Research Paper Botany

Pollen Sterility in *Eichhornia crassipes* (Mart) Solms.

Agadi S. N.

Associate Professor, Developmental Biology and Histochemistry laboratory, Department of Botany, Karnataka University, Dharwad-580003

ABSTRACT

The present studies on the developing anthers of *Eichhornia crassipes* is a small attempt to fill the gap created by the insufficient literature on hydrophytic plants, and to identify any peculiar feature (s) associated with microsporogenesis of anthers of *Eichhornia crassipes*.In *E.crassipes* presence of staminodes, along with fertile ones, is encountered in some flowers (present study). Since all the developing stamens are exposed to uniform external environmental conditions, it is unlikely that the environmental conditions are causative for induction of male sterility only in few stamens. Therefore, most probably the physiological alterations in certain stamens may bring about male sterility. The aberrant physiological conditions of the stamens are reflected in cellular structures as well as in altered localization of histochemical substances.

KEYWORDS

INTRODUCTION

The reviews on male sterility reveal that male sterile anthers exhibit aberrant morphological, anatomical and physiological features (Kaul, 1988, Hegde and Isaacs, 1992; Smith et al., 2002 and Agadi & Hegde, 2003, Agadi1996). Stamens of cytoplasmic male sterile carrot may exhibit either a petalloid or a carpelloid phenotype, depending on nuclear background (Hanson and Bentolila, 2004). In rice male sterility occurs either at tetrad/microspore stage/pollen stage (Pradhan 1992).

More often male sterile mutants have recognizable defects in tapetal structure. Tapetal cells may exhibit hypertrophy (Horner and Rogers, 1974; Sun and Ganders, 1987; Agadi and Hegde, 2003), precocious degeneration (Agadiet et al., 2002), or delayed degeneration (Kattiet et al., 1994). Cell organelles may breakdown in the tapetum (Smith et al., 2002), Unsynchronized development in the same anther locule (Gadi 2006), or there may be irregular deposition of Ubisch bodies (Warmke and Lee, 1977) or sporopollenin (Greybosch and Palmer, 1987). In mutant rice, anther wall layers lack tapetum (Nonomuraet et al., 2003).Effects of male sterility on reproductive cells are also not uncommon. They may be in the form of aberrant cytokinesis (Johns et al., 1992), degraded plastids and rudimentary intine in pollen grains (Smith et al., 2002), abnormal exine (Agadi and Hegde, 2003), defective synthesis of dissolution of callosic wall (Wei et al., 1996), abnormal carbohydrate metabolism (Khattra and Singh, 1989), low activities of enzymes (Binoet et al., 1986; Sawhney and Bhadula, 1987), defective amino acid composition (Nakashima, 1975; Tripathi-et al., 1981), uncontrolled secretion of sporopollenin (Ahokas, 1978) and high levels of free polyamines (Rastogi and Sawhney, 1990a, 1990b).

Molecular studies on cytoplasmic male sterility reveal that microsporogenesis is highly sensitive to mitochondrial mutation (Hanson and Bentolila, 2004).

MATERIALS AND METHODS

Flower buds of *Eichhornia crassipes* (Mart) Solms.were collected from pond in the Karnatak university campus, Dharwad. Collected flower buds were fixed in FAA fixative for 12 hours. Employing standard microtechnique procedures, the fixed flower buds were dehydrated, infiltrated and embedded in paraffin wax. 4 µm thick transverse sections of flower buds were taken with the help of auto microtome, stained and microphotographed.

OBSERVATION

EICHHORNIA CRASSIPES(MART.) Solms.

The infertile anthers of *E. crassipes* manifest morphological/ histochemical deviations. Among them, the most significant deviation is absence of storage carbohydrates in the connective and wall layers at sporogenous stage (Fig.1A). The other difference noticed is lack of synchronous development male reproductive cells in different locules of the same anther. Some locules are still at sporogenous stage while adjacent ones are already showing degenerated microspores (Fig.1A).

In the young sterile anther, no significant changes are observed in the sporogenous cells and tapetum which becomes periplasmodium (Fig.1A). But unlike fertile anther, not only the periplasmodium becomes rich in cytoplasmic polysaccharides during meiosis, but also peritapetal membrane differentiates prematurely between periplasmodium and anther wall layers (Fig.1B). Again unlike fertile anther, dyads and microspore tetrads, and also periplasmodium, lack cytoplasmic polysaccharides (Fig.1C,1D). The intersporal walls of dyads and tetrads are insignificant (Fig.1C,1D). But similar to fertile anther, peritapetal membrane persists in the locule (Fig.1C).

