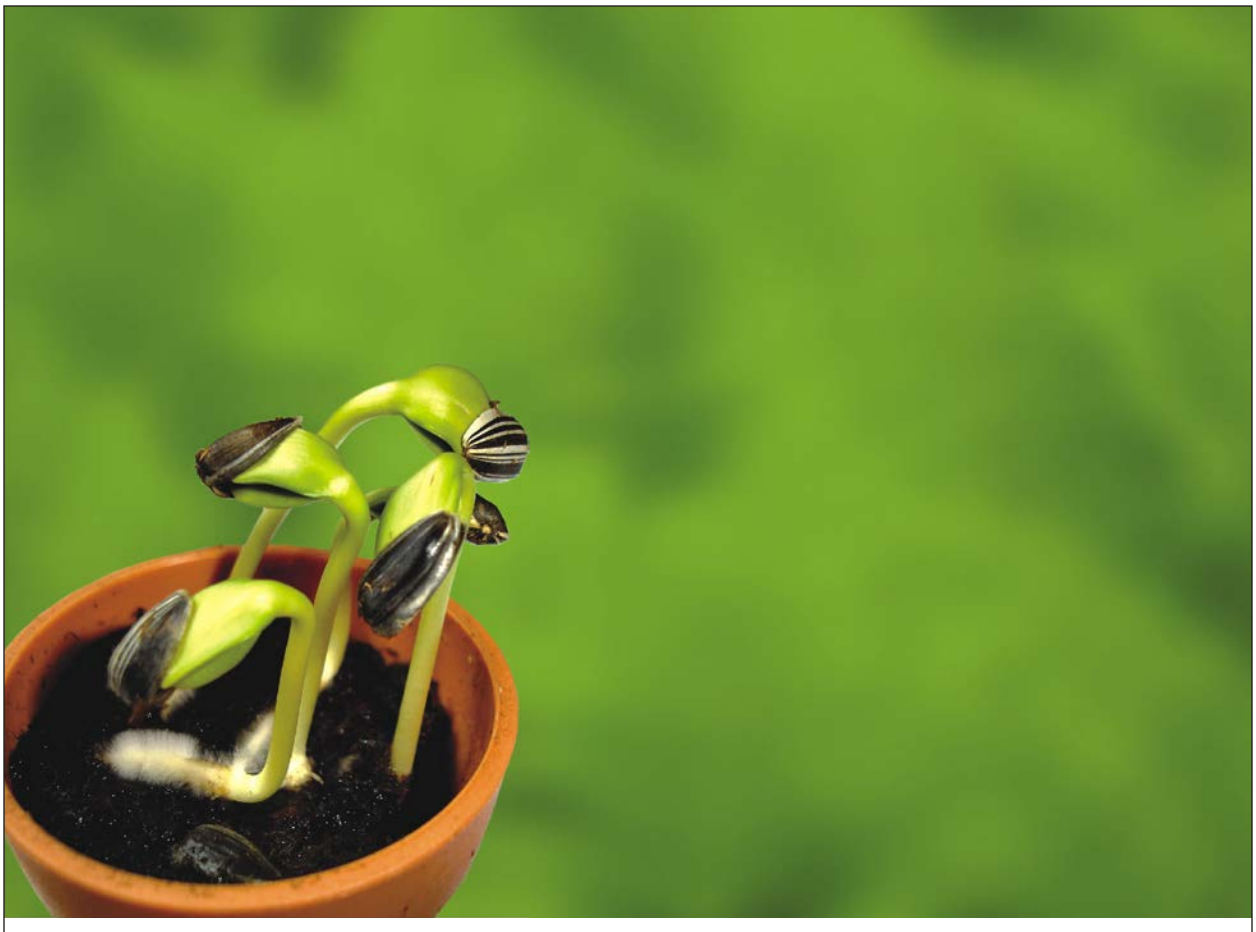

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Fertility Restoration and Cytological Studies of Pollen in Pearl Millet (*Pennisetum glaucum* (L.) R.Br.) Male Sterile and Fertile Lines

[1]M. Vetriventhan, [1]A. Nirmalakumari, [1]K. Ganeshamurthy,
[2]D. Sudhakar, [1]S. Ganapathy and [1]T.S. Raveendran
[1]Centre for Plant Breeding and Genetics,
[2]Centre for Plant Molecular Biology and Biotechnology,
Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

