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Studies on heterosis involving diverse cytoplasmic male sterility systems in pearl millet

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Abstract

Investigation was undertaken to quantify the magnitude of heterosis of alloplasmic- isonuclear lines of pearl millet in two environments (normal and late sown crop). Three alloplasmic isonuclear pearl millet lines (81A₁, 81A₄, 81A₅ and their corresponding maintainer lines) were crossed with eight diverse pollinators in a line x tester design to study the nature and magnitude of heterosis for grain yield and its eight component traits. The extent of heterosis varied in each cross for all the characters studied. The high magnitude of heterosis was not observed for any of the trait; medium level of heterosis was exhibited for grain yield per plant, dry fodder yield per plant, harvest index and effective tillers per plant. While, low amount of heterosis was recorded for days to 50 % flowering, 1000-grain weight, plant height, panicle length and panicle girth. Among 48 crosses, eight (Environment 1) and seven (Environment 2) hybrids exhibited significant and positive heterosis over standard check (HHB-197) for grain yield. The direction and magnitude of heterosis varied from cross to cross in two different environments. This indicates environmental influence on the expression of hybrid vigour. The hybrid combination 3×11 (81A₅×ERC) expressed significant positive heterosis for grain yield, effective tillers, panicle girth, harvest index in one or both the environments. The hybrids namely 1×13 (81A₁×99HS141) for days to 50% flowering, 1000-grain weight, panicle girth and 4×12 (81B₁×H77/833-2) for dry fodder yield, effective tillers, panicle length expressed high heterosis for these productivity traits. The other crosses namely 1×10 (81A₁×MRC) exhibited desirable heterosis for grain yield, effective tillers, panicle girth, panicle length and harvest index; 3×14 (81A₅×HTP 92/80) for grain yield, 1000- grain weight and harvest index. These crosses involved the 81A₁ and 81A₅ cytoplasmic sources. This indicated the superiority of these two cytoplasmic sources to produce heterotic hybrids.

Keywords: Pearl millet, heterosis, diverse cytoplasmic sources

Introduction

Pearl Millet [*Pennisetum glaucum* (L.) R. Br.] is an important cereal crop grown in tropical semi-arid regions of the world primarily in Africa and Asia. It is a highly nutritious cereal crop with wide agroecological adaptation primarily grown for grain production, but also valued for its fodder (both stover and green forage). It is nutritionally very rich, supplying highest number of calories than all other cereals, having high protein, fibre and fat content (NIN, 2003) [13].

It is a highly cross pollinated crop, and single-cross hybrids generally give 20–30% more yield than open pollinated varieties (Rai *et al.* 2006) [16]. Hybrid cultivar development in pearl millet became possible with the discovery of cytoplasmic- genic male-sterility (Burton, 1958) [4]. The use of CMS (cytoplasmic-genic male sterility) in pearl millet paved the way for grain yield augmentation with the development and release of first grain hybrid HB-I (Tift 23 A x BIL-3B) by Athwal (1965) [3].

Several other sources of male-sterility inducing cytoplasmic sources besides A₁ (Burton, 1965) [5] such as A₂, A₃ (Burton and Athwal, 1967) [6], PT732A (Appadurai *et al.*, 1982) [2], ex-Bornu (Gero) (Aken'ova, 1985) [1], *violaceum* (Marchais and Pernes, 1985) [12], A₄ (Hanna, 1989) [8] and A₅ (Rai, 1995) [14] have been reported in pearl millet. Since then, large number of hybrids have been developed and commercialized in India, largely based on the A₁ CMS system except two (GHB 316 on A₃, HHB 216 on A₄ CMS system) hybrids. It has been shown that the A₄ and A₅ CMS systems are good alternative to the A₁ system in terms of the agronomic performance of pearl millet hybrids (Rai *et al.*, 2001; Rai *et al.*, 2009) [17, 15].

The isonuclear lines have also been established in the background of several diverse CMS sources, which provides an opportunity for studying cytoplasmic effects on the expression of different characters. In any hybrid breeding programme based on several CMS sources, the information on relative magnitude of heterosis in cytoplasmically diverse hybrids helpful in determining relative chance and quantifying the magnitude and direction of heterosis in hybrids carrying different cytoplasm in pearl millet.

