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Microsporogenesis in Male Sterile and Male Fertile *Crotalaria pallida* Ait.

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Male sterility in the genus *Crotalaria* was reported for the first time by Kempanna and Sastry (1958) in a population of *C. striata* DC. Subsequently it was observed in *C. mucronata* Desv. (Edwardson 1967), *C. juncea* L. (Mitra 1970, 1976) and *C. pallida* Ait. (Gupta 1975). Self-incompatibility and physiology of pollen tube growth in self-incompatible *C. pallida* was also studied by Gupta and Rani (1975a, 1975b). The present study of microsporogenesis and development of male gametophyte in cytoplasmic male sterile and male fertile *C. pallida* was undertaken to determine precisely when does the abortion take place and what cellular events are responsible for male sterility.

Material and methods

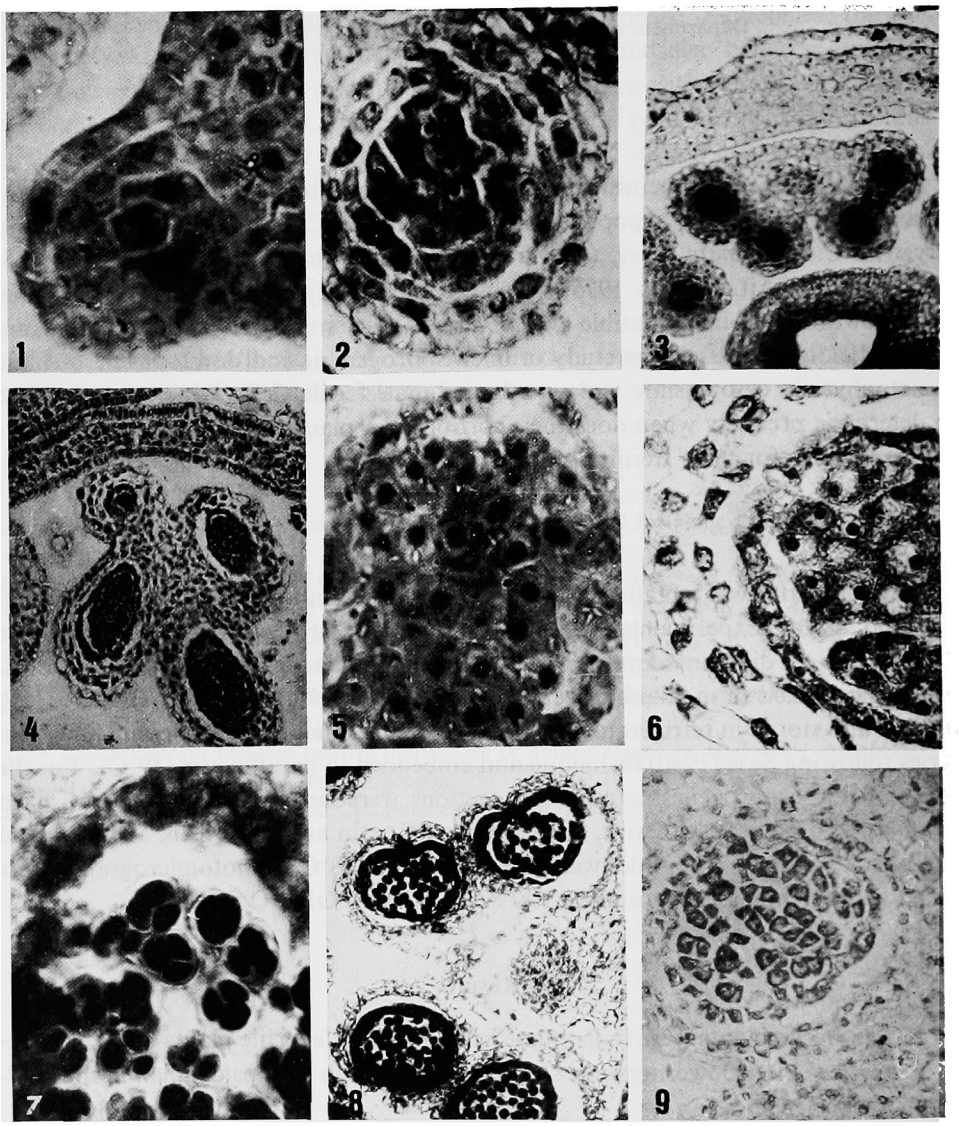
The material was obtained in the form of seeds and the plants were raised in the field at the Experimental Research Farm, Meerut University, Meerut. The flower buds of different developmental stages of male sterile and male fertile *C. pallida* were fixed in methanol: acetic acid (3 : 1) for 24 hours; transferred to 70% alcohol and stored in refrigerator. They were dehydrated through alcohol and xylol series following Sass (1940) technique and embedded in paraffin wax (59°C). For the study of microsporogenesis, transverse sections were cut at 6–10 μ thickness, fixed to slides with Haupl's adhesive, stained with saffranin and fast green, and mounted in Canada balsam. The slides were dried in oven at 60°C. Photomicrographs were taken with Olympus Camera using 120 ASA, 35 mm, ORWO film.

