Embryological studies in three species of *Cymbopogon* Spreng (Poaceae)

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Abstract. The embryology of *Cymbopogon nardus* var. *confertiflorus*, *C. martinii* var. *Motia* and *C. parkeri* has been studied. *C. nardus* and *C. martinii* have been observed to be seed-sterile owing to failure of fertilization while in *C. parkeri*, the seed-setting is only about $8 \cdot 00\%$.

1. Introduction

The genus Cymbopogon belongs to the tribe Andropogoneae of the subfamily Panicoideae and it is represented by 24 species in the Indian sub-continent (Bor 1960). A number of species of *Cymbopogon* yield essential oils and are used in perfumery. The embryology of *C. martinii* and *C. nervatus* has been worked out by Brown and Emery (1958) although of a preliminary nature. The present paper deals with the embryology of *Cymbopogon nardus* (L.) Rendle var. *Confertiflorus* (Steud.) Stapf ex Bor, *C. martinii* (Roxb.) Wats var. *Motia* and *C. parkeri* Stapf, to find the nature of seed sterility observed in different species.

2. Materials and methods

The material of *C. nardus* and *C. martinii* was collected from the Botanical Gardens of the Panjab University while that of *C. parkeri* from Shiwalik hills in the months August to November. Conventional methods of dehydration and embedding were used. The sections were cut at 5–10 μm and stained with safranin and fast green. For studying the growth of the pollen tube in the style, the method given by Khoshoo and Vij (1963) has been followed. For this purpose the gynoecia were fixed in 1 : 3 acetic alcohol, after 3, 6, 9 and 24 hrs of pollination. After half an hour the styles were transferred to 30% ethyl alcohol for preservation. The ovaries were transferred to 1% solution of acid fuchsin for about 20 min. Subsequently, the styles were cleared in lactic acid at 60° C and the whole mounts were made in pure lactic acid.

3. Observations and discussion

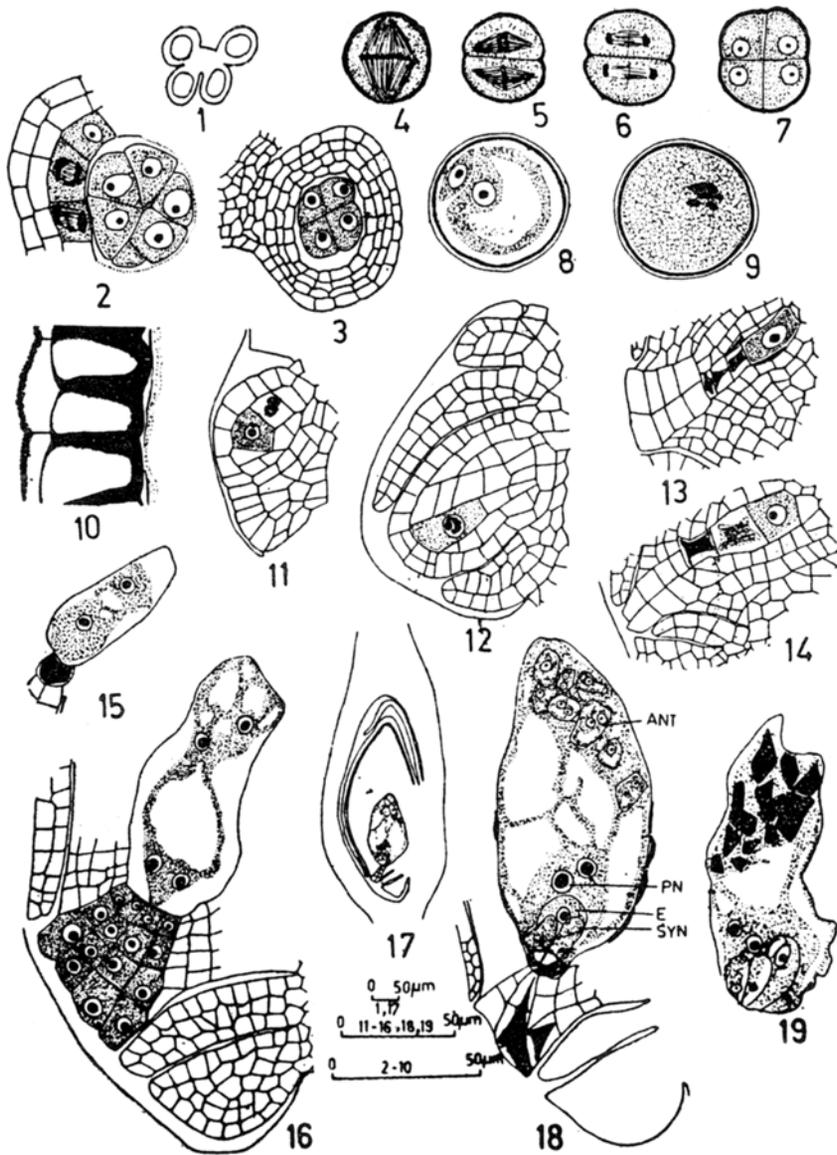### 3.1. *Microsporogenesis and male gametophyte*

