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Identifying Promising Pearl Millet Hybrids Using AMMI and Clustering Models

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ABSTRACT

A set of 27 pearl millet (*Pennisetum glaucum* (L.) R. Br.]) hybrids that newly developed using A_1 cytoplasmic male-sterile lines, were evaluated over three (two wet and one dry) crop seasons (hereafter refer to as environments) in Randomized Complete Block Design (RCBD) with two replications to predict genotype by environment (G × E) interaction for grain yield and its component traits, and to identify the high yielding stable hybrids through AMMI and cluster analysis method for possible adaption. Analysis of variance showed significant genetic variation for all studied traits exists. The Additive Main Effects and Multiplicative Interaction (AMMI) analysis indicated that genotype, environment and G × E interaction highly significant for grain yield and other traits. However, G × E interaction component explained very low magnitude (3.87%) towards total genetic variation, while genotype alone contributed much higher magnitude (8.04%) in AMMI model and found TNBH 05 45 was an ideal hybrid for all three environments for grain yield (34% over best control). Diversity analysis showed seven diverse clusters following Euclidean distance coefficient of 0.91 and found TNBH 05 03 and TNBH 05 45 hybrids are promising. Based on these two models, TNBH 05 03, TNBH 39 and TNBH 05 45 were identified for stable performance *per se* in all the environments, and could be used for subsequent advanced testing and hybrid breeding programmes for possible release within regions.

Keywords

Additive main effects and Multiplicative interaction, Grain yield, Pearl millet, Stability.

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Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a climate-smart cereal, mostly cultivated on arid and semi-arid region of Africa and India, primarily for food and little area for fodder purpose (Khairwal *et al*., 1999). Pearl millet is also referred as "Crop of Camel", because of its ability to avoid and tolerate the drought condition. In India, pearl millet grown on 9.61 million hectares with the production of 10.37 million tones and productivity of 1079 kg ha^{-1} in 2011-12 (Anonymous, 2012). In India, although area under pearl millet is declining but the production remains the same, in fact, increased productivity over the years. This is simply because of domination of hybrid cultivations. It's mainly planted in rainy season crop and has wide range of yielding potential and best for grain and fodder in dryland farming. The basic cause of differences between cultivars in their yield stability is the wide occurrence of G × E interactions and its influences on genetic expression, *i.e.*, the ranking of genotypes depends on the particular environmental conditions in which they grown. These interactions of genotypes with the environments cannot be neglected and still be partly understood. For instance, grain yield highly influenced by genotype, environment and G × E interaction since the effect of genotype and environment was ascertained their yield potential expression (Falconer and Mackay, 1996). Environment interaction has negative impingement of heritability of the genotype and the G × E usually elaborates the procedure of selecting superior genotypes as well as yield and adaptation of cultivar. Several statistical models have been developed to minimize the effect of the G × E interaction in selected varieties and to predict phenotypic responses to environmental changes. However, most statistical stability approaches are not able to provide an accurate and complete variety response pattern for this interaction, due to genotype responses to environmental variation is multivariate relations and most stability indices have univariate responses (Crossa, 1990).

Conversely, AMMI (Additive Main Effect and Multiplicative Interaction) is a most widely used model to explain G × E interaction of multi-environment cultivar trial and it distinguishing the genotype into narrow or wider adaptation (Crossa *et al*., 1990). Grouping based on genotypes and environments with similar interaction and yield response by the cluster analysis. Although AMMI is an additive model but analyses of results are shown in graphs, so called biplot (Gauch and Zobel, 1997). The present study aimed to evaluate the performance of pearl millet hybrids and phenotypic stability in reducing the G × E interaction effects and make the appropriate selection for stable high-yielding hybrids.