Results

Male sterile plants were indistinguishable from male fertile plants during vegetative growth. After flowering male sterile plants develop clumps of small fleshy seedless pods.

The anther is tetrasporangiate with a persistent epidermis. Two to three layers of hypodermal archesporial cells differentiate in each of the four lobes (Fig. 1). These are characterized by their large size, dense cytoplasm and conspicuous nuclei. Wall development follows the dicotyledonous pattern. Each sporangium has four wall layers. The outermost is epidermis, followed by endothecium, middle layer and the tapetum (Fig. 2).

The study of microsporogenesis in male fertile and male streile *C. pallida* revealed that the development initially follows a normal course in both the cases. The archesporial cell divides to give rise to wall initial and primary sporogenous cells. Primary sporogenous cells divide repeatedly to give rise to microspore mother cells. Development of microspore mother cells in male fertile and male sterile *C.*



Figs. 1–9. 1, a young anther showing archesporial cell. 2, an anther lobe showing wall layers and microspore mother cells. ×1500. 3, an anther showing four lobes (*C. pallida* male fertile). ×600. 4, an anther showing four lobes (*C. pallida* male sterile). ×600. 5, an anther lobe showing microspore mother cells (*C. pallida* male fertile). ×1500. 6, an anther lobe showing microspore mother cells (*C. pallida* male sterile). ×1500. 7, an anther lobe showing tetrahedral arrangement of pollens (*C. pallida* male fertile). ×1500. 8, a mature anther (*C. pallida* male fertile). ×600. 9, a mature anther lobe (*C. pallida* male sterile). ×600.

pallida is same. In male fertile *C. pallida*, microspore mother cells undergo meiosis and produce tetrads which are predominantly tetrahedral in their arrangement (Fig. 7). In male sterile *C. pallida* due to failure of meiosis, in microspore mother cells no tetrads are formed (Fig. 9). Microspore mother cells separate and develop a wall around themselves. Photomicrographs of microsporogenesis and the development of anther in both male fertile and male sterile *C. pallida* are shown in Figs. 1–9.

Discussion

During the present study, microsporogenesis was studied in male sterile and male fertile *C. pallida* in order to locate the disturbances in microsporogenesis which lead to male sterility. Cytoplasmic male sterility and gynodioecy in this species was earlier demonstrated from our Cytogenetics Laboratory at Meerut University (Gupta and Rani 1975a, 1975b, Gupta 1975). Male sterility was also reported earlier in *C. striata* (Kempanna and Sastry 1958), *C. mucronata* (Edwardson 1967) and *C. juncea* (Mitra 1970, 1976).

