Cytological Mechanism of 2n Microspore Formation in Garden Asparagus

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Abstract. Microsporogenesis was studied in 42 randomly chosen F₁ plants of garden asparagus (Asparagus officinalis L. cv. UC 157) (2n = 2x = 20) that had been previously screened for production of pollen of heterogeneous size. At the tetrad stage, the average frequencies of tetrads, triads, and dyads were 58.9%, 15.4%, and 25.9%, respectively. Dyads and triads originated from the lack of chromosome migration toward opposite poles at anaphase II in either one or both cells of a microsporocyte, followed by the absence of cytokinesis in telophase II. The resulting 2n microspores were, therefore, genetically equivalent to second meiotic division restitution products. The observation that all plants examined produced 2n microspores in high frequencies is taken as an indication that the modified meiosis in these plants is under genetic control.

The genus Asparagus includes 150 species of herbaceous perennial and tender woody shrubs and vines grown as ornamentals, except for garden asparagus, which is cultivated for its edible shoots. The species in this genus form a polyploid series with 2x, 4x, and 6x somatic chromosomes (x = 10).

Hybridization attempts between the garden asparagus (2n = 2x = 20) and its related polyploid species have been largely unsuccessful, although the basis for this failure is not known (McCollum, 1988). Therefore, a potentially valuable source of germplasm for breeding purposes remains unexploited.

Hybridization in interploid crosses may be prevented by postfertilization breeding barriers such as endosperm failure. This problem may be circumvented if 2n gametes (gametes or gametophytes with the unreduced chromosome number) are formed. This type of gamete can be produced by modifications of meiosis (Mendiburu and Peloquin, 1976), pre- or postmeiotic chromosome doubling, fusion of postmeiotic 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 (Hermsen, 1984; Mendiburu and Peloquin, 1976).

There is abundant experimental information indicating that 2n gamete formation in angiosperms has an important genetic component (Harlan and de Wet, 1975). However, except for a few well-studied species such as potatoes (Solanum spp.), alfalfa (Medicago spp.), and corn (Zea mays L.) the cytological mechanisms of 2n gamete formation and their genetic control are largely unknown (Camadro, 1986).

The production of polyploids via the functioning of 2n gametes has been termed "sexual polyploidization" (Mendiburu and Peloquin, 1976). The objective of this study was to investigate the occurrence of 2n gametes in garden asparagus to assess the feasibility of using the sexual polyploidization

Fig. 1. Pollen of heterogeneous size: large (1) and small (s) (× 97.5).
normal tetrad of n microspores (x 1250).

Fig. 2. Normal tetrad of n microspores (x 1250).

approach to hybridize it with its related polyploid species.

Screening for 2n pollen was carried out in F1 plants of the hybrid 'UC 157' grown in the field at Balcarce. Pollen samples of 42 plants were stained on a glass slide with acetocarmine glycerol jelly (Marks, 1954) and observed with a microscope at x 125.

Thirty-six of the plants examined produced pollen of heterogeneous size, large and small (Fig. 1). The frequency of large pollen per plant, estimated > 200 grains per sample, ranged from 5% to 48%. The large pollen grains were presumably 2n pollen.

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

As in other Monocotyledoneae, cytokinesis in Asparagus occurs successively at telophase I and telophase II, giving rise to a tetrad of n microspores (Fig. 2). In the 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 many meiocytes, and, consequently, chromosomes failed to migrate toward opposite cell poles (Fig. 3). At telophase II, meiocytes with four, three, or one large microspore or dyads of large microspores were formed (Figs. 4 and 5). In more than 200 meiocytes examined per plant, the average frequencies of tetrads, triads, and dyads were 58.9% ± 10.1%, 15.4% ± 2.3%, and 25.9% ± 12.1%, respectively.

In the F1 plants of 'UC 157' studied, 2n microspores originated by a cytological mechanism genetically equivalent to the restitution of the second meiotic division products (Second Division Restitution (SDR)). This mechanism is identical to those observed in Prunus cerasus L. (Mashkina, 1979) and Lolium perenne L. (Sala et al., 1989).

In Lolium, the SDR 2n pollen was functional in 4x × 2x crosses, giving rise to tetraploid progeny. In Asparagus, 2n microspores developed into large pollen grains (2n pollen); however, it has not been determined if such pollen grains are functional in interplloid crosses. Other SDR cytological mechanisms leading to functional 2n pollen have been described in Rames thyriflorus × Rames acetosa F1 hybrids (Swietlinska, 1960; Swietlinska and Zuk, 1965), Brassica campestris L. (Stringham, 1970), and tuber-bearing Solanum (Iwanaga and Peloquin, 1982; Mok and Peloquin, 1975).

Most mutants affecting the meiotic process are recessive (Camadro, 1986). All plants examined in this study produced 2n microspores through the same cytological mechanism. This result indicates that 2n microspore production in these F1 plants of 'UC 157' is under simple genetic control and that the parents of the F1 hybrid from which they were derived were, possibly, homozygous for the gene(s) involved. Further, the variability in 2n microspore frequency observed in various plants may be explained by incomplete penetrance of the gene(s) involved and environmental influences on gene expression.

A wider search should be undertaken in other cultivars to establish if 2n pollen production is a common phenomenon in A. officinalis. Nevertheless, it can now be concluded that 2n pollen could be used to incorporate related polyploid germplasm into an asparagus breeding program if hybridization in interplloid crosses is prevented by postfertilization breeding barriers such as endosperm failure.

**Literature Cited**


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