Materials and Methods

Plant material and field trial

Field trial was comprised of 25 pearl millet hybrids including two checks (X7 and NBH 163). X7 is a public hybrid released from TNAU and NBH 163 is a private seed company-bred popular high-yielding hybrid and both were largely grown in India. All these hybrids were derived from A_1 CMS lines. Hybrid trial was planted in Randomized Complete Block Design (RCBD) with two replication and row-to-row spacing 45 cm during summer season (dry) at Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. After 15 days of planting, thinning was done to maintain plant to plant spacing at 15 cm. similar planting practice were followed in subsequent two rainy seasons. The recommended packages of practices were followed during entire crop season to grow good crop. Data were recorded on days to 50% flowering, plant height, number of productive tillers per plant, panicle length, panicle girth, grain yield per plot, seed set percentage and days to maturity. At or after physiological maturity all the plots were harvested manually and hand threshed for recording grain yield/plot and same was converted into t ha^{-1}.

Statistical analysis

Field data on aforementioned traits were subjected to analysis of variance and additive main effects and multiplicative interaction (AMMI) was worked out using the statistical package GENSTAT release 14.1 (Payne *et al*., 2011). The AMMI stability value (ASV) was calculated as previously described by Purchase *et al*., (2000). The AMMI model does not make provision for a quantitative stability measure, such a measure is essential in order to quantify and genotypes ranking according to their yield stability. In effect, the ASV is distance from zero in a two dimensional scatter gram of IPCA1 scores against IPCA2 scores. Since the IPCA1 score contributes more to GE sum of square, it has to be weighted by the proportional difference between IPCA1 and IPCA2 scores to compensate for the relative contribution of IPCA1 and IPCA2 total GE sum of squares. Cluster analysis performed using UPGMA method for estimating the diversity and grouping the hybrids upon its performance over three environments using $NTSYS_{pc}2.0$ (Rolf, 1998).

Results and Discussion

Genetic variability among hybrids

Analysis of variance was carried out to partition the total variances into its components following AMMI model and it revealed that highly significant genotypic differences exist among all the traits (Table 1). AMMI analysis of variance for stability of grain yield was obviously showed that genotypes ($p<0.01$), environments ($p<0.01$) and G × E ($p<0.05$) pattern were highly significant, showing the wider range of diversity among the hybrids. Total genotypic variation indicated that main effects of genotype, environment and G × E interaction accounted for 8.04%, 88.03% and 3.87% variation for grain yield. The G × E interaction was highly significant and it was further partitioned into two interaction principal component axes (IPCA) with the contribution of 76.89% and 23.11%, respectively. The interaction of principal component in axis-2 (IPCA2) mean sum of squares were non-significant for grain yield which is highly expected as it was much reduced magnitude compared to IPCA1. The mean performances of hybrids for each trait and wide genetic variation of hybrids for different traits was observed for days to 50% flowering (38-57 days), plant height (125-204 cm), number of productive tillers (2-8 no), panicle length (15-29 cm), panicle girth (5.2-11.1 cm), grain yield (0.19-2.40 tha^{-1}), seed set percentage (0-95 percentage) and days to maturity (80-96 days). For potential genetic variability, two folds variations were observed each for panicle girth (5.2-11.1 cm) and panicle length (15-29 cm) and four fold variations were observed for number of productive tillers (2-8). These hybrids can be up scaled as trait-specific hybrids for the regional adoption. The trial mean grain yield over the environments was 1.35 tha^{-1} and it was ranged from 1.09 tha^{-1} (TNBH 05 47) and 1.69 tha^{-1} (TNBH 05 45) (Table 2). In E1, mean grain yield was 1.83 tha^{-1} and it varies between 1.30 tha^{-1} (NBH 163) and 2.25 tha^{-1} (TNBH 05 45) and E2 has 1.69 tha^{-1} mean grain yield and ranges from 2.05 tha^{-1} (TNBH 05 39) and 1.35 tha^{-1} (TNBH 05 53). In E3, mean grain yield was 0.51 tha^{-1} and the yield range among the hybrids are 0.20 tha^{-1} (TNBH 05 53) and 0.90 tha^{-1} (TNBH 05 41). Based on the mean grain yield, TNBH 05 45, TNBH 05 08 and TNBH 05 03 were the best hybrids (Table 3).

