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CYTOHISTOLOGICAL STUDIES ON MALE STERILITY IN DIFFERENT CYTOPLASMIC GENETIC MALE STERILE LINES OF RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Cytological, cytohistological and morphological causes for pollen breakdown in two cytoplasmic genetic male sterile lines MS 577A and IR 58025A were carried out in comparison with their corresponding maintainer lines (MS 577B and IR 58025B) and a restorer line (Vajram). The anther sac layers, i.e. the inner tapetum, middle endothecium and outer epidermis showed slight variation in their thickness at all stages among the lines but showed normal behaviour in development in both the cytoplasmic genetic male sterile lines, IR 58025A and MS 577A, as well as their maintainer lines (IR 58025B and MS 577B) and the restorer (Vajram). In IR 58025A, the critical stage of breakdown was observed to be at binucleate stage whereas in MS 577A pollen grains were morphologically similar to its maintainer line but failed to fertile in selfing. The reason for pollen breakdown may be incompatibility between nuclear and plasma genes.

Key words: Cytology, histology, CGMS, rice.

The use of cytoplasmic genetic male sterility in commercial exploitation of heterosis in rice is steadily increasing. It can be used to avoid emasculation for large scale production of hybrid seed. However, there are many reports about the breakdown of male sterility either after a few generations or in different environments. The causes for breakdown of male sterility have been studied the cytohistological and morphological aspects of male sterility using two cytoplasmic genetic male sterile lines (A), their maintainer lines (B), and a restorer line (R).

MATERIALS AND METHODS

Five lines, which included two promising cytoplasmic genetic male sterile lines (A), IR 58025A, MS 577A, their maintainer lines (B) IR 58025B, MS 577B and a restorer line (R),

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Vajram were used in the study. Young spikelets at appropriate stage were taken for cytological observations from each genotype and fixed in acetic acid + ethanol (1:3). Acetocarmine smears were prepared to study the different stages of microsporogenesis and microgametogenesis [6] leading to pollen breakdown.

Histological studies were conducted from flower primordial stage to anthesis in the spikelets fixed in acetic acid + ethanol (1:3). Permanent slides of sections of spikelets at different stages were made using the common micrometric techniques [7]. Naturally shed pollen (300–400 pollen grains) was collected and stained with acetocarmine to study pollen sterility. The pollen grains taking dark stain and no stain were counted as fertile and sterile, respectively. The size of different anthers tissues, viz., tapetum, endothecium and epidermis in male sterile and fertile plants were measured at various stages of microsporogenesis and microgametogenesis with the help of standard occular micrometer.

RESULTS AND DISCUSSION

The development of anthers and anther sac layers in the above five lines was almost similar until the formation of microspores. Both IR 58025A and MS 577A had no detectable differences in the development of tapetum, endothecium and epidermis during the development of anther sac layers. Further the anther sac layers increased maximum at the tetrad formation stage in A, B and R lines (Table 1), which progressively degenerated and finally formed a thin layer before dehiscence. This study confirms normal behaviour of the anther sac layers in the male sterile lines, which were very similar in all respects to their corresponding maintainer and restorer lines without any hypertrophy. Similar observations were reported in rice earlier [1–3].

The development of microspores in all the lines were normal up to the first mitotic division. After that the microspores of all the lines, including MS 577A (Fig. 1: 1–4) increased in size rapidly with the accumulation of cytoplasm. IR 58025A was an exception. In contrast to this normal development, the microspores showed moderate increase in size and were located at the periphery nearer to tapetum (Fig. 1: 5, 6) but there was no accumulation of cytoplasm in IR 58025A (WA cytoplasmic source). Therefore, it was concluded that the first signs of breakdown in IR 58025A appears after the first mitotic division during the development of male gametophyte. Earlier reports also show that in several male sterile lines, breakdown occurs just before or soon after the first mitotic division of microspore development [1–3].

