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Fertility restoration studies on diverse cytoplasm sources in Pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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Abstract

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the most widely grown drought tolerant coarse grain cereal cultivated in semi-arid tropical environments of south Asia and sub-Saharan Africa. It is also consumed as feed and fodder for livestock. 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 cytoplasm (A₁, A₄ and A₅) as female parents. In the current study, out of twenty testers, 8 testers viz., ICMR 15455-2, ICMR 15455-3, ICMR 15024, ICMR 15037, ICMR 15038, ICMR 19631, ICMR 20405 and ICMR 20444 were considered as the strong restorers of the fertility on A₁ and 9 testers viz., BRBL 1136R, ICMR 15455-2, ICMR 15455-3, ICMR 15024, ICMR 15038, ICMR 15082, ICMR 19631, ICMR 16409 and ICMR 16410 on A₄ based male sterile lines. No testers showed strong fertility restoration on A₅ cytoplasm. Three testers ICMR 15024, ICMR 19631 and ICMR 15097 showed high restoration of fertility (>80%) on all three A₁, A₄ and A₅ based male sterile lines. Identification of restorers for diverse cytoplasmic male sterile sources opens up new avenues in pearl millet hybrid breeding. It brings out the possibility of developing successful hybrid combinations in diverse cytoplasmic background other than routinely used A₁ cytoplasm and to solve the risk of emergence of diseases.

Keywords: Pennisetum, pearl millet, fertility restoration, restorer

Introduction

The presence of exploitable male sterility mechanisms in pearl millet has enabled the commercial utilization of heterosis and hybrid vigor to enhance productivity. The discovery of Tift 23A₁, a cytoplasmic-nuclear male sterility (CMS) and its effective application in producing male sterile lines for commercial pearl millet hybrids production marks a significant milestone in the improvement of pearl millet (Athwal, 1965; Burton and Athwal, 1967 and Burton and Powell, 1968) [2, 4, 3]. In the 1970s, when hybrids based on the Tift 23A₁ cytoplasm faced susceptibility to downy mildew infection, research endeavors in pearl millet breeding centers were intensified. Their focus was on diversifying the genetic foundation of male sterile lines in the A₁ cytoplasmic system and investigating alternative CMS systems within pearl millet. Burton and Athwal (1967) [4] employed A₁, A₂ and A₃ CMS sources for hybrid development while Marchais and Pernes (1985) [8] identified two other sources (A₁ and A₄) derived from distinct accessions of *Pennisetum glaucum monodii*, a wild relative of cultivated pearl millet. Additionally, a completely novel A₅ cytoplasmic male sterility system was created by Rai (1995) [9]. This development showcased the ongoing efforts to explore and enhance male sterility mechanisms within pearl millet for the purpose of breeding improved hybrids.

Although various cytoplasmic male sterility systems exist within pearl millet, there is a notable scarcity of comprehensive research on the identification of restorers for these diverse cytoplasm. Among the extensively evaluated systems like A₂ and A₃, none demonstrated superior performance compared to the A₁ CMS system in relation to one or more crucial pearl millet traits. While the A₄ and A₅ sources displayed high stability, their practicality is limited due to the absence of appropriate restorers (Rai *et al.*, 2006) [10]. Therefore, it is imperative to focus on this aspect in order to effectively utilize the array of sterile sources in creating new pearl millet hybrids. Developing suitable restorers is vital for the continued advancement of commercial hybrid varieties.

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The availability of cytoplasmic genetic male sterile lines in this crop facilitates the practical exploitation of heterosis for commercial purposes and the large scale production of hybrid seeds. The application of heterosis at a commercial level in pearl millet is considered a significant advancement in enhancing its productivity.

