Proc. Indian natn. Sci. Acad.
Vol. 43, Part B, No. 5, pp. 175-183 (1977)
Plant Embryology

THE MODE OF REPRODUCTION IN *BOTHRIOCHLOA ODORATA* (LISBOA) A. CAMUS AND *PASPALUM DISTICHUM* LINN.

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(*Received* 2 *August* 1977)

The present investigation deals with two perennial grass species *Bothriochloa odorata* (Andropogoneae) and *Paspalum distichum* (Paniceae). The former is a tetraploid taxon with 2n=40 while the latter is hexaploid with 2n=60. *B. odorata* yields an aromatic oil with medicinal properties while *P. distichum* possesses slender rhizomes with extensive stolons and is used as a soil binder on the banks of streams.

Both taxa develop aposporous embryo sacs with pseudogamous mode of reproduction. The megaspore mother cell degenerates as such or soon after the formation of a tetrad. One to five aposporous embryo sacs arise from the cells of the nucellus. In *B. odorata*, unreduced embryo sacs are four nucleate with one egg, two synergids and a polar nucleus or one egg, one synergid and two polar nuclei. In *P. distichum*, the mature aposporous embryo sacs are eight nucleate with the usual organisation.

The embryo starts developing from the diploid egg cell. It fails to reach maturity in the ovules where there is no endosperm formation. In *B. odorata*, the endosperm is triploid and is formed as a result of fusion between the diploid polar nucleus and a male gamete. In *P. distichum*, it is pentaploid, as both the polar nuclei take part in fertilization.

INTRODUCTION

The family Gramineae is specially noteworthy for the high frequency with which seed production by apomixis occurs in various taxa. Nygren (1967), *reviewed apomixis* in angiosperms and listed 91 grass species belonging to 28 genera, which show apomixis of one kind or another. Out of these 61 species are aposporous, 16 diplosporous and 10 viviparous. In the remaining species, the condition is doubtful.

In the subfamily Panicoideae, unreduced embryo sacs with a few exceptions such as *Dichanthium annulatum* (Reddy & D'cruz, 1969*a*) and *Paspalum secans* (Snyder, 1957), are typically four nucleate, although sexual species in the same genus or sexual forms of the same species have Polygonum-type of embryo sac. Such a relationship between reduced and unreduced embryo sacs does not exist in the subfamily Pooideae and the apomictic species always have eight nucleate embryo sacs.

In the apomictic species, multiple embryo sacs are formed due to the activity of a number of aposporous initials. Polyembryony has been reported in many species of the grass family. This may be due to the multiple embryo sacs as in *Dichanthium*

Presented at the Indo-Soviet Symposium on "Embryology of Crop Plants" held at Delhi, from March 2-6, 1976 (Convener : Prof. B. M. Johri, FNA) *annulatum* (Reddy & D' cruz, 1969*b*) and *Poa pratensis* (Nielsen, 1946). In *Trypsacum dactyloides* (Farquharson, 1955), the extra embryos arise from synergids within the same embryo sac or from the egg or synergid of a second embryo sac.

Most of the aposporous species are pseudogamous and the endosperm may be triploid or pentaploid. But in *Paspalum secans* (Snyder, 1957), the endosperm is hexaploid because the divisions in the microspore mother cells are mitotic. In most of the diplosporous species the endosperm has double the chromosome number of the mother plant. This is brought about by an autonomous development of the central nucleus as in many species of *Calamagrostis* (cf. Nygren, 1967). Few other diplosporous species such as *Poa alpina* (Hakansson, 1943), are pseudogamous. In such cases, fertilization of the central nucleus is essential for the development of the endosperm.