In MS 577A (Korean cytoplasmic source), it was observed that the pollen grains were morphologically similar to that of its maintainer line B (Fig. 1: 7) but panicles on selfing did not produce any seed (Fig. 1: 8), which indicates that the pollen grains, although appearing normal, were sterile. This finding is in agreement with that of [8]. At this stage, the reasons

Table 2. Range and mean values of the characters of spikelet morphology in A, B and R lines of rice

Variety	Anther length (mm)		Anther width (mm)		Pollen diameter (μm)		Fertilty (%)	
	range	mean	range	mean	range	mean	range	mean
IR 58025A	1.58–2.22	1.89	0.30–0.54	0.394	23.1–30.0	26.6	0.5–3.0	1.2
IR 58025B	1.62–2.37	1.99	0.30–0.68	0.429	38.5–53.9	45.9	96.6–98.1	98.3
MS 577A	1.68–2.14	1.98	0.27–0.66	0.384	30.8–38.5	34.0	3.8–7.7	4.3
MS 577B	1.92–2.84	2.24	0.33–0.60	0.462	30.0–46.2	42.3	95.8–97.8	96.5
Vajram	1.89–2.34	2.25	0.30–0.66	0.454	38.5–53.9	46.2	98.3–99.0	98.7

chromosomes was normal in all the A, B and R lines (Table 3). This rules out any role of chromosomal aberrations in including pollen sterility in the CMS lines [1–3].

Based on the above observations, it may be concluded that:

a) The behaviour of anther sac layers was normal in both the sources of male sterility.

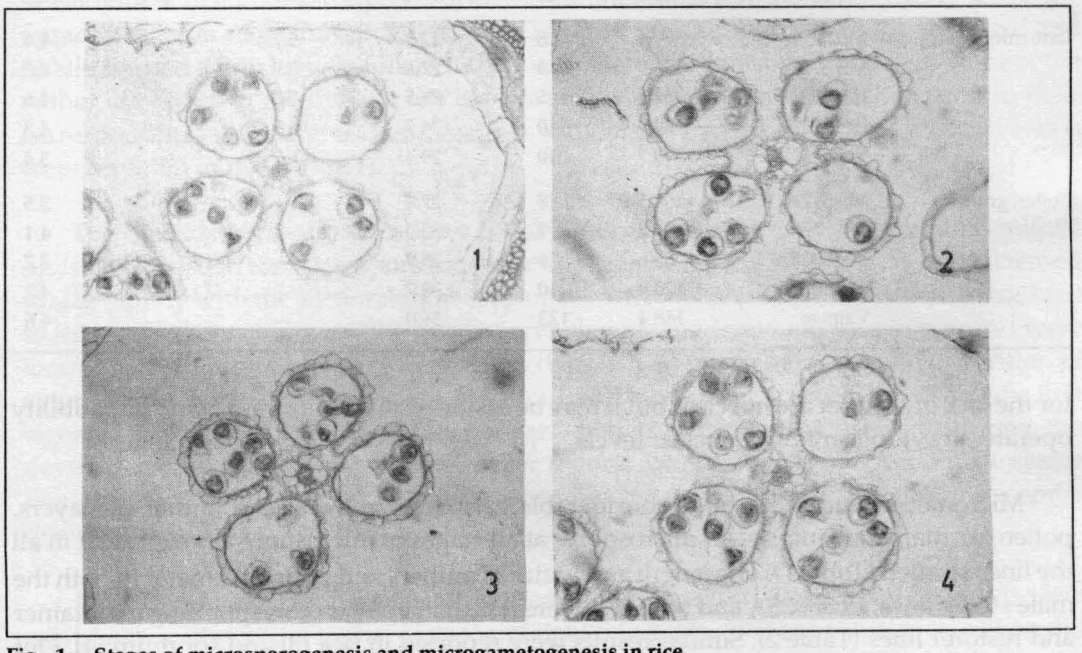

Fig. 1. Stages of microsporogenesis and microgametogenesis in rice.
Late microspore stage with richly stained microspores and degenerating anther sac layers in:
1. MS 577A, 2. MS 577B, 3. Vajram, 4. IR 58025B.

