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Fertility restoration studies involving three diverse cytoplasmic-nuclear male sterility systems in Pearl millet

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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 were available and found to be highly stable, their utility is restricted due to non-availability of suitable restorers. The investigation on fertility restoration reaction of the various lines revealed that out of the 105 lines, 38 exhibited satisfactory (> 60 %) seed setting on A_1, 63(50%) on A_4 cytoplasm and 47 lines (44.76 %) on A_5 cytoplasm, acted as restorers. 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. Among the three sources of male sterility the seed set percentage was highest on A_1 (78.51) followed by A_4 (76.10) and A_5 (68.72) in *kharif* season. However, during summer only on A_4 source the seed set per cent was >70%. The proportion of restorers exhibiting >80 % seed set was highest on A_4 followed by A_5 and A_1 across *kharif* and summer seasons. The mean seed set percentage (across the seasons) on A_4 was highest (73.50 %) followed by on A_1 (71.60%) and 66.60% on A_5.

Key words: CMS, restorers, seed set percentage, pearl millet, male sterility.

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.], is primarily grown in India for grain and fodder production (both green and stover). The use of cytoplasmic genetic male sterility system has made it feasible to exploit heterosis on commercial basis in pearl millet. The extensive use of single source of genetic male sterility for commercial exploitation of heterosis had raised the fear of occurrence of its potential vulnerability to pest and diseases during 1970's. This led to the identification of alternative CMS sources A_1, A_2 [1, 2], A_4 [3] and A_5, [4] and development of hybrids based on these sources. Identification of restorers on diverse sources of cytoplamic-nuclear male sterility is a pre-requisite for utilizing alternate cytoplasm for commercial exploitation of heterosis and to evade the risk associated with the use of single source. However, systematic studies on identification of restorers on diverse cytoplasm are meager. Though the A_4 and A_5 sources were found to be highly stable [5] and their utility is restricted due to non-availability of suitable restorers [6]. Hence, the work in this direction is essential to make use of diverse sterile sources in the development of new Pearl millet hybrids and their commercial exploitation. The main objective of the present investigation was to evaluate the germplasm lines for their restoring ability on diverse CMS sources to identify stable restorers and to characterize the cytoplasmic differences based on restoration pattern.

Material and methods

The experimental materials consisting of three male sterile lines of Indian origin with different cytoplasmic sources *viz*., A_1 [Tift 23D2A, Cytoplasm source (A_1) backcrossed to 81B], A_4 [Hanna's *monodii*, cytoplasm source (A_4) backcrossed to 81B green] and A_5 [LSGP, cytoplasm source (A_5) backcrossed to 81 B] as female and 105 germplasm collections representing the major pearl millet growing regions of the world as male parents were used for crossing programme during 2004. The 105 hybrids produced on each of the three diverse cytoplasm (totally 315 F_1 hybrids) were evaluated for fertility restoring ability to identify stable restorers on A_1, A_4 and A_5 cytoplasm. The hybrids were grown in two rows of 4 m length with intra row spacing of 15 cm

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and inter row spacing of 50 cm at Regional Agricultural Research Station, Bijapur, Karnataka during 2005 summer and *kharif* by following the recommended package of practices. Five plants in each treatment were selfed by covering with brown paper bag before flowering and the same plants were used for recording seed set data. At maturity, number of seeds were counted out of total number of spikelet per ear head and seed set percent was calculated and the restoration with less than 60% seed setting was considered as partial [7].

Results and discussion

Results of fertility restoration reaction of the various lines revealed that of the 105 lines, 38 (36.19%) exhibited satisfactory (> 60 %) seed setting on A_1 (Table 1), 63 (60%) genotypes on A_4 and 47 lines (44.76%) on A_5 cytoplasm. These results revealed 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.

Among the three sources of male sterility the seed set percentage was highest on A_1 (78.51) followed by A_4 (76.10) and A_5 (68.72) in *kharif* season. However, during summer only on A_4 source the seed set per cent was >70 (Table 2 and Fig. 8). These results indicated the scope of utilization of A_4 as an alternative source of male sterility to develop commercial hybrids for both *kharif* and summer reasons.

