



Research Article

Identification and Screening of Restorers and Maintainers for different CMS Lines of Pearl Millet (*Pennisetum glaucum* (L.) R.Br.)

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Abstract :

For the evolution of new pearl millet hybrids with diverse cytoplasmic background suitable for specific areas and condition, there is a need for diversified CMS sources among locally adaptable lines and identification of locally adaptable restorers. The present study was undertaken to screen the male sterile and restorer lines among the crosses of five male sterile lines and thirty inbreds of pearl millet. Five isogenic alloplasmic male sterile lines (ICMA 94111A, 81A, ICMA 88004A, PT 5054A and 732A) were crossed with thirty inbreds in line x tester design, so as to obtain 150 F₁ hybrids. F₁'s were categorized as restorers (>80 % pollen fertility), partial restorers (20 to 79 % pollen fertility), partial maintainers (10-19 % pollen fertility) and maintainers (<10 % pollen fertility). Among the 150 hybrids studied, 43 complete sterile and 24 complete fertile hybrids were identified. 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 latter expressed sterility with the male sterile lines 81A, 732A, ICMA 94111A and PT 5054A. In case of 732A, maximum restorers were observed and 10 inbreds showed complete fertility and only one inbred showed complete sterility. The identified restorers and maintainers could be utilized for development of new restorer lines/hybrids and CMS lines, respectively in future.

Key words:

Pearl millet, male sterile lines, restorers, maintainers, pollen sterility, spikelet fertility.

Introduction

Pearl Millet (*Pennisetum glaucum* (L.) R.Br.) belongs to the family Poaceae and possess $2n = 2x = 14$ chromosomes. It is commonly grown in the arid and semi-arid regions of Africa and India as a staple food for millions of people. It is particularly adapted to nutrient-poor soil and low rainfall conditions, yet it is capable of rapid and vigorous growth under favourable conditions (Maiti and Bidinger, 1981). Pearl millet is a potential alternative grain crop for areas of the Great Plains with sandy soil, low rainfall, and a short growing season since dwarf hybrids with good yield potential have been developed. The occurrence of male sterility in pearl millet was first reported by Kajjari and Patil (1956). Burton (1951) observed a wide range of seed set in the self fertile inbred lines of pearl millet. Krishnaswamy (1962) described several kinds of sterility in pearl millet, namely, complete sterility, partial sterility, female sterility, tip sterility, gappiness in panicles and headless plants. Discovery of A1 cytoplasmic genic male-sterility (CGMS) system and breeding of a

commercially viable male-sterile line (A-line) Tift 23A (Burton, 1958 and 1965) is a landmark in pearl millet hybrid cultivar development. In 1961 and 1962, two new sources of cytoplasmic male sterility in pearl millet were discovered at Ludhiana, India (Burton and Athwal, 1969). A cytoplasmic male sterile line developed from the first of these, observed in a selfed progeny of an African variety was designated as L 66A. Another cytoplasmic male sterile line developed from the second source which appeared in the selfed progeny of an outcross that had a mixture of yellow and slate grey seeds was designated as L 67A. A 'CMS' source was identified in Large Seeded Gene Pool designated as LSGP-66 at ICRIAS (Rai, 1995). A set of 22 new male sterile lines of diverse base constituting six isocyttoplasmic and eleven alloplasmic lines were synthesized (Hepziba, 1996). ICMA 5, a new source of pearl millet cytoplasm A₅ for male sterility was identified (Rai *et al.*, 1998). For evolution of new hybrids suitable for specific area and locality, there is need for diversified CMS sources from local adaptable lines and identification of local adaptable restorers. 732 A belongs to Bellary cytoplasm, isolated at MBS, Coimbatore is a different source which had been derived from PT 819, an inbred line originated from Andhra Pradesh, India. In this study,

efforts were made to identify the maintainers and restorers for the development of potential hybrids from the crosses between thirty inbreds and five diverse cytoplasmic sources.

Materials and methods

Five male sterile lines and thirty inbreds of pearl millet obtained from pearl millet unit, Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore were chosen for the study. ICMA 94111A and 81A were having A₁ cytoplasm, ICMA 88004A and PT 5054A has A₄ cytoplasm and 732A belongs to Bellary cytoplasm. The five male sterile lines of diverse origin and thirty inbreds were raised during *Rabi*, 2005 in adjacent plots to facilitate easy crossings. Five isogenic alloplasmic male sterile lines were crossed with thirty inbreds (testers) in line x tester design, so as to obtain 150 F₁ hybrids. Artificial selfing and crossing operations were carried out as suggested by Rao *et al.* (1949). 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 dusted with pollen collected from the male parent and again covered. Pollination was effective between 8.00 am to 11.00 am.

