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STUDIES IN LIBYAN GRASSES VIII. APOMIXIS IN *PENNISETUM DIVISUM* SENSU LATO[1] AND *P. SETACEUM* (FORSSK.) CHIOV.

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Abstract

The Libyan material of *Pennisetum divisum* sensu lato and *P. setaceum* (Forssk.) Chiov was studied embryologically. Both the species demonstrated facultative apomixis. The frequency of apomictic and multiple embryo-sacs in *P. divisum* was found to be much higher than in *P. setaceum*. However, polyembryony was observed in *P. setaceum* only.

Introduction

Pennisetum divisum sensu lato and *P. setaceum* (Forssk.) Chiov from Libya were studied embryologically. The latter species with 2n = 27 chromosomes was studied earlier by Narayan (1962). The material of *P. setaceum* used in the present study, however, has n = 18 chromosomes.

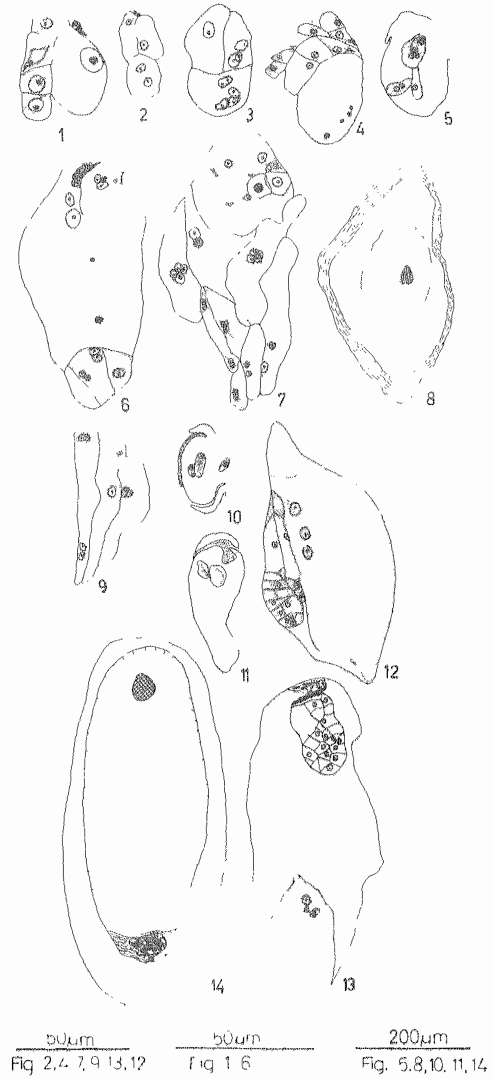
Materials and Methods

Floral buds at their different stages of development were collected from the nursery of local grasses of the Department of Botany, Al-Faateh University, Tripoli, Libya.

The material was preserved in 3:1, alcohol acetic and, dehydrated in alcohol-xylol series and serial sections were cut at 7-9 μ thickness. These were stained in safranin and light green. The study was confined to the female gametophyte only.

Results

The female gametophyte: The ovule in *P. divisum* is anatropous, bitegmic and crassinucellate. The archesporial cell directly functions as megaspore mother cell, which



Fgs. 1-8. *Pennisetum divisum* sensu lato. Fig.1. Showing one linear megaspore tetrad and one functional megaspore. Fig.2. 2-healthy megaspores and a 2-nucleate embryo sac. Fig.3. One healthy megaspore and two embryo sacs developing from the same tetrad. Fig.4. 4-nucleate embryo sac and a group of megaspore tetrads. Fig.5. Three embryo sacs. Fig.6. Entry of pollen tube and one of the antipodal cells is 4-nucleate. Fig.7. 9-embryo sacs. Fig.8. L.S. ovary wall and ovule with a well developed sexual embryo and cellular endosperm.

