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HISTOLOGICAL AND HISTOCHEMICAL CAUSES OF MALE STERILITY IN TWO CYTOPLASMIC-GENIC MALE STERILE LINES OF RICE (*ORYZA SATIVA* L.)

H. S. YOGEESHA* AND M. MAHADEVAPPA

University of Agricultural Sciences, Bangalore 560065

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ABSTRACT

Histological and histochemical changes during microsporogenesis were studied in the CMS anthers of V20A and Pragathi A rices and their maintainers. Both the CMS lines differed from their respective maintainers in post-meiotic stages only. The tapetal and endothecial abnormalities were the main histological causes of male sterility in both CMS lines. The CMS anthers had lower insoluble polysaccharides, proteins and RNA content than their maintainer counterparts.

Key words: Histological, histochemical, male sterility, tapetum, endothecium.

The cytoplasmic–genic male sterility (CMS) system is widely used in hybrid seed production of open-pollinated crops. The discovery of cytoplasmic male sterility and a pair of fertility restoring genes [1] led to exploitation of hybrid vigour a self-pollinated crop like rice. The patterns of pollen abortion in the CMS lines and their morphological features display different genetic backgrounds, conducive to hybrid rice production [2]. However, few studies have tried to understand the causes of male sterility in the various cytosterility systems. The present study has been undertaken to find the histological and histochemical reasons for cytoplasmic genic male sterility in rice.

MATERIALS AND METHODS

Two CMS lines, viz. V20A (WA cytosterile); Pragathi A (577A cytosterile), and their maintainer lines (V20B and Pragathi B, respectively) were raised at the Main Research Station, University of Agricultural Sciences, Hebbal, Bangalore during 1990 summer. Samples were taken at successive stages of anther development from primordial initiation

*Present address: Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi 110012.

to anthesis and fixed separately in carnoy B solution (6 parts of ethanol + 3 parts of chloroform + 1 part of acetic acid by volume) for localization of proteins and RNA, and in formalin, acetic acid and ethanol in the ratio of 1:1:18 by volume (FAA) for the localization of total insoluble polysaccharides. Subsequently, the materials were dehydrated using different ethanol:butanol grades (1:3, 1:1, 3:1) and embedded in paraffin 58–60°C using the paper boat technique [3]. Sections of 8 μm thickness were taken and subjected to the histochemical tests listed in Table 1. Successive stages of microsporogenesis and pollen development of CMS lines were compared with those of their respective maintainer lines under light microscope for histological and histochemical differences.

Table 1. Histochemical tests adopted for different cellular metabolites in rice pollen

Metabolite	Test	Colour
Total insoluble polysaccharides	Periodic acid Schiff's (PAS) method [3]	Magenta
Insoluble proteins	Mercuric bromophenol blue method [4]	Deep Blue
RNA	Toluidine blue test [5]	Light purple

RESULTS AND DISCUSSION

HISTOLOGICAL CHANGES

The anther development in both CMS and their maintainer lines was similar until the completion of meiotic division. The anther primordia differentiated into tetrasporangiate of isodiametric cells. A row of isodiametric cells in each sporangium got differentiated into archesporial cells. Later, each archesporial cell gave rise to inner sporogenous mass of cells and outer wall layers, viz., outer endothecium, a middle layer and inner tapetum, all enclosed in a layer of epidermal cells (Fig. 1: 1), The sporogenous mass later differentiated into conical shaped pollen mother cells (Fig. 1: 2) which gave rise to isobilateral tetrads (Fig. 1: 3). Until late meiosis, no histological differences were visible in the CMS and maintainer lines. The CMS lines differed from their respective maintainer lines in tapetum and endothecium development and functioning from the microspore stage onward. The tapetum, being the nutritive layer, showed gradual disintegration after tetrad formation in anthers of both maintainer lines. The rate of disintegration was rapid at late microspore stage (Figs. 2: 1, 3: 1) and completely disintegrated at pollen maturity (Figs. 2: 3, 3: 3). In contrast, the tapetum in the CMS anthers of the line V20A showed disintegration at the microspore stage, the disintegration rate was slower than in its fertile counterpart and resulted in abortion of microspore (Fig. 2: 2). The thickness of tapetum at microspore stage was 5.9 μm and 7.6 μm in the fertile and CMS anthers, respectively. The aborted microspores and pollen grains were irregular and small while the normal pollen grains were spherical