Abstract: An experiment comprised of 5 male sterile lines and 30 inbred testers of pearl millet and 150 hybrids was conducted at Millet Breeding Station, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore during 2005-2007. The fertility restoration ability of thirty diverse inbred pollinators on different cytoplasmic sources were studied. Among the one hundred and fifty hybrids studied, forty one hybrids were complete sterile and twenty four hybrids were recorded fertile. The two inbreds namely PT 5259 and PT 5773 contributed sterility with four male sterile lines out of five male sterile lines used. The former expressed sterility with ICMA 88994A, 81A, ICMA 94111A and PT 5054A, the later expressed sterility with the male sterile lines 81A, 732A, ICMA 94111A and PT 5054A. The A line PT 5054A expressed sterility in fifteen hybrids out of inbreds studied and it was followed by 81A with in fourteen inbreds. The 732A cytoplasm expressed sterility with only one inbred (PT5773) and also it was registered complete fertility with most of the inbreds than other male sterile lines. Developmental variation in the male sterile and male fertile lines of pearl millet was studied in five developmental stages viz., PMC, dyad, tetrad, soon after microspore release and before dehiscence of anther and it was found that, break down of fertility in the sterile anthers of pearl millet was post meiotic, in specific soon after the release of tetrad in the developmental stage.

Key words: Cytoplasmic male sterility, microsporogenesis, pearl millet, *Pennisetum glaucum*, pollen degeneration, tapetum

INTRODUCTION

Taxonomically pearl millet (*Pennisetum glaucum* (L.) R.Br.) belongs to the family Poaceae, sub-family Panicoideae, section Penicillarium and possesses 2n = 2x = 14 chromosomes. It is a cross-pollinated annual C_4 crop species that originated in western Africa and was introduced to eastern Africa and the Indian sub continent some 2000 years ago. India is the largest producer of this crop, both in terms of area (9.1 m ha) and production (7.3 m t), with an average productivity of 780 kg ha^{-1} during the last five years. Nearly 50% of the millet area is under hybrid cultivars. Earlier reports of occurrence of male sterility in pearl millet were by Kajjari and Patil (1956) and Burton (1958). The discovery of cytoplasmic male sterility in pearl millet (Burton, 1958), led to the production of high-yielding commercial hybrids using the male sterile line, Tift $23A_1$. Subsequently, two additional sources of cytoplasmic male-sterility designated $L66A_2$ and $L67A_3$ were identified (Burton and Athwal, 1967). In fact, a number of male-sterile lines were developed by genome substitution of Tift $23A_1$ by repeated

Corresponding Author: M. Vetriventhan, Centre for Plant Breeding and Genetics,
Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

back-crossing to several maintainers (Burton, 1965; Pokhriyal *et al*., 1974; Athwal *et al*., 1976). The use of male sterile line Tift 23A, in producing high yielding hybrids failed to last long due to downy mildew epidemics. Hence, to obviate the dangers of genetic uniformity and disease susceptibility, attempts have been made in pearl millet to intensity the research on cytoplasmic and genetic diversification of male sterile lines (Kumar and Andrews, 1984; Rai and Singh, 1987). Both nuclear and cytoplasmic diversity will have practical utility in exploitation of heterosis. Altering the male sterile lines by incorporating the nuclear genome of alternate maintainer lines may lead to genetic and cytoplasmic diversity, thereby providing a wider base for further exploitation. With the availability of number of male sterile lines in diverse cytoplasmic background, the scope for developing superior hybrids has enlarged. However, information on the performance of these male sterile lines in combination with specific restorer is a pre-requisite in selecting suitable parents for the yield and its contributing characters to develop a high yielding commercial hybrid.

Comparative studies of pollen sterility/fertility and meiotic studies in four temperature-sensitive genic male sterile (TGMS) lines, along with restorer line RHRBI-458 of pearl miller, were conducted studied by Shinde and Mehetra (2003). The TGMS lines (RHRBTS-9862, RHRBT-9865, RHRBT-9869 and RHRBTS-9872) had major portion of their anthers is completely sterile class (97.8 to 99.7% under high temperature conditions of >38.7° PMC of earlier tillers), while the major portion of their anthers was classified into completely fertile class (from 97.7% in low temperature of <34.2°). The fertile line RHRBI-458 had 99.1 to 100% completely fertile anthers under both environments.

Microsporogenesis of cytoplasmic male sterile lines and male fertile lines was studied in order to identify the stage of breakdown of fertility during microsporogenesis which leads to male sterility in pearl millet. Fertility/sterility pattern of hybrids (Rai and Hash, 1990) and mitochondridal DNA-RFLP pattern (Chhabra, 1995) have been used to characterize these CMS sources. Microsporogenesis pattern has been used to characterize CMS differences in maize (Lee *et al*., 1979). Pearl millet studies of A_1 system CMS lines show strong effect of the genetic background on microsporogenesis. For example, Burton (1958) indicated that microsporogenesis breakdown occurs mainly during tetrad formation. Influence of a range of cytoplasm on microsporogenesis and anther development in pearl millet using six isonuclear A-lines having five cytoplasm (A_1,A_2,A_3,A_4 and A_5) and the nuclear genome of 81 B were studied by Chhabra *et al*. (1997).