Studies have indicated that cytoplasm exhibits pronounced effect on heterosis and combining ability (Young and Virmani, 1990; Yadav, 1994) [24-25, 22]. Heterosis breeding was ideal for increasing yield in pearl millet (Ramamoorthi and Nadarajan, 2001) [18]. Commercial exploitation of hybrid vigour in pearl millet has resulted in a substantial improvement in the productivity in the country as a whole and in a few states in particular but there is still a need to improve the grain yield to make this crop more economically viable. Therefore, the present investigation was conducted to study the extent of hybrid vigour in F₁ for grain yield and its components involving alloplasmic isonuclear lines of pearl millet.

Materials and Methods

The material for present study consisted of three CMS lines (A-lines), their corresponding maintainers (B-lines) and eight restorers and the details are presented in **Table 1**. Three male sterile lines and their corresponding three maintainer lines were crossed with eight restorers in line x tester fashion at ICRISAT, Hyderabad, during off season crop (January- April 2013). The forty eight pearl millet hybrids, thus, produced and their parents along with the standard check HHB-197 were grown in two environments (two dates of sowing) E₁ - Planting 15th July (Normal sowing) and E₂ Planting 24th July (Late sowing) during *kharif* 2013. The experiment was raised in a Randomized Block Design with three replications in each of the environments at CCSHAU, Hisar with a plot size of 2row x 4 m x 0.5 m with 10-12 cm intra- row spacing. All the recommended agronomic practices were followed to raise a good crop.

During *kharif* 2013, total rainfall of 594.3 mm was recorded with a total of 24 rainy days. The total rainfall was below normal and distribution was also abnormal. The highest rainfall (154.2 mm) in a single week was recorded on 33rd week. The weekly mean minimum temperature (June to November) varied from 16.3°C to 27.5°C, whereas, the maximum temperature was between 31.3°C to 38.7°C and both were normal. The mean weekly morning relative humidity ranged between 78 to 96%.

Table 1: Male sterile lines, their corresponding maintainers and restorers lines used in the study:

Sr. No.	Lines	Source
1.	81A ₁	ICRISAT
2.	81A ₄	ICRISAT
3.	81A ₅	ICRISAT
4.	81B ₁	ICRISAT
5.	81B ₄	ICRISAT
6.	81B ₅	ICRISAT
Testers		
7.	H77/29-2	HAU
8.	TCH 26-1	HAU
9.	G 73- 107	HAU
10.	MRC/10/61 (MRC)	ICRISAT
11.	ERC/99/35 (ERC)	ICRISAT
12.	H77/833-2	HAU
13.	99HS141	HAU
14.	HTP 92/80	HAU

Results and Discussion

In the commercial exploitation of hybrid vigour, excess of F₁ over standard check (standard heterosis), is of significance. Hence, in the present investigation, the extent of heterosis over standard check hybrid (HHB 197) for grain yield and eight yield component traits is discussed. The magnitude of

heterosis in terms of per cent increase or decrease in the performance of the hybrids over the standard check HHB-197 was studied and the results are presented. The analysis of variance of the data performed (including check values) for the various characters studied in two environments are presented in **Table 2**. The mean squares due to treatments (hybrids and standard check) were highly significant for all the characters studied.

Grain yield is of economic importance for which considerable degree of heterosis was registered in a number of crosses. Among the 48 crosses studied in two environments, 8 in E₁ and 7 in E₂ exhibited significant positive heterosis manifested significant positive standard heterosis. Environment wise percent standard heterosis ranged from -31.11 to 24.07 and -31.98 to 22.67 with mean values -8.91 and -6.57 in E₁ and E₂, respectively (**Table- 4**). The crosses namely, 1×8 (E₁, E₂), 3×11 (E₂), 3×14 (E₂), 6×10 (E₁) and 1×10 (E₂) expressed significant heterosis over the check HHB-197 (**Table- 3**). This result indicated that the favorable combination of yield contributing characters resulted in a higher proportion of cross combinations showing significant positive standard heterosis. Vetriventhan *et al.* (2008) [21] also observed high magnitude of heterosis for grain yield.