Cytohistological studies on male sterile and male fertile plants are available in several cases including *Capsicum annuum* (Horner and Rogers 1974), *Pennisetum typhoides* (Reddy and Reddi 1970, 1974), *Helianthus annuus* (Horner 1977) and soybean (Albertsen and Palmer 1979). Role of tapetal development in pollen fertility has been reviewed by several workers. It has been shown in several cases that behaviour of tapetum differs in male sterile and male fertile plants so that tapetum persists causing male sterility (Reddy and Reddi 1974, Ohmasa *et al.* 1976, Lee *et al.* 1979). Contrary to these earlier observations and in accordance with earlier observations in soybean (Albertsen and Palmer 1979), no major difference in tapetum was observed in male sterile and male fertile in our studies. The only difference recorded related to staining intensity which was more intense in male fertile than in male sterile. This suggests that there must be some enzymes or other chemical compounds which persist in case of male fertile causing sterility. Such an observation was earlier made by Lee *et al.* (1979) in corn. However, disintegration of tapetum takes place almost at the same time in male fertile and male sterile plants as earlier reported by Albertsen and Palmer (1979) in soybean and complete degeneration does not take place till very late.

Differences have also been reported in endothecial development in male fertile and male sterile strains (Pritchard and Hutton, 1972) and it was shown that thick endothecium gradually becomes attenuated to facilitate dehiscence in male fertile sorghum (Webster and Singh 1964), orchard grass (Fillion and Christie 1966) and pearl millet (Reddy and Reddi 1970, 1974). In sterile material no such phenomenon was obterved. Contrary to this in the present investigation no significant differences were observed in male fertile and male sterile anthers which is in agreement with observations made by Albertsen and Palmer (1979) in soybean.

In different studies on male sterility, it has been shown that sterility may or may not be associated with meiotic irregulatrities. For instance, some meiotic irregularities were observed in male sterile *Crotalaria striata* (Kempanna 1960) and sweet pepper (Novak 1971). However, no meiotic abnormalities were observed by

Duvick (1965) in male sterile corn. In the present study, normal tetrads were produced in male fertile but on tetrads at all were produced in male sterile anthers, an observation which was earlier made by Albertsen and Palmer (1979) in soybean. This may be attributed to failure of cytokinesis which was earlier reported in *Melilotus alba* (Castelter 1925) and *Pisum sativum* (Gottaschalk and Kaul 1974). In soybean, Albertsen and Palmer (1979) found that microspore mother cells each with four nuclei developed a wall around themselves after meiosis and behaved like a coenocytic microspore. Somewhat similar observations were made during the present study also.

It can, therefore, be concluded that in male sterile *Crotalaria pallida*, sterility is caused due to some biochemical rather than morphological changes in tapetum and due to failure of cytokinesis after meiosis in microspore mother cells. As a matter of fact, it has been concluded earlier by several workers (Edwardson 1970, Heslop-Harrison 1972, Laser and Larsten 1972) that nothing definite is known about causes of cytoplasmic male sterility. It is possible that there are several events which determine cytoplasmic male sterility and that these events differ in different cases so that no generalisations are possible.

Summary

A comparative study of microsporogenesis in male fertile and male sterile *C. pallida* was conducted. The developmental pattern for fertile microsporogenesis was compared with the developmental pattern in sterile plants to determine the time of microsporogenesis breakdown. The first abnormality was observed in the tapetal cells. Although normal tetrads were observed in male fertile plants, no tetrads were observed in the male sterile plants. It is suggested that sterility is due to differences in tapetal behaviour and failure of cytokinesis in male sterile type.

References

Albertsen, M. C. and Palmer, R. G. 1979. A comparative light and electron-microscopic study of microsporogenesis in male sterile (ms_1) and male fertile soybeans (*Glycine max* (L.) Merr.). Amer. J. Bot. **66**: 253–265.

Castelter, E. F. 1925. Studies on the comparative cytology of the annual and biennial varieties of *Melilotus alba*. Amer. J. Bot. **12**: 270–286.

Duvick, D. N. 1965. Cytoplasmic pollen sterility in corn. Adv. Genet. **13**: 1–56.