MATERIALS AND METHODS

Five male sterile lines and thirty inbreds of pearl millet were chosen for the study. The materials were obtained from pearl millet unit, Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Among the five male sterile lines used as female parents, ICMA 94111A and 81A are having A_1 cytoplasm, ICMA 88004A and PT 5054A have A_4 cytoplasm and 732A belongs to Bellary cytoplasm, a different source which had been derived from PT 819, an inbred line originated from Andhra Pradesh, India. The thirty inbreds were used as testers. Artificial selfing and crossing operations were carried out. To ensure selfing, the panicles of the parents, just before the emergence of stigma were covered with butter paper bags and fastened well with a piece of twine at the base to prevent contamination. After complete protrusion of stigma, the panicle of the seed parent was pollinated with pollen collected from the desired male parent and again covered. Pollination was effective between 8.00 to 11.00 am. The resultant hybrids along, their parents were raised in Randomized Block Design with three replications during Kharif 2006. Each hybrid was accommodated in one row of 45×15 cm. Uniform and recommended cultural practices were followed. At 75% anthesis, plants were scored for pollen shedding in early hours between 7.00 to 9.00 a.m. by tapping the unbagged ears and observed for pollen cloud. In all fertile/sterile plants anthers from florets that will open on the following day were collected and squeezed with a needle in a drop of acetocarmine 2% stain and studied under microscope. Completely round and well stained pollen grains

Table 1: Dehydration of anthers gradually by using TBA dehydration series

Alcohol % (mL)	TBA (mL)	Ethanol 95 % (mL)	Distilled water (mL)	Duration (h)
50	1.0	4.0	5.0	2-3
70	2.0	5.0	3.0	12
85	3.0	5.0	1.5	12
95	4.5	4.5	1.0	12
100	7.5	2.5	0.0	12

were counted as fertile while, the shriveled, unstained or partially stained ones were considered as sterile. Counts were taken in each cross and fertility/sterility was expressed in percentage.

Anther Anatomy Study Through Sectioning

Paraffin sectioning was done as described by Johanson (1940). For this purpose, anthers were collected in F_1 sterile hybrid and F_1 fertile hybrid. The panicles were fixed in formalin (40% formaldehyde) : acetic acid : alcohol (1:1:18) solution for 24 h at 4°C. The anthers were dissected out from the spikelet and transferred to 50, 60 and 70%, ethanol each at one hour intervals. The anthers were gradually dehydrated in Tertiary Butyl Alcohol (TBA) series as shown in the Table 1.

After dehydration the anthers were given soaked in complete TBA twice at 6 h intervals. During the final change, a pinch of eosine was added to stain the specimen superficially so as to locate them inside the wax while embedding stage.

The specimen was transferred to TBA : liquid paraffin (1:1) for 2-3 h to a beaker containing three-fourth of solid wax (melting point 56-58°C). An additional quantity of TBA : liquid paraffine mixture was again added to the specimen and the beaker was kept at 60-65°C for 12 h. Two soakings were done with paraffine wax at 6 h intervals. The specimen was embedded in glycerin coated paper boat and the wax was rapidly cooled in ice water to get wax blocks which were stored at 4°C.

Sections of 10-12 μ thickness were cut and the ribbons were stretched out by placing them in warm water (40°C). These ribbons were cut into appropriate size and received on a micro slide coated with Haupt's adhesive. The slides were flooded with 4% formalin and kept in warm plate (40°C) to get maximum stretching of ribbons. Then the excess fluid on the slide was drained and the slides were shade dried for 10-12 h in a dust free chamber.

The slides were passed through the following series:

- Xylene-l, xylene-ll, xylene ethanol (1 : 1)-30 min each
- Ethanol-100, 95, 70, 50, 30 and 10% and distilled water-10 min each
- Ferrous ammonium sulphate (4%), 1% Erlich's hematoxyline (ripened for 30 days) - 30 min each
- Washed in running tap water (alkaline) for 10-15 min
- Ethanol 10, 50, 70 and 100% - 5 min each
- Xylene : ethanol (1:1), xylene-l, xylene-ll - 15 min each

Then the slides were mounted in DPX mountant and left undisturbed for 24 h for examination. Sample were examined under a light microscope and photographed.