Low of heterosis has been observed in fodder yield, which is an important component of pearl millet being a dual-purpose crop. The cross 1×14 (14.61 %) recorded the maximum standard heterosis for higher dry fodder yield in E₂ environment followed by 4×12 (12.33 %). The results on per cent heterosis for dry fodder yield revealed that most of the crosses were poor combinations with negative significant values. Environment wise percent standard heterosis ranged from -41.49 to 10.73 and -49.03 to 14.61 with mean values -10.29 and -7.98 in E₁ and E₂, respectively.

Early flowering was treated as desirable attribute and accordingly negative estimates of heterosis were considered to be desirable. The results on per cent heterosis for days to 50 per cent flowering revealed that out of 48 crosses as many as 7 in E₁ and 4 in E₂ showed significant negative values of heterosis. The hybrid 4×13 was noted for negative and significant heterosis in both the environments. The crosses 1×9, 3×8, 6×8 in E₁ and 1×13 exhibited significant negative values of heterosis in E₂. Environment wise percent standard heterosis ranged from -8.62 to 25.86 and -12.23 to 11.87 with mean values 9.65 and 2.47 in E₁ and E₂, respectively. Chotaliya *et al.*, (2009) [7] and Vaghasiya *et al.*, (2009) [20] also observed heterosis to some extent for this trait.

None of the hybrids were found to be exhibit significant positive heterosis for plant height in both of the environments over the standard check. The most of the crosses exhibited negative values for heterosis. Environment wise percent standard heterosis ranged from -31.20 to 11.30 and -27.63 to 12.73 with mean values -4.11 and -9.09 in E₁ and E₂, respectively.

The effective tillers considered an important yield component trait. The cross combinations 1×9, 2×13, 3×11 and 4×12 proved to be favorable combinations in both the environments and 1×10 in E₂. The results on percent heterosis revealed that out of 48 crosses as many as 17 in E₁ and 11 in E₂ showed significant positive values of heterosis. Environment wise percent standard heterosis ranged from -44.58 to 39.76 and -34.21 to 36.84 with mean values -4.99 and -7.81 in E₁ and E₂, respectively.

Test weight being an important yield attributing character, use of parents with high test weight in breeding programme may be most desirable. The results on per cent heterosis for 1000-

grain weight revealed that out of 48 crosses as many as 4 in each of E₁ and E₂ showed significant positive heterosis. Environment wise percent standard heterosis ranged from -36.59 to 10.71 and -30.41 to 13.41 with mean values -18.80 and -16.52 in E₁ and E₂, respectively. The highest standard heterosis for 1000-grain weight was observed in cross 5×7 (22.89 %) in E₁ and 15.79% in E₂. The crosses 1×13, 2×10, 3×14 and 5×12 exhibited significant positive heterosis in both the environments and 1×11 in E₂. Heterosis for test weight in pearl millet was also reported by Chotaliya *et al.* (2009)^[7] and Vaghasiya *et al.* (2009)^[20].

The estimates of heterosis for panicle girth revealed that crosses 1×10, 1×13, 3×11 and 5×8 exhibited significant positive values of heterosis in both of the environments and 6×10 in E₂. Environment wise percent standard heterosis ranged from -49.68 to 11.86 and -36.36 to 15.49 with mean values -16.06 and -17.21 in E₁ and E₂, respectively.

The results on percent heterosis for panicle length revealed that out of 48 crosses as many as 5 in E₁ and E₂ exhibited significant positive heterosis. The cross 1×10 exhibited significant positive values in both of the environments. The other desirable crosses in one of the two environments were 2×7 (E₂), 2×13 (E₂), 4×12 (E₂) and 6×13 (E₁). Environment wise percent standard heterosis ranged from -19.76 to 25.21 and -35.03 to 21.97 with mean values -6.86 and -11.81 in E₁ and E₂, respectively.

