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Research Article

Heterosis in relation to genetic diversity in pearl millet (*Pennisetum glaucum L.*)

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Abstract: In the advent of failure of some transgenics at the field level and concerns due to biosafety and bioethical issues, there much thrust on natural plant genetic diversity as a means of adaptive value. Plant breeding basing on natural genetic diversity has gained momentum along with marker assisted breeding technologies for increased production and sustenance to various kinds of stresses. In the present investigation, divergence classification (DC) – a method devised by Arunachalam and Bandyopadhyay (1984) were used to classify 28 Pearl millet genotypes to draw a limit of parental diversity in expressing maximum heterosis. Mahalanobis D^2 (D^2 Values) statistic was used to measure the genetic diversity and then the genotypes were classified in to four divergence classes based on mean and standard deviation of all D^2 values. According to divergence classification DCI involved most distantly and DCIV the most closely related parents where as DCII and DCIII involved the medium divergent parents. A scoring system was adopted to work out the relative order of importance of the divergence classes. The overall scores for each divergence classes based on the proportion of crosses (q) showing significant heterosis (heterosis over better parent) in desired direction and mean (y) of such crosses for ten characters were carried out to rank the divergence classes. According to the scoring system, the most desirable class would be with the lowest total score. Results clearly showed the superiority of class DCIII followed by DCII, as both the classes received low overall score and maximum number of heterotic cross combinations. In conclusion it can be said that divergence classification appears to be effective in clubbing the pearl millet genotypes for parental diversity and suggested that pearl millet parents with intermediate diversity would be used to produce heterotic cross combination.

Keywords: Pearl Millet, Genetic diversity, Heterosis, Divergence Classification

Introduction

Pearl millet (*Pennisetum glaucum* L.) is a staple food crop in arid and **semi-arid** areas of Africa and Asia (Khairwal *et al.,* 1999). Pearl millet is capable of producing a reliable yield under the marginal environments and simultaneously responds to good management conditions. About 89.5% of the cultivated area under pearl millet is rainfed, i.e. without irrigation (All India Coordinated Research Project-Pearl Millet, 2016). The total area under pearl millet peaked from 9.02 million hectares in 1950-51 to 13.93 million hectares in 1973-74. After 1973-74 (The era of green revolution) the area under this crop started decreasing and has reached to the level of 7.12 million hectares in 2015-16 (AICRP-PM, 2017). In spite of area shrinkage, the production of pearl millet increased constantly to 8.06 m.t. in 2015-16 (AICRP-PM, 2017). As an average of latest data of four years (2012-13 to 2015-16) Rajasthan, Uttar Pradesh, Maharashtra, Haryana and Gujarat accounted for 94.82% of total area under Pearl millet and contributed to 87.70% of total production (AICRP-PM, 2017).

In the cultivar development greater emphasis has been given on genetic diversification of both seed and pollinator parents. As a result, improvement in grain productivity has further increased to 20 kg/ha/year. To meet the demand of the growing population and we would have to intensify the research efforts.

Heterosis breeding has been recognized as the most suitable breeding methodology for obtaining quantum jumps in the production and productivity of pearl millet. Selection of suitable parents and assessment of degree of heterosis in the resulting crosses forms an important step (Salagarkar and Wali, 2016). Precise information on the nature and degree of genetic diversity helps the plant breeder to choose the diverse parents for purposeful hybridization to improve the yield and quality (Vajire *et al.,* 2017). Genetic diversity is a pre-requisite for any crop improvement programme, as it helps in the development of superior recombinants (Manonmani and Fazlullah Khan, 2003). Pearl millet is endowed with a rich reservoir of genetic variability for various yield components, adaptation and quality traits (Berwal and Khairwal,

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1997). The higher genetic distance between parents, the higher heterosis in progeny can be observed (Lahbib *et al.,* 2012). However, when divergent parents are crossed, heterosis was not observed always. It has also been demonstrated that genetic diversity is necessary for significant levels of heterosis, but is not sufficient to guarantee it (Cress, 1966). Therefore, establishing the possible limits to parental divergence within which there are reasonably high chances for the occurrence of heterosis is an important issue that needs to be addressed. In the present investigation the multivariate analysis methods such as Mahalanobis D^2 statistic (Mahalanobis, 1936) and divergence classification (Arunachalam and Bandyopadhyay, 1984) were used to classify the genotypes and to draw the limits of parental divergence in expressing maximum heterosis.