RESULTS AND DISCUSSION

Fertility Restoration Studies

Fertility restoration pattern of F_1 hybrids developed from crosses between male sterile lines possessing different CMS sources and a set of inbreds have been used for the classification of CMS sources in pearl millet (Burton and Athwal, 1967; Akenova and Chheda, 1981; Hanna, 1989; Rai and Hash, 1990). Based on pollen fertility and seed set in the five lines and thirty inbreds were classified as non restorers when there was less than 15%, while the others were classified as restores. Such classification based on seed set has been adopted by Hanna (1989).

The fertility restoration ability of thirty diverse inbred pollinators on different cytoplasmic sources were studied and the results are given in the Table 2. Among the one hundred and fifty hybrids studied, forty one hybrids were complete sterile and twenty four hybrids were fertile (Table 3). Among thirty inbreds, seven inbreds showed sterility with ICMA 88004A (PT 5232, PT 5259, PT 5426, PT 5440, PT 5446, PT 5450, PT 5455) and six testers restored the fertility (PT 5297, PT 5423, PT5427, PT 5433, PT 5447 and PT 5575). Out of thirty testers, twelve showed sterility with 81 A (PT 5130, PT 5164, PT 5173, PT 5200, PT 5232, PT 5446, PT 5450, PT 5451, PT 5455, PT 5627, PT 5656 and PT 5773) and only three testers restored the fertility in 81A. In case of 732A, 10 inbreds restored the fertility (PT 5172, PT 5181, PT 5423, PT 5433, PT 5446, PT5502, PT 5627, PT 5636, PT 5656 and PT 5809) and only one inbred showed sterility with 732A.

Among the thirty inbreds, six inbred showed sterility with ICMA 94111A (PT 5164, PT 5259, PT 5294, PT 5440, PT 5446 and PT 5773) and four inbreds restored the fertility (PT5173, PT 5181, PT 5232 and PT 5423). Fifteen inbreds showed sterility with PT 5054A (PT 5164, PT 5181, PT 5232, PT 5259, PT 5260, PT 5426, PT 5427, PT 5433, PT 5440, PT 5451, PT 5455, PT 5480, PT 5627, PT 5636 and PT 5773) and only one tester restored the fertility with PT 5054A. The testers PT 5164, PT 5232, PT5259, PT5440, PT 5446 and PT 5455 showed sterility with three male sterile lines out of five and the testers PT 5171, PT5181, PT 5297, PT 5427 and PT 5433 restored the fertility with two male sterile lines out of five male sterile line used. PT 5423 restored the fertility with ICMA 88004A, ICMA 94111A and 732A.

Anther Anatomy Studies Through Sectioning

Anther development and microsporogenesis in male sterile and fertile lines were studied through sectioning. Developmental variation in the male sterile and male fertile lines of pearl millet were studied

Table 2: The fertility restoration ability of thirty diverse inbred pollinators from different cytoplasmic sources

Lines/Testers		PT 5130	PT 5164	PT 5172	PT 5173	PT 5181	PT 5200	PT 5232	PT 5259	PT 5260	PT 5294
ICMA 88004 A	ss	24.88	27.85	16.99	17.87	51.11	23.99	0.00	0.00	8.67	40.21
		P	P	P	P	P	P	S	S	P	P
81 A	ss	0.00	0.00	8.24	0.00	9.00	0.00	0.00	0.00	44.91	68.98
		S	S	P	S	P	S	S	S	P	P
732 A	ss	69.20	23.46	85.39	55.66	85.21	41.39	14.46	18.60	28.41	37.30
		P	P	F	P	F	P	P	P	P	P
ICMA 94111 A	ss	69.22	0.00	70.72	89.74	90.25	45.16	84.77	0.00	78.16	0.00
		P	S	P	F	F	P	F	S	P	S
PT 5054 A	ss	70.01	0.00	85.67	15.06	0.00	42.22	0.00	0.00	0.00	40.17
		P	S	F	P	S	P	S	S	S	P
Lines/Testers		PT 5297	PT 5423	PT 5426	PT 5427	PT 5433	PT 5440	PT 5446	PT 5447	PT 5450	PT 5451
ICMA 88004 A	ss	87.73	89.19	0.00	84.00	80.34	0.00	0.00	89.15	0.00	13.34
		F	F	S	F	F	S	S	F	S	
81 A	ss	91.80	25.25	30.81	84.34	21.10	76.74	0.00	10.01	0.00	0.00
		F	P	P	F	P	P	S	P	S	S
732 A	ss	36.82	85.69	72.22	48.21	88.77	46.08	85.66	48.31	65.87	23.58
			F			F		F	P	P	P
ICMA 94111 A	ss	29.96	86.76	69.07	69.74	49.85	0.00	0.00	57.75	67.49	12.06
			F	P	P	P	S	S	P	P	P
PT 5054 A	ss	73.95	21.71	0.00	0.00	0.00	0.00	40.55	39.22	68.09	0.00
		P	P	S	S	S	S	P	P	P	S
Lines/Testers		PT 5455	PT 5480	PT 5502	PT 5575	PT 5627	PT 5636	PT 5656	PT 5773	PT 5809	PT 5823
ICMA 88004 A	ss	0.00	68.93	33.49	82.79	37.74	34.41	30.23	40.63	77.25	57.27
		S	P	P	F	P	P	P	P	P	P
81 A	ss	0.00	14.94	7.30	0.00	0.00	15.05	0.00	0.00	36.63	84.64
		S	P	P	S	S	P	S	S	P	F
732 A	ss	60.95	15.20	94.84	23.02	85.60	89.68	84.31	0.00	97.83	68.67
		P	P	F	P	F	F	F	S	F	P
ICMA 94111 A	ss	63.00	78.28	43.40	65.31	52.80	50.37	57.75	0.00	56.06	50.61
		P	P	P	P	P	P	P	S	P	P
PT 5054 A	ss	0.00	0.00	33.63	48.66	0.00	0.00	16.12	0.00	43.82	38.96
		S	S	P	P	S	S	P	S	P	P