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. Earlier, sorghum genotypes showing above 60 per cent seed set were broadly grouped as restorers which were classified into different categories based on their restoration ability [8]. In the present investigation, a total of 105 genotypes were evaluated for their restoration ability on three diverse sources of male sterility. Further these lines were grouped into six categories depending upon seed set percentage (Table 3) by following the classification model developed by Biradar [8]. The proportion of genotypes falling in different restorer classes and seed set percentage in two seasons is presented in Table 4. Mean seed set percentage (average of two seasons) observed in different restoration classes on three diverse source of male sterility is presented in Fig. 1.

The values presented in Table 4 indicated that the proportion of restorers exhibiting >80 % seed set was highest on A_4 followed by A_5 and A_1 across *kharif* and summer season. When the seed set per cent in summer alone was considered, similar trend was seen. However, in *kharif* season the highest proportion was observed on A_4 followed by A_1 and A_5. The mean seed set percentage (across the seasons) on A_4 was highest (73.50 %) followed by on A_1 (71.60%) and 66.60% on

Table 1. Mean seed set percentage in F_1 hybrids derived from three male sterile lines having diverse cytoplasm with 105 male parents

	Diverse cytoplasm								
	A_1			A_4			A_5		
Item	K	S	M	K	S	M	K	S	M
Mean	78.51	63.70	71.60	76.10	70.95	73.50	68.72	63.29	66.01
S.D.	33.5	37.70	27.23	39.9	36.70	38.00	36.3	33.6	34.87
No. of restorers among 105 lines		67.00			42.00			57.00	

K*: Kharif*, S: Summer, M :Mean

Table 2. Mean seed set percentage observed in two different seasons on three diverse sources of male sterility in pearl millet

Cytoplasm	Seasons			
	Kharif		Summer	
	Mean	Range	Mean	Range
$81A_1$ (Tift 23 D2A)	78.51	50.0-95.0	64.88	30.5-88.0
$81A_4$ (Hannas monodii)	76.10	40.0-96.0	70.75	35.0-91.6
$81A_5$ (LSFP)	68.72	30.0-95.0	63.29	30.0-91.0

Table 3. Classification of restoration based on mean seed set percentage (average of two seasons)

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	-	-	7	91.41	3	91.53
II. High restoration	>80-90	14	85.65	23	89.83	10	84.37
III. Moderate restoration	>60-80	18	70.46	17	70.79	18	68.82
IV. Partial restoration	>10-60	6	45.88	16	49.20	17	47.66
V. Low restoration	<10	-	-	-	-	-	-
VI. Maintainers	Zero	67	-	42	-	57	

A_5 (Table 5). This clearly indicates that A_4 cytoplasmic sources can be comfortably exploited for development of commercial hybrids.

Stability of restoration on different cytoplasmic source

The seasons suited for pearl millet cultivation are mainly *kharif* and summer. The temperature variation in *kharif* season is less, which generally provides good vegetative growth and promotes grain filling in pearl millet, while in summer variation in temperature is more and erratic. Keeping these contrasting features of the seasons, it was felt necessary to examine restoration reaction of each genotype over two seasons. It is evident from the present study that there is a reduction in proportion of lines showing high restoration and mean seed set percentage from *kharif* to summer (Table 5).

Based on change in proportion of restorers and over all seed set percentage observed in each season, it is evident that A_4 cytoplasm is less sensitive to environmental fluctuation as compared to A_1 and A_5 cytoplasmic sources. Thus, A_4 appears to be more stable source of male sterility followed by A_5 and A_1 cytoplasm. Further A_4 cytoplasm is more suited for development of hybrids for summer season.

From the Table 4, it is clear that there is reduction in number of lines showing > 90 per cent seed set from *kharif* to summer. This effect was noticed in all the cytoplasmic sources. However, this trend of decrease in proportion of restorer was not so obvious in lower classes of restoration (60-80%). This indicates that the restoring ability of strong restorers appear to be more sensitive to environmental fluctuation than in the moderate restorer. Such studies are not available in pearl millet. However, in sorghum Biradar [8] observed similar phenomenon.