AMMI stability value (ASV)

AMMI biplot analysis is a predominant method to find the G × E interaction for grain yield. In AMMI, the mean of genotypes which are greater than grand mean and PCA scores almost zero considered as a general adaptability over the environment. In AMMI biplot, (Figure 1) the genotypes with high mean performance and large value of IPCA scores are conceived as specific adaptability to environment. However, the quantitative measure of stability will not be provided by AMMI analysis, therefore, Purchase *et al*., (2000) proposed an ASV measure to quantify and classify genotypes according to their *per se* potential in that ASV is the distance of the varieties from point zero of the scatter diagram (IPCA1 *vs*. IPCA2). Although the IPCA1 score contributes more to the total sum of squares for the G × E interaction, it must be weighted by the relative difference between the scores of IPCA1 and IPCA2 in order to compensate for the proportional contribution of IPCA1 and IPCA2 to the total sum of squares of the interaction. Therefore, hybrids TNBH 05 10 and TNBH 05 45 recorded with lower ASV scores, were considered to be stable entries (Table 3). The ASV parameter has been successfully used in several studies to find stable performers (Mallikarjuna *et al*., 2015).

Hybrid cluster analysis

Cluster analysis using UPGMA method of 27 pearl millet hybrids with the Euclidean distance coefficient of 0.91, showed that all these hybrids are distinguished based on genetic distance and grouped into 7 clusters (Table 4, Figure 2). This would imply that there is a substantial genetic diversity among the hybrids which will be contributed by the hybrid parentage. For instance, all the female parents of these hybrids are ICRISAT-bred inbred having A_1 cytoplasmic male sterility, while, the pollinators of these hybrid come from bred-locally or from derivatives of germplasm maintained at Department of Millets, TNAU. Therefore, male counterpart expected to contribute much diversity because of two reasons; first it's deserve different origin or diversity group, secondly all these pollinators are being a germplasm derivatives its holding several loci for its heterozygosity and will not behave like inbred lines. Biggest cluster was cluster IV and smallest clusters were cluster III, V and VI. Cluster I includes three hybrids NBH 163, TNBH 05 47 and TNBH 05 20. The hybrids of cluster II were X7, TNBH 05 63. None of the hybrids within cluster I and II had similar parentage. Cluster III had only one hybrid TNBH 05 12. Cluster IV is the major cluster (14 hybrids) that includes TNBH 05 56, TNBH 05 53, TNBH 05 40, TNBH 05 57, TNBH 05 33, TNBH 05 58, TNBH 05 44, TNBH 05 42, TNBH 05 19, TNBH 05 55, TNBH 05 36, TNBH 05 13, TNBH 05 10 and TNBH 05 04. In this cluster, six hybrids had only one female parent ICMA 93111 very common and four hybrids had one common female parent ICMA 94111 while, ICMA 91666 also a female parent of four hybrids. Cluster V and VI had only one hybrid in each cluster, TNBH 05 39 and TNBH 05 14 respectively.

Table.1 Analysis of variance for grain yield and it component traits stability using AMMI model

		Mean sum of squares							
Source of variation	**df**	**DTF**	**PH**	**NPT**	**PL**	**PG**	**DTM**	**S.S%**	**GY**
Treatment	80	30.96**	691**	1.50**	9.36**	2.81**	16.17**	1435**	0.81**
Genotype (G)	26	26.85**	732**	1.91**	10.45**	2.66**	12.33**	2382**	0.20**
Environment (E)	2	679.57**	11130**	3.41**	146.69**	59.80**	311.34**	5594**	28.60**
G × E	52	8.07**	268**	0.84**	3.54*	0.70**	6.74**	801**	0.05*
IPCA 1	27	12.22**	315**	1.20**	3.81*	0.97**	8.00**	1109**	0.07**
IPCA 2	25	3.59**	218*	0.45ns	3.25ns	0.40**	5.37*	469**	0.02ns
Replication	2	3.32ns	7*	1.94**	6.05*	0.16ns	11.32*	750**	0.21**
Error	78	1.69	12	0.47	2.00	0.15	3.13	93	0.03

