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Second meiotic division restitution (SDR) 2n pollen formation in diploid and hexaploid species of *Asparagus*

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Summary

Plants of two cultivars of 2x *A. officinalis* L. and two related ornamental species, 2x *A. plumosus* Baker and 6x *A. densiflorus* (Kunth) Jessop cv. Sprengeri were screened for production of pollen of heterogeneous size. Twenty four plants out of thirty-one studied produced this type of pollen, with frequencies of 3% or more large pollen. Some of these plants also produced giant grains. The numerically unreduced pollen arose by the lack of chromosome migration at Anaphase II in either one or both cells of a meiocyte, followed by the absence of cytokinesis in cells with abnormal chromosome behavior, a Second Meiotic Division Restitution (SDR) cytological mechanism. Double unreduced pollen resulted from a similar mechanism acting at both Anaphase I and Anaphase II stages. The high frequency of plants which produced 2n pollen in significant amounts is taken as an indication that the modified meiosis observed is under genetic control.

Introduction

The genus *Asparagus*, which belongs to the family Liliaceae, Class Monocotyledoneae, is composed of 150 species of herbaceous perennial and tender woody shrubs and vines. Many species are grown as ornamentals or for their biochemical properties, except for the garden asparagus, *A. officinalis* L., which is cultivated for its edible shoots. There are diploid, tetraploid and hexaploid species in the genus, with a basic chromosome number of x = 10.

The garden asparagus, a diploid species, is susceptible to two important fungal diseases caused by *Fusarium* spp. and *Stemphylium* spp. Resistance to these pathogens has been detected in two related polyploids, *A. densiflorus* (Kunth) Jessop cvs. Sprengeri and Myersii. However, interspecific hybridization attempts within and between ploidy levels have been largely unsuccessful, although the basis for this behavior is not known (McCollum, 1988). A potentially valuable source of germplasm, therefore, remains unexploited for breeding purposes.

In nature, polyploids can originate either asexually, by spontaneous chromosome doubling of somatic tissues, or sexually, by functioning of gametes or gametophytes with the unreduced chromosome number (2n gametes). This type of gametes can be produced by modifications of the meiotic process (Mendiburu & Peloquin, 1976), pre- or post-meiotic chromosome doubling, fusion of post-meiotic n nuclei, or from apomeiotic cells of the ovule (Asker, 1980; Hermsen, 1984). The various modes of 2n gamete formation may have different genetic consequences, which become evident when factors such as genetic variability,

inbreeding, heterosis and epistasis in the newly formed polyploids are considered (Mendiburu & Peloquin, 1976; Hermsen, 1984).

For many years spontaneous chromosome doubling was regarded as the major cause of polyploidization (Winge, 1917). In a large number of genera, however, polyploids have arisen sexually, following interspecific crosses, crosses between cytotypes within a species, or spontaneously, in open pollinated species (Harlan & de Wet, 1975; Camadro, 1986). The phenomenon of sexual polyploidization can be unilateral or bilateral according to whether one or both parents contribute the numerically unreduced gamete (Mendiburu & Peloquin, 1976). There is abundant experimental information indicating that 2n gamete formation in Angiosperms has an important genetic component. However, except for a few well studied species—such as potatoes, alfalfa, corn—the cytological mechanisms of 2n gamete formation and their genetic control are largely unknown (Camadro, 1986).

As a preliminary step to assess the feasibility of using sexual polyploidization to incorporate germplasm from related polyploid species into *A. officinalis* ($2n = 2x = 20$), Camadro (1992) examined 42 plants of the commercial cultivar UC 157 F_2 and found that 36 of them produced from 3% to 48% 2n pollen by a Second Meiotic Division Restitution (SDR) mechanism. These results were taken as an indication that the modified meiosis leading to SDR 2n pollen formation was under genetic control.

It was therefore predicted that if genetically controlled 2n pollen had been instrumental in the polyploid evolution of the genus *Asparagus*, this type of pollen should be found in diploids and in their related polyploids. To test this prediction, a 2n pollen search was undertaken in plants of garden asparagus and a related ornamental species, *A. densiflorus* cv. Sprengeri ($2n = 6x = 60$), and the cytological mechanism involved in the formation of the numerically unreduced pollen was determined.