The resultant hybrids along with their parents were raised in Randomized Block Design with three replications during *Kharif*, 2006. Each hybrid was accommodated in one row with row spacing of 45 cm and plant to plant spacing of 15 cm. Uniform and recommended cultural practices were followed to raise agronomically good managed crop. At 75 per cent anthesis, 150 F₁ hybrids 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 2 per cent acetocarmine stain and studied under microscope. Completely round and well stained pollen grains were counted as fertile while, the shrivelled, unstained or partially stained ones were considered as sterile. Counts were taken in each cross and fertility/sterility was expressed in percentage. The pollen fertility was calculated as follows:

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains examined}} \times 100$$

Genotypes were categorized as restorers (>80% pollen fertility), partial restorers (20 to 79% pollen fertility), partial maintainers (10-19% pollen fertility) and maintainers (<10 % pollen fertility).

Five randomly selected emerging ear head from each F₁ hybrid were bagged to avoid out crossing before anthesis. The seed set percentage of hybrids were calculated on the basis of five randomly selected earheads from each F₁ at the time of maturity. Number of seeds/cm² were counted randomly in each ear heads in five locations in selfed and open pollinated earheads and expressed as percentage. Percentage of seed set directly proportional to the fertility/sterility of the hybrids. The percentage of seed set was calculated as given below:

$$\text{Seed set (\%)} = \frac{\text{Number of grains/cm}^2 \text{ in a selfed ear head}}{\text{Number of grains/cm}^2 \text{ in open pollinated ear head}} \times 100$$

The male parents of F₁ hybrids were classified as restorers (>80 % seed set), partial restorers (20-79 % seed set), partial maintainers (10-19 % seed set) and maintainers (<10 % seed set) on the basis of the seed set in selfed and open pollinated ear head of the hybrids.

Results and discussion

The fertility restoration ability of thirty diverse inbred pollinators on different cytoplasmic sources was studied. Among the one hundred and fifty hybrids studied, forty three hybrids recorded complete sterility and twenty four hybrids recorded complete fertility (Table 1 and Table 2).

Among thirty inbreds, seven inbreds showed sterility with ICMA 88004A (PT 5232, PT 5259, PT 5426, PT 5440, PT 5446 (Fig 2), PT 5450, PT 5455) and six inbreds restored the fertility (PT 5297, PT 5423, PT5427, PT 5433, PT 5447 and PT 5575). Out of thirty testers, fourteen showed sterility with 81A (PT 5130, PT 5164, PT 5173, PT 5200, PT 5232, PT 5259, PT 5446, PT 5450, PT 5451, PT 5455, PT 5575, PT 5627, PT 5656 and PT 5773) and only three inbreds (PT 5823) 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 (PT 5773) 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 inbred (PT 5172) 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 (Table 2).

Among five male sterile lines used, PT 5054A expressed sterility with most of the inbreds used in the study (50 %) followed by 81A (46.67 %). The male sterile line 732A showed sterility with only one inbred (3.33 %) restored the fertility with ten inbreds (33.33 %) followed by ICMA 88004A which restored fertility in six hybrid (20.00 %) (Figure 1).

Conclusion

In the present study, out of one hundred and fifty hybrids of five male sterile lines with thirty inbred testers, forty three expressed complete sterility and twenty four recorded complete fertility. 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 30 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 registered complete fertility with most of the inbreds than other male sterile lines.

Among five male sterile lines used, the maximum proportion of maintainers observed in PT 5054A (50 %) followed by 81A (46.67 %) which could be utilized for the development of new male sterile lines. The male sterile line 732A showed sterility with only one inbred (3.33 %) and maximum proportion of restorer were observed (33.33 %) followed by ICMA 88004A which restored fertility in six hybrids (20.00 %). Most of the testers showed partial restorer or maintainers based on the result from pollen sterility and seed set.

The hybrids which showed sterility in F₁ generation will be backcrossed with respective pollen parent to develop alloplasmic and isogenic male sterile line for the locally adapted conditions for the development of new hybrids in future. The tester which is selected as maintainer is based on seed set (0 % seed set) under selfed condition to ensure complete sterility and also pollen fertility < 10 % (Table 2). The identified restorers and maintainers could be utilized for development of new restorer lines/hybrids and CMS lines, respectively in future.