Microsporogenesis in sterile anthers terminates at tetrad stage. Unusual large vacuoles appear in periplasmodium that is poor in polysaccharides (Fig.1E). The anther wall layers, except peritapetal membrane, collapse (Fig.1E). Microspores become permanently arrested in tetrads (Fig.1F). The cytoplasm of the spore depletes. Subsequent to the consumption of periplasmodium, microspores within tetrads have rich carbohydrates (Fig. 1F). Peritapetal membrane persists between endothecium and locular contents (Fig.1F). Endothecial cells show very thick, carbohydrate in the radial walls (Fig.1F). The disintegration of microspores is accompanied by further deposition of carbohydrates in the wall material (Fig.2A). Gradually, the disintegrated mass of microspores becomes amorphous (Fig.2B). The sterile anther is indehiscent and its locule contains degenerated remnants of carbohydrate deposition (Fig.2C). Even in the mature anther peritapetal membrane persists (Fig.2B).

DISCUSSION

The role of pollen grain as a male partner in sexual reproduction of seed plants was established by the end of the 19th century (Maheshwari, 1950, 1963). It is in the anther, the fertile part of the stamen, microsporogenesis and pollen grain formation occur. The anther is a specialized heterogeneous cell system and is involved in protection, nutrition and dispersal of pollen.

Pollen sterility

The selective damage to male reproductive organs suggests that development of anther requires very precise growth conditions. Even slight alteration in external and internal environment may lead to pollen sterility. Anther development in cotton (Meyer, 1969) and rice (Nishiyama, 1984) is very sensitive to temperature conditions. In wheat combination of abnormal temperature and water deficit is deleterious for anther development (Sainiet et al., 1984). In wheat water deficit alone, during male meiosis, can cause pollen sterility (Koonjulet et al., 2005). In Zea mays, male sterility is induced when plants are grown in short days, deficiency of molybdenum (Moss and Heslop-Harrison, 1968, Agarwalaet et al., 1979). In several crop plants copper deficiency causes male sterility (Dell, 1981). Or deficiency of calcium brings the same effect in rice (Tianet et al., 1998).

Since, not all the stamens in a flower are sterile, development of staminodes in E. crassipes may not help in prevention of selfing (present study). But plants that produce staminodes have other advantages. For some species, there is sound evidence that female plants produce more seeds than hermaphrodite ones. It is implicated that female plants can invest more energy, saved by the suppression of male organs, in seed production (Hanson and Bentolila, 2004). Maximum seeds are produced in those species where anther development is suppressed at very early stage than in the species where disruption occurs late in the development of anther. In the latter case, staminodes are produced only after considerable energy has been expended (Hanson and Bentolila, 2004).

Cellular events of male sterility in *E.crassipes*

Male sterility is expressed only during post-meiotic phase. The circumstantial evidences point out to the defective carbohydrate metabolism and abnormal behavior of the tapetum as cause for male sterility (present study). The lack of storage carbohydrates in the connective and endothecium results in the significant drop in the energy status of the anther. On the contrary, fertile anthers show abundant starch storage in the connective and endothecium until the starch-filling stage of pollen grains and differentiation of endothecial fibrous thickenings. According to Clement et al. (1996), during microspore stage, energy demand of the anther reaches its peak. It is quite understandable because in male reproductive organ every spore produced is a potential male gametophyte.

All cellular events associated with male sterility in *E.crassipes* resemble those in *Potamo get on richard sonii* (present study). The significant difference between fertile and sterile anthers of *E. crassipes* is absence of starch storage in the connective and endothecium of the latter, at sporogenous stage. But absence of starch storage, at sporogenous stage, cannot be presumed as deficiency in carbohydrate supply, because development of sterile anther is normal up to tetrad formation. The absence of starch storage can be implicated only as absence of starch synthesis in the connective and endothecium. Perhaps all the photosynthates received by the anther are transported to anther locule.

Continued absence of starch storage, during post-meiotic phase, certainly represents aberrant carbohydrate metabolism. In fertile anthers, presence and subsequent loss of starch storage in the endothecium and connective correlate with the developmental events of pollen grains and differentiation of endothecial thickenings. The arrest of micro gametogenesis and failure of differentiation of endothecial thickenings in sterile anthers, therefore, can be correlated with lack of storage carbohydrates. This implies the importance of carbohydrates in the development of anther.