Materials and Methods

The material of *Bothriochloa odorata* was collected from Kalka hills and that of *Paspalum distichum* from Chandigarh plains. *B. odorata* is a tetraploid with 2n=40 and *P. distichum* is hexaploid (2n=60). Mehra *et al.* (1968) carried out a cytological study of these taxa. They reported irregular meiosis in *P. distichum*. Meiosis was found to be normal in *B. odorata*. *B. odorata* contains an aromatic oil with medicinal properties while *P. distichum* possesses slender rhizomes with extensive stolons and is used as a soil binder on the banks of streams.

For sectioning inflorescences at various stages of development were fixed in FAA (50% ethyl alcohol, 90 ml, acetic acid, 5ml and formaldehyde, 5ml). The florets were dehydrated in tertiary Butyl alcohol — ethyl alcohol series (Johansen, 1940), and embedded in paraffin. Sections were cut from 8-15 μ depending upon the size and age of florets. For staining safranin — fast green combination and Hiedenhain's hematoxylin were used.

For the study of microsporogenesis in anthers and cytology of the endosperm, material was fixed in acetic alcohol (three parts ethyl alcohol and one part acetic acid). Young anthers were squashed in a drop of 1% aceto-carmine. The ovaries after anthesis were stained and hydrolysed in a warm 9:1 solution of aceto-carmine and N. HCl. The endosperm tissue was dissected out of the ovules and squashed in a drop of 1% aceto-carmine. Voucher specimens have been deposited in the Harbarium, Department of Botany, Panjab University, Chandigarh.

Results

Bothriochloa odorata

Megasporogenesis and female gametophyte : A single hypodermal megaspore mother cell is distinguished in the young ovules of this species. This may degenerate as such or it may undergo the meiotic divisions to form a linear tetrad. These tetrads soon degenerate and 3-4 nucellar cells become conspicuous due to their large size and prominent nuclei (Fig. 1). These are the aposporous initials. These initials enlarge due to vacuolation (Fig. 2). Though a number of aposporous initials are formed, the one near the micropylar end develops into a mature embryo sac. Its nucleus undergoes a mitotic division and the two nuclei thus formed remain at the micropylar region of the embryo sac (Fig. 3). Another mitotic division in each of these two nuclei results in a four nucleate embryo sac. These four nuclei organize either into an egg, two synergids and a polar nucleus (Fig. 4) or an egg, one synergid and two polar nuclei. The former condition was of more frequent occurrence and was observed in about 70% of the ovules studied. In some ovules the two polar nuclei were of unequal size (Fig. 5) and in a few cases one of the two polar nuclei gave a degenerating appearance. Due to the functioning of a number of aposporous initials multiple embryo sacs were observed in a number of ovules. In many ovules an embryo sac was organized in the region of the ovular attachment (Fig. 6). At the time of fertilization the number of four nucleate embryo sacs in different ovules varied from one to three. Eight nucleate embryo sacs were rarely observed (2 ovules out of a total of 70). These embryo sacs possessed an egg, two synergids, two polar nuclei and an antipodal complex of 4-6 cells, each with two to three nuclei.

Fertilization and pseudogamy : Fertilization of the polar nucleus was observed in a number of ovules. Figure 7 shows one of the male gametes in the process of fusion with the polar nucleus. Syngamy was not observed indicating that the egg develops parthogenetically. In most of the ovules a single aposporous embryo sac develops further, although they possessed multiple embryo sacs. The rest of the embryo sacs gradually degenerate and disappear. In many ovules one to three globular proembryos were also observed (Fig. 8). These embryos occurred in the micropylar or chalazal region of the embryo sac. Such ovules showed the absence of endosperm formation and the polar nucleus or nuclei could still be observed. These embryos never reach maturity. The mature caryopsis always revealed a single embryo and the cellular endosperm (Fig. 9). The seed set was observed to be about 30% in this species.

Chromosome number in the endosperm — A cytological study of the endosperm reveals about sixty chromosomes (Fig. 10). This number is obviously derived by a fusion between the unreduced polar nucleus having 40 chromosomes and a male nucleus with the reduced number of 20 chromosomes. These observations support the occurrence of pseudogamous mode of reproduction in this species.