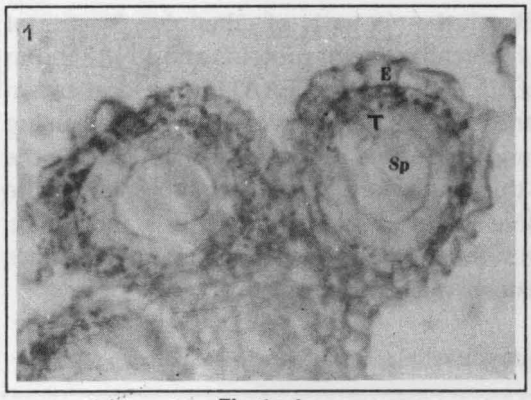

Fig. 1 : 1

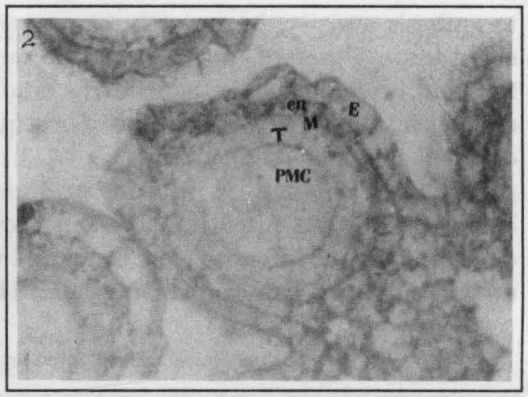

Fig. 1 : 2

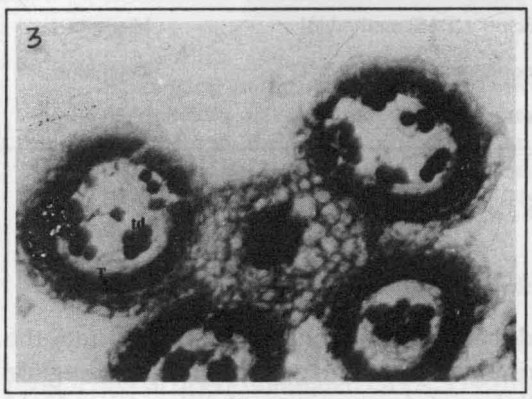

Fig. 1 : 3

Fig. 1. **Cross sections of anthers (x1250) showing: 1) sporogenous stage; 2) pollen mother cells stage; and 3) tetrads formation stage.**

SP—sporogenous mass; T—tapetum layer; E—epidermis; en—endothecium; M—middle layers; PMC—pollen mother cell; and td—tetrad.

and larger. The tapetal behaviour in Pragathi A did not differ much from its maintainer line. However, disintegration of tapetum was slightly delayed, leading to microspore abortion (Fig. 3: 2). The size of tapetum at microspore level was 6.5 and 7.2 μm in Pragathi B and Pragathi A, respectively. A few pollen grains in the anthers of Pragathi A were spherical while others had abnormal shape. Improper nourishment of the developing microspores due to abnormal tapetum behaviour leads to pollen abortion [6–9]. Tapetal abnormalities like tapetal hypertrophy, hill and baloon types, vacuolation of tapetal cells, tapetal periplasmodium and intratapetal syncytium are known to occur. However, no such abnormalities were observed in the tapetum of V20A or Pragathi A. Functional abnormality, such as, slow disintegration might have led to improper nourishment of the developing microspores and their abortion. The thin layer of tapetum at the pollen grain stage in V20A had enough nutrients. This shows that only the starvation of microspores resulted in abortion. Pan et al. [10] reported that the tapetal degeneration was normal but was slightly delayed in Tienrei 409A CMS line. Normal development of tapetum layer was reported in