ss: Seed set percent, F: Fertile, S: Sterile, P: Partially

Table 3: Pollen sterility and seed set per cent of hybrids

Lines/Testers		PT 5130	PT 5164	PT 5172	PT 5173	PT 5181	PT 5200	PT 5232	PT 5259	PT 5260	PT 5294
ICMA 88004 A	ss	24.88	27.85	16.99	17.87	51.11	23.99	0.00	0.00	8.67	40.21
	ps	90.46	83.09	78.65	86.83	80.37	71.78	94.71	94.78	94.53	71.55
81 A	ss	0.00	0.00	8.24	0.00	9.00	0.00	0.00	0.00	44.91	68.98
	ps	97.35	98.01	89.24	95.32	87.22	97.71	97.71	92.71	30.89	30.74
732 A	ss	69.20	23.46	85.39	55.66	85.21	41.39	14.46	18.60	28.41	37.30
	ps	52.05	93.08	10.00	83.85	3.97	56.76	56.67	34.09	79.00	82.43
ICMA 94111 A	ss	69.22	0.00	70.72	89.74	90.25	45.16	84.77	0.00	78.16	0.00
	ps	34.16	81.83	89.95	83.16	8.31	17.25	11.00	81.46	86.94	81.46
PT 5054 A	ss	70.01	0.00	85.67	15.06	0.00	42.22	0.00	0.00	0.00	40.17
	ps	75.17	96.06	26.57	67.88	97.03	81.83	94.46	95.21	97.03	87.10
Lines/Testers		PT 5297	PT 5423	PT 5426	PT 5427	PT 5433	PT 5440	PT 5446	PT 5447	PT 5450	PT 5451
ICMA 88004 A	ss	87.73	89.19	0.00	84.00	80.34	0.00	0.00	89.15	0.00	3.34
	ps	0.99	31.33	95.00	24.53	8.00	97.68	62.68	15.00	98.54	95.01
81 A	ss	91.80	25.25	30.81	84.34	21.10	76.74	0.00	10.01	0.00	0.00
	ps	0.99	65.68	50.57	8.31	60.10	48.99	95.33	97.03	97.71	98.38
732 A	ss	36.82	85.69	72.22	48.21	88.77	46.08	85.66	48.31	65.87	23.58
	ps	38.55	12.33	81.97	79.23	61.72	32.28	16.52	74.50	89.95	71.78
ICMA 94111 A	ss	29.96	86.76	69.07	69.74	49.85	0.00	0.00	57.75	67.49	12.06
	ps	81.83	8.88	27.57	33.82	96.32	97.71	90.00	77.90	65.76	60.96
PT 5054 A	ss	73.95	21.71	0.00	0.00	0.00	0.00	40.55	39.22	68.09	0.00
	ps	5.46	76.52	95.01	95.69	94.75	79.36	81.83	80.29	78.24	96.36
Lines/Testers		PT 5455	PT 5480	PT 5502	PT 5575	PT 5627	PT 5636	PT 5656	PT 5773	PT 5809	PT 5823
ICMA 88004 A	ss	0.00	68.93	33.49	82.79	37.74	34.41	30.23	40.63	77.25	57.27
	ps	97.71	96.88	86.20	3.31	82.67	77.71	78.76	85.79	30.98	17.90
81 A	ss	0.00	4.94	7.30	0.00	0.00	15.05	0.00	0.00	36.63	84.64
	ps	89.32	88.90	66.90	97.03	97.04	58.49	95.00	98.00	46.87	7.67
732 A	ss	60.95	15.20	94.84	23.02	85.60	89.68	84.31	0.00	87.83	68.67
	ps	78.74	53.80	2.42	83.59	47.25	2.65	8.31	97.33	2.98	87.76
ICMA 94111 A	ss	63.00	78.28	43.40	65.31	52.80	50.37	57.75	0.00	56.06	50.61
	ps	37.94	30.01	31.40	39.52	79.95	21.49	23.55	93.33	82.15	48.88
PT 5054 A	ss	0.00	0.00	33.63	48.66	0.00	0.00	16.12	0.00	43.82	38.96
	ps	97.71	94.46	65.68	64.58	98.38	96.36	76.42	97.71	94.13	85.12