The post meiotic event (s) in the locule of sterile anthers can be attributed to abnormal behavior of the periplasmodium. During meiosis, it becomes prematurely rich in cytoplasmic polysaccharides. This carbohydrate is presumably utilized for the premature formation of peritapetal membrane. The subsequent loss of carbohydrates in the periplasmodium substantiates this. The peritapetal membrane possibly blocks the supply of nutrients into the locule, as evidenced by reduced carbohydrate content in periplasmodium during dyad or tetrad formation. This also has a serious consequence, because periplasmodium is unable to supply sufficient nutrients needed for the synthesis of intersporal walls of tetrads. In sterile anthers intersporal walls of tetrads are very insignificant (present study).

The inability of tapetum to supply nutrients for the development of spores is evident by the depletion of cytoplasmic contents of microspores in tetrads. The periplasmodium soon starts degenerating and the breakdown products are also not diverted for the nutrition of spores. The heavy deposition of sporopollenin-like substance on the microspore tetrads suggest most of the breakdown products are utilized for the synthesis of sporopollenin. The increase in sporopollenin deposition with progress in the degeneration of periplasmodium substantiates this contention.

The sterile anthers are indehiscent. The abnormal tapetum or pollen grains may not be responsible for anther indehiscence because dehiscence does not require signals derived from locular contents (Goldberg et al., 1993). Cell ablation experiments have shown that a functional stomium region is essential for dehiscence which is lacking in the sterile anthers of *E. crassipes* (present study). Also, is the possibility that, the sterile anthers are Jasmonic acid (JA)deficient.JA is required for the expression of genes involved in water transport in anthers (Scott et al., 2004). Involvement of ethylene signaling is also envisaged in anther dehiscence (Rieu et al., 2003).

By and large histochemical studies on the developing anthers of *E. crassipes* fall in line with those of terrestrial plants. In *E. crassipes* environmental conditions in few stamens, abnormal behavior of the tapetum and malnutrition causes the male sterility.

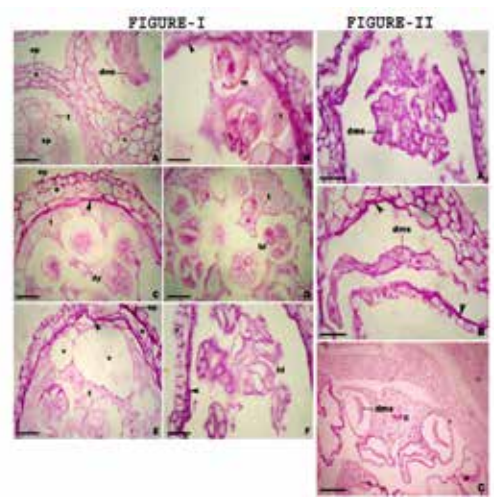

FIGURE-1

E. crassipes anther sections stained for carbohydrate (ep = epidermis; e=endothecium; sp= sporogenous cells; m=meiocytes; dy = dyads;

td = tetrads; t = tapetum; dms = degenerating microspores; c = connective; v =vacuole).Bar: 20µm

A. Sterile anther showing unsynchronized growth. One locule contains sporogenous cells and other the degenerating microspores. Anther wall layers and connective lack starch storage.
B. Periplasmodium becomes polysaccharide-rich. Note thick PAS-positive peritapetal membrane (arrows).
C,D.-Dyads, tetrads and periplasmodium are impoverished of cytoplasmic polysaccharides. The intersporal walls of tetrads are indistinct. Peritapetal membrane is PAS- positive (arrow). Anther wall layers lack starch storage.
E. Vacuoles of different sizes appear in periplasmodium. An-

ther wall layers collapse, but peritapetal membrane is intact (arrow).

F. Microspores are surrounded by thick carbohydrate deposition, show depleted cytoplasm and remain in tetrad condition. Endothecial cells possess thick carbohydrate in radial walls. Peritapetal membrane persists (arrow).