*,** significant at P<0.05 and P<0.01, respectively

Table.2 Mean performance, and stability parameters for grain yield of 27 pipeline hybrids

S.No	Genotype	Mean			Pooled Mean	PCA 1	PCA 2
		E1	E2	E3			
1	TNBH 05 03	2.17	2.02	0.66	1.62	0.14	0.08
2	TNBH 05 04	1.87	1.82	0.49	1.40	0.05	0.14
3	TNBH 05 08	2.07	1.97	0.86	1.64	-0.09	0.00
4	TNBH 05 10	1.83	1.72	0.55	1.37	-0.03	0.02
5	TNBH 05 12	1.55	1.37	0.70	1.21	-0.36	-0.25
6	TNBH 05 13	1.97	1.76	0.35	1.36	0.21	0.06
7	TNBH 05 14	1.82	1.72	0.75	1.43	-0.19	-0.06
8	TNBH 05 19	1.87	1.75	0.39	1.34	0.12	0.09
9	TNBH 05 20	1.50	1.47	0.29	1.19	-0.08	0.08
10	TNBH 05 33	1.82	1.51	0.55	1.30	-0.05	-0.21
11	TNBH 05 36	2.02	1.78	0.39	1.40	0.22	0.03
12	TNBH 05 38	1.95	1.92	0.82	1.56	-0.14	0.03
13	TNBH 05 39	2.22	2.05	0.47	1.58	0.31	0.16
14	TNBH 05 40	1.85	1.47	0.33	1.22	0.12	-0.17
15	TNBH 05 41	1.98	1.88	0.90	1.58	-0.19	-0.06
16	TNBH 05 42	1.80	1.69	0.38	1.29	0.07	0.09
17	TNBH 05 44	1.85	1.58	0.42	1.29	0.06	-0.09
18	TNBH 05 45	2.25	2.00	0.82	1.69	0.06	-0.07
19	TNBH 05 47	1.44	1.47	0.35	1.09	-0.16	0.08
20	TNBH 05 53	1.80	1.35	0.34	1.16	0.07	-0.27
21	TNBH 05 55	2.00	1.77	0.46	1.41	0.15	0.01
22	TNBH 05 56	2.03	1.49	0.33	1.28	0.25	-0.26
23	TNBH 05 57	1.89	1.78	0.20	1.29	0.27	0.21
24	TNBH 05 58	1.87	1.61	0.42	1.30	0.09	-0.07
25	TNBH 05 63	1.34	1.63	0.49	1.15	-0.32	0.27
26	X 7	1.45	1.67	0.64	1.26	-0.35	0.17
27	NBH 163	1.30	1.26	0.30	0.95	-0.24	-0.02
	Environment mean	1.83	1.69	0.51	1.35		
	PCA 1	0.65	0.08	-0.74			
	PCA 2	-0.35	0.59	-0.24			
	CV (%)		14.2%				