ss: Seed set, ps: Pollen sterility

in five developmental stages viz., PMC, dyad, tetrad, soon after microspore release and before dehiscence of anther (Fig. 1).

Microsporogenesis of cytoplasmic male sterile lines and male fertile lines was studied in order to identify the stage of breakdown of fertility during microsporogenesis which leads to male sterility in pearl millet. The major reason of the sterility was the abnormal behavior of the tapetal cells and bad timing of callose activity in anther. This result was conformed by the findings of Azhaguvel (1997) and Chhabra *et al.* (1997). In pollen mother cell, there is no variation in the wall layer in both male sterile line and male fertile lines. After the tetrad stage a very weak reaction and tapetum degeneration in the male fertile anthers were observed. The similar results were observed by Reddy and Reddi (1974) and Azhaguvel (1997). Microspores in sterile lines collapsed soon after their release from the tetrads. Similar observation has been recorded by Singh and Sharma (1963), Reddy and Reddi (1974) and Azhaguvel (1997).

In male fertile lines, tapetum thickness continued to increase and the central callose splitted along the sporocyte walls, following this, sporocytes migrated towards the inner tapetal wall carrying callose tips on their inner face. Callose breakdown occurred after the tetrad stage when young microspores were liberated. Microspores were oriented in a ring along the densely stained tapetum. Empty looking pollen grains slowly filled with starch granules and the tapetum degenerated. At this stage thickness of epidermis and endothecium was reduced. This result was conformed by the finding of Chhabra *et al.* (1997). Densely stained pollen grains observed in the male sterile lines in the contrast to the faintly stained irregular pollen grains in the male sterile lines. A similar result was observed by Azhaguvel (1997) and Chhabra *et al.* (1997).

