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# Fertility restoration studies in different cytoplasms of pearl millet [*Pennisetum glaucum* (L.) R. BR.]

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**ABSTRACT**

Pearl millet [*Pennisetum glaucum* (L.) R. Br.], hybrids grown widely in India are all based on $A_1$ CMS source. Though alternative sources of CMS are available and found to be highly stable, their utility is restricted due to non-availability of suitable restorers. The present investigation carried out using three different cytoplasms ($A_1$, $A_4$ and $A_5$) as female and $A_1$ cytoplasm as male showed differential restoration of fertility. The fertility restoration was moderate to partial for both $A_4$ (16% to 52%) and $A_5$ (20%) cytoplasms. The male parent PPMI 69 showed complete fertility restoration with ICMA 99555 belonging to $A_4$ cytoplasm. Identification of restorers for diverse cytoplasmic male sterile sources opens up new avenues in pearl millet hybrid breeding.

**Key words:** *Pennisetum*, pearl millet, fertility restoration, restorer, maintainer.

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] (2n=14), is a drought tolerant warm season cereal crop grown as staple food grain. It is the major source of feed and fodder on about 25 million hectares in Asia and Africa. It is grown primarily under very hot and dry conditions on infertile soils of low water-holding capacity where other crops fail completely. In India, pearl millet is the fourth most important cereal crop, widely grown by the resource poor farmers in the arid and semiarid regions, particularly in the states of Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana, which account for nearly 95 percent of the acreage under pearl millet.

Availability of exploitable male sterility system in pearl millet has made it possible to commercially exploit heterosis and hybrid vigour to increase productivity. The discovery of Tift $23A_1$, cytoplasmic-nuclear male sterility (CMS) and its successful utilization in breeding male-sterile lines of commercial pearl millet hybrids is a landmark in pearl millet improvement (Athwal,1965; Burton,1965; Burton and Powell, 1968). When hybrids based on Tift $23A_1$ cytoplasmic background succumbed to downy mildew infection in 1970s, research efforts were intensified at IARI, New Delhi, several other pearl millet breeding centers and ICRISAT (International Crops Research Institute for Semi-Arid Tropics), Patancheru to diversify the genetic base of male-sterile lines in the $A_1$ cytoplasmic system and to explore other CMS systems in pearl millet. Burton and Athwal (1967) used $A_1$, $A_2$ and $A_3$ CMS sources for developing hybrids while two other sources ($A_v$ and $A_4$) derived from different accessions of *P. glaucum* SSP. violaceum = monodii, a wild relative of cultivated pearl millet were identified by Marchais and Pernes (1985) and a totally new and different $A_5$ cytoplasmic male sterility system was developed by Rai (1995).

The important features that contribute to the commercial viability of any male sterility system are- complete male sterility of A-lines, high levels of male-fertility restoration of their hybrids, stability of these two characteristics across environments, and high frequency of maintainers in a diverse range of breeding materials (Rai *et al.* 2001). Though, different cytoplasmic male sterility systems are available in pearl millet, systematic studies on identification of restorers on these diverse cytoplasms are meager. Amongst those that were evaluated extensively (i.e., $A_2$ and $A_3$ systems),

**Table 1. Male and Female parents from different cytoplasmic sources**

| S. No | Cytoplasm | Female parent (Male sterile lines) | Male parent |
|---|---|---|---|
| 1. | $A_1$ | MS 5141A, MS 843 A, ICMA 88004, ICMA 89111, ICMA 91444, ICMA 92111, ICMA 93333, ICMA 95111 | D-23, PPMI 69, PPMI 493, PPMI 741, PPMI 761 |
| 2. | $A_4$ | ICMA 98333, ICMA 99222, ICMA 99555, ICMA 00777, ICMA 04888 | |
| 3. | $A_5$ | ICMA 07999 | |

none performed any better than the $A_1$ CMS system with respect to one or more of the mentioned features. Though the $A_4$ and $A_5$ sources were found to be highly stable, their utility is restricted due to non-availability of suitable restorers (Rai *et al.* 2006). Hence, work in this direction is essential to make use of diverse sterile sources in the development of new Pearl millet hybrids with suitable restorers for further commercial exploitation.