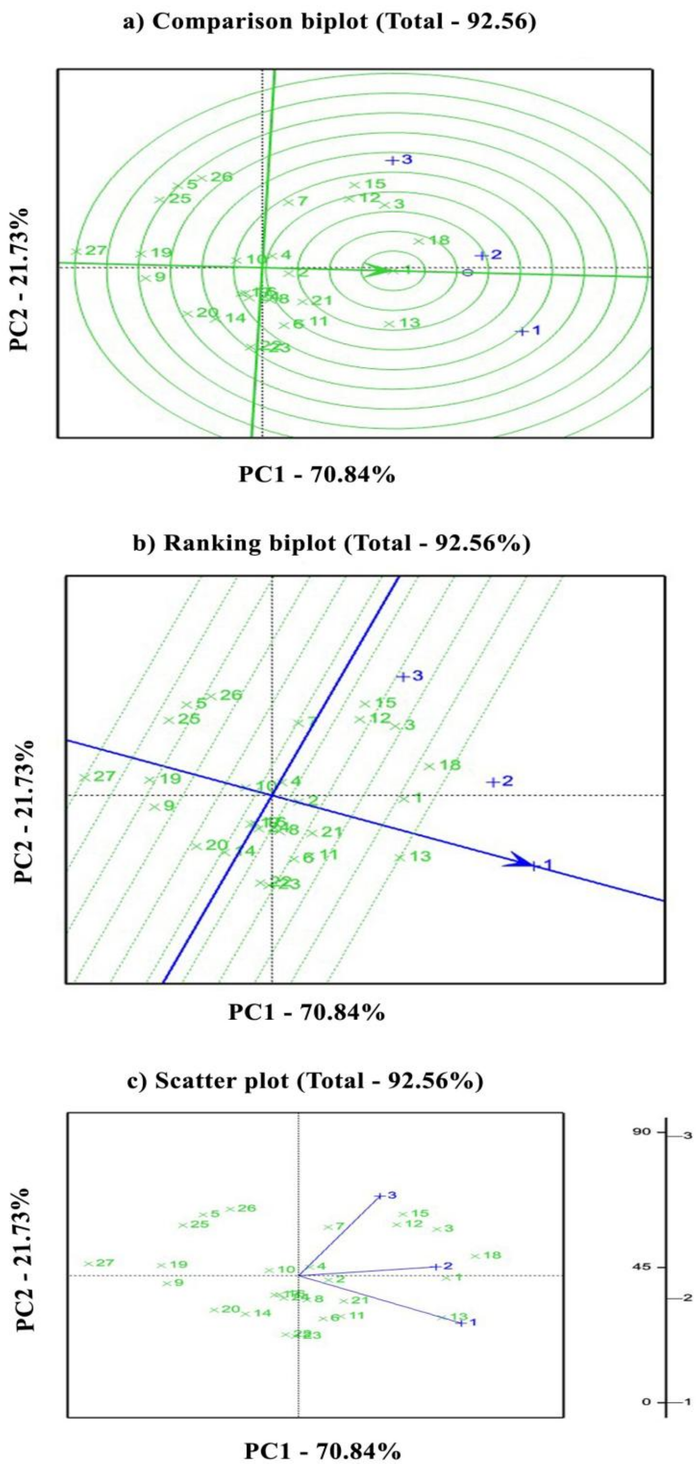
Table.3 Ranking of 27 hybrids by mean performance, IPCA 1 scores and AMMI stability value (ASV) for grain yield

S.No	Genotype	Mean	Rank	IPCA 1 score	Rank	ASV	Rank
1	TNBH 05 03	1.62	3	0.14	13	0.47	15
2	TNBH 05 04	1.40	10	0.05	3	0.19	3
3	TNBH 05 08	1.64	2	-0.09	9	0.30	8
4	TNBH 05 10	1.37	11	-0.03	1	0.10	1
5	TNBH 05 12	1.21	22	-0.36	27	1.25	27
6	TNBH 05 13	1.36	12	0.21	19	1.05	24
7	TNBH 05 14	1.43	7	-0.19	17	0.63	17
8	TNBH 05 19	1.34	13	0.12	11	0.40	12
9	TNBH 05 20	1.19	23	-0.08	8	0.27	7
10	TNBH 05 33	1.30	14	-0.05	2	0.21	4
11	TNBH 05 36	1.40	9	0.22	20	0.73	19
12	TNBH 05 38	1.56	6	-0.14	13	0.46	14
13	TNBH 05 39	1.58	5	0.31	24	1.01	23
14	TNBH 05 40	1.22	21	0.12	11	0.42	13
15	TNBH 05 41	1.58	4	-0.19	17	0.63	17
16	TNBH 05 42	1.29	16	0.07	6	0.24	6
17	TNBH 05 44	1.29	16	0.06	4	0.21	4
18	TNBH 05 45	1.69	1	0.06	4	0.13	2
19	TNBH 05 47	1.09	26	-0.16	16	0.30	8
20	TNBH 05 53	1.16	24	0.07	6	0.30	8
21	TNBH 05 55	1.41	8	0.15	15	0.50	16
22	TNBH 05 56	1.28	19	0.25	22	0.89	21
23	TNBH 05 57	1.29	16	0.27	23	0.93	22
24	TNBH 05 58	1.30	14	0.09	10	0.30	8
25	TNBH 05 63	1.15	25	-0.32	25	1.13	25
26	X 7	1.26	20	-0.35	26	1.18	26
27	NBH 163	0.95	27	-0.24	21	0.79	20