Paspalum distichum

Megasporogenesis and female gametophyte: In this species also a hypodermal megaspore mother cell is differentiated in the nucellus of young ovules as usual. A peculiar condition observed was that the megaspore mother cells did not develop beyond the early prophase stage. One to three nucellar cells, surrounding the megaspore mother cell become prominent due to their large nuclei and dense cytoplasm (Fig. 11). These aposporous initials enlarge considerably due to vacuolation and ultimately one of them takes up the position which was earlier occupied by the megaspore mother cell (Fig. 12). The nucleus of the aposporus initial undergoes a mitotic division and the two nuclei thus formed move to the opposite poles (Fig. 13). The degenerating remains of the megaspore mother cell could be seen in the micropylar region of the enlarging aposporous initial or a two nucleate embryo sac in a number of ovules (Fig. 13). In this position the nuclei undergo two successive mitotic divisions resulting in the formation of an eight nucleate embryo sac with an egg, two synergids, two polar nuclei and three antipodals (Fig. 14). The antipodals become enlarged and contain 1-3 nuclei. Though a number of aposporous embryo sacs start developing, only one of them reaches the mature eight-nucleate stage.

Fertilization and pseudogamy: A number of stigmas were examined after anthesis and pollen grains were observed germinating on the stigma and the pollen tubes growing into the stigmatic hairs. The process of triple fusion was also observed. The egg, however, develops parthogenetically. Figure 15 shows a portion of the pollen tube lying close to the egg in the micropylar region. What appears to be a male nucleus can still be observed in the pollen tube. The division of the primary endosperm nucleus precedes that of the egg.

The egg apparatus in the ovules which do not receive a pollen tube, gives degenerated appearance. The egg might undergo a few divisions in such ovules. The embryonal mass, however, degenerates because no endosperm is formed without the stimulus of a male gamete. The mature caryopsis always had a single embryo with cellular endosperm (Fig. 16). The seed set was found to be about 34% in this species.

