloquin (1977) to emphasize that 2n gametes have been the major instrument in polyploid evolution of the tuber-bearing Solanums, and that somatic chromosome doubling of species and interspecific hybrids has been of limited importance.

The literature, however, is rather equivocal on the mode of origin of polyploids, as Harlan and de Wet (1975) have pointed out. Information on the occurrence and frequency of 2n gametes in natural populations of higher plants, plus their genetic determination, cytological basis,

genetic consequences and role in sexual polyploidization and introgression is lacking.

A group of wild species from Northwest Argentina, belonging to the series *Tuberosa*, *S. spgazzini* Bitt. (spg<sup>1</sup>, 2n = 2x = 24), *S. gourlayi* Hawkes (grl<sup>2</sup>, 2n = 2x = 24 and 2n = 4x = 48), *S. oplocense* Hawkes (opl<sup>3</sup>, 2n = 6x = 72), and *Cuneolata*, *S. infundibuliforme* Phil. (ifd<sup>4</sup>, 2n = 2x = 24), was seen as suitable material to investigate the mode of origin of polyploids in the genus *Solanum*. Several ploidy levels exist in this group of species, hybrids have been recorded in nature, and introgression of 2x ifd into 4x grl and 6x opl has been detected (Hawkes 1978; Okada 1974, 1979). Furthermore, Hawkes and Hjerting (1969), based on morphological observations, have suggested that these species are closely related but, to date, the nature of their relationship still remains ill defined.

The present report is mainly concerned with the occurrence, frequency and mode of formation of 2n pollen in the diploid species spg and ifd and in a diploid race of grl.

## Materials and Methods

Seeds of 26 introductions of ifd (2n = 2x = 24), 23 of spg (2n = 2x = 24), 8 of grl (2n = 2x = 24), 32 of grl (2n = 4x = 48) and 18 of opl (2n = 6x = 72) were obtained from the Interregional Potato Introduction Station (IR-1), at Sturgeon Bay, Wisconsin. Twelve to fourteen seedlings of each introduction were grown in a greenhouse and in the field.

The screening for 2n pollen in the diploids was based on the size differences between n and 2n pollen of a clone (Quinn et al. 1974). Although pollen size differs with species and even within species, the fraction of large pollen grains could be easily and rapidly estimated. Open flowers of diploid clones were collected in the field and in the greenhouse into coin envelopes. Following transportation to a laboratory, the pollen was vibrated onto glass slides, stained with acetocarmine glycerol jelly (Marks 1954) and observed at 400x magnification. Clones with 5% of more stainable pollen in the large class were considered to produce 2n pollen. The occurrence of diplandroids was confirmed in several clones by seed set following 4x-2x or 6x-2x crosses, using 4x grl and 6x opl as the female parents.

To study the mechanism of 2n pollen formation, young flower buds were fixed in 3:1 (absolute ethanol:glacial acetic acid) at room temperature, for 24-48 hrs., and then stored in 70% ethanol until used. Slides were prepared by the standard acetocarmine squash technique. Chromosome behavior was examined at M.II, A II, T II and the tetrad stage.

## Results

The results of screening for plants producing 2n pollen are summarized in Table 1. Only those introductions in which at least five plants were examined are reported. Twenty-

Table 1. Frequency of introductions and plants with 2n pollen

Series	Species	Introduction			Plants		
		No. tested	w/2n pollen		No. tested	w/2n pollen	
			No.	%		No.	%
Tuberosa	spg	22	13	59.1	238	20	8.4
Tuberosa	grl	8	5	62.5	76	16	21.1
Cuneolata	ifd	26	14	53.9	235	32	13.6

two introductions of spg, 26 of ifd, and 8 of grl, which comprised 238, 235 and 76 clones respectively, were tested. The percentage of introductions with plants producing significant amounts of 2n pollen ( $\geq 5\%$ ) was high for the three diploid species. The percentage of 2n pollen produced by these plants varied from 5% to almost 50% of the stainable pollen. The overall numbers of plants producing 2n pollen was high, if one takes into account that the sample size was small for almost all introductions. These diploids were also characterized by high male fertility.