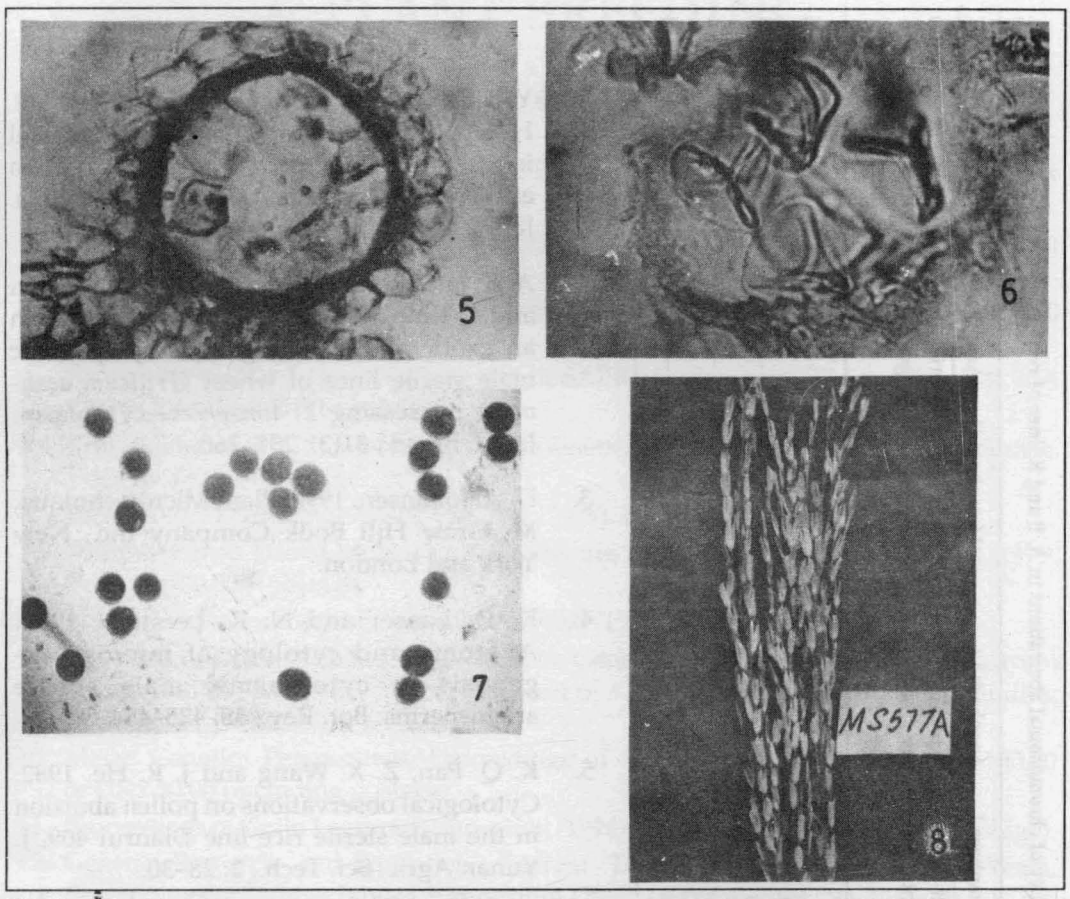

Fig. 1 (contd.)
5. IR 58025A. 6. Unstained sterile pollen grains in IR 58025A. 7. Stained pollen incapable of fertilization in MS 577A. 8. Panicle with unfilled grains of MS 577A.

b) Meiosis was also normal in both the CMS lines.

c) Morphological variations existed among the anthers of the male sterile lines and their corresponding maintainer and restorer lines.

d) In IR 58025A, binucleate stage was the critical stage of breakdown, whereas in MS 577A, no breakdown was observed. However, its pollen grains, which were morphologically similar to its maintainer line, failed to fertilize on selfing. This pollen breakdown may be due to incompatibility between nuclear and plasma genes.

e) Biochemical studies may help in explaining the breakdown in the CMS lines precisely.

Table 3. Frequency of chromosomal aberrations in A, B and R lines of rice

Variety	No. of plants	No. of PMCs	Prophase-I		Metaphase-I		Anaphase-I		Telophase-I laggards	Metaphase-II		Anaphase-II		Telophase-II micronuclei	Total	Frequency (%)
			fragments	univalents	multivalent	univalents	bridges	laggards		multivalents	univalents	bridges	laggards			
IR 58025A	5	374	—	—	3	—	—	1	—	—	—	—	—	—	4	1.86
IR 58025B	5	446	—	1	1	—	—	—	—	—	—	—	—	—	2	0.44
MS 577A	5	386	—	—	2	—	—	—	—	—	—	—	—	—	2	0.51
MS 577B	5	412	—	—	—	—	—	1	—	—	—	—	—	—	1	0.24

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