The present investigation was taken up to identify restorers and maintainers for different cytoplasmic backgrounds in pearl millet.

**Table 2. Percent seed set and pattern of fertility restoration**

| % seed set | Type of restoration |
|---|---|
| > 90 | Strong restoration(SR) |
| 80-90 | High(HR) |
| 60-80 | Moderate(MR) |
| 10-60 | Partial(PR) |
| < 10 | Low(LR) |
| 0 | Maintainer(M) |

## Materials and Methods

The material comprised of fourteen cytoplasmic male sterile lines belonging to different cytoplasmic sources like $A_1$ (8), $A_4$ (5) and $A_5$ (1) as female parents and five restorers coming from $A_1$ background as male parents (Table 1). All these parents were crossed in a line x tester mating design in the rainy season of 2009. The hybrids along with their parents were grown in a randomized block design with three replications during rainy season of 2010 and were evaluated for fertility restoring ability to identify restorers and maintainers on $A_1$, $A_4$ and $A_5$ cytoplasm.

The hybrids were grown in three rows of 4 m. length with intra row spacing of 15 cm and inter row spacing of 75 cm in the farm of Division of Genetics in Indian Agricultural Research Institute, New Delhi following the recommended package of practices. The spikes of five plants in each treatment were selfed with parchment paper bag before flowering and the same plants were used for recording seed set per cent. The percentage of seed set was worked out by following the procedure given by Kishan and Borikar (1989) in Table 2.

## Results and Discussion

The data from the selfing studies show the percent seed set in the specific cross combinations involving different cytoplasms with the $A_1$ male gametes as all the testers belonged to $A_1$ cytoplasm. The results are presented in the Table 3. The data shows that the seed set values ranged from 0 to >90%. The seed set depends on the aspects like compatibility of the cytoplasm of the female gamete i.e. egg with the nuclear background of the male gamete. As the cytoplasmic background is different in different male sterile lines the variation observed is valid. The reason for extreme variation in the different lines of $A_1$cytoplasms could be due to differential interaction of the nuclear background of the male gamete with the cytoplasm of the female gamete in the particular line.

The extent of restoration among different cytoplasms is presented in Table 4. Results of fertility restoration reaction of the various lines revealed that - of the 70 hybrids, strong restoration was observed only in two crosses of $A_1$ cytoplasm (MS 843 A and ICMA 93333 with PPMI 761), high restoration was recorded in

**Table 3. Percent seed set observed in different cross combinations involving $A_1$, $A_4$ and $A_5$ cytoplasms of Pearl millet**

| PARENTS | D 23 | PPMI 69 | PPMI 493 | PPMI 741 | PPMI 761 |
|---|---|---|---|---|---|
| 5141A ($A_1$) | 47.5 (PR) | 30.5 (PR) | 35.1 (PR) | 47 (PR) | 19.5 (PR) |
| 843A ($A_1$) | 40 (PR) | 16 (PR) | 37 (PR) | 25 (PR) | 91 (SR) |
| ICMA88004 ($A_1$) | 65 (MR) | 45 (PR) | 67 (MR) | 52 (PR) | 62 (MR) |
| ICMA89111 ($A_1$) | 40 (PR) | 41 (PR) | 27 (PR) | 23.5 (PR) | 9.5 (LR) |
| ICMA91444 ($A_1$) | 70 (MR) | 0 (M) | 27.1 (PR) | 0 (M) | 0 (M) |
| ICMA92111 ($A_1$) | 3.7 (LR) | 25.8 (PR) | 27 (PR) | 2.8 (LR) | 0 (M) |
| ICMA93333 ($A_1$) | 87 (HR) | 33 (LR) | 20 (PR) | 68 (MR) | 93 (SR) |
| ICMA95111($A_1$) | 32 (PR) | 36 (PR) | 37 (PR) | 29 (PR) | 29.5 (PR) |
| ICMA98333 ($A_4$) | 16 (PR) | 71.1 (MR) | 10.2 (LR) | 43.2 (PR) | 22.2 (PR) |
| ICMA99222 ($A_4$) | 66.0 (MR) | 30.2 (PR) | 6 (LR) | 69.2 (MR) | 4.9 (LR) |
| ICMA99555 ($A_4$) | 30.3 (PR) | 82 (HR) | 22 (PR) | 64 (MR) | 22 (PR) |
| ICMA00777 ($A_4$) | 50.5 (PR) | 2.9 (LR) | 18.5 (PR) | 0 (M) | 0 (M) |
| ICMA04888 ($A_4$) | 2.1 (LR) | 39.2 (PR) | 37.1 (PR) | 51 (PR) | 56.5 (PR) |
| ICMA07999 ($A_5$) | 0 (M) | 39.2 (PR) | 74 (MR) | 0 (M) | 0 (M) |