The introductions with plants producing 2n pollen along with the number of plants tested and the number of plants with 2n pollen are listed in Table 2.

Meiosis was studied in three clones of ifd, two clones of grl and one clone of spg, belonging to the plant introductions Oka 4348, Oka 4550, Oka 4568, Hof 1553, Hof 1800 and Oka 4052 respectively, which were known to produce 2n pollen in high frequencies (20-30%). The cytological event leading to 2n pollen formation in these clones was the parallel orientation of the A II spindles during microsporogenesis.

## Discussion

### Occurrence and Frequency of ps

Parallel spindles at A II, which result in meiotic products genetically equivalent to first division restitution (FDR) gametes, are under control of the Mendelian recessive *ps* (Mok and Peloquin 1975a, b). This allele has been found in diploid and triploid *S. chacoense* Bitt. and triploid *S. commersonii* Dun. (Mok, Peloquin and Tarn 1975), which belong to the series *Commersoniana*, and in *Phureja*-haploid *Tuberosum* hybrids (Mok and Peloquin 1975a, b). If 2n gametes have been important in the evolution of polyploids among tuber-bearing *Solanums*, one would expect to find *ps* in polyploids as well as in diploids. Dyads as well as fusion of metaphase plates during the second

1,2,3,4 Abbreviations according to Simmonds (1963)

**Table 2.** Introduction with plants producing 2n pollen

Species	Introduction	No. plants tested	No. plants w/2n pollen
spg	Oka 4032	10	2
	Oka 4036	10	1
	Oka 4052	12	1
	Oka 4056	9	1
	Oka 4201	14	1
	Oka 4266	8	2
	Oka 4267	14	1
	Oka 4268	8	1
	Oka 4269	7	2
	Oka 4847	10	1
	Oka 4902	19	4
	Oka 4930	16	1
	Hof 1754	18	2
	grl	Hof 1553	16
Hof 1636		5	1
Hof 1800		10	4
Oka 4307		11	2
Oka 4868		8	1
ifd	Oka 3853	9	1
	Oka 3960	6	2
	Oka 3966	11	3
	Oka 4313	5	1
	Oka 4348	14	2
	Oka 4451	7	1
	Oka 4488	16	3
	Oka 4505	5	1
	Oka 4531	9	3
	Oka 4550	12	1
	Oka 4568	10	4
	Oka 4881	18	4
	EBS 2948	6	2
	Hof 1626	12	4

meiotic division have been reported in several cultivars (Bleier 1931; Oppenheimer 1933; Stelzner 1943; references in den Nijs and Peloquin 1977). Recently Souter, Benke and Peloquin (1979) have found that dyads in the cultivar 'Sebago' are due to parallel spindles at Ana II. The allele *ps* has also been found in haploids of the variety 'Katahdin', which is one of 'Sebago's' parents (Mok and Peloquin 1975b). Experimentally, tetraploids quadruplex for *ps* have been obtained in matings between *Phureja*-haploid *Tuberosum* hybrids (Mok and Peloquin 1975b).

Even though the presence of *ps* has been reported in diploid, triploid and tetraploid Solanums, information on its frequency in nature is lacking. The results of this investigation suggest that *ps* is widely distributed in natural populations of 2x spg, 2x grl and 2x ifd. If the originally sampled populations were in Hardy-Weinberg equilibrium for this locus, then the frequency of the *ps* allele could be roughly estimated by assuming either that only the six plants studied are homozygous recessive, *psps*, or that all

plants which produced 2n pollen are of this genotype. Taking this into consideration, the frequency of *ps* would be either 0.16 or 0.46 in grl, 0.11 or 0.37 in ifd and 0.07 or 0.29 in spg. The frequency is very high with either assumption.

It is intriguing to consider how *ps* is maintained in diploid populations. A unique mutation without selective advantage cannot produce a permanent change in the population. With normal mutation rates, recurrent mutation alone can produce only very slow changes in gene frequency. Even though on an evolutionary time scale these changes might be important, mutation alone, as Falconer (1976) has stated, is most unlikely to be a cause of evolutionary change. Selection chiefly determines whether a gene spreads through the population or remains a rare abnormality, unless the mutation rate is very much higher than average. The allele *ps*, thus, could be neutral. However, one is inclined to suspect that selection is operating. If this occurs, likely alternatives to the hypothesis of neutrality, which could account for the high frequency of *ps* in nature, are: 1) heterozygosity per se is advantageous for the diploids, 2) *ps* is pleiotropic, 3) *ps* is closely linked with 'desirable' genes (genes for vigor, fertility, etc.) and 4) certain feedback mechanisms between tetraploids and diploids exist in the population.