Overall seed set percentage was highest in *kharif* than in summer season. However, when mean seed set percentage was examined in different restoration classes, the pattern of reduction of seed set from *kharif* to summer was more in the class of strong restorers on all the three cytoplasm. The other classes with low seed set percentage did not show this kind of sensitivity to seasonal differences. It is very difficult to give exact reason for differential sensitivity of different restoration classes.

Though majority of the lines showed reduction in seed set from *kharif* to summer some unique lines *viz*., IP-8726, IP-9416, IP-9440, IP-19321, IP-6510, IP-15355 showed high restoration even in summer. After confirming their consistency particularly during summer season, these lines can be specifically used for developing hybrids for summer season.

Choice of restorers

Pearl millet hybrids are mainly grown in *kharif* and summer seasons. The seasonal condition in *kharif* and summer are distinctly different. Of the two seasons, summer is characterized by very hostile environment with high temperature and dry weather. If the hybrids are to be recommended for cultivation in both the seasons and to realize the complete yield potentiality, it is necessary that concerned male parent should have consistently high restoring ability in both the seasons. Based on the percentage of seed set observed in both seasons, the strong and consistent restorers observed in the study are listed in the Table 6. It is evident from the Table 6 that these genotypes can be used as parents of hybrids as well as donors for transferring restorer genes. Study also revealed that, the restorer IP-16096 exhibited restoring fertility on A_1 and A_4 cytoplasm and IP-8276 showed restoring ability on A_4 and A_5

cytoplasmic source and these lines can be used to develop alloplasmic hybrids.

In addition, 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 it gives scope for selecting restorers with good combining ability and adaptation to diverse agro climatic conditions.

Geographic distribution of male sterility restorers and maintainers

In the present investigation, restorers and maintainers on A_1, A_4 and A_5 cytoplasm represented eleven different countries (Table 7). The distribution pattern of restorers and maintainers across these countries revealed interesting observation. Among the 26 lines of Indian origin, 65.4 % were restorers and remaining were maintainers. Similarly, out of 12 lines from Namibia 91.7 % were restorers. On the other hand out of 12 lines from Zimbabwe only 16.7 % were restorers and remaining were maintainers. These results gave a clue to search for restorers/ maintainers from different geographical areas. Earlier, Appa Rao *et al.* [9] identified large number of restorers on Tifton source of male sterility in West Africa and maintainers in India.

In addition, identification of restorers/maintainers from different countries 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 it gives scope for selecting restorers with good combining ability and adaptation to diverse agro climatic conditions.

Common restorer on diverse cytoplasmic sources

In the present study, very high proportion of restorers were noticed on A_4 (47), followed by A_5 (37) and A_1 (31) (Table 7). On the other hand, the common restorers on A_4 and A_5 (31) were highest (Table 8 and Fig.2) followed by A_1 and A_4 (28) and A_1 and A_5 (2). Over all, a total of

Table 4. Proportion of lines representing different restoration classes and seed set percentage in two season

Restoration category	A_1		A_4		A_5	
	Kharif	Summer	*Kharif*	Summer	*Kharif*	Summer
I. Strong restorers (>90%)						
i) No.of lines	7	-	20	2	6	2
ii) Proportion	6.66	-	19.04	1.90	5.71	0.95
iii) Mean seed set %	92.60	-	92.86	91.60	93.71	91.00
II. High restorers (>80-90%)						
i) No.of lines	14	9	13	20	9	6
ii) Proportion	13.33	8.57	12.38	19.04	8.57	5.71
iii) Mean seed set %	86.82	84.51	86.07	85.16	86.55	85.38
III. Moderate restorers (>60-80%)						
i) No.of lines	12	16	18	22	16	21
ii) Proportion	11.42	15.23	17.14	20.95	15.23	20.00
iii) Mean seed set %	66.96	74.03	70.11	72.18	65.15	70.11
IV. Partial restorers (>10-60%)						
i) No.of lines	5	13	12	19	17	19
ii) Proportion	4.76	12.38	11.42	18.09	16.19	18.09
iii) Mean seed set %	54.80	51.57	48.20	51.52	49.70	43.24
V. Low restorers (<10%)						
i) No.of lines	-	-	-	-	-	-
ii) Proportion	-	-	-	-	-	-
iii) Mean seed set %	-	-	-	-	-	-
VI. Maintainers (0%)						
i) No. of lines	67	67	42	42	57	57
ii) Proportion	63.80	63.80	40.0	40.0	54.28	54.28
iii) Mean seed set %	-	-	-	-	-	-