SR = strong restorer; HR= high restorer; MR= Moderate restorer; PR = Partial restorer, LR- Low restorer and M = Maintainer

**Table 4. Variation in extent and type of restoration in different cytoplasms of Pearl millet**

| Name of the cytoplasm | Strong restoration | High restoration | Moderate restoration | Partial restoration | Low restoration | Maintainer |
|---|---|---|---|---|---|---|
| $A_1$ cytoplasm | 2 (5%) | 1 (2.5%) | 5 (12.5%) | 24 (60%) | 4 (10%) | 4 (10%) |
| $A_4$ cytoplasm | - | 1 (4%) | 4 (16%) | 13 (52%) | 5 (20%) | 2 (8%) |
| $A_5$ cytoplasm | - | - | 1 (20%) | 1 (20%) | - | 3 (60%) |
| | 2 (2.85%) | 2 (2.85%) | 10 (14.29%) | 38 (54.26%) | 9 (12.86%) | 9 (12.86%) |

ICMA 93333 of $A_1$ cytoplasm with D 23 and ICMA 99555 of $A_4$ cytoplasm with PPMI 69.

Moderate restoration was observed in 5 crosses of $A_1$ cytoplasm, 4 crosses of $A_4$ cytoplasm and one cross of $A_5$ cytoplasm. Partial restoration was recorded in 24 combinations with $A_1$ cytoplasm, 13 combinations with $A_4$ cytoplasm and one combination involving $A_5$ cytoplasm. Low restoration was observed in 4 crosses involving $A_1$ cytoplasm and 5 crosses of $A_4$ cytoplasm. No restoration was observed in 4 crosses of $A_1$ cytoplasm, 2 crosses of $A_4$ cytoplasm and three crosses of $A_5$ cytoplasm. The comparative results indicate that moderate to partial restoration is possible in all the cytoplasms. Among the $A_4$ and $A_5$ restoration is more possible in $A_4$ than in $A_5$ as the extent of no restoration is only 8% in $A_4$ as compared to that of 60% in $A_5$. These results reveal that frequency of restoration on $A_4$ cytoplasm is quite high compared to $A_1$ and $A_5$ indicating the possibility of developing hybrids on $A_4$ source (Andrews and Rajewski, 1994; Rai, 1995; Rai *et al.* 1998). This may be ascribed to the following reasons like different backgrounds of the female parents into which the $A_1$ cytoplasm has been introgressed and variable interaction of the nuclear and cytoplasmic organelle genomes i.e. chloroplast and mitochondrial genomes with the nuclear genome.