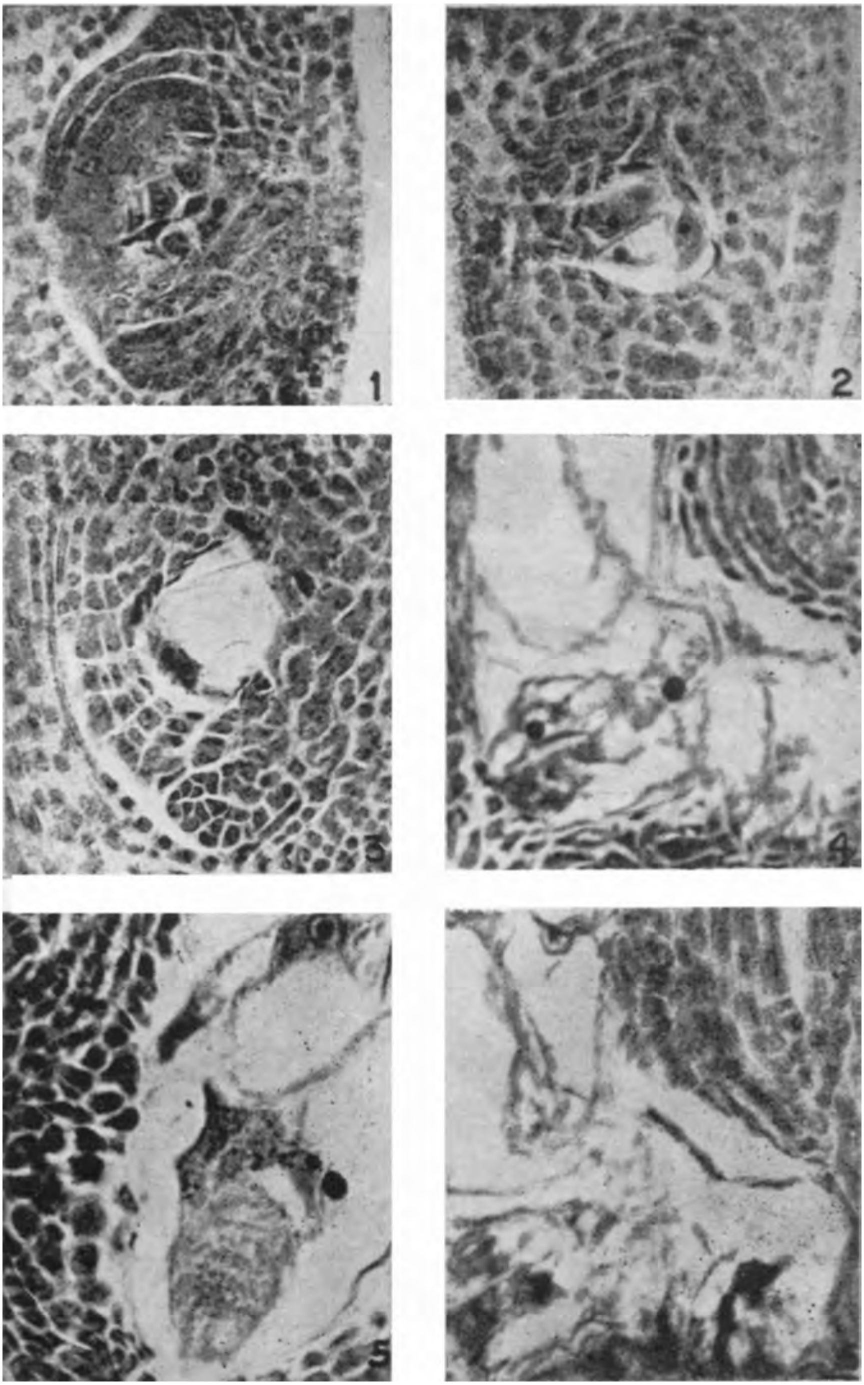
Chromosome number in the endosperm—The endosperm of this species revealed about 150 chromosomes (Fig. 17), which is obviously derived by a fusion between two polar nuclei (each with 60 chromosomes) and a male nucleus with 30 chromosomes. This is an evidence of the occurrence of pseudogamy in this species.

Discussion

Agamospermy has previously been reported in ten species of *Bothriochloa* (Brown & Emery, 1956, 1958; Harlan *et al.* 1964) and six species *Paspalum* (cf Nygren, 1967). All these species are aposporous. In *Bothriochloa odorata*, the megaspore mother cell or its products degenerate at a very early stage. The embryo sacs are formed from the nucellar cells. In *Paspalum distichum*, the megaspore mother cells do not divide further and degenerate as such.

In *Bothriochloa ischaemum*, Brown and Emery (1956), observed that megaspore mother cells regularly degenerate, either as a result of a physical crushing by the enlarging aposporus embryo sac initials or in the absence of such initials, by the action of some unknown sterility factor. In case of *Dichanthium annulatum* (Reddy & D'Cruz, 1969*a*), the megaspore mother cell degenerates in many ovules, followed by the formation of aposporous initials. In few ovules, the megaspore mother cell divided in a normal manner and such ovules are free from aposporous embryo sacs. In *Paspalum secans*, Snyder (1957) reported that mega spore mother cells frequently collapse without dividing. When meiosis does occur, it is marked by complete or almost complete chromosome asynapsis or desynapsis and is highly irregular. The products of division degenerate at various stages, and both cytological and breeding evidence indicates that functional gametophytes are produced very rarely, if ever, from the megaspores. In case of *Setaria leucopila* and *S. villosissima* (Emery, 1957), indications of apospory were not usually apparent until sexual embryo sacs were in two to four-nucleate stage.