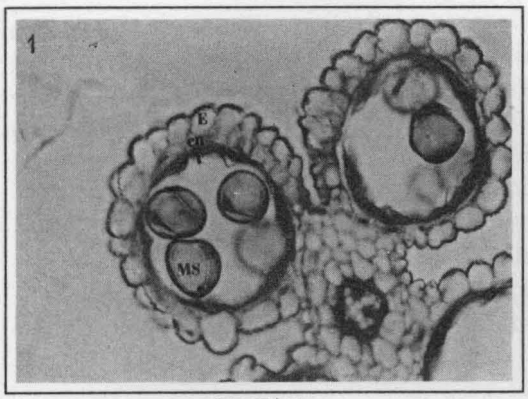

Fig. 2 : 1

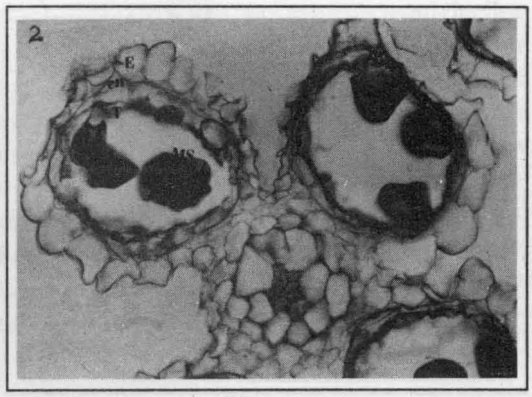

Fig. 2 : 2

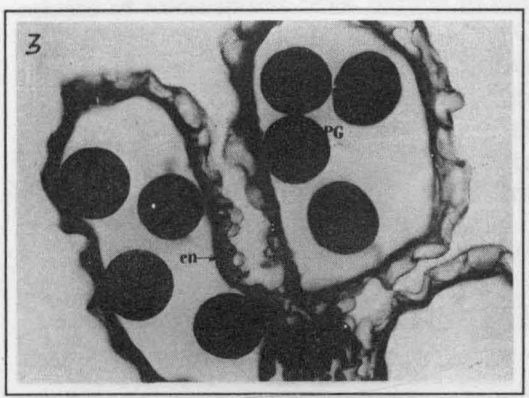

Fig. 2 : 3

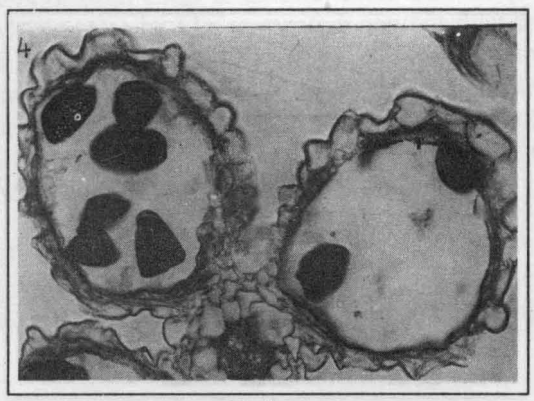

Fig. 2 : 4

Fig. 2. **Cross section of anthers (x500) of V20A and B showing: 1) normal microspore development in V20B; 2) abnormal microspore development in V20A; 3) well formed pollen grains in V20B; and 4) aborted pollen grains in V20A anther with a thin layer of tapetum.**

MS—microspore; T—tapetum; en—endothecium; PG—pollen grain; and E—epidermis.

Kwang-Shan 3A, a WA type male sterile line [11]. Cheng and Huang [12] did not observe tapetal abnormalities in the functional male sterile rices. It may be mentioned that early degeneration of tapetum has also been reported in the CMS anthers [6, 13].

The endothecium in the anthers of both maintainer lines developed into fibrous thickenings at pollen maturity stage (Figs. 6, 10) while it was enlarged in the anthers of CMS lines (V20A and Pragathi A), as in the fertile anthers, but developed into subnormal fibrous thickenings, resulting in nondehiscence of anthers (Figs. 2: 4, 3: 4). The importance of endothecium and epidermis in anther dehiscence has been emphasised by [12], who reported that strongly thickened fibrous endothecium with a particular orientation pattern and slightly thick walled epidermal cells resulted in nonseparation of the

