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Research Article

Genetic diversity in seed and restorer parents in relation to grain yield and its component traits in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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Abstract
Genetic diversity in the breeding materials is an essential component to improve the efficiency of any crop improvement programme. In present study, 150 seed and restorer parents were evaluated for grain yield and its component traits at two diverse agro-ecologies CCSHAU-Hisar and ICRISAT-Patancheru. Analysis of variance revealed significant differences among hybrid parents for all traits. Clustering of hybrid parents clearly partitioned almost all the B- and R-lines into separate groups indicating B- and R-lines to be genetically distant from each other. Further, seed (B-lines) and restorer (R-lines) parents were found distributed in four clusters each, and hybrid parents having same plant type (having similar set of specific traits) were found in same cluster. Significant numbers of hybrid parents having common parent in their pedigrees were found in same cluster. Cluster B-IV of B-lines and cluster R-III of R-lines had higher grain yield and above average performance for other grain linked traits. Grain yield was found to have highly positive significant correlation with all the traits under study for both B- and R-lines, except for effective tillers plant^{-1}. Stover yield followed by panicle girth and panicle length had highest positive direct effect on grain yield.

Key words
Hybrid parents, genetic divergence, clustering, association, path analysis

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is extensively cultivated for grain as well as fodder in the dry areas of South Asia, particularly India and Africa. The crop can adapt to diverse and marginal agro ecological conditions, hence is grown in environments of low and erratic rainfall, high temperature and low soil fertility. India is the largest producer of pearl millet with annual production of 9.25 million tonnes of grains from 7.8 million ha (Anonymous, 2016). The significant contribution of hybrids towards yield improvement of pearl millet in India led to adoption of hybrid technology at large scale and presently about 70 % of pearl millet cultivated area is under hybrids. This has enhanced the productivity to the present level of 1270 kg ha^{-1} which is 412% higher than the productivity (305 kg ha^{-1}) during pre-hybrid phase 1951-1955 (Gupta *et al*., 2015). Continuous breeding efforts have helped in developing a wide diverse range of hybrids adapted to different agro-climatic zones of India using genetically and cytoplasmically diverse sources of seed as well as restorer parents.

Phenotypic as well as genetic variability in the hybrid parents is a prerequisite to develop high yielding hybrids in pearl millet for different agro ecological zones. In past, several workers have documented the range and magnitude of phenotypic diversity in hybrid parents of pearl millet (Izge *et al*., 2006; Abuali *et al*., 2012; Singh *et al*., 2015). Pearl millet breeding programs in public and private sector including ICRISAT have developed large range of genetically diverse hybrid parents (both seed and restorer parents) utilizing diverse array of breeding materials. It is important to understand the patterns of genetic diversity in these hybrid parents to further formulate plant breeding strategies in pearl millet hybrid programs. One approach could be clustering of available germplasm into groups through precise phenotyping to formulate suitable breeding strategy in this crop. Hence, the present study was undertaken to assess the extent of genetic diversity among hybrid parents based on grain yield and its component traits, and to assess the levels of indicators of diversity existing for these traits to help breeders in development of higher yielding hybrids of pearl millet.

Materials and methods

A set of 150 hybrid parents, comprising of 75 seed (B-lines) and restorer parents (R-lines) each of diverse pedigrees was selected from ICRISAT Patancheru and CCS HAU Hisar pearl millet breeding programs. Each of these sixty seed and restorer parents from ICRISAT breeding program were coded from B01 to B60 and R01 to R60 respectively, while rests of each 15 seed and restorer parents from CCS HAU Hisar were coded as B61 to B75 and R61 to R75 respectively. This set of hybrid parents were evaluated in alpha lattice design with three replications in the rainy season (June to September) of 2014 under two agro-ecologically diverse environments, CCS HAU, Hisar (Env-1; 29^{0}15' N Latitude, 75^{0}7' E Longitude and 215.2 m altitude) and ICRISAT, Patancheru (Env-2; 17°53' N Latitude, 78°27' E Longitude and 545 m altitude). B- and R-lines were planted as separate blocks in each replication, side by side, to avoid growth suppressive effect of R-lines (generally taller in height than B-lines). The main block for each replication had 10 sub blocks with a size of 8 entries per block. Each entry was grown in two rows of 4m length at both the locations. Rows were spaced at 75 cm and 50 cm at ICRISAT, Patancheru and CCS HAU, Hisar respectively. Recommended agronomic practices were followed for good crop growth.