Materials and methods

Pollen samples of *A. officinalis* cvs. Geynlim (18 plants) and Mary Washington (six plants) were collected in commercial fields in the province of Buenos Aires, Argentina. Those of *A. densiflorus* cv.Sprengeri ($2n = 6x = 60$) were collected from six plants pot-grown in a screenhouse at the Balcarce Agricultural Exp. Sta., I.N.T.A., in the same province. One plant of *A. plumosus* Baker ($2n = 2x = 20$), another ornamental species which was flowering at the time, was also included in the screening. Samples of each plant were stained on a glass slide with acetocarmine glycerol jelly (Marks, 1954) and observed with a microscope at 500×.

To examine microsporogenesis, flower buds were fixed in 3:1 (95% ethanol: glacial acetic acid) for 24 h, and kept in 70% ethanol in a refrigerator until used. Fixed buds were stained with Snow carmine (Snow, 1963) for one week and intensified with haematoxylin (Núñez, 1968). Slides were observed under a light microscope, at 1250×.

Results

The results of the pollen screening are presented in Table 1. In cv. Geynlim, pollen grains of heterogeneous size were produced by 15 plants out of 18 examined. The percentage of large size grains varied between 3% and 5% in eight plants, and more than 5% in the remaining seven. Four of the latter had also giant grains in low frequencies (less than 1%). In cv. Mary Washington, all plants produced pollen of heterogeneous size, with 5% or more large pollen. The only plant of *A. plumosus* examined produced more than 5% large pollen. In cv. Sprengeri, only two plants out of the six

Table 1. Results of pollen screening in plants of 2x *Asparagus officinalis* L., 2x *A. plumosus* Baker and 6x *A. densiflorus* (Kunth) Jessop cv. Sprengeri

Material	No. plants		
		With heterogeneous pollen	
	Total analyzed	3–5%[1]	> 5%
A. officinalis			
cv. Geynlim	18	8	7[2]
cv. Mary Wash.	6	0	6
A. plumosus	1	0	1
A. densiflorus			
cv. Sprengeri	6	0	2[3]

[1]Large pollen
[2]4 plants with <1% giant pollen
[3]1 plant with 1.5% giant pollen

examined produced pollen of heterogeneous size. One of them had only 10% fertile pollen, with roughly equal frequencies of large and small grains, and the other had 23% large pollen and 2% giant grains (Fig. 1).

Microsporogenesis was examined in three plants of cv. Geynlim which produced pollen of heterogeneous size, one plant of *A. plumosus*, and in all plants of cv. Sprengeri. The events observed in the three species were similar, as described below.

In *Asparagus* spp., cytokinesis occurs successively at Telophase I and Telophase II, giving rise to a tetrad of n microspores (Fig. 2). In all plants examined, meiosis was normal in all meiocytes until Anaphase II. At this stage, however, spindle formation did not occur in either one or both cells of a relatively large number of meiocytes and, consequently, chromosomes failed to migrate towards opposite cell poles (Fig. 3). In a few meiocytes, absence of chromosome migration was observed at both Anaphase I and Anaphase II. At Telophase II, meiocytes with four, three, two or one groups of chromosomes were observed (Fig. 4a, b). Cytokinesis did not occur in cells in which chromosome disjunction did not take place and, consequently, triads of two small and one large microspore, dyads of large microspores and monads were formed (Fig. 5a, b).

More than 150 meiocytes were examined per plant (Table 2). In cv. Geynlim, the average frequencies of tetrads, triads and dyads were 55%, 18% and 26%, respectively; 1% monads were also observed in a plant that produced giant pollen grains. The corresponding frequencies were 66%, 11% and 16% in cv. Sprengeri and 61%, 15% and 21% in *A. plumosus*, with 7% and 4% monads, respectively. Monads developed into giant grains with double unreduced chromosome numbers.

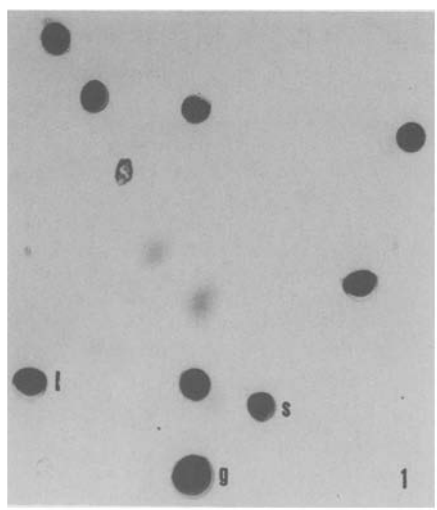

Fig. 1. Pollen of heterogeneous size in *Asparagus densiflorus* (Kunth) Jessop cv. Sprengeri; ×500 (g = giant; l = large; s = small).

