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Male Sterility in *Potamogeton richardsonii* L

KEYWORDS: Tapetum, Meiocytes, Sporopollenin, Male sterility.

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ABSTRACT *Male sterility in flowering plants is very important, for production of hybrid seeds.Hydrophytic plants possess unique morphological and anatomical features. But researchers are neglected the developmental aspects of reproductive structure in hydrophytes. Even slight alteration in external and internal environment may lead to pollen sterility. Here is a small attempt to study the reproductive structure of Potamogeton. The Potamogetonrichardsonii L. manifestation of male sterility are 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).*

INTRODUCTION

Hydrophytic plants possess unique morphological and anatomical features. These features include thin and /or dissected waxy leaves, poorly developed mechanical tissue, presence of large air cavities etc. But information on the occurrence of unique features, if any, in reproductive structures is very scanty.

Most of the literature on hydrophytic plants is concerned about floral construction, with special reference to stylar conditions and pollination (Barrett, 1980, Barrett and Harder, 1992), structure of pollen exine and its relation to pollination (Pettitt and Jermy, 1975, Takahashi, 1994).

The current investigation is encompassing complicated morphogenetic processes and abnormalities involved in the development of reproductive structures.

AIMS and Scope of the Present Work

The main objective of the present study is to provide a comprehensive account on microsporogenesis by using the result of present study in the background of hypothesis and conceptions advanced by other workers.

For the present study *Potamogetonrichardsonii* is chosen for the following reasons.

1. By and large, studies on microsporogenesis have been neglected in hydrophilous plants.
2. Till this date, histochemical studies on developing anthers in this plant is not made.
3. To ascertain unique features during microsporogenesis and gametogenesis, if any, in this plant.

MATERIALS AND METHODS

Flower buds of *Potamogetonrichardsonii* L. were collected from the Kelegeri pond of Dharwad. Flower buds were fixed in FAA for 12 hours. Employing standard microtechnique procedures. The fixed flower buds were embedded in paraffin wax, 4µm thick transverse sections were taken with the help of automatic microtome. Sections were stained with PAS, and microphotograped.

RESULTS

Potamogetonrichardsonii L.

Some anthers of *P. richardsonii* are sterile. During initial stages of development, these anthers are indistinguishable from fertile anthers. The development of sterile anthers is normal up to completion of meiosis. During post-meiotic phase sterile anthers show number of abnormalities. The development of microspores terminates as soon as they are released from tetrads although their cytoplasm shows polysaccharides (Fig. 1A). Initial growth of microspores is associated with the depletion of their polysaccharide content (Fig. 1B). Microspores adhere to one another and their cytoplasm begins to condense (Fig. 1C). Sterile microspores lack well defined wall and form a clump (Fig. 1D). As anther locule enlarges the parietal periplasmodium encircles the microspore clump and pushes it to the periphery of the locule (Fig. 1E and 1F). Concurrently, heavy PAS-positive material deposits around each degenerating microspore (Fig.1E, 1F and 1G). Finally clump of microspores is absorbed within the locule making it empty (Fig. 1G, 1H).

The peritapetal membrane makes its appearance in normal way (Fig. 1A, 1C, 1D). But what is unusual is its persistence even in the locule of mature anther (Fig. 1E, 1F, 1G).

The abnormal features are also noticed in the anther wall layers and connective. During post-meiotic phase, the sterile anthers completely lack carbohydrate storage, both in the connective (Fig. 1A, 1B) and endothecium (Fig.1E, 1F, 1G, 1H). Endothecium lacks fibrous thickenings (Fig.1H). Sterile anther is also defective in being indehiscent (Fig. 1H).

DISCUSSION

POLLEN STERILITY

Formation of defective reproductive organs, specially the male ones, is not uncommon in nature. 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 water deficit alone, during male meiosis, can cause pollen sterility (Koonjul et al., 2005). In maize male sterility caused by the deficiency of molybdenum (Agarwala et al., 1979) and deficiency of calcium brings the same effect in rice (Tian et al., 1998).

In P. richardsonii 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,

Since, not all the stamens in a flower are infertile, development of staminodes in *P. richardsonii* may not help in prevention of selfing (present study). But plants that produce staminodes have other advantages. For some species, there is

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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 &Bentolila, 2004).

The reviews on male sterility reveal that male sterile anthers exhibit aberrant morphological, anatomical and physiological features (Kaul, 1988, Hegde& Isaacs, 1992, Smith et al., 2002). In male sterile

Nicotianatabacum and Lycopersiconesculentum stamens exhibit pistilloidy (Bhadula&Sawhney, 1989, Hegde et al., 1992, Hegde et al., 1996, Mazzucato et al., 1999). Stamens of cytoplasmic male sterile carrot may exhibit either a petalloid or a carpelloid phenotype, depending on nuclear background (Hanson &Bentolila, 2004).