Materials and Methods

Experimental material consisted of 22 lines and 6 testers, which have been collected from TriMurti Plant Sciences Private Ltd., Hyderabad. (Table 1). These lines were multiplied in kharif 2014. 132 cross combinations were made in a Line X tester mating design of Kempthorne (1957) during Kharif 2015. The parents and F_1'S were grown in a randomized block design with three replications at R&D Station, TriMurti Plant Sciences Private Ltd., Hyderabad. Experimental plot consisted of five-meter long rows were spaced 60 cm apart and plants with in rows were spaced 15 cm apart. Each entry was grown in one row for recording observations. The observations were recorded on 12 quantitative characters. Recommended package of practices for pearl millet cultivation was followed for growing the experimental material.

The divergence analysis among the parents were carried out using Mahalanobis D^2 statistic (Mahalanobis, 1936). Divergence classification of the genotypes were done based on mean and standard deviation of all D^2 values, as given by Arunachalam and Bandyopadhyay (1984). The method was devised to delineate the parental divergence into four divergence classes (DCI, DCII, DCIII, and DCIV). The mean (m) and standard deviation (s) of the D^2 values were calculated and then divergence classes were defined as follows:

DCI : D^2 > or = (m+s).
DCII : D^2 < (m+s) and > or = m.
DCIII: D^2 > or = (m-s) and < m.
DCIV: D^2 < (m - s).

Table 1. Experimental material and their characters

Lines	Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13
Females														
L1	610001	55	143.5	30	14.5	7.8	6	49.8	3.5	0.39	0.22	32.4	17.5	7.6
L2	610004	57	112.1	31	13.8	8.5	6	49.4	3.3	0.88	0.50	121.4	30.8	9.7
L3	610005	53	105.3	24	15.3	9.5	6	50.1	3.5	0.16	0.09	116.6	10.2	9.9
L4	610006	53	76.7	26	13.2	7.5	4	46.2	3.5	0.03	0.02	113.6	30.6	2.4
L5	610009	64	140.6	28	17.2	11.3	5	48.2	3.5	0.23	0.13	34.9	23.0	9.8
L6	610011	54	79.7	28	24.1	10.5	4	52.4	3.4	0.35	0.20	25.1	10.9	9.9
L7	610012	63	88.4	29	23.9	11.5	6	63.1	3.5	0.47	0.27	39.1	18.2	8.8
L8	610016	53	72.5	29	19.3	10.7	5	51.8	3.5	0.27	0.15	30.1	17.0	9.5
L9	610017	52	55.7	28	16.8	8.4	4	55.4	3.7	0.15	0.08	114.2	33.1	8.4
L10	610019	52	122.5	29	15.8	10.5	4	46.4	3.7	0.57	0.33	103.7	24.9	11.7
L11	610020	51	61.3	28	12.8	7.6	4	62.2	4.0	0.22	0.12	28.3	19.5	8.8
L12	610021	53	66.0	29	15.2	7.7	6	63.7	3.2	0.11	0.06	16.8	10.1	6.8
L13	610022	56	93.0	28	23.3	8.6	6	67.4	3.4	0.28	0.16	39.4	15.7	8.2
L14	610023	54	73.8	27	20.3	7.7	4	66.7	3.8	0.18	0.10	21.0	9.4	4.7
L15	610025	56	64.8	29	12.8	8.6	5	69.8	3.4	0.20	0.11	94.0	31.5	9.2
L16	610026	65	64.3	30	22.5	11.4	5	64.0	3.5	0.36	0.21	17.7	8.7	8.2
L17	610031	54	154.9	30	22.4	10.5	6	70.0	3.5	0.36	0.20	30.3	18.5	12.4
L18	610032	54	157.3	28	21.2	9.9	5	67.8	4.4	0.42	0.24	24.6	15.3	13.2
L19	610033	52	158.3	28	25.0	10.5	5	72.3	3.5	0.44	0.25	24.6	14.3	12.2
L20	610034	55	81.2	29	15.5	7.7	4	56.0	3.6	0.43	0.25	14.7	8.1	7.4
L21	610038	62	155.6	30	25.8	9.5	4	67.1	3.6	0.29	0.16	88.4	25.7	11.4
L22	610048	54	77.5	28	21.2	8.6	5	63.5	3.4	0.22	0.13	95.7	22.0	6.8
Testers														
T1	640025	63	156.6	29	22.6	8.5	4	73.1	3.5	0.71	0.411	50.0	16.0	8.6
T2	640042	56	168.1	26	28.3	10.5	4	59.8	3.6	0.24	0.151	93.9	22.5	6.9
T3	640047	63	151.8	28	28.1	10.6	4	79.4	3.4	0.53	0.329	30.3	16.7	10.0
T4	640070	67	167.7	28	26.3	10.4	4	61.3	3.6	0.47	0.291	25.5	12.2	9.4
T5	640127	68	153.7	28	27.0	10.5	3	59.9	3.4	0.36	0.220	32.7	16.7	7.5
T6	640138	58	154.7	27	26.7	10.1	4	41.2	3.5	0.63	0.388	36.2	16.3	11.1

