Indian J. Genet., **42** : 75-81 (1982)

CHARACTERIZATION OF DIVERSE SORGHUM MALE STERILE POLLEN GRAINS BY SCANNING ELECTRON MICROSCOPY*

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ABSTRACT

Pollen grain shape and size, exine sculpture and internal structure as revealed by scanning electron microscopy of pollen from diverse sorghum cytoplasmic-genetic male sterile and their maintainer lines showed distinct differences VZM2 pollen showed resemblence to GI pollen but differed from both CK 60 and M 35-1 pollen. CK60 pollen exine sculpture showed differences from that of M35-1 pollen. The pollen from make sterile exine lines were more or less devoid of protein and starch particles, The grouping of sorghum lines based on pollen structure corresonds to the grouping based on genetic and biochemical studies.

All commercial hybrids of sorghum developed todate are based on milo cytoplasmic male sterility. There is need to identify and characterize alternative sources of male sterility to safeguard against any possible disease hazards. Rao (1962), Hussaini and Rao (1964). and Nagur and Menon (1974) have reported the occurrence of cytoplasmic genetic male steriles from India which are different from milo-kafir system.

Recent studies have shown characteristic differences in soluble protein and esterase isoenzyme patterns among different cytoplasmic-genetic male steriles of Indian origin (Tripathi, Mehta and Rao. 1981b,c). In addition, differences in amino acid composition of anther protein from diverse male steriles and their maintainers have been observed (Tripathi, Mehta and Rao, 1981a).

In the present study pollen from diverse cytoplaimic genetic male sterile lines of sorghum have been studied by scanning electron microscopy.

MATERIALS AND METHODS

Seeds of four male steriles (CK 60A, VZM 2A, GIA and M35-1A) and their maintainer lines were grown at IARI, New Delhi, during 1978. Mature anthers were collected and the pollen grains were fixed by tearing the anthers in 6.5% glutaldehyde solution in cacodylate buffer, pH 7.2, for 24 hours at 4°C.

To observe the internal structure of the pollen grains by SEM, the anthers were ground at 4°C to break the pollen grains. The material was later fixed for 24 hours at 4°C.

The samples after fixation were washed in cacodylate buffer for 15 min. and transferred in 1% osmium tetraoxide solution at 25°C in cacodylate buffer (pH 7.2). The material was post fixed for 4 hours and finally washed with buffer. The samples were dehydrated by usual procedure with a graded series of alcohol and later mounted on SEM stubs and room dried. The material was

* Part of Ph. D. thesis submitted by the senior author to P.G. School, IARI, New Delhi, India.

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later vacuum coated with gold in a coating unit. The mounted stubs were rotated while being coated. The gold coated pollen grains were examined in Cambridge Stereoscan S 4-10 scanning electron microscope for their (i) shape and size (ii) exine ornamentation (iii) fractured pollen grain surface and interior, at 15-20 KV accelerating voltage in the secondary emission mode at large working distance.

Results and Discussion

Pollen Shape And Size

The pollen grains of M 35-1A and M 35-1B were similar and oval in shape and largest in size (15.0$\mu \times 8.6\mu$). The pollen grains of GIA and GIB were the smallest and spheroidal in shape. On comparing A *vs.* B lines B pollen were larger in size (13.4$\mu \times$ 8.9 μ) than A pollen (11.1 $\mu \times 8.4\mu$) in GI. The pollen of VZM2 A and VZM2 B were also almost identical to GI A in their shape and size. CK 60 B pollen was intermediate in size (12.7 $\mu \times 8.8\mu$) between M35-1 and GI. In VZM2 A, pollen were polyhedral and shrunken in shape (Fig. 1 E).

Exine Sculpture

The exine organization of sorghum pollen grains are shown in Fig. 2. In CK 60A the exine distribution was completely irregular and a symmetrical but its maintainer line CK 60B showed very clear grouping in the exine. It had on an average 10.66 processes per aggregation (Table 1). The exine organisation in M 35-1A and M35-1B showed more or less uniform distribution. There was less aggregation of exines in M35-1A line than M35-1B. The density of exine processes was much more in VZM2 A as compared witn VZM2 B. In GI B line the grouping processes were having on an average 2.84 processes per aggregation. The aggregations were distinctly separate in CK 60B as compared to GIB.