Table.4 Cluster analysis of Pearl millet hybrids for grain yield

Cluster number	Entries	Parentage		Mean grain yield (tha^{-1})
		Female	Male	
1	3	ICMA 91666 , ICMA 93111	PT 2582, PT 6042	1.04
2	2	ICMA 94111, L111A	PT6029, PT 1890	1.20
3	1	ICMA91666	PT6038	1.21
4	14	ICMA 94111, ICMA 93111, ICMA 93111, ICMA 94111, ICMA 93111, ICMA 94111, ICMA 93111, ICMA 93111, ICMA91666, ICMA 94111, ICMA 93111, ICMA91666, ICMA91666 and ICMA91666	PT 6017, PT 6056, PT 6028 PT 6018, PT 6019, PT 6021, PT 6035, PT 6030, PT 2199, PT6013, PT6022, PT6040, PT6032 and PT6018	1.31
5	1	ICMA 93111	PT 6017	1.58
6	1	ICMA91666	PT6042	1.43
7	5	ICMA 93111, ICMA 93111, ICMA 93111, ICMA91666 and ICMA91666	PT6029, PT6025, PT 6036, PT6029 and PT 6017	1.62

Figure.1 AMMI - biplot for mean of genotypes and environment score for 27 hybrids at three locations



a) Biplot of genotype and environment IPCA1 score versus means.
b) Biplot of genotype and environment IPCA2 score versus means.

Figure.2 Hierarchical clustering of 27 pearl millet hybrids into 7 clusters by Unweighted Pair Group Method using Arithmetic Average (UPGMA)

