

Apomixis in *Capillipedium huegelii* (Hack.) Stapf (Gramineae)

S P CHODA and RAVINDER K BHANWRA

Department of Botany, Panjab University, Chandigarh 160 014

(Received 3 December 1979)

The mode of reproduction in *Capillipedium huegelii* belonging to tribe Andropogoneae has been studied. The megaspore mother cell or the products of its meiotic division disorganise in most of the ovules followed by the formation of 4-nucleate unreduced embryo sacs from the nucellar cells. Eight-nucleate *Polygonum* (reduced) type of embryo sac is also formed in some ovules. The unreduced egg cell develops parthenogenetically but the fertilization of the polar nucleus is essential for the formation of endosperm.

Key Words : Gramineae, Apomixis, Apospory, *Capillipedium huegelii*

Introduction

The genus *Capillipedium* comprises 11 species distributed in the warmer regions of the world (cf. Airy Shaw 1973). In India, it is represented by 8 species (Bor 1960). Apomixis has been previously reported in *Capillipedium parviflorum* (Brown & Emery 1958), *C. spicigerum* (Ceralier & Harlan 1957) and in many species of the related genera, *Bothriochloa* and *Dichanthium* (see Ceralier & Harlan 1957, Brown & Emery 1958, Harlan et al. 1964, Knox & Heslop-Harrison 1963, Gupta 1968, Reddy & D'Cruz 1969a, b, Tothil 1968, Sunil & Dewet 1970, Choda & Bhanwra 1977). The present investigation deals with the mode of reproduction in *Capillipedium huegelii* which belongs to the tribe Andropogoneae of the subfamily Panicoideae.

Capillipedium huegelii is a perennial grass endemic to central and western India. The panicles are contracted with nodes and the

callus densely bearded. The plant climbs and weaves its way over and through other grasses and shrubs and roots at the nodes.

Material and Methods

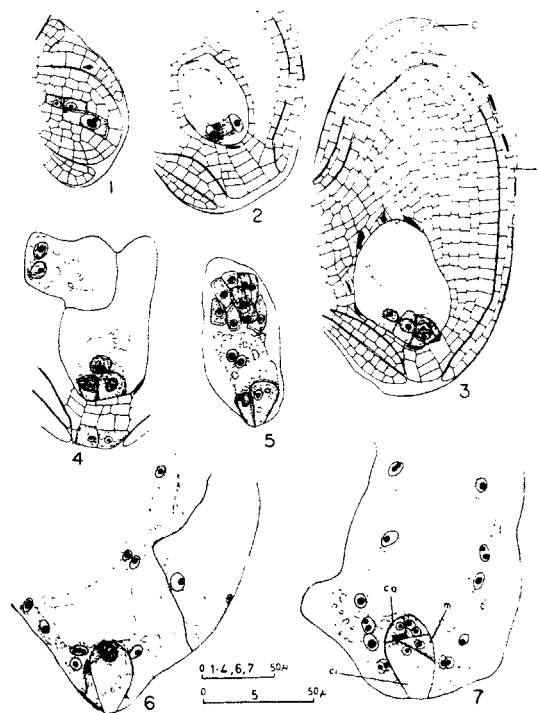
The material for study was collected from road side in Kalka hills during August-October. The inflorescences at different stages of development were fixed in FAA (50% ethyl alcohol, 90 ml; acetic acid, 5 ml and formalin, 5 ml). The florets were dehydrated in tertiary butyl alcohol—ethyl alcohol series (Johansen 1940) and embedded in paraffin wax. Sections were cut at a thickness of 6-12 μ and were stained using safranin—fast green or hematoxylin. Voucher specimens of the material used have been deposited in the Herbarium, Department of Botany, Panjab University, Chandigarh.