The estimates of heterosis for harvest index revealed that crosses 1×7 and 3×11 exhibited significant positive values for heterosis in both the environments and 1×10 (E₂), 6×10 (E₂) and 3×14 (E₂). Environment wise percent standard heterosis

ranged from -11.49 to 18.84 and -14.00 to 28.33 with mean values 0.85 and 0.99 in E₁ and E₂, respectively. Hybrids displayed desirable heterosis for almost all the characters except plant height. The desirable heterosis has also been reported by Dangaria *et al.* (2009a)^[7-15-20]; Vagadiya *et al.* (2010)^[19] and Jethva *et al.* (2012)^[9] in pearl millet. Low heterosis may also be obtained for some characters and some crosses as also reported by Lakshmana *et al.* (2010)^[11]. Heterosis in cross pollinated crops such as in pearl millet is an important phenomenon and also reported by Yadav (1999)^[23]; Vagadiya *et al.* (2010)^[19] and Kathale *et al.* (2013)^[10] for grain yield and its components characters.

The direction and magnitude of heterosis varied from cross to cross in two different environments. This indicates environmental influence on the expression of hybrid vigour. The hybrid combination 3×11 (81A₅×ERC) expressed significant positive heterosis for grain yield, effective tillers, panicle girth and harvest index. The hybrids namely 1×13 (81A₁×99HS141) for days to 50% flowering, 1000-grain weight, panicle girth and 4×12 (81B₁×H77/833-2) for dry fodder yield, effective tillers, panicle length expressed high heterosis for these productivity traits. The other crosses namely 1×10 (81A₁×MRC) exhibited desirable heterosis for grain yield, effective tillers, panicle girth, panicle length and harvest index; 3×14 (81A₅×HTP 92/80) for grain yield, 1000-grain weight and harvest index; 6×10 (81B₅×MRC) for grain yield, panicle girth and harvest index. These crosses involved the 81A₁ and 81A₅ cytoplasmic sources. This clearly revealed superiority of these two cytoplasmic sources to produce heterotic hybrids.

Table 2: Analysis of variance for Randomized Block Design for some quantitative traits in two environments

Source of variation	d.f.	Grain yield (g/plant)		Dry fodder yield (g/plant)		Days to 50% flowering	
		E ₁	E ₂	E ₁	E ₂	E ₁	E ₂
Replication	2	7.70	0.37	19.85	219.50	13.35	12.21
Treatment	48	35.51**	29.03**	388.90**	407.40**	64.51**	14.39**
Error	96	9.75	9.57	51.80	42.68	5.10	91.40

Source of variation	d.f.	Plant height (cm)		Effective tillers (No./plant)		1000-grain weight (g)	
		E ₁	E ₂	E ₁	E ₂	E ₁	E ₂
Replication	2	577.09	90.27	0.01	0.05	0.43	0.02
Treatment	48	744.93**	677.85**	0.73**	0.50**	2.60**	1.95**
Error	96	91.40	76.68	0.10	0.06	0.39	0.17

Mean squares							
Source of variation	d.f.	Panicle girth (cm)		Panicle length (cm)		Harvest Index (%)	
		E ₁	E ₂	E ₁	E ₂	E ₁	E ₂
Replication	2	0.004	0.03	15.17	1.33	4.30	6.12
Treatment	48	0.38**	0.38**	13.12**	14.82**	9.20**	21.50**
Error	96	0.05	0.05	1.79	1.81	3.97	3.67

** significant at P= 0.01

E₁ and E₂ = Normal and late sown crops, respectively

Table 3: Estimates of heterosis (%) of top five hybrids and their *per se* performance (in parentheses) for different characters in two environments

Character	Hybrids	Pedigree	Mean		Heterosis over standard check		Env. mean	Number of crosses showing significant desirable heterosis	
			E ₁	E ₂	E ₁	E ₂		E ₁	E ₂
Grain yield (g/plant)	1×8	81A ₁ × TCH 26-1	30.65	28.53	6.44* (30.65)	8.30* (28.53)	(29.59)	8	7
	3×11	81A ₅ × ERC 99/35 (ERC)	28.91	28.05	0.37 (28.91)	6.48* (28.05)	(28.48)		
	3×14	81A ₅ × HTP 92/80	23.89	30.07	-17.04* (23.89)	14.12* (30.07)	(26.98)		
	6×10	81B ₅ × MRC/10/61 (MRC)	30.51	26.03	5.93* (30.51)	-1.21 (26.03)	(28.27)		