Tapetal cells may exhibit hypertrophy(Horner & Rogers, 1974; Sun & Ganders, 1987;Agadi&Hegde, 2003), precocious degeneration (Agadi et al., 2002) or delayed degeneration (Katti et al., 1994). Cell organelles may breakdown in the tapetum (Smith et al., 2002), or there may be irregular deposition of Ubisch bodies (Warmke& Lee, 1977) or sporopollenin (Greybosch& Palmer, 1987). In mutant rice, anther wall layers lack tapetum (Nonomura et al., 2003).

Effects of male sterility on reproductive cells are also not uncommon. They may be in the form of abnormal exine (Agadi&Hegde, 2003), defective synthesis of dissolution of callosic wall (Wei et al., 1996), abnormal carbohydrate metabolism (Khattra& Singh, 1989), defective amino acid composition (Nakashima, 1975, Tripathi et al., 1981), uncontrolled secretion of sporopollenin (Ahokas, 1978) and high levels of free polyamines (Rastogi&Sawhney, 1990a, 1990b).

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

Cellular events of male sterility in *P.richardsonii*

The persistence of periplasmodium also has undesirable effects. The lack of starch filling in the pollen grains and failure of endothecial thickenings can be related to persistence of periplasmodium. Because these two events occur during post-degradation period of periplasmodium in fertile anthers (present study). Because of lack of pollen wall, microspores clump together and persistent periplasmodium encircles such clump and pushes it towards the one side of the anther locule. It is speculated that the degenerating periplasmodium secretes sporopollenin-like wall material which deposits on the surface of degenerating microspores. Sporopollenin is PAS–positive and stains greenish with toluidine blue. Thus, instead of supplying nutrients to the developing microspores, the periplasmodium produces excess of sporopollenin-like material.

The persistence of peritapetal membrane, even in the mature anther, may have serious consequence. This membrane might function as a barrier, blocking the transport of nutrients from the connective and endothecium.

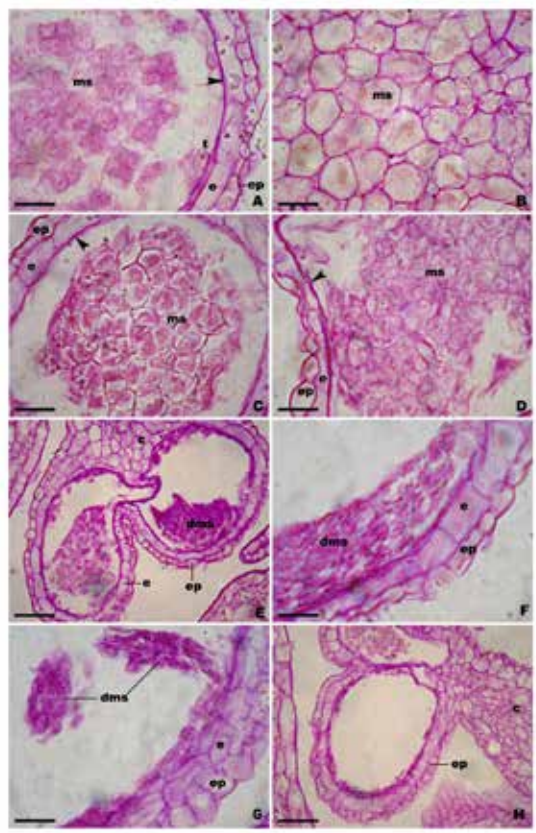
The sterile anthers are indehiscent. The abnormal tapetum or pollen grains may not be responsible for anther indehiscence because dehiscence does not require signals derived from locular contents (Goldberg et al., 1993). Cell ablation experiments have shown that a functional stomium region is essential for dehiscence which is lacking in the sterile anthers of *P.richardsonii* (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).

CONCLUSION

The present studies on the developing anthers of *P. richardsonii* is an attempt to fill the lacuna created by the inadequate literature on hydrophilous plants,and is also an attempt to identify any peculiar feature (s) associated with microsporogenesis of anthers of *P. richardsonii*

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

FIGURE - 1



8. FIGURE-1

Potamogeton richardsonii

anther sections tested for insoluble polysaccharides

(ms = microspores; t = tapetum; e = endothecium; ep = epidermis; ptm = peritapetal membrane , dms = degenerating microspores; c = connective; ptm = peritapetal membrane).

Bar : E = 50µm; others = 20µm

A,B. Sterile anther showing microspores impoverished of polysaccharide. Thin, smooth exine is PAS-positive. Endothecium and epidermis are devoid of starch storage.

C. Sterile anther. The under-developed microspores clump together. Epidermis and endothecium lack starch storage.

D. Sterile anther showing microspore clump undergoing degeneration.

E. Sterile anther lacks starch storage in the connective and endothecium. Degenerating microspores become amorphous.

F. Degenerated microspore mass is gradually absorbed in the intact locule. Endothecium and epidermis lack starch storage.

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G. The size of degenerating microspore clump further reduces.
H. Mature sterile anther showing empty indehisced locule. Endothecium lacks fibrous thickenings. Connective is devoid of starch storage.

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