Table 5. Proportion of restorers representing different restoration classes and seed set percentage on three cytoplasmic sources in two season

Restoration category	Diverse cytoplasmic sources								
	A_1			A_4			A_5		
	K	S	M	K	S	M	K	S	M
i) > 80%	31.40	23.8	27.6	48.6	41.9	45.25	29.5	27.6	28.55
ii) < 60%	4.80	12.4	5.73	11.4	18.1	14.75	16.2	18.1	17.15
iii) Mean seed set %	78.51	63.7	71.60	76.0	70.95	73.50	66.72	63.29	66.01
Total number of lies tested	105	105	-	105	105	-	105	105	-

K: *Kharif*, S: Summer, M: Mean

Table 6. List of best restorers showing consistently high restoring ability across seasons

Source of cytoplasm	Seasons		
	Kharif	Summer	Mean
1. A_1 cytoplasm			
1. IP-10186	92.00	85.00	88.50
2. IP-10085	91.00	81.00	86.00
3. IP-14038	93.60	85.00	89.30
4. IP-9306	93.60	81.60	87.60
5. IP-12682	95.00	85.00	90.00
6. IP-16096	85.00	85.00	85.00
7. IP-13833	90.00	85.00	87.50
2. A_4 cytoplasm			
1. IP-9140	92.00	85.00	88.50
2. IP-9149	94.00	91.60	92.80
3. IP-15829	95.50	96.00	95.80
4. IP-8276	92.00	90.00	91.00
5. IP-9416	90.50	90.00	90.30
6. IP-15304	95.00	85.00	90.00
7. IP-15273	92.00	85.00	88.50
8. IP-16096	90.60	84.00	87.30
9. IP-7440	92.60	90.00	91.30
10. IP-19321	95.00	91.60	93.30
11. IP-12768	92.00	86.00	89.00
12. IP-12901	98.00	81.00	89.50
13. IP-18742	92.00	89.00	90.50
3. A_5 cytoplasm			
1. IP-15857	92.66	85.00	88.80
2. IP-6510	95.00	91.00	93.00
3. IP-8276	92.60	88.00	90.30
4. IP-15355	95.00	90.00	92.50
5. IP-10839	92.00	85.00	88.50

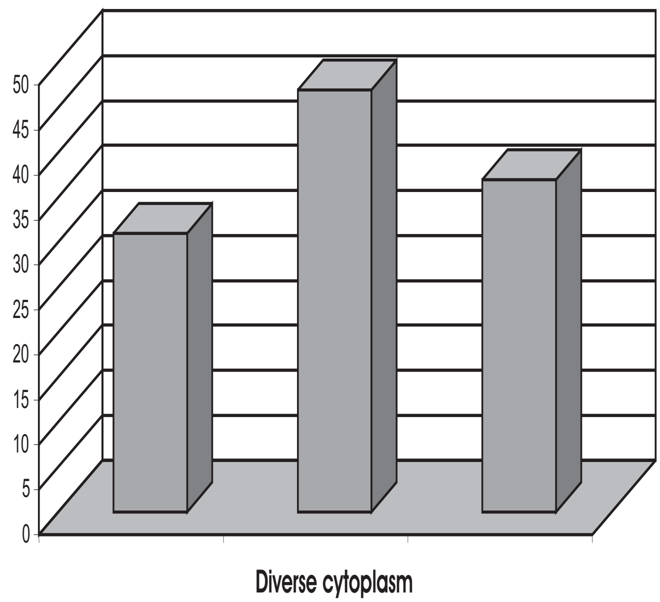

Fig. 1. Distribution of sterility maintainers/fertility restorers on diverse cytoplasm in pearl millet