Five representative random plants of each entry in each replication were tagged and used for recording plant height (cm), number of effective tillers plant^{-1}, panicle length (cm), and panicle girth (cm). Days to 50% flowering was recorded on plot basis when the main panicle in 50% of the plants exhibited stigma exertion. All panicles of a plot were harvested separately for each entry and threshed to record grain yield (kg). Stover yield was recorded after 15 days of sun drying of leftover plant of each entry (kg). A sample of 400 seeds (in two lots each of 200 seeds) were recorded for weight and multiplied by a factor of 2.5 to estimate 1000 seed weight (g). Genetic diversity was assessed following multivariate analysis suggested by Mahalanobis (1936). The hybrid parents were grouped into different clusters according to Ward's method (Ward 1963). Pooled analysis of variance across environments were performed using replicated data to test the significance of genotype (G), environment (E) and genotype x environment interaction (GEI) using MIXED procedure of SAS (SAS Institute Inc. 2015), considering replication, genotype and environment as fixed source of variation. Individual environment residual variances were modeled into combined analysis using repeated statement in MIXED procedure. BLUE’s (Best Linear Unbiased Estimators) for genotypes x environments were estimated from combined analysis of variance. Using SAS/Stat module heritability, genetic advance, coefficient of variation and correlation coefficient were estimated for different traits. Association analysis of traits and path coefficients were analysed separately for B- and R-lines considering grain yield as dependent variable and other observed traits as independent variables.

Results and discussion

Genetic variability parameters: Mean performance of all the traits was higher at Patancheru than Hisar, except for days to flowering, plant height and panicle length. CCS HAU, Hisar being located at higher latitude than ICRISAT, Patancheru it had longer days with higher day and night temperature during rainy season (July to September, 2014) in comparison to ICRISAT, Patancheru (Fig. 1), which resulted into longer vegetative growth period in short day crop like pearl millet leading to delayed flowering and taller plants at Hisar. This was in agreement with the findings of Carberry and Campbell (1985) in pearl millet that dry matter production is higher and harvest indices is lower under extended day length conditions. Analysis of variance based on grain yield and its component traits across both the locations indicated significant variation for all the traits in the present set of hybrid parents (Table 1). Further, the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were moderate (10-20%) to high (>20%) for all the traits under study (Table 2), except GCV for days to flowering (9.84, among B-lines) again indicating availability of sufficient genetic variability for all the traits. Similar results on genetic variability for different yield linked traits in pearl millet hybrid and parental material were also reported by Vidyadhar *et al.* (2006), Vinodhana *et al.* (2013) and Chaudhary *et al* (2015). The PCV was higher than GCV for effective tillers per plant, grain yield and stover yield indicating significant effect of environment on their expression. Moreover, narrow difference between PCV and GCV for panicle length and panicle girth was observed, which suggested stability of these traits over environments. All the traits exhibited high heritability (>60 %) along with high genetic advance as per cent of mean (19.05 to 74.12%) except grain yield, indicating most of these yield component traits under the influence of additive genes. The results are similar to the findings of Vinodhana *et al.* (2013) and Singh *et al.* (2014) on hybrid parents of pearl millet which reported high heritability and genetic advance for most of these yield component traits.