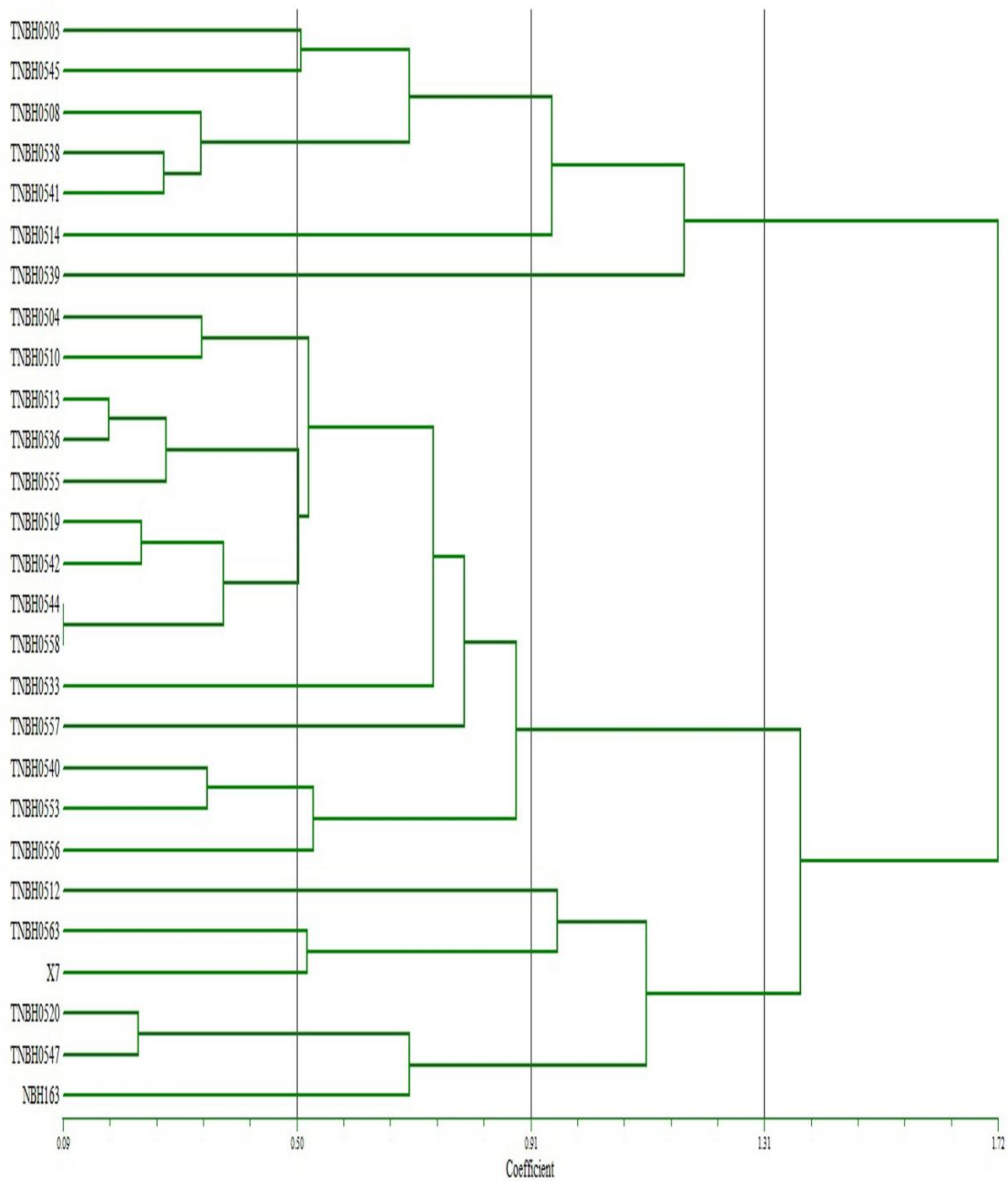
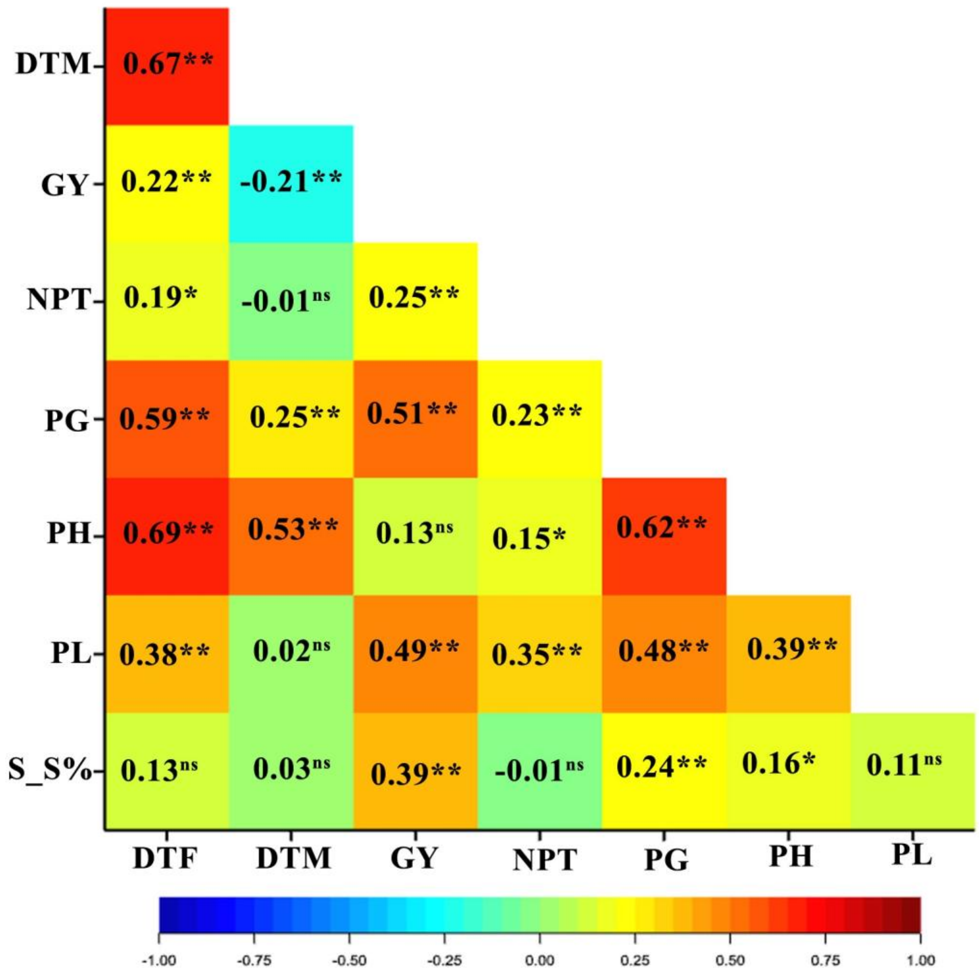