Figs. 1-6. *Bothriochloa odorata*. 1, Degenerating tetrad and aposporous initials. x 480; 2, Enlarging aposporous initials. x 480; 3, Two nucleate aposporous embryo sac. x 480; 4, Four nucleate aposporous embryo sac with the egg, synergid (the second synergid was present in the adjacent section), and a polar nucleus. x 480. 5, A portion of the four nucleate embryo sac with unequal polar nuclei. x 480; and 6, An aposporous embryo sac in the region of the ovular attachment. x 480.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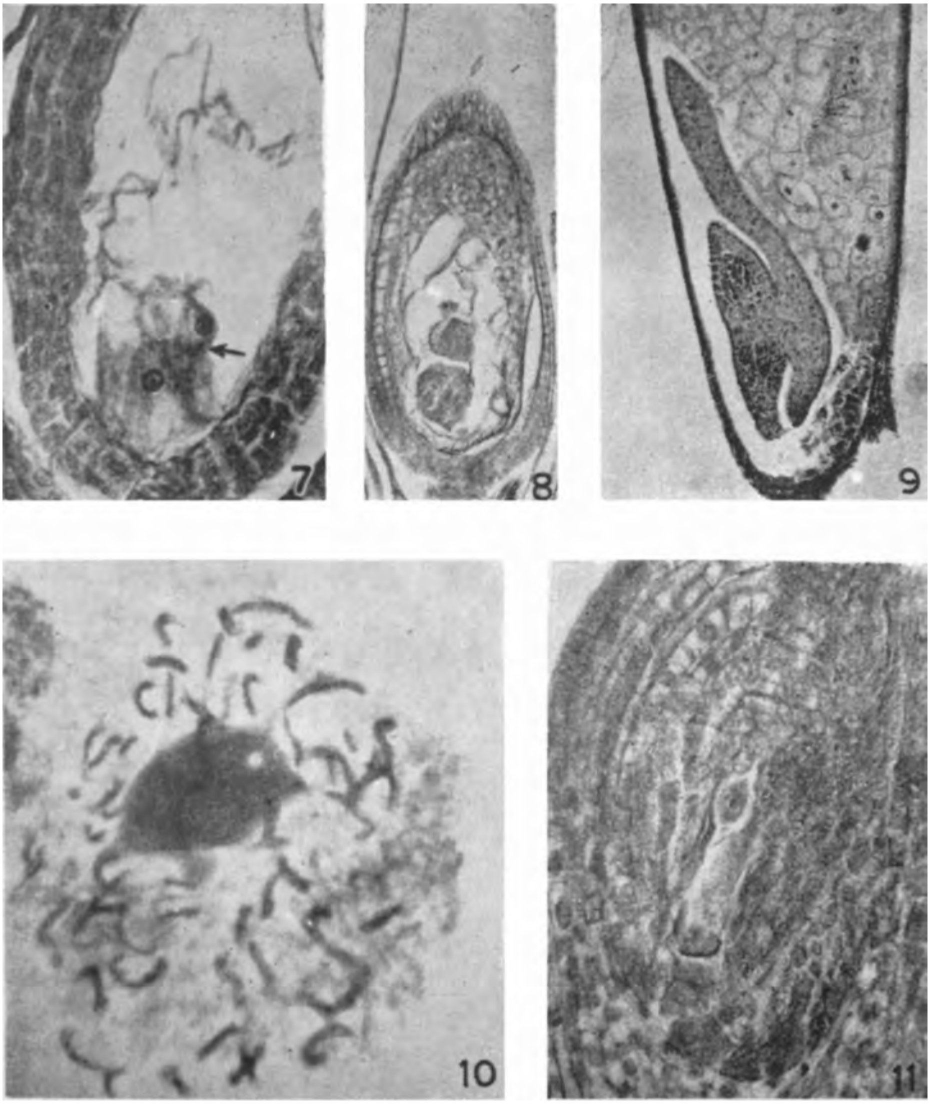
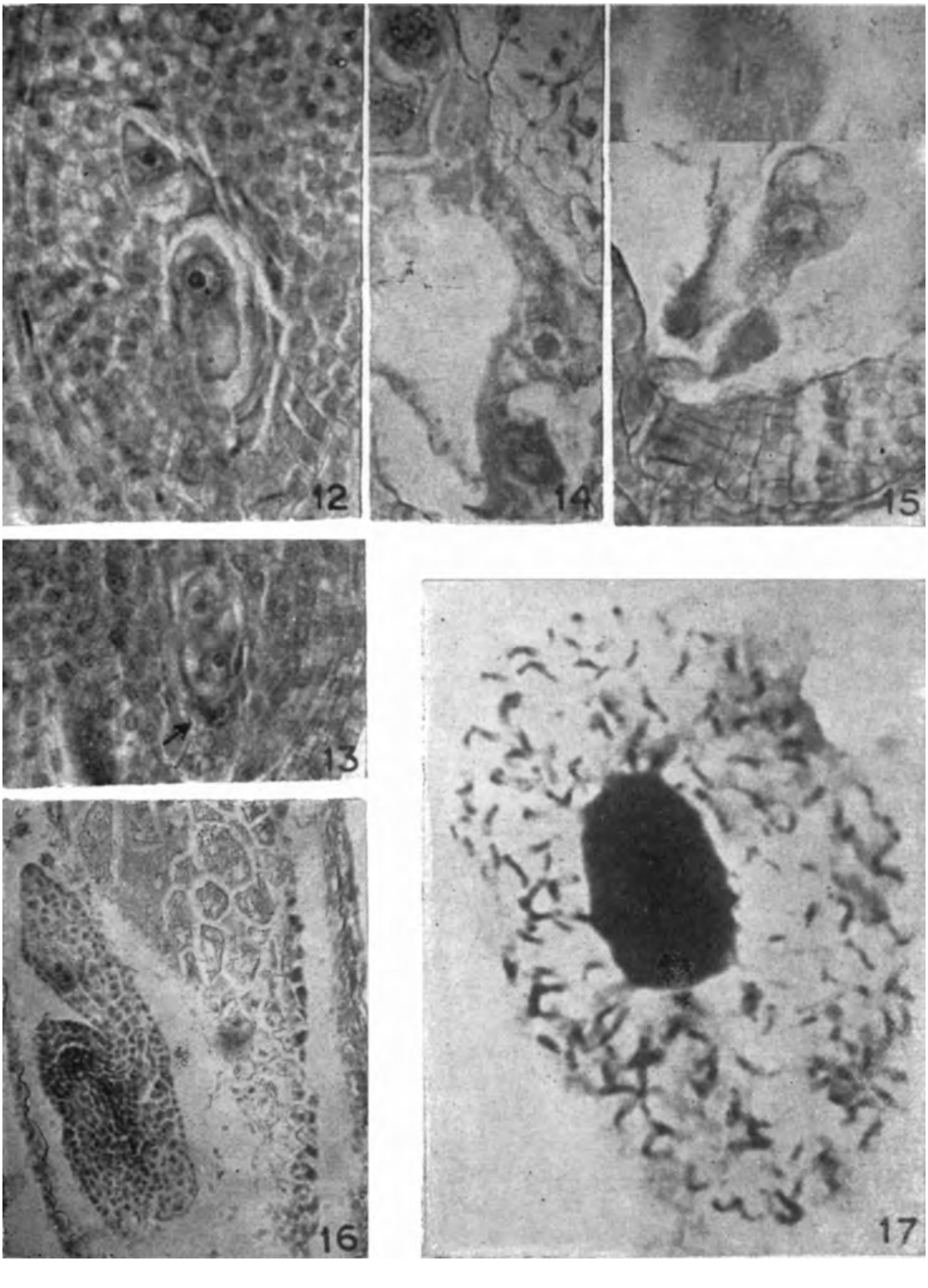