Clustering pattern of hybrid parents: The genetic diversity analysis based on grain yield and its seven component traits clearly partitioned all the seed (B-lines) and restorer parents (R-lines) into clear-cut separate groups with very few (12%) cross-overs indicating B- and R-lines as two separate broad based gene pools (Fig 2). This clustering of seed and restorer parents into two groups might be due to trait specific breeding approach followed by pearl millet breeding programs in the past for the development of B- and R-lines. Seed and restorer parents in pearl millet breeding programs have been bred for a specific set of traits separately in B- and R-lines programs. For instance, maintainer or B-lines are generally bred for short height (<100 cm) and larger seed size while restorer or R-lines are generally bred for taller height (150–180 cm), more tillers, relatively small seed size, and profuse pollen production (Rai *et al.*, 2006). This practice of trait based breeding approach was evidenced from trends shown by these traits in this study also, as R-lines were found taller with lesser seed size than B-lines, while B-lines had more tillering than R-lines (Table 2).

Based on the genetic distances between hybrid parents, 75 seed and 75 restorer parents grouped into four clusters each, indicating presence of significant genetic diversity in this set of hybrid parents. The cluster B-I, B-II, B-III and B-IV of seed parents had 28, 16, 9, and 22 B-lines (Fig 3), while cluster R-I, R-II, R-III and R-IV of restorer parents had 31, 8, 18, and 18 R-lines, respectively (Fig 4). The mean values for different traits at cluster level indicated that hybrid parents having same plant type (having same set of specific traits) pooled into same cluster (Table 3). Cluster B-II and Cluster R-I had large number of lines with adaptation for arid type ecology (as being bred at CCS HAU-Hisar) as was also reflected from these clusters having lines with earliest maturity and highest number of tillers plant^{-1} (Table 3). Most of the arid type restorer parents having small and thin panicles with early maturing traits and were grouped into clusters R-I and R-II which might be the reason for lines with lowest panicle length, panicle girth and days to 50% flowering found in cluster R-I and R-II. Similar trend of clustering based on phenotypic traits was reported earlier by Chaudhary et al., (2015) and Shanmuganathan et al. (2006) in pearl millet. Lines in cluster R-III showed higher panicle length, panicle girth and stover yield, while lines in cluster B-III and R-III had higher 1000-seed weight. Parents in cluster B-I, B-IV and cluster R-III had higher grain yield.

Also, many hybrid parents from the same cluster were found to share common parent in their pedigrees. For instance, out of 28 B-lines in cluster B-I, 11 and 5 lines had HHVBC (breeding material with larger and thicker panicles) and 843B respectively, as common parent; and these HHVBC-type panicle traits dominated this cluster as evidenced from the highest mean value for panicle length (19.8 cm) and panicle girth (2.8 cm) in this cluster. Also, ten B-lines sharing the ICMB 01222 (thick panicle and erect plant type line) and five lines sharing ICMB 93333 as a common parent in their parentage and grouped in cluster B-I. Cluster R-II comprised five R-lines having Mandore Restorer Composite (MRC; composite developed at ICRISAT using lines having arid type ecology) derived hybrid parents in their parentage along with two restorers from CCS HAU, Hisar. Similarly Shanmuganathan *et al.* (2006) grouped 104 pearl millet accessions in to 12 groups based on grain yield and its component traits. Such grouping of pearl millet lines in different clusters provides opportunity to select trait specific diverse parents (Chaudhary *et al.*, 2015).

Association and contribution of yield attributes: Grain yield being a dependent variable, its improvement can be achieved by selection exercised on its component traits. In present study, grain yield had significant positive correlation with all yield components (except effective tillers per plant) in both B- and R-lines (Table 4). Earlier Singh *et al.* (2014) and Singh *et al.* (2015) also reported significant high correlation between yield and its most of component traits in hybrids and hybrid parents in pearl millet. The effective tillers had significant negative correlation with grain yield. In contradiction, some earlier studies on hybrids showed positive correlation between tillers and grain yield (Singh *et al.*, 2014; Izge *et al.*, 2006). Stover yield had highest positive direct effect while days to 50% flowering had highest negative direct effect on grain yield for both B- and R-lines indicating possibilities of development of high yielding hybrids with higher stover yield in early maturing backgrounds. Panicle length and girth also had higher positive direct effect on yield (Table 5). Similar results were reported by Mohammad *et al.* (2003) and Singh *et al.* (2014) in pearl millet. Plant height and days to 50% flowering had the highest indirect effect towards grain yield through stover yield for both B- and R-lines. Though, 1000 seed weight had negative direct effect on grain yield but was compensated by indirect positive effects through panicle girth and stover yield.