Internal Structure

In order to get information about the cytoplasmic material, apparently the starch granules and protein matrix in pollen grains, the broken fragment of the crushed pollen were studied. Internal structure of pollen from different lines is shown in Fig. 3. CK 60A pollen showed many cavities with more protein matrix and little amount of starch granules embeded in it. In CK 60B the cavities were few and large with very small protein material but much more aggregated starch granules which were spherical to oval in shape (Fig. 3B). The GIA and GIB pollen showed almost similar structure with much more protein matrix inside them. The amorphous protein material was covering the starch granules which appeared much smaller in size, than in CK 60B and M35-1B pollen. In M35-1A line the cavities were larger thanin CK 60A, but in M35-1B line there was no cavity and it was densly packed. The starch granules were more in number in M35-1B pollen as compared with all other pollens and were of bean and capsule shape. The protein material was less in it.

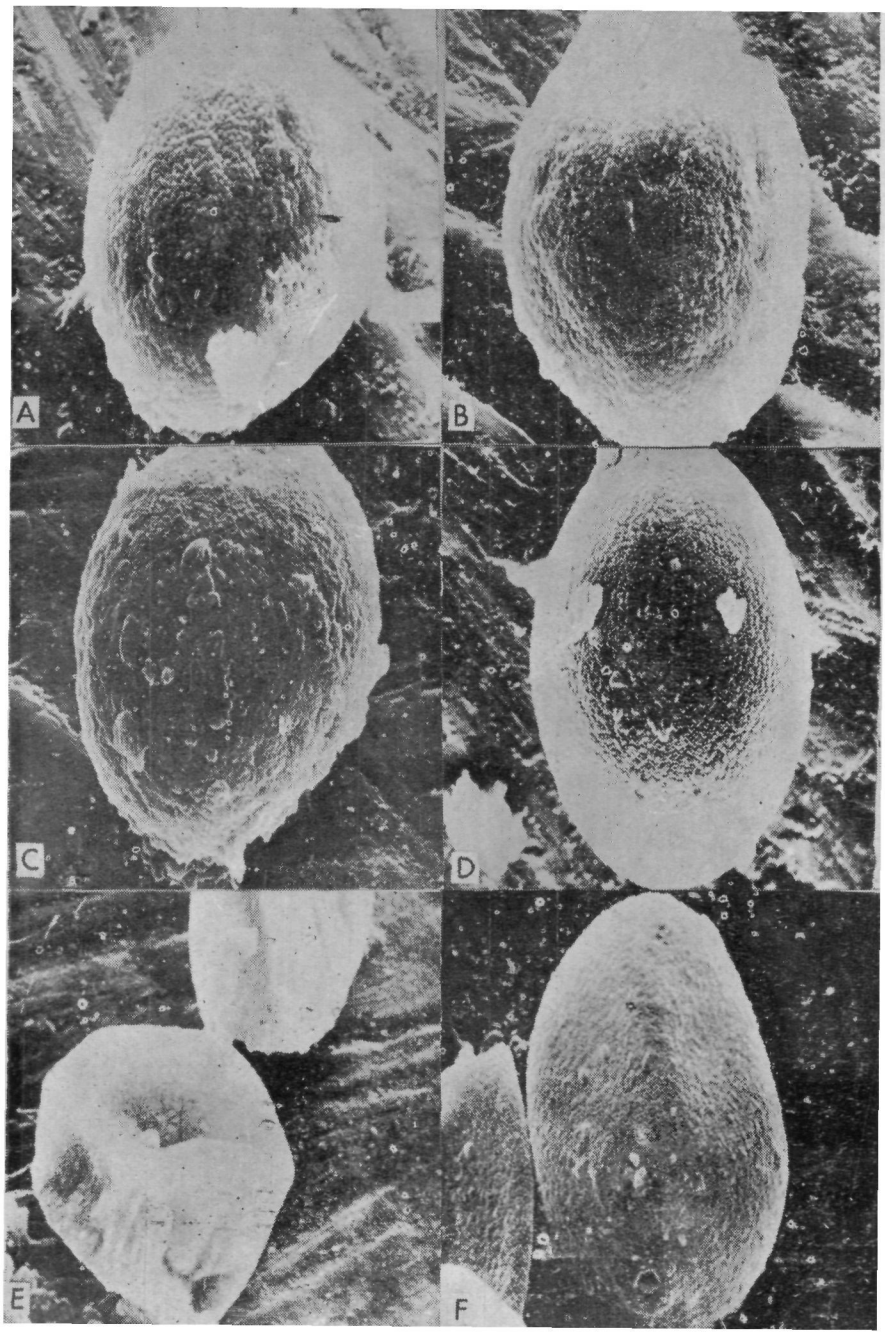

Fig. 1 Scanning electron micrograph showing pollen size and shape :
A. CK60 (× 5885) B. GIB (× 5885) C. VZM2B (× 5885)
D. M35-1B (× 5491) E. VZM2A (× 4634) F. M35-1A (× 5512)

TABLE 1

Pollen size and exine ornamentation in diverse male sterile of sorghum and their maintainer lines

Line	Pollen size (μ)		No. aggregation with X processes per aggregation where											Total No. Processes	Mean No. Processes per aggregation
	L	W	X=1	2	3	4	5	6	7	8	9	10	15		
CK 60A	—	—	5	3	1	—	—	—	—	—	—	—	—	14	1.55
CK 60B	12.7	8.8	—	—	—	—	—	—	1	—	—	1	1	32	10.66
M35-1A	15.0	8.6	18	1	—	—	—	—	—	—	—	—	—	20	1.05
M35-1B	14.7	9.2	19	2	2	—	—	—	—	—	—	—	—	29	1.26
VZM 2A	11.5	9.4	29	4	3	2	—	—	—	—	—	—	—	54	1.40
VZM 2B	13.1	9.1	11	2	—	2	1	1	1	—	—	—	—	41	2.27
G 1A	11.1	8.4	—	—	—	—	—	—	—	—	—	—	—	—	—
G 1B	13.4	8.9	7	—	2	2	—	—	—	2	—	—	—	37	2.84