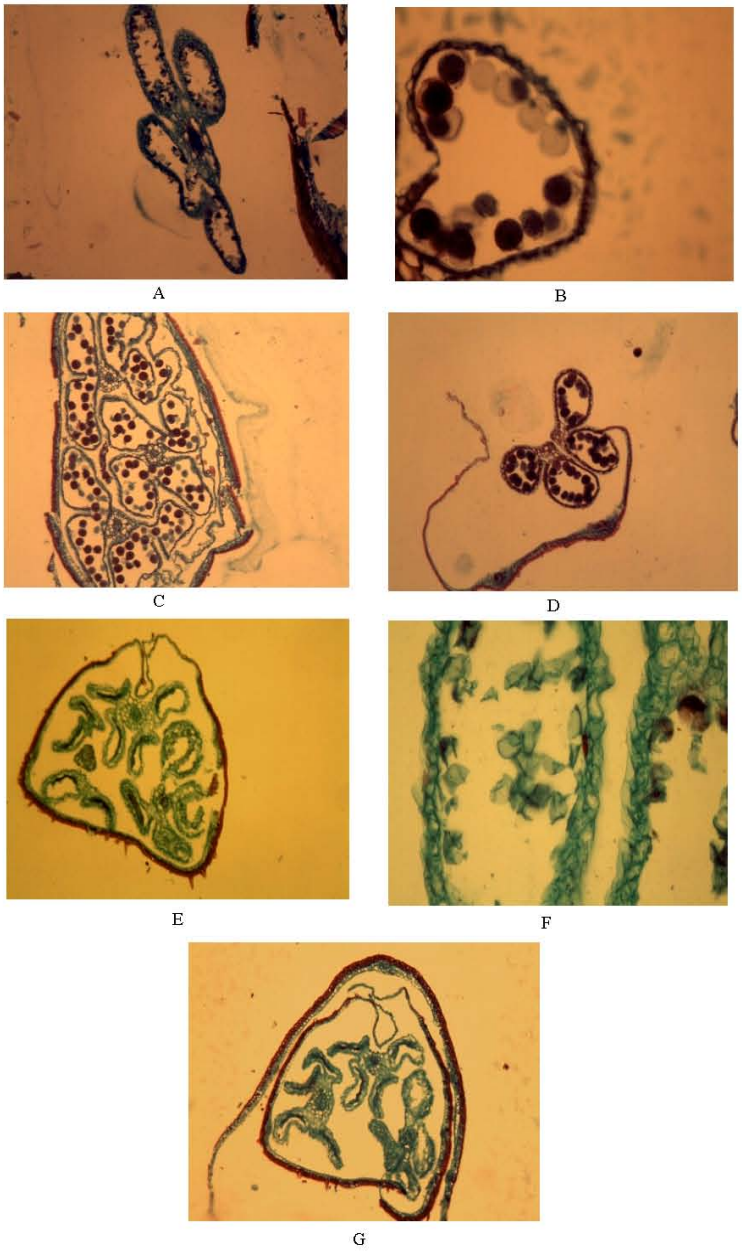

Fig. 1: Transverse sections of fertile and sterile anthers representing microsporogenesis from young sporogenous stage to pollen maturity. A: Microspores were oriented in a ring along the densely stained tapetum; B: Empty looking pollen grains slowly filled with starch grainules; C and D: Well filled pollen grains with starch granules and fully developed exine and intine were seen at the anther maturity; E: In advanced stages of anther development cytoplasmic contraction of microspores and microspores started to shrinking; F and G: Microspores degenerated due to the inability of the fibrous tapetum to supply nutrition to developing microspores

Anther Development and Microsporogenesis in Male Fertile Lines

The results showed that the intensity of protein reactions was similar in all the wall layers. Prior to initiation of meiosis, young anthers contained sporogeneous tissue surrounded by tapetum, followed by a middle layer, an endothecium and the epidermis. Callose deposition started at the locule only after this stage, became conspicuous at the onset of meiosis. Tapetum thickness continued to increase and the central callose splitted along the sporocytes walls. Following this, the sporocytes migrated towards the inner tapetal wall carrying callose tips on their inner face. Callose breakdown occurred after the tetrad stage when young microspores were liberated. Microspores were oriented in a ring along the densely stained tapetum (Fig. 1A). Empty looking pollen grains slowly filled with starch granules and the tapetum degenerated (Fig 1B). At this stage thickness of epidermis and endothecium was reduced. Well filled pollen grains with starch granules and fully developed exine and intine were seen at the anther maturity (Fig. 1C, D).

Anther Development and Microsporogenesis in Male Sterile Lines

At the dyad stage, the tapetum became tick and highly vacuolated. Young microspores remained adhered to the tapetum, but later, microspores formed a ring and migrated towards the locule interior. In advanced stages of anther development cytoplasmic contraction of microspores and tapetal hypertrophy occurred and microspores started to shrink (Fig. 1E). Ultimately, microspores degenerated completely. Tapetal cytoplasm turned fibrous and microspores degenerated due to the inability of the fibrous tapetum to supply nutrition to developing microspores (Fig. 1F, G).

Tapetum cells increased in male fertile and sterile lines but declined in other wall layers. Soon after the release of microspore stage, all layers showed a very weak reaction and tapetum degenerated in the male fertile anther. The male sterile anthers were recorded very in high colour intensity in the tapetal layer and microspore collapsed immediately after separation from tetrad. Unlike the fertile line, in sterile lines, the tapetum at this stage persisted. Before dehiscence of anther stage, the male fertile lines showed that the pollen grains were densely stained.

CONCLUSION

In the present study, out of one hundred and fifty hybrids consisting of five male sterile lines and thirty inbred testers were studied, forty three hybrids expressed sterility and twenty four hybrids complete fertile. The hybrids expressed the sterility would be utilized for maintainer line development by backcrossing with the respective inbred lines and the inbred which restored the fertility of the male sterile line will be exploit for commercial hybrid vigour.