In conclusion, significant genetic diversity was observed between seed and restorer parents of pearl millet which revealed that seed and restorer parents are genetically distinct from each other and exist as separate gene pools in pearl millet. B- and R-lines clusters were identified representing specific set of traits, which can help breeder to select parents for crossing programs. Furthermore, stover yield, plant height, panicle girth and panicle length appeared to be the prominent characters and should be given maximum consideration as selection indices for grain yield improvement in pearl millet.

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Table 1. Analysis of variances for grain yield and its component traits in seed (B-lines) and restorer (R-lines) parents evaluated during Rainy season of 2014 at two locations (CCS HAU, Hisar and ICRISAT, Patancheru)

Source of variation	df	Days to 50% flowering	Plant height	Effective tillers plant^{-1}	Panicle length	Panicle girth	1000-seed weight	Stover yield	Grain yield
B-lines									
Environment	1	58.27	15.21	0.32	0.39	0.01	3.24	0.14	35.77
Replication (Env)	4	1.44	2.15	0.37	0.7	0.01	0.03	0.04	1.99
Block (Env × Rep)	30	0.39**	12.61**	0.04*	0.22**	0.01	0.05	0.01	1.22*
Genotype	74	27.26**	255.20**	0.26**	9.16**	0.15**	2.04**	1.21**	16.66**
G × E	74	5.19**	64.74**	0.19**	1.13**	0.01**	0.88**	0.53**	39.71**
Residual	266	8.58	78.15	0.18	2.1	0.05	1.97	0.43	18.17
Total	449	101.1	428.1	1.4	13.7	0.2	8.2	2.4	113.5
R-lines									
Environment	1	23.3	74.28	0.29	1.68	0.01	0.01	0.03	9.64
Replication (Env)	4	0.85	21.01	0.02	0.43	0.01	0.01	0.07	0.89
Block (Env × Rep)	30	0.08	9.14**	0.01	0.09	0.01	0.01	0.03	2.1
Genotype	74	33.05**	292.27**	0.26**	19.24**	0.25**	0.02**	1.20**	12.12**
G × E	74	9.63**	96.03**	0.36**	1.04**	0.02**	0.01**	2.23**	29.08**
Residual	266	5.74	91.92	0.14	4.22	0.02	0.01	1.43	20.94
Total	449	72.7	584.7	1.1	26.7	0.3	0.1	5.0	74.8

*, ** Significant at 0.05, 0.01 levels of probability, respectively

Table 2. Variability parameters for grain yield and its component traits in pearl millet based on two locations (CCS HAU, Hisar and ICRISAT)

Traits	Mean	GCV	PCV	Heritability (h^2_{bs})	GA (% of mean)
B-lines					
Days to 50% flowering	53.91	9.84	10.44	0.89	19.05
Plant height (cm)	116.63	13.70	14.71	0.87	26.25
Effective tillers plant^{-1}	2.70	22.10	27.34	0.65	36.72
Panicle length (cm)	18.84	16.12	16.76	0.92	31.87
Panicle girth (cm)	2.66	14.44	14.92	0.94	28.74
1000-seed weight (g)	9.27	15.52	17.68	0.77	28.01
Grain yield (kg ha^{-1})	1829.00	22.32	33.72	0.44	30.36
Stover yield (kg ha^{-1})	2742.70	40.25	44.94	0.80	74.12
R-lines					
Days to 50% flowering	53.33	10.91	11.74	0.86	20.85
Plant height (cm)	148.71	11.44	12.49	0.84	21.56
Effective tillers plant^{-1}	2.15	25.75	35.04	0.54	38.91
Panicle length (cm)	19.62	22.38	22.89	0.96	45.00
Panicle girth (cm)	2.50	20.16	20.62	0.96	40.52
1000-seed weight (g)	8.40	18.51	20.16	0.84	34.94
Grain yield (kg ha^{-1})	1919.51	18.15	27.78	0.43	24.37
Stover yield (kg ha^{-1})	3599.00	31.07	44.72	0.48	44.38