Observations

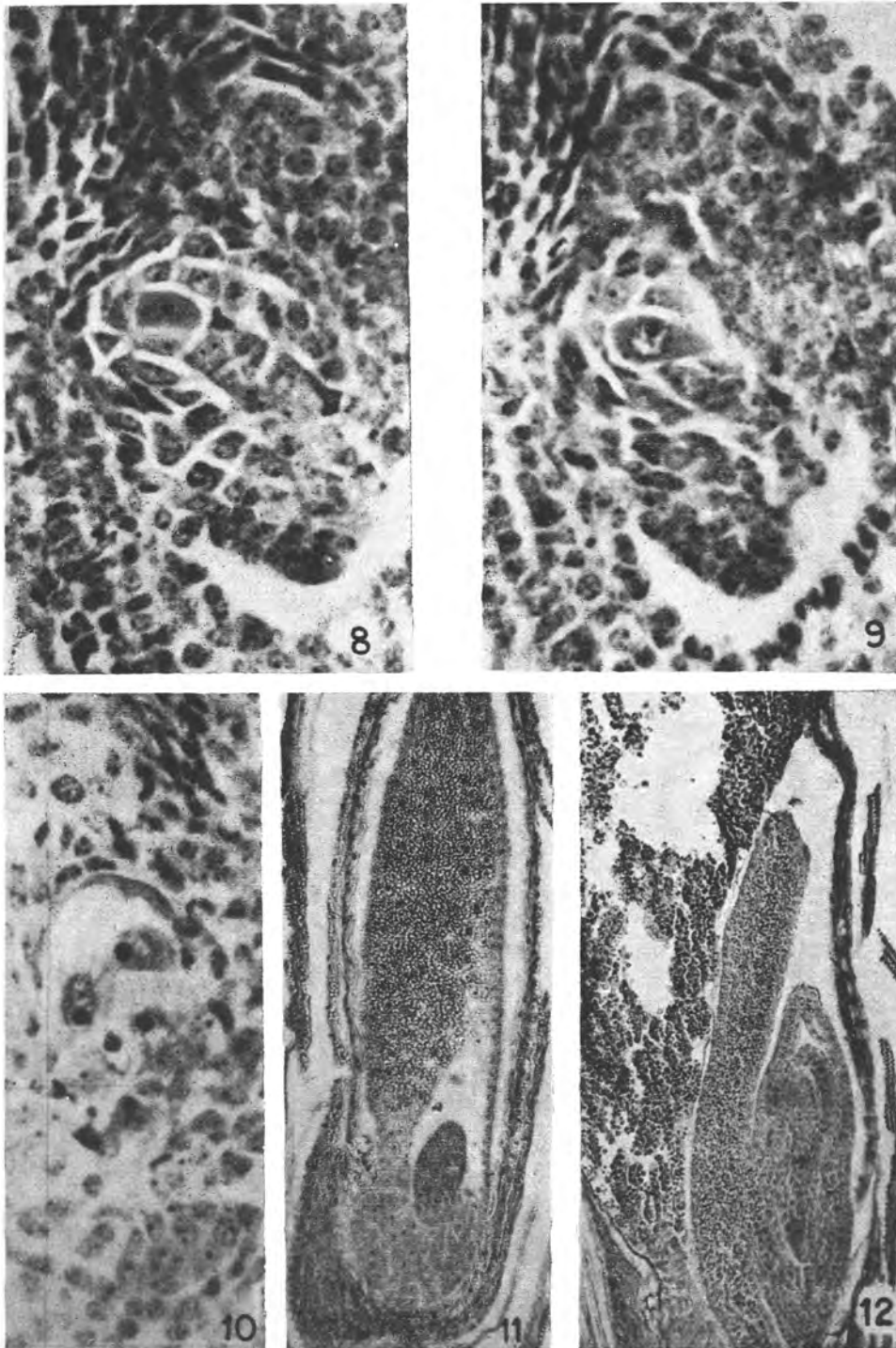
Ovule: The ovary contains a single ovule, which is sessile, bitegmic, pseudocrassinucellar and hemianatropous (figure 3). It is attached to the ventral inner surface. At megaspore mother cell stage, the ovule is placed at an angle of about 90° to the longitudinal axis of the ovary. At the time of fertilization the ovule becomes hemianatropous with the micropyle directed towards the base of the ovary (figure 3). The inner integument is mostly composed of two layers of cells and it does not grow beyond the nucellus (figure 3). The outer integument is also made up of 2 to 3 layers of cells. On the upper side of the ovule, the outer integument is poorly developed and covers only about one-third of the ovule. Both the integuments degenerate after fertilization and do not take part in the formation of the seed-coat (figures 11,12). The nucellar epidermis in the region of the micropyle undergoes 2 to 3 periclinal divisions to form a parietal tissue (figures 2-4).