Figs. 7-11. *Bothriochloa odorata.* 7, Embryo sac showing an egg and a male nucleus in the process of fusion with the polar nucleus. x 480; 8, Two globular embryos and polar nuclei. x 100; 9, Mature caryopsis with an embryo and the cellular endosperm. x 100; 10, An endosperm nucleus showing about 60 chromosomes. x 1560; and 11, Degenerating megaspore mother cell and an aposporous initial in *Paspalum distichum.* x 480.



Figs. 12-17. *Paspalum distichum*. 12, Two enlarged aposporous initials. x 480; 13. Two nucleate aposporous embryo sac and degenerating remains of the megaspore mother cell (indicated by an arrow). x 480; 14, An eight nucleate embryo sac showing the egg, degenerated synergid, polar nucleus and antipodals (the second synergid and the polar nacleus were present in the adjacent section). x 480; 15, Embryo sac showing the egg. portion of the pollen tube with an undischarged male nucleus and the primary endosperm nucleus undergoing first mitotic division. x 480; 16, Mature caryopsis with an embryo and the cellular endosperm. x 100; and 17, An endosperm nucleus showing about 150 chromosomes. x 1760.

In *B. odorata*, unreduced embryo sacs are four nucleate, a condition which has been observed in majority of the members belonging to the sub-family Panicoideae. In *P. distichum*, the unreduced embryo sacs have been observed to be eight-nucleate. Such a behaviour has also been reported in *Paspalum secans* (Snyder, 1957). In *B. odorata*, the four-nucleate sacs possessed either one egg, two synergids and a polar nucleus or one egg, one synergid and two polar nuclei. The former type is however of more frequent occurrence as has also been reported in *Themeda triandra* and *Bothriochloa ischaemum* (Brown & Emery, 1956). There are many species in the Panicoideae, such as *Cenchrus setigerus* (Fisher, Bashaw, & Holt, 1954), *Dichanthium annulatum* (Reddy & D'Cruz, 1969a). *Panicum maximum* (Warmke, 1954), *Pennisetum ciliare* (Snyder, Hernandez & Warmke 1955) and *Pennisetum orientale*, *P. villosum*, *P. clandistinum* & *P. setaceum* (cf Emery, 1957) which possess four nucleate embryo sacs regularly with one polar nucleus.

In *B. odorata*, the reduced embryo sacs are of rare occurrence and in *P. distichum* they were not observed. According to Harlan *et al* (1964), all apomicts in the *Bothriochloa-Dichanthium* complex, which they investigated had some sexual potential, but only a part, and sometimes none, of this potential was realized. Thus the degree of sexuality as determined by segregation and progeny studies was found to be 6.5% in a tetraploid of *Dichanthium annulatum* from Israel, 21.5% in a tetraploid of this very species from India, 1.7% in *D. papillosum*, 10.2% in *Bothriochloa grahmii*, and none in case of *B. ischaemum* var. *songarica*. Facultative apomixis has also been observed in many other species such as *Apluda mutica* (Murty, 1973), *Dichanthium annulatum* (Reddy & D'Cruz, 1969 *D. aristatum* (Knox, Heslop-Harrison, 1963); *Panicum maximum* (Warmke, 1954); *Setaria leucopila* (Emery, 1957) and *Pennisetum ciliare* (Snyder, Hernandez & Warmke, 1955). According to Nygren (1967), complete obligate apomicts are very rare.

Both these species are pseudogamous i.e. the fertilizatian of the polar nucleus or nuclei is essential for the development of the endosperm. The fertilization of the polar nucleus in *B. odorata* and polar nuclei in *P. distichum* was observed. These observations are further supported by the chromosomal counts in the endosperm nuclei. Most of the species so far investigated in this family are pseudogamous. Autonomous development of the endosperm has been reported in some species of *Calamagrostis*, e.g. *C. canadensis*, *C. chalybaea*, *C. crassiglumis*, *C. inexpensa*, *C. langsdorffi*, *C. lapponica*, *C. purpurascens* and *C. purpurea* (cf. Nygren, 1967).