Table 3. Mean for different traits in different clusters of seed and restorer parents in pearl millet

Clusters	No. of lines	Days to 50% flowering	Plant height (cm)	Effective tillers / plant	Panicle length (cm)	Panicle girth (cm)	1000-seed weight (g)	Stover yield (kg ha^{-1})	Grain yield (kg ha^{-1})
Seed parents (B-lines)									
B-I	28	55	111	2.4	19.8	2.8	8.4	2740	1860
B-II	16	49	113	3.3	16.9	2.5	9.6	2262	1710
B-III	9	52	121	2.4	17.7	2.7	11.8	3406	1740
B-IV	22	57	125	2.7	19.3	2.5	8.8	2824	1913
Restorer parents (R-lines)									
R-I	31	50	143	2.0	18.6	2.6	8.3	3462	2016
R-II	8	46	129	3.4	15.0	1.8	6.7	2615	1431
R-III	18	58	152	1.9	22.3	2.7	9.1	4568	2156
R-IV	18	57	164	1.8	20.6	2.4	8.8	3303	1734

Table 4. Correlation coefficients among grain yield and its component traits in B- and R-lines in pearl millet

Traits	Plant height	Effective tillers/ plant	Panicle length	Panicle girth	1000-seed weight	Stover yield	Grain yield
B-lines							
Days to 50% flowering	0.37**	-0.35**	0.49**	0.52**	-0.21*	0.55**	0.47**
Plant height		-0.13	0.17	0.16	0.17	0.67**	0.60**
Effective tillers plant^{-1}			-0.24*	-0.66**	-0.17	-0.35**	-0.39**
Panicle length				0.31**	-0.12	0.26*	0.33**
Panicle girth					0.28*	0.41**	0.48**
1000-seed weight						0.25*	0.19*
Stover yield							0.82**
R-lines							
Days to 50% flowering	0.69**	-0.52**	0.34**	0.41**	0.22*	0.54**	0.27*
Plant height		-0.47**	0.53**	0.3**	0.38**	0.58**	0.44**
Effective tillers plant^{-1}			-0.34**	-0.66**	-0.63**	-0.32**	-0.28*
Panicle length				0.16	0.35**	0.3**	0.33**
Panicle girth					0.59**	0.3**	0.34**
1000-seed weight						0.26*	0.28*
Stover yield							0.71**

*, ** significant at 5 and 1 per cent level, respectively

Table 5. Direct (main diagonals) and indirect path coefficients of grain yield attributes estimated in set of B- and R-lines in pearl millet