Apospory: There is a single hypodermal archesporial cell in the nucellus which increases in size and functions as the megaspore mother cell (figure 1). The megaspore mother cell divides meiotically so as to form a linear tetrad of megaspores of which the chalazal one functions (Fig. 8). Simultaneously 1-6 cells of the nucellus become conspicuous owing to their large size and prominent nuclei (figures 1,9,10). These are the aposporous embryo sac initials. Like most other members of this tribe the aposporous embryo sac initials develop into 4-nucleate embryo sacs consisting of either one egg cell, 2 synergids and a single polar nucleus (figure 4) or into one egg, one synergid and two polar nuclei, the latter condition occurring only in about 3.22% embryo sacs. Figure 4 shows two embryo sacs in the same ovule, the one near the micropyle is

4-nucleate while the second one is still 2-nucleate. Although many ovules possess a number of aposporous embryo sac initials but only one or two of these reach the mature 4-nucleate stage. In some instances the entire nucellus gets absorbed by the development of aposporous embryo sacs.



Figures 1-7 *Capillipedium huegelii* (II, inner integument; OI, outer integument; ca, ci, m, tiers of the proembryo). 1, V. S. of a young ovule showing megaspore mother cell and 2 aposporous embryo sac initials; 2, V. S. ovule at 2-nucleate stage of the aposporous embryo sac development; 3, V. S. mature ovule showing 4-nucleate aposporous embryo sac with egg, two synergids and a single polar nucleus; 4, Two aposporous embryo sacs, the micropylar 4-nucleate and chalazal at 2-nucleate stage of development; 5, Eight-nucleate embryo sac with proliferated antipodal cells; 6, A transverse division in the unreduced egg cell and nuclear endosperm. Also note the remains of another aposporous embryo sac. 7, Embryo sac showing 6-celled proembryo and nuclear endosperm



Figures 8–12 *Capillipedium huegellii*. 8, 9, Adjacent sections of ovule showing functional megaspore, remnants of degenerating megaspores and one aposporous embryo sac initial ($\times 550$); 10, V. S. ovule showing 4-aposporous embryo sac initials ($\times 550$); 11, V. S. young *carvovis* showing globular proembryo and endosperm ($\times 150$); 12, V. S. *carvovis* showing

In a few ovules *Polygonum* type of embryo sac has been noticed. Such an embryo sac contains one egg cell, two synergids, two polar nuclei and up to 9 antipodal cells which have been formed by the secondary multiplication of the original three cells (figure 5). The smaller size of the nuclei in each of these embryo sacs gives a clear indication of their reduced nature. The 4-nucleate and 8-nucleate embryo sacs have not been observed in association with each other within the same ovule.

Pseudogamy: The endosperm fails to develop in such ovules where polar nucleus or nuclei are not fertilized. However, the fertilization of the polar nucleus has been observed in a few ovules. In such cases the endosperm nuclei reveal about 60 chromosomes in each cell. This number can only be derived by the fusion between the unreduced polar nucleus with 40 chromosomes and a male gamete with 20 chromosomes.

The primary endosperm nucleus divides first. At the time of first division in the unreduced egg there are about 16 endosperm nuclei. The endosperm becomes completely cellular at the globular stage of the proembryo (figure 11). The cells of the endosperm are full of starch grains.