Edwardson, J. R. 1967. Cytoplasmic male slterility and fertility restoration in *Crotalaria mucronata*. J. Hered. **58**: 266–268.

— 1970. Cytoplasmic male sterility. Bot. Rev. **36**: 341–420.

Fillion, W. G. and Christie, B. R. 1966. The mechanism of male sterility in a clone of orchard grass (*Dactylis glomerata* L.). Crop. Sci. **6**: 345–347.

Gottschalk, W. and Kaul, M. L. H. 1974. The genetic control of microsporogenesis in higher plants. Nucleus **17**: 133–166.

Gupta, P. K. 1975. Cytoplasmic male sterility in self-incompatible *Crotalaria pallida* Ait. Incompatibility. Newsletter **5**: 13–14.

— and Rani, S. 1975a. Self-incompatibility in *Crotalaria pallida* Ait. Ibid. **5**: 4–8.

— and — 1975b. Physiology of pollen tube growth in self-incompatible *Crotalaria pallida* Ait. Ibid. **5**: 9–12.

Heslop-Harrison, J. 1972. Sexuality of Angiosperms. Physiology of Development: From Seeds to Sexuality in Plant Physiology (F. C. Steward, ed.), Vol. *VIC*: 133–289. Academic Press, New York.

Horner, H. T. Jr. 1977. A comparative light and electron microscopic study of microsporogenesis in male-fertile and cytoplasmic male-sterile sunflower (*Helianthus annuus*). Amer. J. Bot. **64**: 745–759.

— and Rogers, M. A. 1974. A comparative light and electon microscopic study of microsporogenesis in male-fertile and cytoplasmic male-sterile pepper (*Capsicum annuum*). Can. J. Bot. **52**: 435–441.

Kempanna, C. 1960. Cytology of male sterile *Crotalaria striata*. Curr. Sci. **29**: 138.

— and Sastry, K. S. K. 1958. Male sterility in *Crotalaria striata*. Curr. Sci. **27**: 181.

Laser, K. D. and Larsten, N. R. 1972. Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperm. Bot. Rev. **38**: 425–454.

Lee, S. L. J., Gracen, V. E. and Earle, E. D. 1979. The cytology of pollen abortion in C-cytoplasmic male sterile corn anthers. Amer. J. Bot. **66**: 656–667.

Mitra, G. C. 1970. Mode of reproduction in Sunn-hemp *C. juncea*. Broteria **39**: 79–85.

— 1976. Male sterility in Sunn-hemp (*Crotalaria juncea* L.). Curr. Sci. **45**: 873–874.

Novak, F. 1971. Cytoplasmic male sterility in sweet pepper (*Capsicum annuum* L.) II. Tapetal development in male sterile anthers. Z. Pflanzenzüchtg. **65**: 221–232.

Ohmasa, M., Watanabe, Y. and Murata, N. 1976. A biochemical study of cytoplasmic male sterility of corn: Alteration of cytochrome oxidase and malate dehydrogenase activities during pollen development. Jap, J. Breed. **26**: 40–50.

Pritchard, A. J. and Hutton, E. M. 1972. Anther and pollen development in male-sterile *Phaseolus atropurpureus*. J. Hered. **64**: 280–282.

Reddy, B. B. and Reddi, M. V. 1970. Studies on the breakdown of male-sterility and other related aspects in certain cytoplasmic male sterile lines of pearl millet (*Pennisetum typhoides* S and H.). Andhra Agric J. **17**: 173–180.

— and — 1974. Cytohistological studies on certain male sterile lines of pearl millet. Cytologia **39**: 585–589.

Sass, J. E. 1940. Elements of Botanical Microtechique. McGraw Hill Book Company, Inc., New York.

Webster, O. J. and Singh S. P. 1964. Breeding behaviour and histological structure of a non-dehiscent anther character in *Sorghum vulgare* Pers. Crop Sci. **4**: 656–658.