There were significant differences in the extent of seed set and fertility restoration among different cytoplasms with $A_1$ testers. This can be explained as the variable interaction of $A_1$ cytoplasm of the male gamete with the eggs of different cytoplasms namely $A_1$, $A_4$ and $A_5$ i.e. cytoplasmic nuclear interactions. Even among the lines of $A_1$ cytoplasm with the $A_1$ testers, large variability is observed in the extent of fertility restoration. The inheritance studies for $A_1$

cytoplasm restoration conducted by Yadav *et al.* (2010) suggested more likelihood of a single-gene control of male sterility and fertility restoration. However, a 3-gene model of male sterility/fertility restoration where dominant alleles at any two of the three duplicate complimentary loci will lead to male fertility could not be ruled out, nor could be ruled out a 2-gene control with duplicate interaction. There was indication of variability even within a highly inbred R-line for fertility restoration gene(s). In another report by Lakshmana *et al.* (2010) on fertility restoration suggested that the frequency of restoration on $A_4$ cytoplasm was quite high compared to $A_1$ and $A_5$ indicating the possibility of developing hybrids on $A_4$ source.

### Classification of Restoration

The seed set percentage recorded on a hybrid represent the restoring ability of a pollen parent. This seed set percentage can vary from 0 to 95 per cent, thus representing wide range of variation in the restoration ability of pollen parent. In the fertility restoration studies conducted in sorghum, those genotypes showing just above 60 per cent seed set were broadly grouped as restorers and these restorers were classified into different categories based on their restoration ability (Kishan and Borikar, 1989; Biradar, 1995). In the present investigation, five inbreds were evaluated for their restoration ability on fourteen diverse sources of male sterility. The genotypes which could restore fertility up to 80% were categorized as potential restorers, those with restoration of fertility from 10 to 80% were categorized as partial restorers and those below 10% restoration were categorized as maintainers. The different categories of restorers for different male sterile cytoplasmic lines is presented in the following Table 5. It is also observed that the male parents are acting as restorers in one cytoplasmic background and as partial restorer in other cytoplasmic background and as maintainer in another. This clearly shows the impact of nuclear and cytoplasmic interactions which affect the fertility status.

In the present study, it is observed that a single male parent is acting as a complete restorer, partial restorer and a maintainer for different cytoplasms as well as same cytoplasm ex - D 23 and PPMI 761 as shown in Table 5. This shows the possibility of utilization of that particular combination and development of new male sterile lines in the future in different backgrounds. This would certainly reduce the risk associated with the use of single cytoplasmic source in the development of hybrids. On the basis of commonness of restoration on different cytoplasm, it is possible to infer the probable cytoplasmic distance existing between the cytoplasms.

Even among the same cytoplasmic source, the restoration of fertility was not same as observed in the $A_4$ cytoplasm where PPMI 69 restored fertility in ICMA 95333 while it maintained sterility in ICMA 99111. From the study it was also clear that the frequency of maintainers was high for $A_4$ cytoplasm compared to $A_5$ cytoplasm.

Similar studies conducted by Rai *et al.* (2006) at ICRISAT in 2002 revealed that $A_4$ and $A_5$ cytoplasms showed stable male sterility. The frequency of maintainers was highest in $A_5$ compared to $A_4$ and $A_1$ and hence provided the greatest opportunities for genetic diversification of A- lines followed by $A_4$ and then $A_1$. In a study conducted by Tara Satyavathi *et al.* (2009), desirable effects of earliness and maturity were obtained in hybrids developed using $A_4$ cytoplasm while desirable heterosis could be obtained for plant height, spike girth, number of nodes, chlorophyll content, relative carotenoids and 1000 grain weight from $A_5$ cytoplasm. Hence the identification of restorers and maintainers for $A_4$ and $A_5$ cytoplasms to develop hybrids opens up new avenues in diversification of cytoplasm for development of hybrids in pearl millet.

### Conclusion

The present study brings out the restoration capabilities of the five male parents tested against fourteen diverse cytoplasmic male sterile lines of pearl millet. Identification of restorers/ maintainers helps to diversify the genetic background of these lines and it is possible to develop male sterile lines in different genetic backgrounds. It is desirable to have male sterile lines with varying maturity and height levels as