1: Days to 50% flowering, 2: Plant Height (cm), 3: Population, 4: Panicle length (cm), 5, Panicle girth (cm), 6: Productive tillers, 7: Leaf length (cm), 8: Leaf width (cm), 9: Head weight/plot (kg), 10: Grain weight/plot (kg), 11: Single panicle weight (g), 12: Single panicle seed weight (g), 13.1000 seed weight (g).

It may be noted that in this classification, DCI and DCIV were extreme divergent classes in either direction. According to this classification, the DCI involved the most distantly and DCIV the most closely related parents. On the other hand, the classes DCII and DCIII involved the medium divergent parents.

After establishment of divergence classes, the number of crosses (n) falling in each divergence class, the proportion of crosses (q) showing significant heterosis (heterosis over better parent) in desired direction and mean (y) for each character over such crosses were worked out. In case of characters such as days to 50% flowering negative direction was considered as desired direction, whereas for all others characters the positive direction was considered as desired one.

Divergence classes were ranked for their relative order of importance on the basis of values of q and y separately. In order to come to a final decision jointly on the ranking based on q and y, a scoring procedure was adopted. The divergence class which gave the highest value of q was allotted a score 1, the next best score would be 2 and so on. The same scoring procedure followed for y. The scores over q and y were added over all characters to obtain a final score for each divergence class.

Results and Discussion

The D^2 values were used to constitute the divergence classes based on the mean and standard deviation of D^2 values (Table 2). The mean of D^2 values (2051.82) and standard deviation (1161.66) were used to form the four divergence classes. So the four divergence classes were then defined by four intervals as follows:

DC=I: D^2 > or = 3213.49

DC=II: D^2 < or 3213.49 and > or = 2051.82

DC=III: D^2 > or = 890.16 and < 2051.82

DC=IV: D^2 < 890.16

Based on the divergence classification, DCIII contained the maximum number of (46 F_1) cross combinations, whose D^2 values ranged between 927.95 - 2030.69. Divergence class, DCII contained 36 F_1 cross combinations followed by DCIV and DCI containing 26 and 24 F_1 cross combinations, respectively. In this experiment, 132 F_1 cross combinations were dealt to get precise result.

Table 2. Divergence classification of Pearl millet genotypes based on mean and standard deviation of **D^2** values.

Divergence classes	Range of D^2 values	No. of crosses	Line X Tester Pairs							
DC I	3237.72 - 4714.62	24	L2T4	L2T5	L3T3	L3T4	L3T5	L3T6	L4T1	L4T3
			L4T4	L4T5	L4T6	L9T3	L9T4	L9T5	L10T5	L11T2
			L11T3	L11T4	L11T5	L12T4	L12T5	L15T4	L15T5	L20T5
DC II	2091.9 - 3181.23	36	L1T5	L2T2	L2T3	L3T1	L4T2	L6T5	L8T2	L8T4
			L8T5	L9T1	L9T2	L9T6	L10T3	L10T4	L11T1	L11T6
			L12T1	L12T2	L12T3	L12T6	L14T2	L14T3	L14T4	L14T5
			L14T6	L15T1	L15T2	L15T3	L15T6	L20T2	L20T3	L20T4
			L22T3	L22T4	L22T5	L22T6				
DC III	927.95 - 2030.69	46	L1T2	L1T3	L1T4	L1T6	L2T1	L2T6	L3T2	L5T2
			L5T6	L6T1	L6T2	L6T3	L6T4	L7T2	L8T1	L8T3
			L8T6	L10T1	L10T2	L10T6	L13T1	L13T2	L13T3	L13T4
			L13T5	L13T6	L14T1	L16T1	L16T2	L16T4	L16T5	L16T6
			L17T2	L17T4	L17T5	L18T2	L18T4	L18T5	L19T2	L19T4
			L19T5	L20T1	L20T6	L21T6	L22T1	L22T2		
DC IV	232.72 – 862.61	26	L1T1	L5T1	L5T3	L5T4	L5T5	L6T6	L7T1	L7T3
			L7T4	L7T5	L7T6	L16T3	L17T1	L17T3	L17T6	L18T1
			L18T3	L18T6	L19T1	L19T3	L19T6	L21T1	L21T2	L21T3
			L21T4	L21T5						

The overall scores based on the significant and desirable heterosis (q) and mean (y) of such crosses for all the characters were given in Table 3. DCIII received an overall score of 29 followed by DCII (35), DCI (67), DCIV (69). According to the scoring system the most desirable class would be with the lowest total score. The results clearly showed the superiority of classes DCIII and DCII. Among the divergence classes DCIII contained the maximum number of heterotic crosses (Table 2) and received the lowest overall score (Table 3) suggesting that this class was the most desirable class followed by DCII. These results agree with those of Arunachalam and Bandyopadhyay (1984).