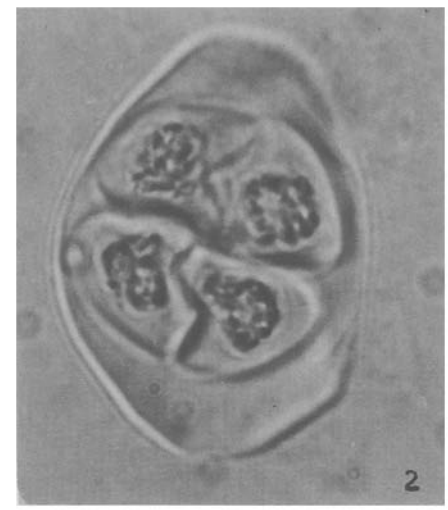

Fig. 2.

Discussion

The modified meiosis observed in plants of 2x *A. officinalis*, 6x *A. densiflorus* cv. Sprengeri, and 2x *A. plumosus* led to the formation of 2n microspores with nuclei genetically equivalent to second meiotic

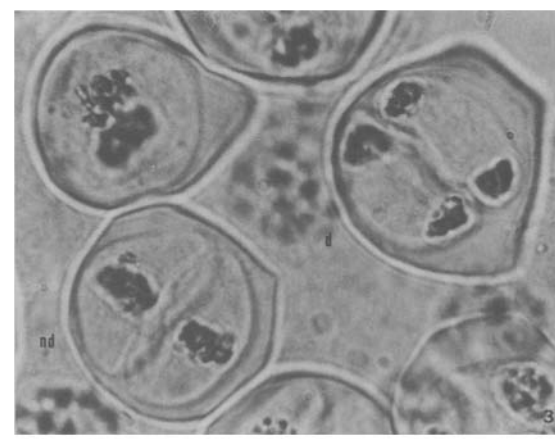

Fig. 3.

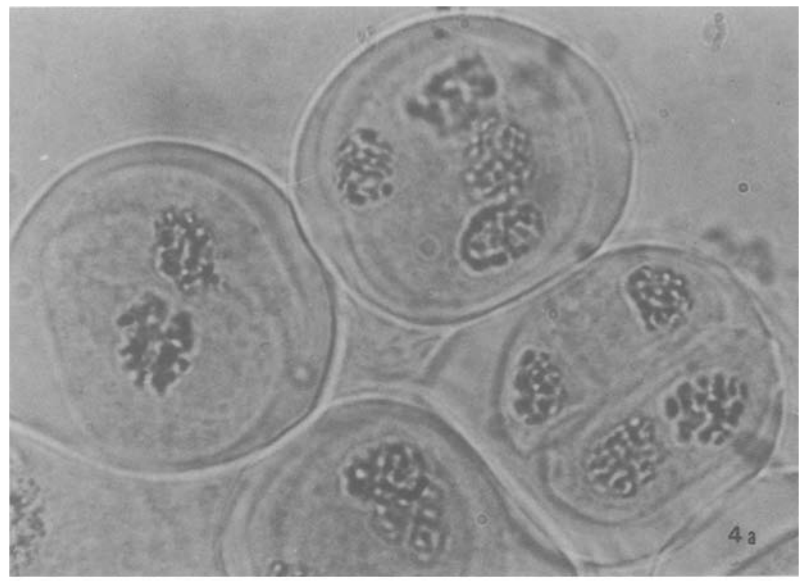

Fig. 4(a).

division restitution (SDR) products. The cytological mechanism involved is identical to the ones reported by Camadro (1992) for cv. UC 157 F_2 of garden asparagus, by Mashkina (1979) in *Prunus cerasus* L., and by Sala et al. (1989) in *Lolium perenne* L. Other SDR cytological mechanisms leading to functional 2n pollen have been described in *Rumex thyrsiflorus* Fingerh. × *Rumex acetosa* L. F_1 hybrids (Swietlinska, 1960; Swietlinska & Zuk, 1965), *Brassica rapa* L. (Stringham, 1970), and tuber-bearing Solanums (Mok & Peloquin, 1975; Iwanaga & Peloquin, 1982).