**Table 5. Classification of the restoration ability of pearl millet inbreds in different cytoplasmic backgrounds**

| Male parent | Complete restorer for | Partial restorer for | Maintainer for |
|---|---|---|---|
| D 23 | ICMA 93333($A_1$) | MS 5141A($A_1$) | |
| | | MS 843A($A_1$) | |
| | | ICMA 88004($A_1$) | ICMA 92111($A_1$) |
| | | ICMA 89111($A_1$) | ICMA 04888($A_4$) |
| | | ICMA 91444($A_1$) | ICMA 07999($A^5$) |
| | | ICMA 95111 ($A_1$) | |
| | | ICMA 98333($A_4$) | |
| | | ICMA 99222($A_4$) | |
| | | ICMA 99555 ($A_4$) | |
| | | ICMA 00777($A_4$) | |
| PPMI 69 | ICMA 99555($A_4$) | MS 5141A($A_1$) | |
| | | MS 843A($A_1$) | |
| | | ICMA 88004($A_1$) | |
| | | ICMA 89111($A_1$) | |
| | | ICMA 92111($A_1$) | |
| | | ICMA 95111($A_1$) | ICMA 91444($A_1$) |
| | | ICMA 98333($A_4$) | ICMA 93333($A_1$) |
| | | ICMA 99222($A_4$) | ICMA 00777(A4) |
| ) | | ICMA 04888($A_4$) | |
| | | ICMA 07999($A_5$) | |
| PPMI 493 | None | MS 5141A($A_1$) | |
| | | MS 843A($A_1$) | |
| | | ICMA 88004($A_1$) | |
| | | ICMA 89111($A_1$) | |
| | | ICMA 91444 ($A_1$) | |
| | | ICMA 92111($A_1$) | |
| | | ICMA 93333($A_1$) | |
| | | ICMA 95111($A_1$) | |
| | | ICMA 99555($A_4$) | ICMA 98333($A_4$) |
| | | ICMA 00777($A_4$) | ICMA 99222($A_4$) |
| | | ICMA 04888($A_4$) | |
| | | ICMA 07999($A_5$) | |
| PPMI 741 | None | MS 5141A($A_1$) | |
| | | MS 843A($A_1$) | ICMA 91444($A_1$) |
| | | ICMA 88004($A_1$) | ICMA 92111($A_1$) |
| | | ICMA 89111($A_1$) | ICMA 00777($A_4$) |
| | | ICMA 93333($A_1$) | ICMA 07999($A_5$) |
| | | ICMA 99222($A_4$) | |
| | | ICMA 99555 ($A_4$) | |
| | | ICMA 04888($A_4$) | |
| PPMI 761 | MS 843A ($A_1$) | MS 5141A($A_1$) | ICMA 89111($A_1$) |
| | ICMA 93333($A_1$) | ICMA 88004($A_1$) | ICMA 91444($A_1$) |
| | | ICMA 95111($A_1$) | ICMA 92111($A_1$) |
| | | ICMA 98333($A_4$) | ICMA 99222($A_4$) |
| | | ICMA 99555($A_4$) | ICMA 00777($A_4$) |
| | | ICMA 04888($A_4$) | ICMA 07999(A5) |

it gives scope for selecting restorers with good combining ability and adaptation to diverse agro climatic conditions. It was observed that the male parent D 23 acts as a complete restorer for ICMA 93333 which belongs to $A_1$ cytoplasm, while it acts as a maintainer for ICMA 92111($A_1$), ICMA 04888($A_4$) and ICMA 07999($A_5$).The other male parent PPMI 69 acts as a complete restorer for ICMA 99555 belonging to $A_4$ cytoplasm and as maintainer for ICMA 91444 and ICMA 93333 of $A_1$ cytoplasm along with ICMA 00777 of $A_4$ cytoplasm. The two male parents PPMI 493 and PPMI 741 could not provide complete restoration to any of the fourteen CMS lines. The male parent PPMI 761 could completely restore fertility in two male sterile lines 843A and ICMA 93333 which belong to $A_1$ cytoplasm. The present study firstly brings out the possibility of developing successful hybrid combinations in diverse cytoplasmic background other than the routinely used $A_1$ cytoplasm. Secondly it also brings out the possibility of use of these male parents in the development of male sterile lines in pearl millet.

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