L = Length
W = Width

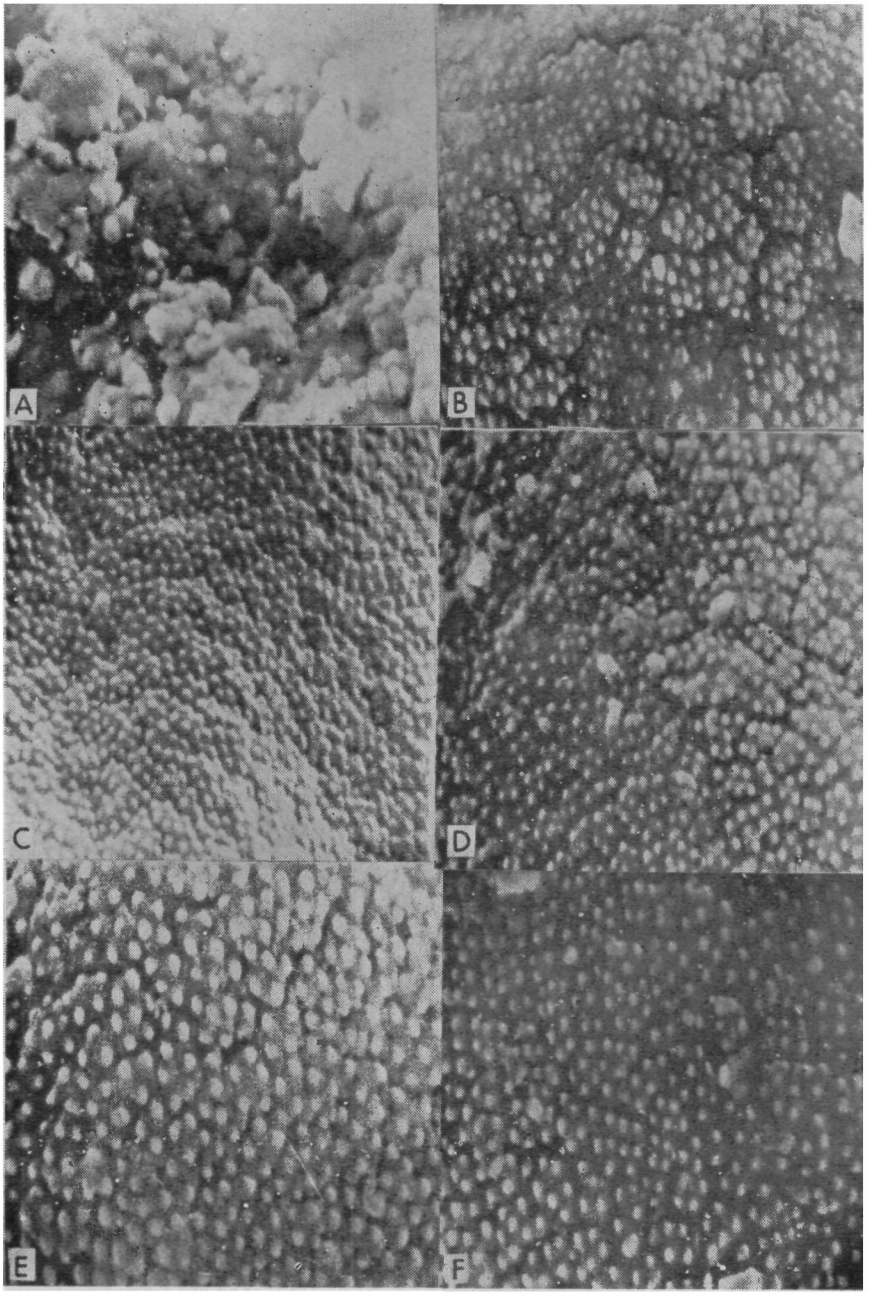

Fig. 2 Scanning electron micrograph showing exine sculpture:
A. CK60A (× 27584) B. CK60B (× 31264) C. V2M2A (× 28391)
D. V2M2B (× 27770) E. M35-1A (× 33862) F. M35-1B (× 31264)