FIGURE-2

E. crassipes anther sections stained for carbohydrates (dms = degenerating microspores; c = connective; ptm = peritapetal membrane) Bar: C = 50µm; others = 20µm

A. carbohydrate rich substance deposits on degenerating microspores.

B. Microspores disintegrate into an amorphous mass. Anther lacks starch storage. Peritapetal membrane persists (arrow).

C. Indehiscent mature sterile anther showing degenerated mass of Microspores. Starch is absent in the connective and endothecium is poorly developed.

REFERENCES

Agadi, B. S. and Hegde, R. R. 2003.The implications of aberrant anatomical and histochemical features in the anthers of male sterile DSF-15 sunflower (Helianthus petiolaris, Nutt.).Helia, 26: 25-38. | Agadi, B. S., Gouda, P., Paramanna, N. and Hegde, R. R. 2002.Anatomical and histochemical studies on pollen degeneration in CMS Helianthus petiolaris, Nutt.Cytologia, 67: 375-382. | Agarwala, S. C., Chataejee, C., Sharma, D. N. and Nautiyal, N. 1979.Pollen development in maize subjected to molybdenum deficiency. Can. J. Bot., 57: 1946-1950. | Ahokas, H. 1978. Cytoplasmic male sterility in barley. II. Physiology and anther cytology of msm 1. Hereditas, 89: 7-22. | Bhadula, S.K. and Sawhney, V.K. 1989. Amylolytic activity and carbohydrate levels during the stamen ontogeny of a male fertile, and a 'gibberellin-sensitive' male sterile mutant of tomato (Lycopersiconesculentum).Jour. Exp. Bot. 40: 789-794. | Clement, C., Burrus, M. and Audran, J. C. 1996. Floral organ growth and carbohydrate content during pollen development in Lilium. Amer. J. Bot. 83(4): 459-469. | Dell, B. 1981. Male sterility and anther wall structure in copper deficient plants. Ann. Bot.48: 599-608. | Goldberg, R. B., Beals, T. P. and Sanders, P. M. 1993. Anther development : Basic principles and practical applications.The plant cell, 5: 1217-1229. | Graybosch, R. A. and Palmer, R. G. 1987.Analysis of a male-sterile character in soybeans. J. Hered. 78: 66-70. | Hanson, M. R. and Bentolila, S. 2004. Interactions of mitochondrial and nuclear genes that affect male gametophyte development. The plant cell, 16(suppl.): S154-S169. | Hegde, R.R.and Isaacs, S.W.1992. Cytology and histochemistry of microsporogenesis in cytoplasmic male sterile plants.In: C.P. Malik (Ed.). Advances in Pollen-Spore Research: XIX. Pollen Physiology and Biotechnology. Today and Tomorrow's Printers and Publishers, New Delhi, pp. 41-63. | Hegde, R.R., Naik, S.S., Katti, R.Y. and Agadi, S.N. 1996. Stamen morphogenesis in cytoplasmic male sterile Nicotianatabacum L.In: C. P. Malik (Ed.). Advances in Pollen-Spore Research: XXI. Pollen Spore Research Emerging Strategies. Today and Tomorrow's Printers and Publishers, New Delhi, pp. 95-119. | 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. | Horner,H.T.Jr. and Rogers, M.A.1974. A comparative light and electron microscopic study of microsporogenesis in male fertile and cytoplasmic male sterile pepper (Capsicum annuum) . Can. J. Bot. 52: 435-441. | Johns, C., Meiqing Lu, Lyznik, A. and Mackenzie, S.1992. A mitochondrial DNA sequence is associated with abnormal pollen development in cytoplasmic male sterile bean plants. The Plant cell, 4: 435-449. | Katti, R.Y., Giddanavar, H.S., ShamalaNaik, Agadi, S.N. and Hegde, R.R.1994. Persistence of callose and tapetum in the microsporogenesis of genic male sterile Cajanuscajan. (L) millsp. withwell formed endothecium. Cytologia, 59: 65-72. | Kaul, M.L.H. 1988. Male sterility in higher plants. Springer-Verlag, Berlin. | Khattra, S.S. and Singh, G. 1989. Histochemical studies in developing male fertile and sterile anthers of Pennisetumtyphoides. Acta. Bot. Ind. 17: 159-162. | Koonjul, P.K., inhas, J.S., Nunes, C., Sheoran, I. S. and Saini, H. S. 2005. Selective ranscriptional down-regulation of anther invertases precedes the failure of pollen development in water-stressed wheat. J. Exp. Bot. 56(409):179-190. | Maheshwari, P. 1950. An Introduction to the Embryology of Angiosperms. McGraw-Hill, New york. p. 453. | Maheshwari, P.