The importance of a delicate chromosomal balance of 2:3:2 between embryo, and the maternal tissue for the successful development of the seed has been discussed by Brink and Cooper (1947). The endosperm is pentaploid in *P. distichum* and triploid in case of *B. odorata*. In spite of this disturbed ratio 2:5:2 between the embryo, endosperm and maternal tissue, a seed set of about 34% was observed in *P. distichum*. The seed set in *B. odorata* was observed to be about 30%. In *Dichanthium annulatum*, Reddy & D'Cruz (1969*a*), made some important observations on unreduced eight nucleate embryo sacs at the time of fertilization. The polar nuclei did not fuse in these sacs, but either both were fertilized individually, or only one functioned, leaving the other to degenerate and thus maintaining the triploid number in the endosperm.

References

Brink, R.A. & Cooper, D.C. (1947). The endosperm in seed development. *Bot. Rev*, **13**, 423-541.

Brown, W. V. & Emery, W. H. P. (1956). Apomixis in the Gramineae, Tribe Andropogoneae : *Themeda triandra* and *Bothriochloa ischaemum*. *Bot. Gaz.*, **118**, 246-253.

———(1958). Apomixis in the Gramineae, Panicoideae. *Amer. J. Bot.*, **45**, 253-269.

Emery W. H. P. (1957). A study of reproduction in *Setaria macrostachya* and its relatives in the southwestern United States and northern Mexico. *Bull. Torrey Bot. Club.*. **84**, 106-121.

Farquharson, L. E. (1955). Apomixis and polyembryony in *Tripsacum dactyloides*. *Amer. J. Bot.*, **42**, 737-743.

Fisher, W. D., Bashaw, E.C. & Holt, E.C. (1954). Evidence for apomixis in *Pennisetum ciliare* and *cenchrus setigenus*. *Agron. J.*, **46**, 401-404.

Harlan, J. R., Brooks, M. H., Borgaonkar, D. S. & DeWet, J. M. J. (1964). Nature and inheritance of apomixis in *Bothriochloa* and *Dichanthium*. *Bot. Gaz.*, **125**, 41-46.

Hakansson, A. (1943). Die entwicklung des embryo sacs and die Besruchtung bei *Poa alpina*. *Hereditas*, **29**, 25-61.

Johansen, D. A. (1940). Plant microtechnique. McGraw-Hill, New York.

Knox, R. B. and J. Heslop-Harrison. (1963). Experimental control of aposporous apomixis in a grass of the Andropogoneae. *Bot. Notiser.*, **116**, 127-141.

Murty, U.R. (1973). Polyploidy and apomixis in *Apluda mutica* L. var. *aristata* (L.) Pilger. *Cytologia*, **38**, 347-356.

Nielsen, E.L. (1946). The origin of multiple macrogametophytes in *Poa alpina*. *Bot. Gaz.*, **108**, 41-50.

Nygren, A. (1967). Apomixis in Angiosperms. *Encyclo. Pl. Physiol.*, **18**, 551-596.

Reddy, P. S. & Cruz, R. D. (1969*a*). Mechanism of apomixis in *Dichanthium annulatum*. *Bot. Gaz.*, **130**, 71-79.

———(1969*b*). Polyembryony in *Dichanthium annulatum*. *Bot. Gaz.*, **130**, 162-165.

Snyder, L. A. (1957). Apomixis in *Paspalum secans*. *Amer. J. Bot.*, **44**, 318-324.

Snyder, L. A., Hernandez, A. R. & Warmke, H. E. (1955). The mechanism of apomixis in *Pennisetum ciliare*. *Bot. Gaz.*, **116**, 209-221.

Warmke, H. E. (1954). Apomixis in *Panicum maximum*. *Amer. J. Bot.*, **14**, 5-11.