Anthers are tetrasporangiate (figure 1) and the anther wall consists of an epidermis, followed on the inner side by the endothecial layer, a single middle layer and the tapetum (figures 2, 3). The epidermal cells in mature anthers show deposition of oil droplets in *C. nardus* and endothecial cells develop fibrous thickenings (figure 10). The middle layer is ephemeral. The tapetum is of the glandular type and its cells become binucleate in *C. nardus* while they remain uninucleate in *C. martinii* and *C. parkeri*. As seen in transections the MMC are disposed in 4 or 5 rows (figures 2, 3). Meiosis in MMC is normal and it is of the successive type leading to the formation of isobilateral microspore tetrads (figures 4–7). The development of the male gametophyte occurs as described in other members of the family and the pollen grains are shed at the 3-celled stage (figures 8, 9). The pollen grains are monocolpate with a thick smooth exine and slightly thinner intine. The pollen fertility is about 90% in *C. nardus* and *C. parkeri* and about 70% in *C. martinii*.

3.2. *Ovary and ovule*

The ovary contains a sessile, bitegmic, pseudocrassinucellar hemianatropous ovule (figures 16, 17). The inner integument is composed of cells 2–3 layers thick but it usually fails to grow over the nucellus so that a definite micropyle is lacking (figure 16). In *Pennisetum typhoideum* (Narayanaswami 1953) and *Capillipedium huegelii* (Choda and Bhanwra 1980) the inner integument does not grow over the nucellus but in most other species of the family the micropyle is formed by the inner integument (Narayanaswami 1954, 1955, 1956). In *Cymbopogon parkeri* where some seed-setting is observed, the inner integument is found to degenerate after fertilization unlike that in *Saccharum officinarum* (Artschwager *et al* 1929) and *Sorghum vulgare* (Artschwager and McGuire 1949), belonging to Andropogoneae where they persist in mature caryopsis.

The outer integument on the upper side of the ovule is 2–3 cell layered but it covers only about a third of the ovule (figure 17), a feature characteristic of the subfamily Panicoideae (Chandra 1963 ; Venkateswarlu and Devi 1964). The outer integument on the lower side of the ovule shows about the same growth as the inner integument and is 2–5 cell layered (figures 16–18).

The nucellar epidermis undergoes 2–3 periclinal divisions in the region of the micropyle (figures 16, 18) by the time the megaspore tetrads are formed. In *C. nardus*, the nucellar cells in this region become conspicuous owing to their large size, dense contents and prominent nuclei (figure 16). The formation of a parietal tissue due to periclinal divisions in the nucellar epidermis near the micropyle has been reported in many species belonging to the subfamily Panicoideae (Chandra 1963 ; Venkateswarlu and Devi 1964). The nucellar tissue is absorbed by the developing embryo and endosperm and is hardly traceable in the mature caryopsis of *C. parkeri* (figures 26, 27).