#### *Evolutionary Significance of Sexual Polyploidization*

Polyploidization can be achieved mainly in two ways: either by somatic doubling of the chromosomes (asexual polyploidization) or by functioning of 2n gametes produced by aberrations in the meiotic process (sexual polyploidization).

The advantages and disadvantages of these two modes of polyploidization become evident when genetic variability, inbreeding, heterozygosity and epistasis in the newly arisen polyploids, as well as the possibility of introgression and gene flow between different ploidy levels, are taken into consideration.

The ultimate success of a plant species in nature depends on its fitness and its genetic flexibility. Many plant species with high fitness have some means of incorporating genetic flexibility. In the tuber-bearing Solanums, asexual reproduction ensures the maintenance of superior genotypes in stable environments and sexual reproduction provides the basis for rapid evolution of new forms under varying environmental conditions. This breeding system combining asexual and sexual reproduction, therefore, provides for high fitness and genetic flexibility. Polyploid complexes of Solanums, as well as some complexes of agamospecies and facultative apomictics, can display great variability and evolutionary potential. Furthermore, largely balanced genotypes, some of them

unrelated, can be transmitted by  $2n$  gametes and compounded at a higher ploidy level (Mendiburu, Peloquin and Mok 1974). Since sexual polyploidization may be preceded by meiosis, there is also opportunity for recombination and innovation. Asexual polyploidization, in contrast, generates isolated individuals which lack genetic variability for natural selection to act upon.

Inbreeding depression in outbreeding species may result in loss of vigor and fertility. This depression has been shown to be strong in asexually propagated plants such as strawberry (Morrow and Darrow 1952) and potato, both at the diploid and tetraploid level (Abdalla 1970; de Jong and Rowe 1971; Krantz 1946). Somatic doubling entails severe inbreeding which is reflected in the vigor, fertility and competitive ability of the new polyploids. The amount of inbreeding generated by sexual polyploidization, on the other hand, depends on the genotype of the parents, the mode of  $2n$  gamete formation and the number of crossovers per chromosome arm. If  $2n$  gametes are formed by FDR, the avoidance of inbreeding at the centromere is maximal (Mendiburu et al. 1974).

The inbreeding depression observed in potato and other polysomic polyploids, such as alfalfa, has been largely attributed to the loss of favorable intralocus interactions (Mendiburu et al. 1974; Busbice and Wilsie 1966). With somatic doubling only one interaction per locus is possible, since a maximum of two alleles can be present at one time. Bilateral sexual polyploidization involving unrelated genotypes, however, provides opportunity for the interaction of four different alleles at a locus, which results in 11 intralocus interactions (Mendiburu et al. 1974). Similarly, interlocus interactions can be compounded in sexual polyploids, but are merely maintained in asexual ones.

Gametophyte heterosis has also been observed in potatoes (Simon and Peloquin 1976). Busbice and Wilsie (1966) have stated that the loss of heterozygosity in gametophytes and gametes may seriously reduce their viability. If the more heterotic gametes succeed in forming the zygotes and if there is a positive correlation between vigor at the gametic level and vigor at the zygotic level, vigorous highly heterozygous tetraploids could be formed by sexual polyploidization within and between sympatric populations. These polyploids which are adapted to their parental habitats may also, by chance, possess new favorable gene combinations. These might provide suitable combinations of physiological and morphological characteristics, enabling them to colonize new areas.

The genetic determination of  $2n$  gamete formation ensures repeated occurrence of sexual polyploidization. The progenitors can be maintained in contact with the new polyploids by unilateral sexual polyploidization (USP). This will result in unidirectional introgression. Gene flow and introgression between all ploidy levels is, thus, envi-

sioned to take place, broadening the genetic base of the polyploid populations.

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