Figs. 9-14 *Pennisetum setaceum* Fig.9. Showing 3-embryo sacs. Fig.10. 3-embryo sacs at different places. Fig.11. Showing one partly extra-ovular embryo sac and two sacs within the ovule. Fig. 12. A 3-nucleate sexual embryo sac and a somatic embryo-like structure. Fig. 13. A micropylar sexual embryo and a 4-nucleate antipodal cell. Fig. 14. A well developed sexual embryo, cellular endosperm and one antipodal cell is embryo-like strcuture.

undergoes meiosis and usually forms a linear tetrad (Fig. 1). Normally the chalazal megaspore becomes functional (Fig. 1) while the three micropylar megaspores degenerate. Sometimes more than one megaspore becomes healthy (Fig. 1-3) resulting in the formation of more than one embryo sac (Fig. 3).

The chalazal megaspore usually develops into an 8-nucleate Polygonum type of embryo sac. Variations, however, are present. Besides the megaspore formed from the archesporium, there is evidence that other somatic cells also become megaspore mother cells and divide meiotically forming a group of tetrads (Fig. 4). One or more cells of these tetrads, however, may degenerate. Furthermore, there is evidence that a number of embryo sacs develop directly from the somatic cells (Fig. 5) and in one ovule up to nine embryo sacs are recorded at their different stage of development (Fig. 7). Occasionally one of the three antipodals becomes 4-nucleate (Fig. 6).

The ovule, megasporogenesis and the development of embryo sac in *Pennisetum setaceum* is similar to that of *P. divisum*. Although it differs from *P. divisum* in few respects. The multiple embryo sacs are not as common in *P. setaceum* as in *P. divisum*. In *P. setaceum* up to three embryo sacs are occasionally observed (Fig. 9). The position of these three embryo sacs indicates that they do not orginate from a single tetrad, since they are neither linear nor T-shaped. The three embryo sacs seem to have originated from somatic tissue. Similarly in Fig. 10 at least 2 embryo sacs appear to be somatic in nature. Furthermore, unlike *P. divisum* partly extra-ovular embryo sacs are observed in *P. setaceum* (Fig. 11).

Besides other differences, the timings of the sexual and somatic embryo sacs are also different. In *P. setaceum* the sexual embryo sac is observed sometimes at its early stages of development, while those from somatic tissue show an embryo like structure (Fig. 12). In another instance the egg of a sexual embryo sac shows a well developed embryo, while at the same time one of its antipodals has become 4-nucleate (Fig. 13). Later this forms a haploid embryo-like structure (Fig. 14).

Fertilization and pollen tube formation is normal is sexual embryo sacs of both species. However, a clear fusion of male nucleus with the egg nucleus is not seen. Pollen tube entry in the sexual embryo sac is observed clearly in *P. divisum*. In the early stages, the development of endosperm is of Nuclear type but later on it becomes cellular in each species. The fully developed embryo as well as endosperm is clearly observed in both species (Fig. 8, 14).

Discussion

Previous studies (Pienaar, 1955; Sharma & De, 1956; Narayan, 1954, 1962; Gildenhuys & Brix, 1959; Gupta, 1971; Davidse & Pohl, 1972; Rangaswamy, 1972; Mehra

& Remanandan, 1973; Khosla & Mehra, 1973; Khosla & Sharma, 1973; Queiros, 1973) as well as our own observations (Faruqi & Quraish, to be published elsewhere) show that both polyploidy and apomixis are quite common in the genus *Pennisetum.*

Although clear meiotic stages in the megaspore mother cell were not observed, yet in both *P. divisum* and *P. setaceum* tetrads were found which were presumably a result of meiotic division. The embryo sacs formed out of these tetrads were regarded as sexual. The sexual embryo sacs were usually healthy. Evidence of fertilization and their subsequent development into an embryo-like structure demonstrated that sexual reproduction is present in both species. Bu' the development of embryo sacs from the somatic cells of the integument or chalazal region as in *P. divisum* and integument or nucellus (partly extra-ovular) as in *P. setaceum* without the formation of a tetrad is a good evidence that apomixis also is present in both species. Moreover, formation of tetrads in some of the somatic cells points out that sexual embryo sacs develop also from somatic tissue.