Table 3. Scores based on heterosis for four divergence classes

DC	n	DFF				PH				PL				PG			
		q	y	a	b	q	y	a	b	q	y	a	b	q	y	a	b
I	24	20	59.2	**3**	4	5	162.8	3	4	2	27. 9	4	2	8	11.3	3	3
II	36	24	58.1	2	2	9	171.7	1	2	6	27.3	2	3	14	11.4	1	2
III	46	26	57.7	1	1	7	172.1	2	1	9	29.1	1	1	11	11.6	2	1
IV	26	19	58.7	4	3	2	165.9	4	3	3	25.6	3	4	7	11.2	4	4

DC	n	PT				HW				GW				SPW			
		q	y	a	b	q	y	a	b	q	y	a	b	q	y	a	b
I	24	1	5.7	3	3	19	1.123	4	2	18	0.653	4	2	5	47.29	4	4
II	36	3	5.8	2	2	28	1.191	2	1	28	0.70	2	1	13	65.72	2	1
III	46	5	5.9	1	1	31	1.117	1	3	31	0.650	1	3	18	58.64	1	3
IV	26	0	0	4	4	22	1.076	3	4	20	0.639	3	4	11	60.45	3	2

DC	n	SPSW				1000 seed weight				Total score (a+b)
		q	y	a	b	q	y	a	b	
I	24	11	25.54	4	4	18	11.61	3	4	67
II	36	26	28.73	2	2	26	12.15	1	2	35
III	46	33	30.32	1	1	22	12.56	2	1	29
IV	26	20	28.33	3	3	10	12.00	4	3	69

DFF: Days to 50% flowering, PH: Plant height, PL: Panicle length, PG: Panicle girth, PT: Productive tillers, HW: Head weight/ Plot, GW: Grain weight/Plot, SPW, Single panicle weight, SPSW: Single panicle seed weight, n = Number of crosses falling in each divergence class; q = The proportion of crosses showing significant heterosis in desired direction; y = The mean of character over such crosses; a = Score on q ; b = Score on y.

According to this divergence classification the intermediate type of parental divergence (DCII and DCIII) are expected to yield maximum number of highly heterotic crosses. Out of total 25 top heterotic crosses (Table 4) examined, 20 belonged to DCII and DCIII. Similarly, Arunachalam and Bandyopadhyay (1984) in rapeseed and groundnut, Ghosh and Gulati (2002) in Indian mustard, Prasad and Singh (1986) in maize, Pal and Ghose (1992) and Ali *et al.* (1995) in *B. napus* reported that the magnitude of heterosis was higher with intermediate parental divergence.

Table 4. Top 25 heterotic crosses

Crosses	Heterosis for Grain yield	Significance	DC
610005*640127	509.68	**	**I**
610020*640042	381.19	**	**I**
610009*640042	380.31	**	III
610017*640042	354.87	**	II
610001*640127	323.46	**	II
610023*640042	321.24	**	II
610034*640070	315.81	**	II
610021*640042	307.08	**	II
610011*640042	306.00	**	III
610022*640127	297.88	**	III
610016*640127	272.16	**	II
610023*640127	267.62	**	II
610006*640042	260.62	**	II
610034*640047	253.60	**	II
610032*640127	244.30	**	III
610016*640042	238.39	**	II
610048*640042	233.63	**	III
610025*640127	231.32	**	**I**
610005*640025	227.94	**	II
610001*640042	224.81	**	III
610038*640042	213.91	**	IV
610031*640127	212.41	**	III
610022*640042	210.49	**	III
610031*640042	207.83	**	III
610011*640138	191.40	**	IV

* and ** Significant at 0.05% and 0.01% level
DC = Divergence classification, Heterosis = Better parent heterosis

Therefore, this method of divergence classification appears to be effective in clubbing the pearl millet genotypes for parental diversity and it is worthwhile to involve parents with intermediate divergence than involving those with extreme divergence to get higher frequencies of heterotic hybrids. Thus, the present study reveals the existence of limits to pearl millet parental divergence for the occurrence of better heterosis.

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