Figures 1–19. Microsporangium, microsporogenesis, male gametophyte, megasporo-genesis and female gametophyte. **1, 3, 11, 16–19.** *Cymbopogon nardus.* **2, 12, 14, 15.** *C. martinii.* **4–10, 13.** *C. parkeri.* **1.** TS of the anther, **2.** TS anther lobe showing periclinal division in the inner secondary parietal layer, **3.** TS anther lobe showing wall layers and sporogenous cells, **4–7.** Stages in microsporogenesis, **8, 9.** 2- and 3-celled pollen grains respectively, **10.** Portion of wall layers showing epidermis and fibrous thickenings of the endothecium, **11.** LS ovule primordium at archesporial cells stage, **12.** LS ovule at megaspore mother cell stage, **13, 14.** Megasporogenesis, **15, 16.** 2- and 4-nucleate embryo sac stages respectively, **17.** VS of the ovary and ovule, **18.** Embryo sac showing egg cell, two synergids, two polar nuclei and antipodal complex of several cells, **19.** Embryo sac showing egg apparatus, polar nuclei and degenerating antipodal cells. (*See explanation of abbreviation in p.* 58).
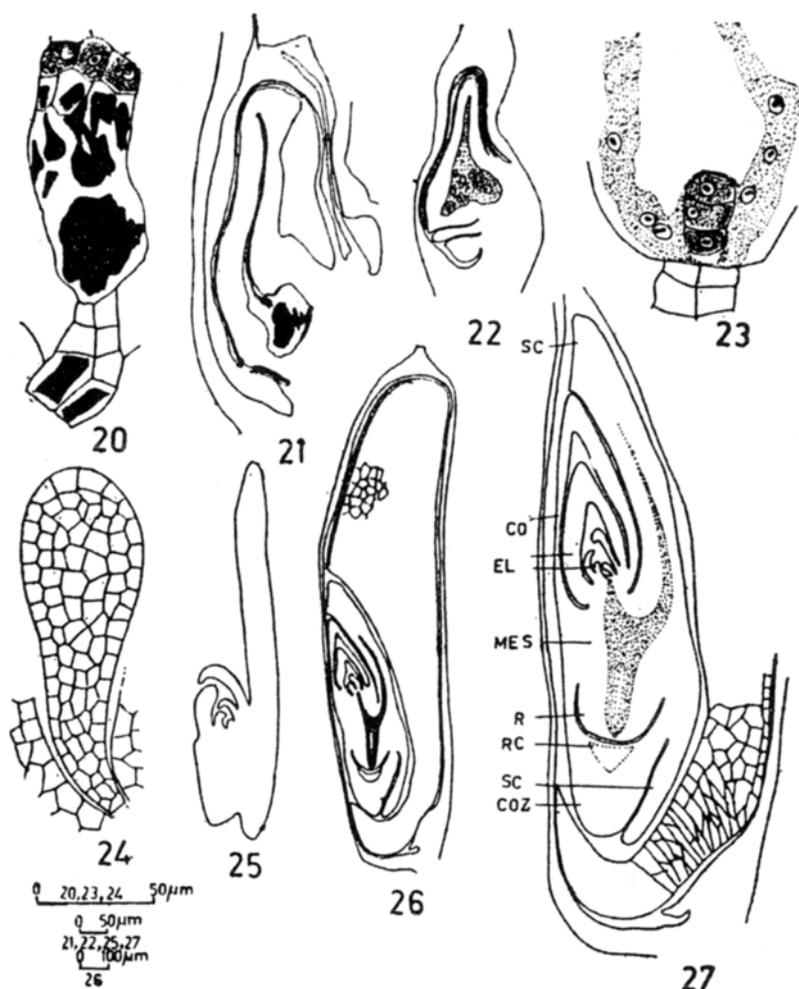
Figures 20-27. Post-pollination development. **20, 22.** *C. nardus*; **21, 23-27.** *C. parkeri.* **20-22.** Degenerating embryo sac and shrivelled ovule; **23-27.** Some stages in caryopsis development.

ANT—antipodal; CO—coleoptile; COZ—coleorhiza; E—egg; EL—embryonic leaf; END—endodermis; EPI—epidermis; IP—inner parietal layer; MES—mesocotyle; PN—polar nucleus; R—radicle; RC—root cap; S—sporogenous tissue; SC—scutellum; SYN—synergid.

3.3. *Embryo sac development*

The single hypodermal archesporial cell is differentiated in the nucellus, which increases in size and functions as the megaspore mother cell (figures 11, 12). It divides meiotically so as to form a linear tetrad of megaspores (figure 13). In *C. martinii*, however, the upper dyad cell degenerates without undergoing division (figure 14). The chalazal megaspore functions and develops into the polygonum type of embryo sac having an egg cell, two synergids, a central cell with its two

polar nuclei and three antipodal cells. The latter proliferate further and form 9–26 cells in *C. nardus*, 12–15 cells in *C. martinii* and 9–16 cells in *C. parkeri*. The multiplication of the 3 antipodal cells is commonly reported in grasses (Venkateswarlu and Devi 1964; Maze and Bohm 1973).

In *C. nardus* and *C. martinii*, the pollen grains germinate on the stigmatic hairs but the pollen tubes fail to reach the embryo sac due to some unknown factor. The embryo sac and the ovule eventually shrivel and undergo disintegration (figures 20–22). Occasionally the ovules become enlarged and they contain endosperm nuclei formed probably due to autonomous divisions of the secondary nucleus, but there is no embryo formation. In *C. parkeri*, however, about 8% seed-set has been noticed. The primary endosperm nucleus starts dividing earlier than the zygote. The endosperm is of the nuclear type (figure 23) as is reported in other grasses. The endosperm becomes completely cellular at globular stage of the proembryo. Figures 23–27 show some of the stages in the development of embryo in the species. The sequence of early development of the embryo could not be traced but the structure of the mature embryo is similar to that described by Reeder (1957) in other members of the tribe Andropogoneae. Seed-sterility in family Poaceae has been previously reported in *Helaria belangeri* and *H. mutica* by Brown and Coe (1951) and in *Digitaria decumbens* by Sheth *et al* (1956).

In *H. belangeri* and *H. mutica*, the degeneration of the female gametophyte may occur any time after megaspore formation and this has been suggested to be the cause of seed sterility. In the present species, however, the degeneration of the female gametophyte occurs only after it has attained maturity.

Poor seed-set as in *C. parkeri* and complete sterility as exhibited by *C. nardus* and *C. martinii* appear to be compensated by the predominance of vegetative propagation as a means of survival; consequent on perturbation of sexuality.

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