Traits	Days to 50% flowering	Plant height	Effective tillers /plant	Panicle length	Panicle girth	1000-Seed weight	Stover yield	Grain yield
B-lines								
Days to 50% flowering	**-0.20**	0.05	0.00	0.06	0.13	0.02	0.40	0.47**
Plant height	-0.07	**0.15**	0.00	0.02	0.04	-0.02	0.48	0.60**
Effective tillers plant^{-1}	0.07	-0.02	**-0.01**	-0.03	-0.17	0.02	-0.25	-0.39**
Panicle length	-0.10	0.03	0.00	**0.12**	0.08	0.01	0.19	0.33**
Panicle girth	-0.10	0.02	0.01	0.04	**0.25**	-0.03	0.30	0.48**
1000-seed weight	0.04	0.03	0.00	-0.01	0.07	**-0.12**	0.18	0.19*
Stover yield	-0.11	0.10	0.00	0.03	0.10	-0.03	**0.72**	0.82**
Residual Effect: 0.27								
R-lines								
Days to 50% flowering	**-0.35**	0.10	0.00	0.05	0.10	-0.02	0.39	0.27*
Plant height	-0.24	**0.15**	0.00	0.08	0.07	-0.03	0.42	0.44**
Effective tillers plant^{-1}	0.18	-0.07	**-0.01**	-0.05	-0.16	0.05	-0.23	-0.28*
Panicle length	-0.12	0.08	0.00	**0.15**	0.04	-0.03	0.22	0.33**
Panicle girth	-0.15	0.04	0.00	0.02	**0.25**	-0.05	0.22	0.34**
1000-seed weight	-0.08	0.06	0.00	0.05	0.15	**-0.09**	0.19	0.28*
Stover yield	-0.19	0.08	0.00	0.04	0.07	-0.02	**0.72**	0.71**
Residual Effect: 0.41								

*, ** significant at 5% and 1%, respectively

Fig. 1. Weekly average (a) day length and (b) temperature during experimental trial at Hisar and Patancheru in rainy season 2014

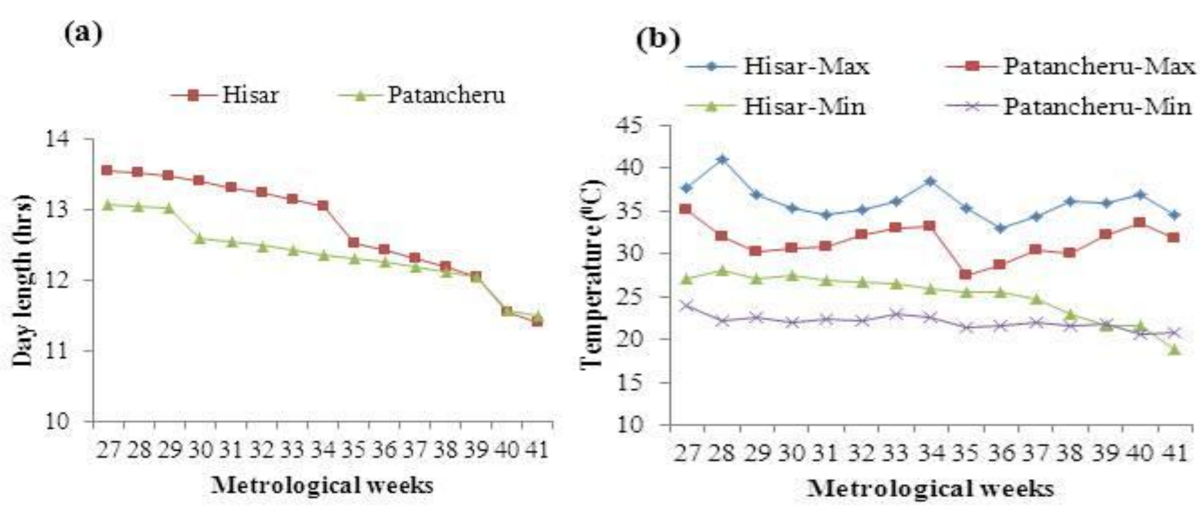

Fig. 2. Clustering pattern of 150 hybrid parents based on yield and its component traits in pearl millet