Normal development of embryo takes place only in those ovules where endosperm is formed. In the absence of pseudogamy, the unreduced egg after undergoing a few divisions degenerates.

The first division in the unreduced egg cell is transverse forming a small terminal cell ca and a large basal cell cb (figure 6). The terminal cell undergoes two vertical divisions at right angles to each other so as to form a quadrant. The plane of division in the basal cell is either oblique forming the cells m and ci (figure 7) or rarely it is vertical. Further divisions are, however, irregular and a globular proembryo is

formed (figure 11). The globular proembryo as in other grasses develops into a mature embryo having the shield-shaped scutellum lying opposite to the endosperm (figure 12). The lower part of the scutellum is free from the coleorhiza, the latter encloses the radicle and the root cap. The shoot apex and the embryonic leaves are enclosed by a cone of coleoptile, In rare cases twin embryos have been observed in one ovule.

Discussion

The mode of reproduction in *Capillipedium huegelii* is aposporic like most of the other species of the tribes Andropogoneae and Paniceae (Brown & Emery 1956, 1958, Nygren 1967, Choda & Bhanwra 1977).

In *Saccharum officinarum* and *S. spontaneum*, diploid megaspores are produced in many ovules as a result of the fusion of 2 nuclei of chalazal megaspores of a tetrad which eventually forms the unreduced embryo sac (Narayanaswami 1940). Recently Shanthamm and Narayan (1977) have reported adventive embryony in *Pennisetum mezianum* and *Cenchrus ciliaris* belonging to the tribe Paniceae.

In the presently studied species the megaspore mother cell or the products of its meiotic division disorganise in most of the ovules but still there are some ovules which have a normal looking megaspore tetrad where the chalazal megaspore is capable of forming a reduced embryo sac. The degeneration of the megaspore mother cell as such has been reported in most of the other species of the tribe Andropogoneae (Brown & Emery 1958).

The unreduced embryo sac in *C. huegelii* is 4-nucleate. This condition is prevalent in most of the members of subfamily Panicoideae. However, in a few cases such as *Saccharum officinarum* and *S. spontaneum* (Narayanaswami 1940), *Tripsacum dacty-*

loides (Farquharson 1955), *Paspalum secans* (Snyder 1957), *P. plicatulum* and *P. guenoarum* (Pritchard 1970) and *P. distichum* (Choda & Bhanwra 1977) the unreduced embryo sacs are 8-nucleate. Unreduced 4-nucleate embryo sacs are also reported in some species of the tribes Chlorideae and Eragrosteae (Brown & Emery 1958) while they are 8-nucleate in the tribes Agrostideae, Aveneae, Festuceae and Triticeae (Nygren 1946, 1949, 1951, Hakansson, 1943, 1944, 1948, Kiellander 1937, Weimarck 1967 a, b, 1970).

The 4-nucleate embryo sacs in *C. huegelii* possess either one egg cell, two synergids and a single polar nucleus or rarely one egg, one synergid and two polar nuclei. These two types of organizations but with a higher frequency of embryo sacs with a single polar nucleus have also been reported in *Bothriochloa ischaemum* and *Themeda triandra* (Brown & Emery 1956), *Apluda mutica* (Murty 1973) and *Bothriochloa odorata* (Choda & Bhanwra 1977). The 4-nucleate embryo sacs regularly possess a single polar nucleus in some species, such as *Panicum maximum* (Warmke 1954), *Pennisetum ciliare* and *Cenchrus setigerus* (Fisher et al. 1954), *Pennisetum orientale*, *P. villosum*, *P. clandestinum* and *P. setaceum* (cf. Emery 1957) and *Dichanthium annulatum* (Reddy & D' Cruz 1969 a). However, in *Setaria leucopila* and *S. villosissima*, the 4-nucleate embryo sacs mostly contain one egg cell, one synergid and 2 polar nuclei (Emery 1957).