(Ed) 1963. Recent advances in the embryology of Angiosperms. Intl. Soc. Plant. Morphol. Delhi. | Mazzucato, A., Testa, G., Biancari, T. and Soressi, G.P. 1999.Effect of gibberellic acid treatments, environmental conditions and genetic background of the expression of the parthenocarpic fruit mutation in tomato.Protoplasma, 208: 18-25. | Meyer, V. S. 1969. Some effects of genes, cytoplasm and environment on male sterility of cotton (Gossypium). Crop Sci., 9: 237-242. | Moss, G. L. Heslop- Harrison, J. 1968.Photoperiod and pollen sterility in maize. Ann. Bot. 32: 833-846. | Nakashima, H. 1975. Histochemical studies on the cytoplasmic male sterility of some crops. IV. Electron microscopic observation in sugar beet anthers. Mem. Fac. Agric. Hokkaido Univ. 9: 247-252. | Nishiyama, I. 1984. Climatic influence on pollen formation and fertilization.In: Tsunoda, S. and Takahashi, N. (Eds.).Biology of Rice. Elsevier, Amsterdom, pp. 153-171. | Nonomura, K. I., Miyoshi, K., Eiguchi, M., Suzuki, T., Miyao, A., Hirochika, H. and Kurata, N. 2003. The MSP1 gene is necessary to restrict the number of cells entering into male and female sporogenesis and to initiate anther wall formation in rice. The plant cell, 15: 1728-1739. | Rastogi, R. and Sawhney, V.K. 1990a. Polyamines and flower development in the male sterile stamenless-2 mutant of tomato (Lycopersiconesculentum Mill.). I. Level of polyamines and their biosynthesis in normal and mutant flowers. Plant Physiol. 93: 439-445. | Rastogi, R. and Sawhney, V.K. 1990b. Polyamines and flower development in the male sterile stamenless-2 mutant of tomato (Lycopersiconesculentum Mill.). II. Effects of polyamines and their biosynthetic inhibitor on the development of normal and mutant floral buds cultured In Vitro. Plant Physiol. 93: 446-452. | Rieu, I., Wolters-Arts, M., Derksen, J., Mariani, C. and Weterings, K. 2003. Ethylene regulates the timing of anther dehiscence in tobacco. Planta, 217: 131-137. | Saini, H. S., Sedgley, M. and Aspinall, D. 1984.Developmental anatomy in wheat of male sterility induced by heat stress, water deficit or abscisic acid.Aust. J. Plant Physiol. 11: 243-253. | Sawhney, V.K. and Bhadula, S.K. 1987. Characterization and temperature regulation of soluble proteins of a male sterile tomato mutant.Biochem.Genet.25: 717-728. | Scott, R. J., Spielman, M. and Dickinson, H.G. 2004.Stamen structure and function. Plant cell, 16(suppl.): S46-S60. | Smith, M.B., Palmer, R.G. and Horner, H.T. 2002.Microscopy of a cytoplasmic male sterile soybean from an interspecific cross between Glycine max and G. soja (Leguminosae). Amer. J. Bot. 89(3): 417-426. | Sun, M. and Ganders, F.R. 1987. Microsporogenesis in male sterile and hermaphroditic plants of nine gynodioecious taxa of HawaiianBidens (Asteraceae). Amer. J. Bot. 74: 209-217. | Tian, H.Q., Kuang, A., Musgrave, M.E. and Russell, S.D. 1998. Calcium distribution in fertile and sterile anthers of photoperiod-sensitive genic male-sterile rice.Planta, 204: 183-192. | Tripathi, D.P., Mehta, S.L. and Rao, N.G.P. 1981. Amino acids in anthers of milo and cytoplasmic genetic male sterile sorghum (Sorghum bicolor L. Moench.) of Indian origin.Theor. Appl. Genet. 59: 113-116. | Warmke, H.E. and Lee, S.L.J. 1977. Mitochondrial degeneration in Texas cytoplasmic male sterile corn anthers. J. Hered. 68: 213-222. | Wei, J., Palmer, R.G. and Horner, H.T. 1996. A combined developmental and genetic study of a new genic male sterile soybean (Glycine max L. Merr.). Amer. J. Bot. 83: Abst. No. 120. |