The two inbreds namely PT 5259 and PT 5773 recorded sterility with four male sterile lines out of five male sterile lines used. The former expressed sterility with ICMA 88994A, 81A, ICMA 94111A and PT 5054A, the later expressed sterility with the male sterile lines 81A, 732A, ICMA 94111A and PT 5054A. Based on the present investigation, the A line PT 5054A expressed sterility with fifteen hybrids out of inbreds studied and it was followed by 81A with fourteen inbreds. The 732A cytoplasm expressed sterility with only one inbred (PT5773) and all others are fertile and/or partial fertile and also it was registered complete fertility with most of the inbreds than other male sterile lines.

Based on these results obtained from microsporogenesis studies, it was found that, break down of fertility in the sterile anthers of pearl millet was post meiotic, in specific soon after the release of tetrad in the developmental stage. Microspore degenerated due to inability of the fibrous tapetum to supply nutrition to developing microspores. Tapetum cells increased in male fertile and sterile lines but declined in other wall layers. The male sterile anthers were recorded very in high colour intensity in the tapetal layer and microspore collapsed immediately after separation from tetrad. Unlike the

fertile line, in sterile lines, the tapetum at this stage persisted. Before dehiscence of anther stage, the male fertile lines showed that the pollen grains were densely stained.

REFERENCES

Akenova, M.E. and H.R. Chheda, 1981. A new source of cytoplasmic genic male sterility in pearl millet. Crop Sci., 21: 984-985.

Athwal, D.S., B.S. Gill and J.L. Minocha, 1976. Diversification of the sources of male sterility in pearl millet. Crop Improv., 3: 96-100.

Azhaguvel, P., 1997. Morphological and molecular characterization of cytosteriles in pearl millet (*Pennisetum glaucum* (L.) R. Br.). M.Sc. (Ag.) Thesis. Tamil Nadu Agricultural University, Coimbatore.

Burton, G.W., 1958. Cytoplasmic male sterility in pearl millet (*Pennisetum glaucum* (L.) R.Br.). Agron. J., 50: 230-230.

Burton, G.W., 1965. Pearl millet Tift 23A released. Crops Soils, 17: 19-19.

Burton, G.W. and D.S. Athwal, 1967. Two additional sources of cytoplasmic male sterility in pearl millet and their relationship to Tift 23A1. Crop Sci., 7: 209-211.

Chhabra, A.K., 1995. Molecular characterization of cytoplasmic nuclear male sterility (CMS) sources and tall/dwarf near-isogenic lines in pearl millet. Ph.D. Thesis. Submitted to CCS Haryana Agricultural University, Hisar 125 004, Haryana, India.

Chhabra, A.K., I.S. Khairwal, K.N. Rai, C.T. Hash and A.K. Murthy, 1997. Influence of cytoplasmic-nuclear male sterility systems on microsporogenesis in Pearl millet (*Pennisetum glaucum* L.) R. Br. Euphytica, 98: 1-10.

Hanna, W.W., 1989. Characteristics and stability of new cytoplasmic-nuclear male sterile source in pearl millet. Crop Sci., 29: 1457-1459.

Johanson, 1940. Plant microtechniques. M.C. Graw-Hill, New Delhi.

Kajjari, N.B. and Patil, 1956. A male sterile bajra. Ind. J. Genet., 16: 146-146.

Kumar, K.A. and D.J. Andrews, 1984. Cytoplasmic male sterility in pearl millet [*Pennisetum americanum* (L.) Leeke]: A review. Adv. Applied Biol., 10: 113-143.

Lee, S.L.J., V.E. Gracen and E.D. Earle, 1979. The cytology of pollen abortion in C-cytoplasmic male-sterile corn anthers. Amer J. Bot., 66: 656-667.

Pokhriyal, S.C., R.R. Patil, R. Dass and B. Singh, 1974. Combining ability of new male sterile lines in pearl millet. Ind. J. Genet., 34: 208-215.

Rai, K.N. and N.B. Singh, 1987. Breeding pearl millet male sterile lines. Paper presented at international Pearl millet workshop, ICRISAT, Patencharu, India.

Rai, K.N. and C.T. Hash, 1990. Fertility restoration in male sterile x maintainer hybrids of pearl millet. Crop Sci., 30: 889-892.

Reddy, B.B. and M.V. Reddi, 1974. Cytohistological studies on certain male sterile lines of pearl millet (*Pennisetum typhoides. S. and H*). Cytologia, 39: 585-589.

Shinde, G.C. and S.S. Mehetre, 2003. The cytological studies of temperature-sensitive genic male sterile lines of pearl millet (*Pennisetum glaucum* (L). R.Br.). Cytologia, 56: 399-403.

Singh, S.P. and Y.P. Sharma, 1963. Preliminary observations on the breeding of pennisetum at B.R. College, Bichpuri, Agra, India. Newslett., 6: 26-28.