In *P. divisum* three categories of embryo sacs were found of which the sexual embryo sac is formed from an archesporium. After the development of megaspore for the proper embryo sac a number of other cells are induced to form linear tetrads whose number ranged from 1-4. Moreover, in these embryo sacs, several cells of chalazal region may form aposporous embryo sacs. The number of embryo sacs in this species could be as many as nine. Narayan (1962) reported nine aposporous initials in *P. setaceum,* but in the Libyan material the maximum number of aposporous embryo sacs in *P. setaceum* is only three. The aposporous embryo sacs when fully formed are 4-nucleate. Although Brown & Emery (1958) have differentiated sexual and apomictic embryo sacs on the basis that the sexual embryo sacs in panicoid grasses are 8-nucleate whereas apomictic ones are 4-nucleate. Gildenhuys & Brix (1959) on the basis of their study on *P. dubium* suggest that in this species even an 8-nucleate embryo sac could be apomictic, whereas a 4-nucleate embryo sac is always so.

The same authors further point out that at the binucleate stage the nuclei in the aposporic embryo sac are usually at one end of the sac. This situation, however, is not alalways true. Also in our own study a close association of the 2 nuclei at binucleate stage is often found, but again similar to *P. dubium* (Gildenhuys & Brix, 1959) in a few sacs such association is lacking (Fig. 10). Narayan (1954) pointed out that aposporic embryo sacs have larger nuclei as compared to haploid embryo sacs. This, however, is not the case either in *P. divisum* or *P. setaceum* where sexual embryo sacs usually show more prominent nuclei than those in the apomictic sacs. Another difference between asexual and apomictic embryo sac was pointed out by Saran & DeWet (1969) who found an opening in the 8-nucleate sexual embryo sac but such an opening was lacking in 4-nucleate aposporous sac in *Dichanthium intermedium.* Such a difference again could not be found either in *P. divisum* or *P. setaceum.* It seems, therefore, that probably the only reliable indicators for the nature of the embryo sac are its initial position and its mode of development.

In *P. divisum*, besides normal, nucellar and integumentary aposporous sacs, sometimes embryo sacs were found also inside the chalazal region. Such embryo sacs of chalazal origin, however, could not be found in *P. setaceum.* Although multiple embryo sacs were noted in both species, yet polyembryony was observed in *P. setaceum* only.

Rosenberg (1907), Stebbins & Jenkins (1939) and Narayan (1962) suggested that physical destruction of either the embryo sac mother cell or the developing megaspore is brought about by the aggressive growth of the aposporous initials. Studies on *Poa pratensis* (Engelbert, 1940; Nielson, 1946), *Pennisetum clandestinum* (Narayan, 1954) as well as our own study on *P. divisum* and *P. setaceum* suggest that both the kinds of embryo sacs could develop simultaneously.

Entry of pollen tube in the sexual embryo sac was observed clearly in *P. divisum* (Fig. 6). It is however, interesting to note that apomictic embryo sacs develop at a latter stage than sexual embryo sacs. However, in *P. setaceum* this is not always true, since an aposporous embryo-like structure is seen at a stage when the sexual embryo sac is at its early stages of development. Presence of both n=18 and 2n=27 chromosomes in *P. setaceum* suggests that similar to *Bothriochloa intermedia* (Faruqi, 1964) and *Hyparrhenia hirta* (Farqui & Quraish, 1978) fertilization of the apomictic embryo sac also takes place in *P. setaceum.*

The species *Pennisetum divisum* and *P. setaceum* seem to have a similar mode of reproduction where both sexual as well as apomictic embryo sacs are formed, but the frequency of apomictic embryo sacs in *P. divisum* is much higher than in *P. setaceum.* Thus facultative apomixis is present in both species.

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