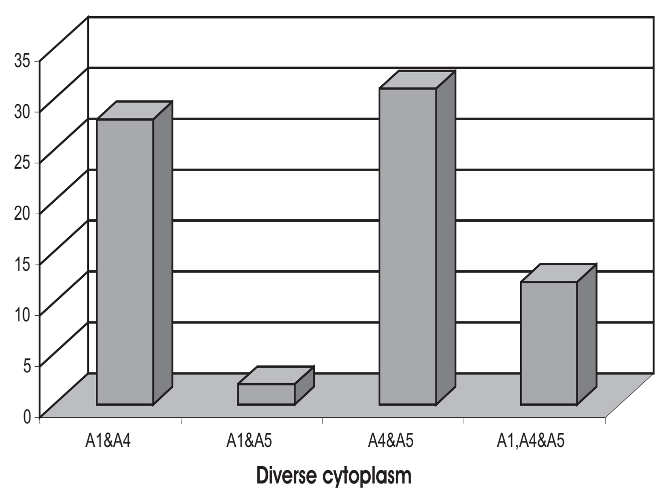

Fig. 2. Common restorers on three diverse sources of cytoplasm

Table 7. Distribution of sterility maintainers/fertility restorers on diverse sources of male sterility in pearl millet

Origin	Total no. of lines evaluated	Number of lines				
		Restorers				Maintainers (on A_1,A_4 &A_5)
		A_1	A_4	A_5	Total	
India	26	10	14	10	17	9
ICRISAT	9	3	4	2	5	4
Togo	6	1	2	2	2	4
Sudan	7	1	1	2	2	5
Tanzania	8	2	3	1	6	2
Mali	8	3	5	3	5	3
Camerron	5	3	3	2	3	2
Zimbabwe	12	2	3	-	5	7
Namibia	12	4	7	10	11	1
Nigeria	5	-	2	2	2	3
Burkino Faso	6	2	2	2	2	4
Ghana	1	-	1	1	1	-
Total	105	31	47	37	61	44

12 lines could restore fertility on all the three sources. These results indicated the scope of developing iso-nuclear alloplasmic hybrids either on all the three sources or combinations of any two sources. 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 cytoplasm. The A_4 cytoplasm appears to be closer to the other two cytoplasm while A_1 and A_5 cytoplasm have wider cytoplasmic distance between them.

Table 8. Common restorers on three sources of cytoplasm

Diverse sources	Restorers	Total
A_1 & A_4	IP-10186, IP-15829, IP-10085, IP-15681,IP-15257,IP-14942, IP-14038, IP-7979, IP-15700, IP-14028, IP-14026, IP-15305, IP-15372, IP-14497, IP-144778, IP-16069, IP-7440, IP-13840, IP-9306, IP-14644,IP-19321, IP-16197, IP-12768, IP-2682, IP-19246, IP-13833, ICMV-221, IP-6545.	28
A_1 & A_5	IP-18625, ICTP-8203	02
A4 & A_5	IP-9140,IP-9286,IP-15856,IP-15899, IP-10394,IP-6510,IP-8276,IP-9416, IP-10811,IP-10085, IP-15681,IP-6417, IP-8429,IP-5275,IP-15335,IP-15273, IP-14497,IP-7440,IP-13840,IP-9306, IP-1695,IP-4779,IP-12779,IP-12768, IP-19338,IP-18742,IP-18657,IP-4169, IP-7468,IP-19067, IP-19243.	31
A_1, A_4 &A_5	IP-10085,IP-15681,IP-15273,IP-14493, IP-7440,IP-13840,IP-9306,IP-14644, IP-19321, IP-12768,IP-13833,ICMV-221	12

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