C. huegelii is pseudogamous and the endosperm is triploid as has also been reported previously in *Pennisetum ciliare* (Snyder et al. 1955), *Dichanthium annulatum* (Reddy & D' Cruz 1969 a) and *Bothriochloa odorata* (Choda & Bhanwra 1977). The

endosperm is reported to be pentaploid in *Setaria leucopila* and *C. villosissima* (Emery 1957), *Tripsacum dactyloides* (Farquharson 1955) and *Paspalum distichum* (Choda & Bhanwra 1977). The endosperm is, however, hexaploid in *Paspalum secans* as the male gametes are also unreduced (Snyder 1957).

C. huegelii is a facultative apomict since many ovules possess reduced 8-nucleate embryo sac which is capable of forming seed through the normal sexual process. Most of the apomictic species in the subfamily Panicoideae are facultative, however, the sexual potential varies from one species to the other.

Twin embryos have been noticed in some of the ovules in *C. huegelii*. Polyembryony has been previously reported in many apomictic grasses, such as *Poa pratensis* (Nishimura 1922, Andersen 1927, Armstrong 1937, Tinney 1940), *Panicum maximum* (Warmke 1954); *Pennisetum ciliare* (Fisher et al. 1954), *Tripsacum dactyloides* (Farquharson 1955), *Dichanthium annulatum* (Reddy & D' Cruz 1969 b) and *Bothriochloa odorata* (Choda & Bhanwra 1977). Polyembryony in the present species results from the functioning of more than one embryo sac in the ovule as is also recorded in *Poa pratensis* (Tinney 1940), *Pennisetum ciliare* (Fisher et al. 1954) and *Paspalum dilatatum* (Smith 1948). Reddy & D' Cruz (1969 b) reported twin embryos due to embryo cleavage as well as due to the functioning of additional aposporous embryo sac in *Dichanthium annulatum*. In *Tripsacum dactyloides*, however, Farquharson (1955) reported that extra embryos are formed from the synergids within the same embryo sac, or from the egg or a synergid of the second embryo sac.