	1×10	81A ₁ × MRC /10/61 (MRC)	24.96	32.32	-13.33* (24.96)	22.67* (32.32)	(28.64)		
Dry fodder yield (g/plant)	4×12	81B ₁ × H77/833-2	76.87	96.90	-6.94 (76.87)	12.33* (96.90)	(86.88)	0	2
	1×14	81A ₁ × HTP 92/80	62.63	98.87	-24.17* (62.63)	14.61* (98.87)	(80.75)		
	3 x 13	81A ₅ × 99HS141	90.97	88.53	10.13	2.63	(6.38)		
	4 x 10	81B ₁ × MRC /10/61 (MRC)	91.27	90.53	10.49	4.95	(7.72)		
	5 x 7	81B ₄ × H77/29-2	91.47	90.20	10.73	4.56	(7.64)		
Days to 50 % flowering	4×13	81B ₁ × 99HS141	44.17	40.67	-8.62* (44.17)	-12.23* (40.67)	(42.42)	7	4
	6×8	81B ₅ × TCH 26-1	46.20	46.00	-4.41* (46.20)	-0.72 (46.00)	(46.10)		
	1×9	81A ₁ × G 73- 107	44.47	45.50	-8.00* (44.47)	-1.80 (45.50)	(44.98)		
	1×13	81A ₁ × 99HS141	47.17	42.93	-2.41 (47.17)	-7.34* (42.93)	(45.05)		
	3×8	81A ₅ × TCH 26-1	45.33	45.17	-6.21* (45.33)	-2.52 (45.17)	(45.22)		
Plant height (cm)	1x 9	81A ₁ × G 73- 107	166.23	146.40	4.68	5.78	(5.23)	0	0
	2x 7	81A ₄ × H77/29-2	184.73	181.20	7.28	7.58	(7.43)		
	4 x 12	81B ₁ × H77/833-2	177.93	189.87	3.33	12.73	(8.03)		
Effective tillers (No./plant)	1×9	81A ₁ × G 73- 107	3.00	3.47	8.43* (3.00)	36.84* (3.47)	(22.63)	17	11
	1×10	81A ₁ × MRC/10/61 (MRC)	2.13	3.20	-22.89* (2.13)	26.32* (3.20)	(2.66)		
	2×13	81A ₄ × 99HS141	3.73	3.13	34.94* (3.73)	23.68* (3.13)	(3.43)		
	3×11	81A ₅ × ERC/99/35 (ERC)	3.07	2.80	10.84* (3.07)	10.53* (2.80)	(2.93)		
	4×12	81B ₁ × H77/833-2	3.87	2.73	39.76* (3.87)	7.89* (2.73)	(3.30)		
1000- grain weight (g)	1×13	81A ₁ × 99HS141	8.67	8.33	3.17* (8.67)	1.63* (8.33)	(8.50)	4	4
	2×10	81A ₄ × MRC/10/61 (MRC)	8.70	8.40	3.57* (8.70)	2.44* (8.40)	(8.55)		
	3×14	81A ₅ × HTP 92/80	8.67	8.27	3.17* (8.67)	0.81* (8.27)	(8.47)		
	5×12	81B ₄ × H77/833-2	8.70	8.23	3.57* (8.70)	0.41 (8.23)	(8.46)		
	1×11	81A ₁ × ERC/99/35 (ERC)	8.33	9.30	-0.79 (8.33)	13.41* (9.30)	(8.81)		

Table contd.....