Materials and Methods

The material for the present study comprises of three female parents from diverse cytoplasmic male sterility sources (A₁, A₄ and A₅) and 20 elite restorers. The three female parents viz., ICMA 98222 (A₁ cytoplasm), ICMA 04777 (A₄ cytoplasm) and ICMA 01777 (A₅ cytoplasm) and the 20 restorers viz., BRBL 1136R, ICMR 15455-1, ICMR 15455-2, ICMR 15455-3, ICMR 15024, ICMR 15037, ICMR 15038, ICMR 15063, ICMR 15097, ICMR 15082, ICMR 16401, ICMR 20365, ICMR 19631, ICMR 20405, ICMR 20444, ICMR 36883, ICMR 15026, ICMR 16411, ICMR 16409, ICMR 16410 were raised during summer 2022-23 at Regional Agricultural Research Station, Vijayapura in adjacent plots with staggered planting to facilitate easy crossing. These three CMS lines were crossed with twenty restorers (testers) in line × tester design (Kempthorne, 1957) ^[6], so as to obtain sixty F₁ hybrids during summer 2022-23. Artificial selfing and crossing operations were carried out as suggested by Rao (1952). To ensure selfing, the panicles of the parents, just before the emergence of stigma were covered with butter paper bag. After complete protrusion of stigma, the panicle of the seed parent was dusted with pollen collected from the male parent and rebagged. Pollination was effective between 8.00 am to 11.00 am.

The resultant hybrids along with their parents and checks were raised in Randomized Complete Block Design with three replications during *Kharif* 2023 at Regional Agricultural Research Station, Vijayapura. Each hybrid was accommodated in two rows of three meter row length in three replications with a row spacing of 45 cm and plant to plant spacing of 15 cm. Randomly selected five competent plants in each treatment (hybrid) were selfed with brown paper bags before flowering and same plants were used for recording the observations. At maturity stage, numbers of seeds were counted out of total number of spikelets per ear head and seed set percentage was calculated (Kishan and Borikar, 1989) ^[7] and classified the percentage seed set as given below.

Per cent seed set	Type of restoration
>90	Strong restoration (SR)
80-90	High restoration (HR)
60-80	Moderate restoration (MR)
10-60	Partial restoration (PR)
<10	Low restoration (LR)
Zero	Maintainers

Results and Discussion

The study evaluated the fertility restoration ability of twenty testers on three diverse CMS lines A₁, A₄ and A₅ based sources during the *Kharif* 2023. The selfing study data illustrate the

percentage of seed set in several cross combinations involving diverse cytoplasm. The outcomes displayed in Table 1 predicted that the values of the seed set ranged from 32 to more than 90 per cent. The compatibility of the cytoplasm of the female gamete or egg with the nuclear background of the male gamete is one factor that affects the seed set. Extreme variation in the different crosses may be caused by distinct interactions between the nuclear background of the male gamete and the female gamete in that particular line. The amount of seed set and fertility restoration varied only little between the different cytoplasm. The percentage of seed set recorded in a cross represents a restoring capability of a pollen parent. The variations in seed set proportion differ from 32 to 98 per cent which reflects wide range of deviation in the restoration capability of pollen parents.

Classification of restoration based on mean seed set percentage is shown in Table 2. Results of fertility restoration reaction of the various testers showed that, 8 out of 20 testers viz., ICMR 15455-2, ICMR 15455-3, ICMR 15024, ICMR 15037, ICMR 15038, ICMR 19631, ICMR 20405 and ICMR 20444 were considered as the strong restorers of the fertility on A₁ and 9 testers viz., BRBL 1136R, ICMR 15455-2, ICMR 15455-3, ICMR 15024, ICMR 15038, ICMR 15082, ICMR 19631, ICMR 16409 and ICMR 16410 on A₄ based male sterile lines. No tester showed strong fertility restoration on A₅ cytoplasm.

Seed set percentage of each of the hybrid was determined and the average seed set value revealed by different fertile F₁'s was calculated and this value was observed to be 95.79 per cent in case of 8 fertile F₁'s considered as strong restorers on A₁ cytoplasm. The mean seed set percentage of 9 fertile hybrids considered as strong restorers on A₄ cytoplasm is 93.67 per cent.

Five testers viz., ICMR 15455-2, ICMR 15455-3, ICMR 15024, ICMR 15038 and ICMR 19631 showed a significant strong restoration of fertility on both A₁ and A₄ cytoplasm. Three testers ICMR 15024, ICMR 19631 and ICMR 15097 showed high restoration of fertility (>80%) on all three A₁, A₄ and A₅ based male sterile lines.

Moderate restoration was recorded in BRBL 1136R, ICMR 15455-1, ICMR 15063, ICMR 36883, ICMR 15026, ICMR 16411 and ICMR 16410 on A₁ cytoplasm (71.69%), ICMR 20405, ICMR 36883, ICMR 15026 and ICMR 16411 on A₄ cytoplasm (69.52%) and BRBL 1136R, ICMR 15455-1, ICMR 15455-2, ICMR 15455-3, ICMR 15037, ICMR 15038, ICMR 15082, ICMR 16401, ICMR 20365, ICMR 36883 and ICMR 16409 on A₅ cytoplasm (72.47%).