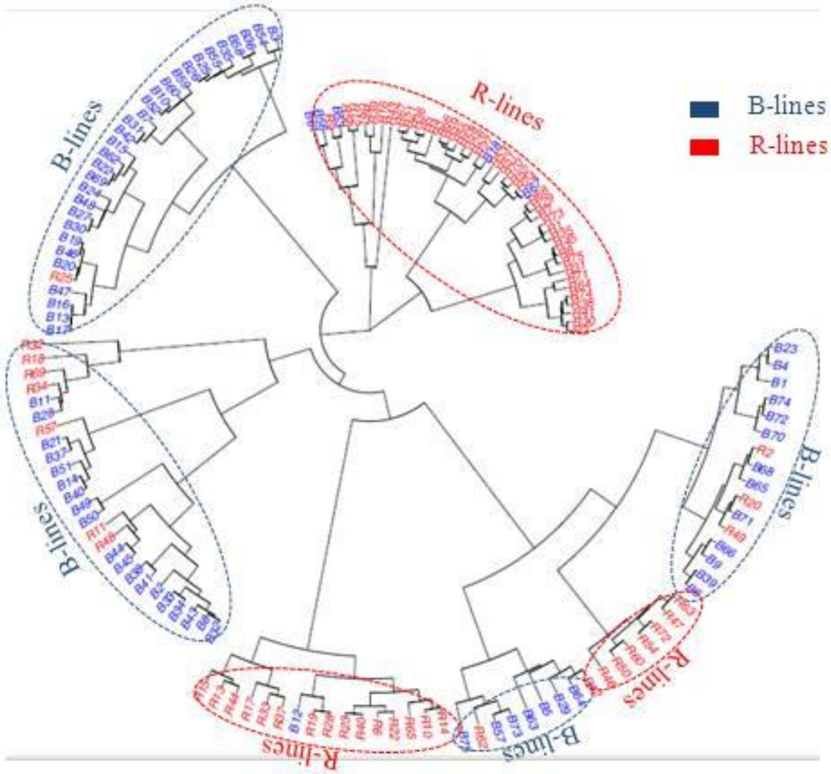

Fig. 3: Clustering pattern of 75 seed parents (B-lines) based on yield and its component traits

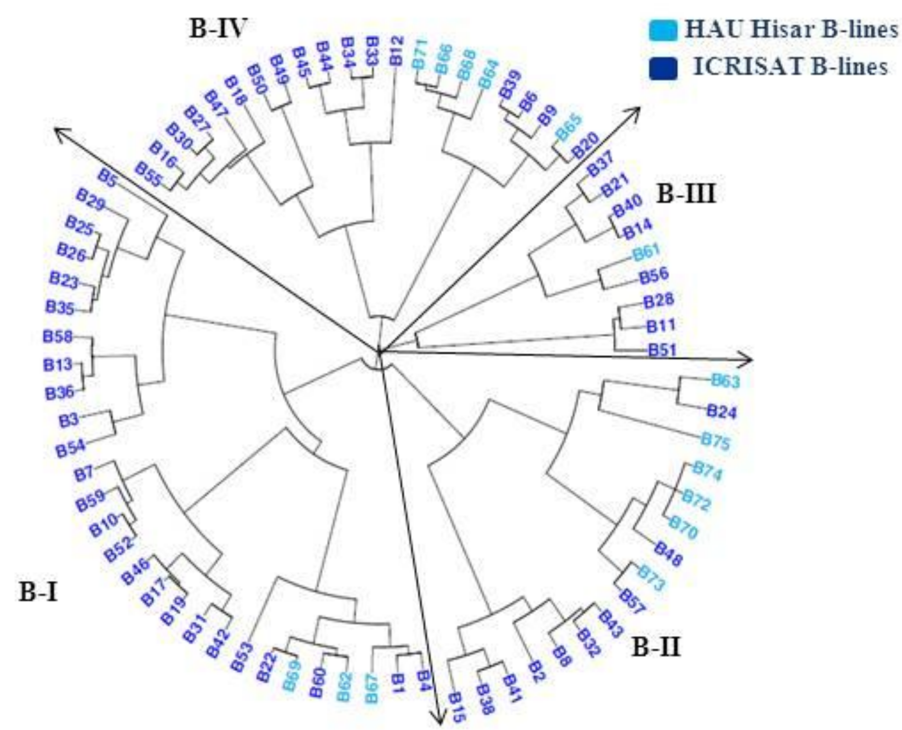

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Fig. 4. Clustering pattern of 75 restore parents (R-lines) based on yield and its component traits