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Table 1. List of inbreds restored fertility with different male sterile lines in F₁ generation and their seed set and pollen fertility per cent

S.No	Inbreds	Male sterile line	Seed set (%)	Pollen fertility (%)
1	PT 5297		87.73	99.01
2	PT 5423		89.19	80.67
3	PT 5427	ICMA 88004 A	84.00	81.47
4	PT 5433		80.34	92.00
5	PT 5447		89.15	85.00
6	PT 5575		82.79	96.69
7	PT 5297		91.80	99.01
8	PT 5427	81 A	84.34	91.69
9	PT 5823		84.64	92.33
10	PT 5172		85.39	90.00
11	PT 5181		85.21	96.03
12	PT 5423		85.69	87.67
13	PT 5433		88.77	80.28
14	PT 5446	732 A	85.66	83.48
15	PT 5502		94.84	97.58
16	PT 5627		85.60	52.75
17	PT 5636		89.68	97.35
18	PT 5656		84.31	91.69
19	PT 5809		97.83	97.02
20	PT 5173		89.74	82.00
21	PT 5181		90.25	91.69
22	PT 5232	ICMA 94111 A	84.77	89.00
23	PT 5423		86.76	91.12
24	PT 5172	PT 5054 A	85.67	81.43

Table 2. List of inbreds expressed sterility with the male sterile lines in F₁ generation

S.No	Inbreds	Expressed sterility in F ₁ generation with	Seed set (%)	Pollen fertility (%)
1	PT 5232		0	5.29
2	PT 5259		0	5.22
3	PT 5426		0	5.00
4	PT 5440	ICMA 88004 A	0	2.32
5	PT 5446		0	7.00
6	PT 5450		0	1.46
7	PT 5455		0	2.29
8	PT 5130		0	2.65
9	PT 5164		0	1.99
10	PT 5173		0	4.68
11	PT 5200		0	2.29
12	PT 5232		0	2.29
13	PT 5259		0	7.29
14	PT 5446	81A	0	4.67
15	PT 5450		0	2.29
16	PT 5451		0	1.62
17	PT 5455		0	9.68
18	PT 5575		0	2.97
19	PT 5627		0	2.96
20	PT 5656		0	5.00
21	PT 5773		0	2.00
22	PT 5773	732A	0	2.67
23	PT 5164		0	8.17
24	PT 5259		0	8.54
25	PT 5294	ICMA 94111A	0	8.54
26	PT 5440		0	2.29
27	PT 5446		0	10.00
28	PT 5773		0	6.67
29	PT 5164		0	3.94
30	PT 5181		0	2.97
31	PT 5232		0	5.54
32	PT 5259		0	4.79
33	PT 5260		0	2.97
34	PT 5426		0	4.99
35	PT 5427	PT 5054A	0	4.31
36	PT 5433		0	5.25
37	PT 5440		0	7.64
38	PT 5451		0	3.64
39	PT 5455		0	2.29
40	PT 5480		0	5.54
41	PT 5627		0	1.62
42	PT 5636		0	3.64
43	PT 5773		0	2.29

Table 3. Percentage and number of inbreds showed fertility and sterility with different cytoplasmic sources

Name of male sterile lines	No. of inbreds showed sterility	%	No. of Inbreds restored the fertility	%
ICMA 88004A	7	23.33	6	20.00
81 A	14	46.67	3	10.00
732A	1	3.33	10	33.33
ICMA 94111A	6	20.00	4	13.33
PT 5054A	15	50.00	1	3.33
Total	43		24	

Fig 1. Percentage of inbreds showed fertility and sterility with different cytoplasmic sources

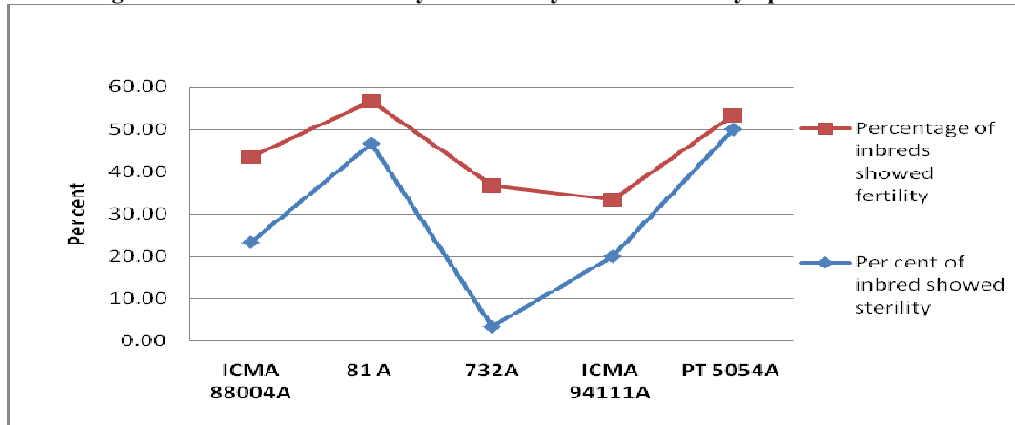


Figure 2. F₁ sterile hybrid from cross between ICMA 8804A and PT 5446