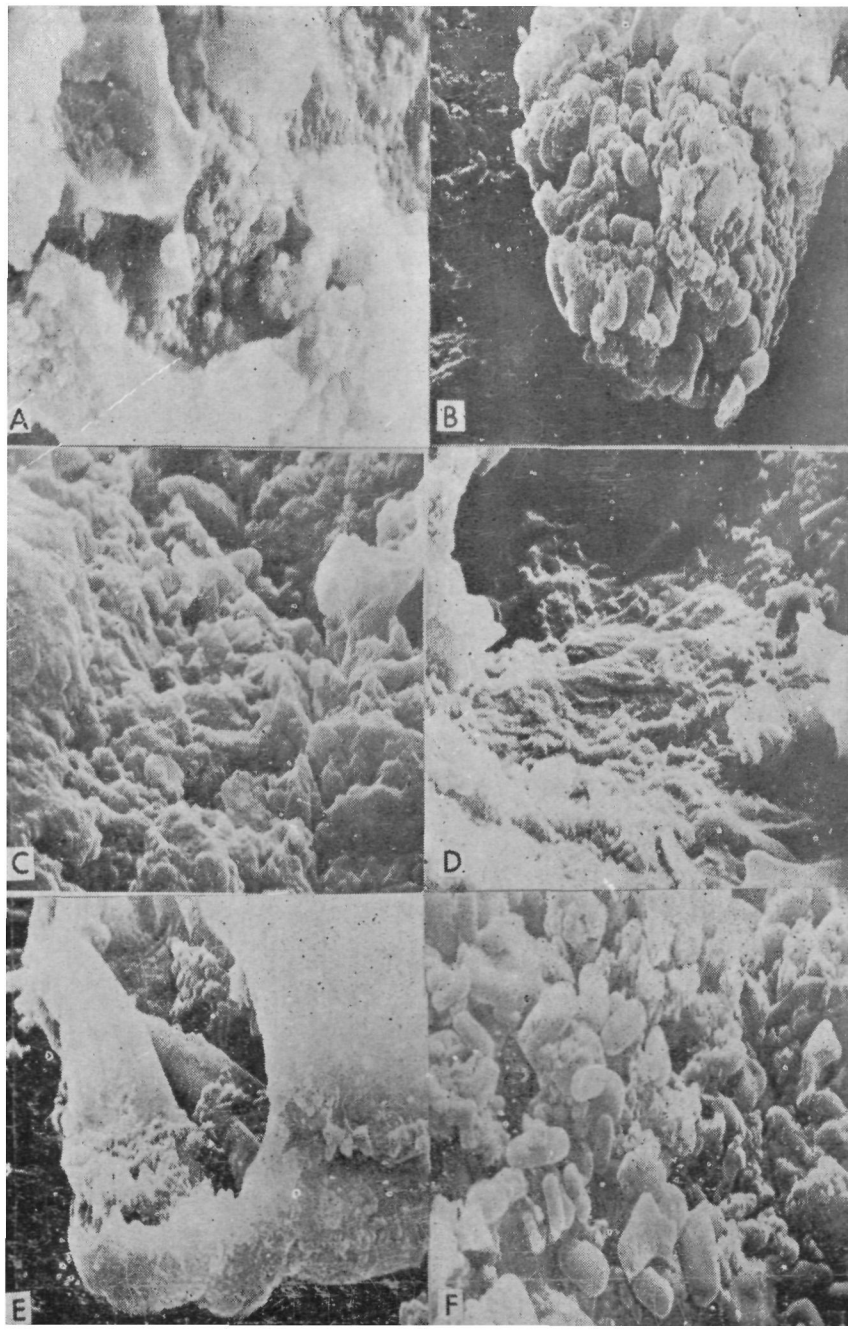

Fig. 3 Scanning electron micrograph showing internal structure of pollen grains :
A. CK60A (× 14130) B. CK60B (× 16805) C. G1A (× 17064)
D. G1B (× 14851) E. M35-1A (× 18966) F. M35-1B (× 16414)

ACKNOWLEDGEMENT

The authors are grateful to Mr. V.B. Lal, Textile Department of IIT, New Delhi, for help in scanning electron microscopy.

REFERENCES

Gornall, R.J. (1977) Notes on the size and exine ornamentation of *Avena* pollen grains. *Ganad J. Bot.* 55 : 2622-29.

Hussaini, S.H. and P.V. Rao. (1964). A note on the spontaneous occurrence of cytoplasmic male sterility in Indian *Sorghum*. *Sorghum Newslett.* 7 : 27-28.

Nagur, T. and M. Menon. (1974). Nature of genotype in different maintainer and restorer lines of *Sorghum*. *Sorghum Newslett.* 18-19

Rao, N.G.P. (1962). Occurnce of cytoplasmic genetic male sterility in some Indian *seorghums*. *Indian J. Genet* 22 : 257-59,

Tripathi, D.P., S.L. Mehta and N.G.P. Rao. (1981a). Amino acids in anthers of *milo* and Indian origin cytoplasmic genetic male sterile sorghums (*Sorghum bicolor* L. Moench) *Theor appl. Genet.*, 59 (In press)

Tripathi, D.P., S.L. Mehta and N.G.P. Rao (1981b) Soluble protein and isoenzymes from seeds of diverse male steriles of sorghum (*Sorghum bicolor* L. Moench.) *Theor. appl. Genet*, 59 (In press)

Tripathi, D.P., S.L. Metha and N.G.P. Rao (1981c). Soluble protein and isoenzymes from anthers of diverse male sterile in sorghum (*Sorghum bicolor* L. Moench) *India t. j. Genet.* (In press.