In *Lolium* (Sala et al., 1991), SDR 2n pollen was functional in 4x × 2x crosses, giving rise to tetraploid progeny. In the *Asparagus* plants analyzed, 2n microspores developed into large size pollen grains (2n pollen) whose functionality in crosses was not determined. However, four tetraploid plants were identified among 200 field grown

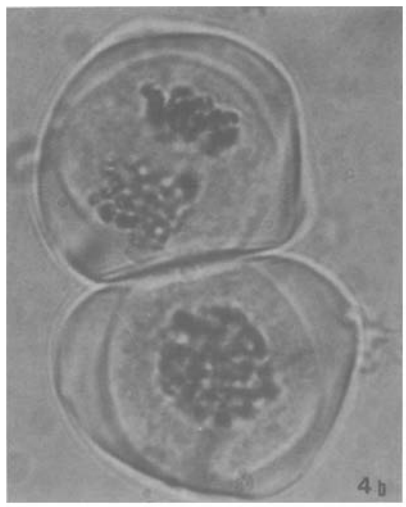

Fig. 4(b).

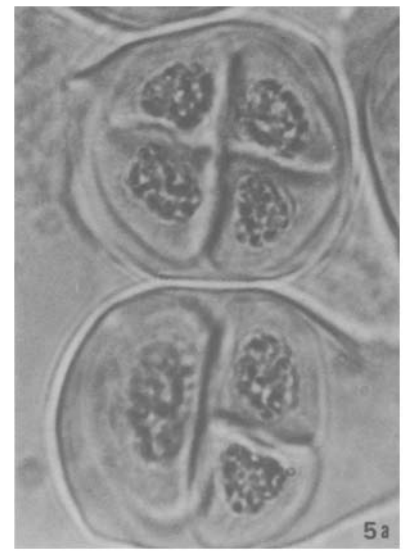

Fig. 5(a).

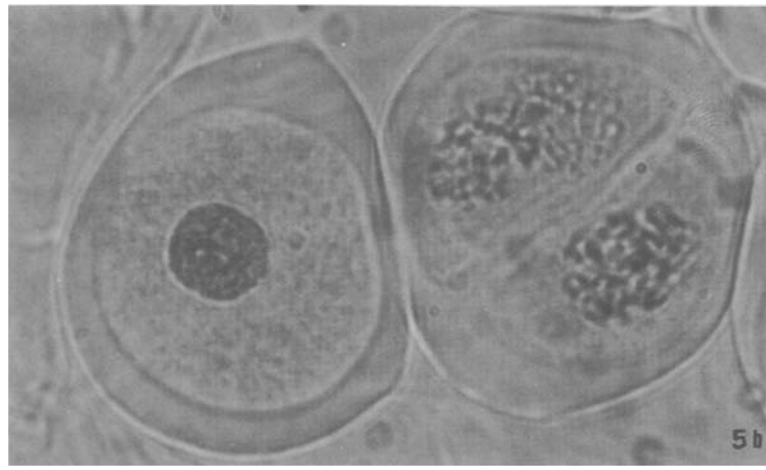

Fig. 5(b).

Figs. 2–5. Microsporogenesis in *Asparagus densiflorus* (Kunth) Jessop cv. Sprengeri, × 1250.
2. Normal tetrad of n microspores
3. Late Anaphase II (d = normal chromosome disjunction; nd = chromosome non-disjunction)
4. Telophase II: (a) meiocytes with two and four groups of chromosomes; (b) meiocytes with one and two groups of chromosomes
5. Tetrad stage: (a) tetrad of n microspores and triad of one 2n and two n microspores; (b) dyad of 2n microspores and monad

plants of cv. UC 157 F_2 produced by open pollination (unpublished results) and Skiebe et al. (1991), working with the same species, obtained 42 triploid and four tetraploid plants from 1168 4x × 2x crosses. They also cite a previous report by Falavigna & Fantino (1985), on the production of polyploids from 4x-2x crosses. These data indicate that 2n pollen in *A. officinalis* is actually functional in interploid crosses.