References

- Airy Shaw H K 1973 *A dictionary of the flowering plants and ferns* (Revised 8th ed. of the late J. C. Willis) Cambridge: University Press.
- Andersen A M 1927 Development of the female gametophyte and caryopsis of *Poa pratensis* and *P. compressa*; *J. Agric. Res.* **34** 1001-1018
- Armstrong J M 1937 A cytological study of the genus *Poa*; *Can. J. Res. C.* **15** 281-297
- Bor N L 1960 Grasses of Burma, Ceylon, India and Pakistan. (Excluding Bambuseae). London, New York, Paris: Pergamon Press.
- Brown W V and Emery W H P 1956 Apomixis in the Gramineae, tribe Andropogoneae; *Themeda triandra* and *Bothriochloa ischaemum*; *Bot. Gaz.* **118** 246-253
- and ——— 1958 Apomixis in the Gramineae, Panicoideae; *Amer. J. Bot.* **45** 253-263
- Celarié R P and Harlan J R 1957 Apomixis in *Bothriochloa*, *Dichanthium* and *Capillipedium*; *Phytomorphology*; **7** 93-102
- Choda S P and Bhanwra R K 1977 The mode of reproduction in *Bothriochloa odorata* and *Paspalum distichum*; *Proc. Indian natn. Sci. Acad.* **43** 175-183
- Emery W H P 1957 A study of reproduction in *Setaria macrostachya* and its relatives in the south-western United States and northern Mexico; *Bull. Torrey Bot. Cl.* **84** 106-121
- Farquharson L I 1955 Apomixis and polyembryony in *Tripsacum dactyloides*; *Amer. J. Bot.* **42** 737-743
- Fisher W D, Bashaw E C and Holt E C 1954 Evidence for apomixis in *Pennisetum ciliare* and *Cenchrus setigerus*; *Agron. Jour.* **46** 491-504
- Gupta P K 1968 Observations on degree of apospory in three members of Andropogoneae; *Current Sci.* **37** 295-296
- Hakansson A 1943 Die Entwicklung des Embryosacks und die Befruchtung bei *Poa alpina*; *Hereditas* **26** 25-61
- 1944 Ergänzende Beiträge zur Embryologie von *Poa alpina*; *Bot. Notiser.* 299-311
- 1948 Embryology of *Poa alpina* plants with accessory chromosomes; *Hereditas* **34** 233-247
- Harlan J R, Brooks H M, Borgaonkar D S and Dewet J M J 1964 Nature and inheritance of apomixis in *Bothriochloa* and *Dichanthium*; *Bot. Gaz.* **125** 41-46
- Johansen D A 1940 *Plant microtechnique*; New York: McGraw-Hill
- Kiellander C L 1937 On the embryological basis of apomixis in *Poa palustris*; *Svensk bot. Tidskr.* **31** 425-429
- Knox R B and Heslop-Harrison J 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* Pilger; *Cytologia* **38** 347-356
- Narayanawami S 1940 Megasporeogenesis and the origin of triploids in *Saccharum*; *Indian J. Agric. Sci.* **10** 534-551
- Nishimura M 1922 Comparative morphology and development of *Poa pratensis*, *Phleum pratense* and *Setaria italica*; *Jap. J. Bot.* **1** 55-85
- Nygren A 1946 The genesis of some Scandinavian species of *Calamagrostis*; *Hereditas* **32** 131-262
- 1949 Apomictic and sexual reproduction in *Calamagrostis purpurea*; *Hereditas* **35** 285-300
- 1951 Form and biotype formation in *Calamagrostis purpurea*; *Hereditas* **37** 519-632
- 1967 Apomixis in Angiosperms; *Encycl. Pl. Physiol.* **18** 551-596
- Pritchard A N 1970 Meiosis and embryo sac development in *Urochloa mosambicensis* and three *Paspalum* species; *Aust. J. Agric. Res.* **21** 649-652
- Reddy P S and D'Cruz R 1969a Mechanism of apomixis in *Dichanthium annulatum* (Forssk.) Stapf; *Bot. Gaz.* **130** 71-79
- and ——— 1969b Polyembryony in *Dichanthium annulatum* (Forssk.) Stapf; *Bot. Gaz.* **130** 162-165
- Shanthamm C and Narayan K N 1977 Formation of nucellar embryos with total absence of embryo sacs in two species of Gramineae; *Ann. Bot.* **41** 469
- Smith B W 1948 Hybridity and apomixis in the perennial grass, *Paspalum dilatatum*; *Genetics* **33** 628-629
- Snyder L A 1957 Apomixis in *Paspalum secans*; *Amer. J. Bot.* **44** 318-324
- , Hernandez A R and Warmke H E 1955 The mechanism of apomixis in *Pennisetum ciliare*; *Bot. Gaz.* **116** 209-221
- Sunil S and deWet J M J 1970 The mode of reproduction in *Dichanthium intermedium*; *Bull. Torrey Bot. Cl.* **97** 6-12
- Tinney F W 1940 Cytology of parthenogenesis in *Poa pratensis*; *J. Agric. Res.* **60** 351-360
- Tothil J C 1968 Variation and apomixis in *Hetero-*

- pogon contortus*, Gramineae; *Bot. Soc. Argent.* **12** 188-201
- Warmke H E 1954 Apomixis in *Panicum maximum*; *Amer. J. Bot.* **41** 5-11
- Weimarck G 1967a Apomixis and sexuality in *Hierochloe australis* and Swedish *H. odorata* on different polyploid levels; *Bot. Notiser* **120** 209-235
- 1967b Apomixis in *Hierochloe monticola* (Gramineae); *Bot. Notiser* **120** 448-455
- 1970 Apomixis and sexuality in *Hierochloe alpina* (Gramineae) from Finland and Greenland and *Hierochloe monticola* from Greenland; *Bot. Notiser* **123** 495-504