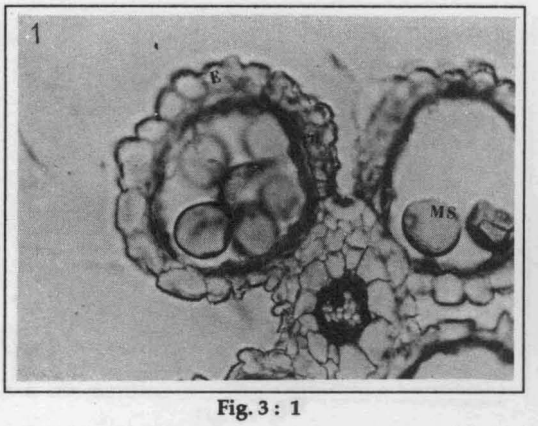

Fig. 3 : 1

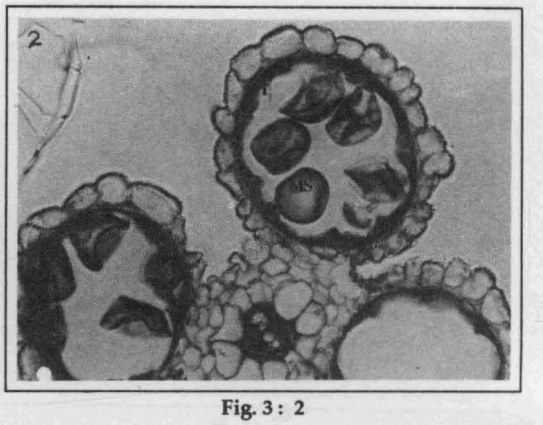

Fig. 3 : 2

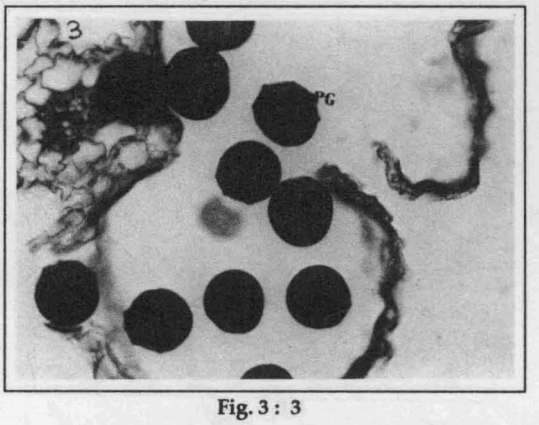

Fig. 3 : 3

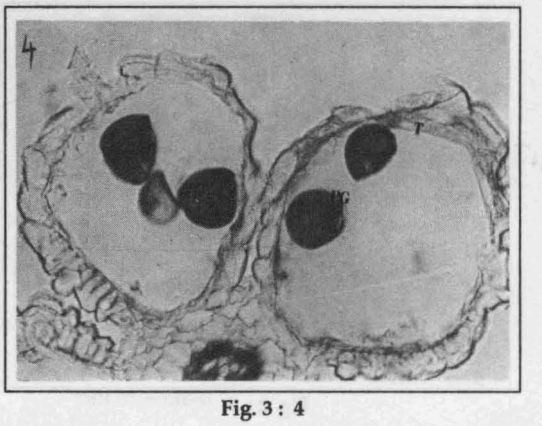

Fig. 3 : 4

Fig. 3. Cross sections of anthers (x500) of Pragathi A and B showing: 1) normal microspores development in Pragathi B; 2) abnormal microspores development in Pragathi A; 3) well formed pollen grains in a Pragathi B anther with locules fused at the time of dehiscence; and 4) aborted pollen grains in a Pragathi A anther with subnormal development of endothecium layer.

MS—microspore; T—tapetum; en—endothecium; E—epidermis; PG—pollen grain.

intermicrosporangial stripes from the underlying parenchyma along each side of the connective tissue. In Pragathi A, anther indehiscence appears to be another cause of male sterility. At pollen ripening stage of the maintainer lines, two microsporangial locules fused through the intervening connective cells to form a single pollen cavity. Similar results were reported in the CMS rice strains 65P2, 61P1 and KR 7 [12].

HISTOCHEMICAL CHANGES

The sporocytes of both maintainer lines showed gradual increase in insoluble total polysaccharide content up to pollen grain formation stage, accompanied by accumulation

of starch grains at pollen maturity, whereas the sporocytes in both CMS lines showed decline in PAS reaction after tetrad formation and had lower starch accumulation at maturity. This was more pronounced in the CMS anthers of V20A than in Pragathi A. The tapetum and endothecium also showed less PAS-positive response after meiosis in both CMS lines compared to their respective maintainers. These observations are in conformity with those reported in *Triticum* [14] and vegetable crops [15]. The absence of starch in the anthers of sterile lines of some crops has been considered to be a cause of abnormal tapetal behaviour due to carbohydrate deficiency, ultimately leading to pollen abortion [15]. This may be true for the V20A CMS line in the present study. However, in Pragathi A, the difference between polysaccharide content of the CMS and maintainer analogues is not enough to ascribe carbohydrates a role in pollen abortion.

The RNA and protein contents were lower in the sporocytes and endothecium after the release of microspores from tetrads in anthers of both CMS lines than their maintainer counterparts. The tapetum layer of V20A CMS line also had lower protein content than in the corresponding maintainer line. Chauhan [14] also observed reduction in total protein content in all the parts of anther in the natural as well as chemically induced male sterile plants of *Triticum* and vegetable crop. Histochemical studies suggest that the inability of tapetum to mobilize important metabolites to the developing microspores causes their starvation and leads to abortion of CMS anthers.

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