Partial restoration was observed in one cross of ICMR 15063 on A₄ cytoplasm (58.04%), two crosses of ICMR 20444 and ICMR 16411 on A₅ cytoplasm (57.63% and 32.41%). These results indicate the scope of utilization of diverse source of cytoplasm as alternative sources of male sterility to generate commercial hybrids.

Similar outcomes were obtained by Amiribehzadi and Satyavati (2012) ^[1], Salagarkar *et al.* (2016) ^[11], Singhal *et al.* (2019) ^[12] and Chandra *et al.* (2022) ^[5]. The fertility restorers that have been identified can be employed for the subsequent creation of new experimental hybrids in pearl millet breeding.

Table 1: Per cent seed set observed in different cross combinations involving A₁, A₄ and A₅ cytoplasm of pearl millet

Sl. No.	Parents	ICMA 98222 (A ₁ cytoplasm)	ICMA 04777 (A ₄ cytoplasm)	ICMA 01777 (A ₅ cytoplasm)
1	BRBL 1136R	77.15 (MR)	97.30 (SR)	77.47 (MR)
2	ICMR 15455-1	68.58 (MR)	87.39 (HR)	67.85 (MR)
3	ICMR 15455-2	93.18 (SR)	90.97 (SR)	70.83 (MR)
4	ICMR 15455-3	98.22 (SR)	93.97 (SR)	74.70 (MR)
5	ICMR 15024	96.97 (SR)	93.45 (SR)	88.67 (HR)
6	ICMR 15037	94.09 (SR)	87.14 (HR)	73.87 (MR)
7	ICMR 15038	95.00 (SR)	95.53 (SR)	71.74 (MR)

8	ICMR15063	74.68 (MR)	58.04 (PR)	82.70 (HR)
9	ICMR 15097	86.50 (HR)	87.19 (HR)	86.92 (HR)
10	ICMR 15082	87.00 (HR)	94.08 (SR)	75.23 (MR)
11	ICMR 16401	86.82 (HR)	89.8(HR)	77.52 (MR)
12	ICMR 20365	86.55 (HR)	83.10 (HR)	72.98 (MR)
13	ICMR 19631	98.45 (SR)	92.48 (SR)	86.90 (HR)
14	ICMR 20405	95.08 (SR)	66.46 (MR)	88.70 (HR)
15	ICMR 20444	95.33 (SR)	86.86 (HR)	57.63 (PR)
16	ICMR 36883	64.81 (MR)	67.27 (MR)	66.96 (MR)
17	ICMR 15026	74.70 (MR)	70.13 (MR)	86.61 (HR)
18	ICMR 16411	76.18 (MR)	74.22 (MR)	32.41 (PR)
19	ICMR 16409	86.06 (HR)	93.85 (SR)	67.97 (MR)
20	ICMR 16410	65.78 (MR)	91.38 (SR)	85.65 (HR)

SR=Strong Restorer; HR=High Restorer; MR=Moderate Restorer; PR=Partial Restorer, LR=Low Restorer and M=Maintainer

Table 2: Classification of fertility restoration based on mean seed set percentage

Restoration classes	Seed set%	Diverse sources of cytoplasm					
		A ₁		A ₄		A ₅	
		No. of restorers	Mean seed set%	No. of restorers	Mean seed set%	No. of restorers	Mean seed set%
I. Strong restoration	>90	8	95.79	9	93.67	-	-
II. High restoration	80-90	5	86.59	6	86.93	7	86.59
III. Moderate restoration	60-80	7	71.69	4	69.52	11	72.47
IV. Partial restoration	10-60	-	-	1	58.04	2	45.02
V. Low restoration	<10	-	-	-	-	-	-
VI. Maintainers	Zero	-	-	-	-	-	-

Conclusion

The present study brings out the restoration capabilities of the twenty male parents tested against three diverse cytoplasmic male sterile lines of pearl millet. Identification of restorers 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. The present study firstly brings out the possibility of developing successful hybrid combinations in diverse cytoplasmic background other than the routinely used A₁ 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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