Character	Hybrids	Pedigree	Mean		Heterosis over standard check		Env. mean	Number of crosses showing significant desirable heterosis	
			E ₁	E ₂	E ₁	E ₂		E ₁	E ₂
Panicle girth (cm)	1×10	81A ₁ × MRC/10/61 (MRC)	2.80	2.56	8.01* (2.80)	8.26* (2.56)	(2.68)	8	8
	1×13	81A ₁ × 99HS141	2.73	2.54	5.44* (2.73)	7.30* (2.54)	(2.63)		
	6×10	81B ₅ × MRC/10/61 (MRC)	2.50	2.73	-3.45* (2.50)	15.49* (2.73)	(2.61)		
	3×11	81A ₅ × ERC/99/35 (ERC)	2.77	2.53	6.72* (2.77)	7.04* (2.53)	(2.65)		
	5×8	81B ₄ × TCH 26-1	2.90	2.67	11.86* (2.90)	12.68* (2.67)	(2.78)		
Panicle length (cm)	1×10	81A ₁ × MRC/10/61 (MRC)	24.27	22.33	8.98* (24.27)	6.69* (22.33)	(23.30)	5	5
	2×7	81A ₄ × H77/29-2	22.46	21.43	0.87 (22.46)	2.39* (21.43)	(21.94)		
	2×13	81A ₄ × 99HS141	22.67	22.13	1.80 (22.67)	5.73* (22.13)	(22.40)		
	4×12	81B ₁ × H77/833-2	22.50	23.33	1.05 (22.50)	11.46* (23.33)	(22.91)		
	6×13	81B ₅ × 99HS141	23.99	21.27	7.72* (23.99)	1.59 (21.27)	(22.63)		
Harvest index (%)	1×7	81A ₁ × H77/29-2	29.64 (32.96)	36.46 (37.12)	7.86* (29.64) (32.96)	28.33* (36.46) (37.12)	(33.05)	15	16

	6×10	81B ₅ × MRC/10/61 (MRC)	26.35 (30.86)	25.58 (30.31)	0.97 (26.35) (30.86)	4.79* (25.58) (30.31)	(25.96)		
	1×10	81A ₁ × MRC/10/61 (MRC)	24.84 (29.87)	33.71 (35.46)	-2.28 (24.84) (29.87)	22.61* (33.71) (35.46)	(29.27)		
	3×14	81A ₅ × HTP 92/80	25.52 (30.30)	32.20 (34.55)	-0.87 (25.52) (30.30)	19.44* (32.20) (34.55)	(28.86)		
	3×11	81A ₅ × ERC/99/35 (ERC)	31.85 (34.30)	30.90 (33.76)	12.21* (31.85) (34.30)	16.70* (30.90) (33.76)	(31.37)		

*significant at P= 0.05

Bold figures in parentheses are angular transformed values

E₁ and E₂ = Normal and late sown crops, respectively

Table 4: Mean and range of heterosis (%)

	Grain yield (g/plant)				Dry fodder yield (g/plant)				Days to 50% flowering			
	E ₁	HC (%)	E ₂	HC (%)	E ₁	HC (%)	E ₂	HC (%)	E ₁	HC (%)	E ₂	HC (%)
Mean	26.23	-8.91	24.62	-6.57	74.10	-10.29	79.38	-7.98	53.00	9.65	47.48	2.47
Minimum	19.84	-31.11	17.92	-31.98	48.33	-41.49	43.97	-49.03	44.17	-8.62	40.67	-12.23
Maximum	35.73	24.07	32.32	22.67	91.47	10.73	98.87	14.61	60.83	25.86	51.83	11.87

	Plant height (cm)				Effective tillers (No./plant)				1000- grain weight (g)			
	E ₁	HC (%)	E ₂	HC (%)	E ₁	HC (%)	E ₂	HC (%)	E ₁	HC (%)	E ₂	HC (%)
Mean	165.12	-4.11	153.12	-9.09	2.63	-4.99	2.34	-7.81	6.82	-18.80	6.85	-16.52
Minimum	118.47	-31.20	121.90	-27.63	1.53	-44.58	1.67	-34.21	5.33	-36.59	5.71	-30.41
Maximum	191.67	11.30	189.87	12.73	3.87	39.76	3.47	36.84	9.30	10.71	9.30	13.41

	Panicle girth (cm)				Panicle length (cm)				Harvest index (%)			
	E ₁	HC (%)	E ₂	HC (%)	E ₁	HC (%)	E ₂	HC (%)	E ₁	HC (%)	E ₂	HC (%)
Mean	2.18	-16.06	1.96	-17.21	20.74	-6.86	18.46	-11.81	30.82	0.85	29.21	0.99
Minimum	1.30	-49.68	1.51	-36.36	17.87	-19.76	13.60	-35.03	27.05	-11.49	24.87	-14.00
Maximum	2.90	11.86	2.73	15.49	27.88	25.21	25.53	21.97	36.32	18.84	37.12	28.33

HC-heterosis over check

E₁ and E₂ = Normal and late sown crops, respectively

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