A high proportion of the plants analyzed in this study (83% in *A. officinalis* and 100% in *A. densiflorus* cv. Sprengeri and *A. plumosus*) and in Camadro's (1992) (86% in *A. officinalis*) produced 2n pollen by the same SDR cytological mechanism. Since these are related species belonging to a polyploid series, the results obtained are considered an indication that 2n pollen formation in them is not the result of accidental disturbances in meiosis but is, rather, under genetic control. On the other hand, no information is available on frequencies of 2n egg production or mode of formation in the genus, although polyploid plants have been obtained in 2x × 4x crosses in garden asparagus (Skiebe et al., 1991).

Table 2. Number of monads, dyads, triads, and tetrads at the tetrad stage in microsporogenesis of 2x *A. officinalis* L., 2x *A. plumosus* Baker and 6x *A. densiflorus* (Kunth) Jessop cv. Sprengeri

Material		Meiocytes analyzed	Monads	Dyads	Triads	Tetrads
A. officinalis						
cv. Geynlim	1	179	2	24	15	138
	2	153	0	36	45	72
	3	190	0	78	36	76
A. plumosus		198	8	41	29	120
A. densiflorus						
cv. Sprengeri	1	174	0	26	41	107
	2	169	3	6	14	146
	3	172	0	14	14	144
	4	200	66	82	12	40
	5	148	0	24	18	106
	6	174	2	18	18	136

Most mutants which affect the meiotic process leading to the formation of numerically unreduced gametes have been found to be recessive. The variability in the frequency of 2n gamete production observed in given genotypes of various Angiosperms has been explained by assuming that the gene(s) controlling the formation of restitution nuclei has incomplete penetrance and that its expression is influenced by the environment (see Camadro, 1986). These observations could also apply to the *Asparagus* species under consideration, although studies of inheritance of the trait and its stability of expression under various environments are needed to draw a conclusion.

In *A. officinalis*, haploids appear spontaneously in polyembryonic seeds in low frequencies (Randall & Rick, 1945; Marks, 1973). It can be envisioned then that, in nature, recurrent polyploidization via the functioning of genetically controlled 2n gametes and haploidization could provide for continuous gene flow and introgression among ploidy levels, in a manner resembling the diploid-tetraploid-haploid cycles reported for the agamospermous *Dichanthium* species (de Wet, 1968). This opens the possibility of broadening the genetic base of the polyploid populations, generating genetic variability upon which natural selection can act. In contrast with sexual polyploidization, chromosome doubling in vegetative tissues and accidental disturbances in meiosis leading to the formation of 2n gametes appear to be isolated events of limited evolutionary potential.

From the point of view of breeding, if hybridization between diploid garden asparagus and its related polyploid species is prevented by post-fertilization barriers, the choice of diploid parents producing 2n gametes might eventually circumvect the problem, as has been the experience with other genera (Johnston et al., 1980). Ploidy level manipulations, then, would have to be performed with the hybrids to produce diploid plants with the desirable traits for their use in breeding.

Alternatively, a breeding program could be carried out at the polyploid level, as Skiebe et al. (1991) have proposed, since the expression of relevant agronomic characters such as spear diameter and yield has a large heterotic component. With a maximum of four alleles per locus, the tetrasomic level is of greater productive potential than the disomic because it can harbor greater diversity per locus. This generates the possibility of promoting heterotic responses not attainable at the lower ploidy level (Mendiburu et al., 1974). In this regard, FDR 2n pollen production is desirable due to its capacity of capturing, transmitting and compounding at a higher ploidy level a large proportion of the heterozygosity present in the diploid parent.

The large proportion of plants producing 2n pollen in high frequencies identified in commercial cultivars makes possible the use of the sexual polyploidization approach. However, all plants analyzed produced SDR 2n microspores, which are highly homozygous. But, since they are formed by independent meiotic processes, the population of spores produced by a plant would present a high level of heterogeneity which could be of value to increase the genetic variability of polyploid populations for breeding purposes.

Asparagus breeding at the polyploid level is still in an early stage of development (Skiebe et al., 1991). Further and intensive breeding work needs to be carried out to test the feasibility of this approach in contrast to conventional diploid breeding, and to assess the advantages of sexual vs. asexual polyploidization in the generation of polyploids.

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