Figure.3 Correlation among yield and yield contributing traits of pearl millet



*,** significant at P<0.05 and P<0.01, respectively

The hybrids of cluster VII includes TNBH 05 41, TNBH 05 38, TNBH 05 08, TNBH 05 45 and TNBH 05 03, of which three hybrids had ICMA 93111 as a common female parents while, two hybrids had ICMA 91666 in its parentage.

The seed parent ICMA/B 91666 is D2 dwarf, late flowering (51 days) with large panicles and large seeds; seed parent ICMA/B 93111 is a D2 dwarf, late flowering but resistant to smut with large panicles, large seeds and stiff stalk that provide lodging resistance in its derived hybrids. ICMA/B 94111 is a non-d2 and 2 days earlier to flower than ICMA 93111 but it has small bristle for its identifications in seed certifications. All these male sterile lines were not much exploited so far in commercial hybrid development in both public and private seed sectors. The most interesting stuff in these hybrids *via* cluster analysis, cluster I, II, III and IV had low yielding and highly adaptable for specific environment, whereas cluster V, VI and VII had grouped as high yielding potential and highly adaptable for all over the environment (Table 4).

Character association and hybrid breeding implication

Correlation among grain yield and yield contributing traits were mostly significant (Figure 3). Grain yield has significantly positive correlation with days to 50% flowering (P<0.01), number of productive tillers per plant (P<0.01), panicle length (P<0.01), panicle girth (P<0.01) and seed set percentage (P<0.01). This indicates that all these traits will enforce selection for higher grain yield. Similar finding in pearl millet were reported (Kumar *et al*., 2014). However, days to maturity were significantly negative association (P<0.01) and plant height was non-significant association to the grain yield indicates that selection of entries for higher yield would leads to early maturity which is more interesting but such result merits further investigation to confirm as some study showing controversial to this statement (Govindaraj *et al*., 2011).

Plant height had positive and highly significant association with days to 50% flowering (P<0.01), days to maturity (P<0.01) and panicle girth (P<0.01) and length (P<0.01) but not correlated with grain yield would suggest that the taller hybrids will have late flowering time and the proportional late physiological maturity while it certainly having long panicle which largely and significantly contributes to its height. For instance, in the present study hybrids are influenced by 173 cm height by its panicle length 25 cm and it was supported with our result (Vinodhana *et al*., 2013).

In conclusion, the present study aimed at to identify promising stable high-yielding hybrids from initial pipeline hybrid trial with wider adaptation, high agronomic performance across environments using stability analysis. Because it can be difficult to identify a most stable hybrid and it is extremely useful for more regional and across regional cultivar recommendations, based on AMMI and Cluster analysis model for hybrid breeding program. Present study identified TNBH 05 03, TNBH 05 39 and TNBH 05 45 hybrids owing to its stable performance for grain yield and its contributing traits across environment and two of these hybrids had female parent ICMA/B 93111 (TNBH 05 39 and TNBH 05 45) and one had ICMA/B 91666 as female (TNBH 05 03) and However, hybrids TNBH 05 03 and TNBH 05 45 found to be most stable and highly adapted across environments through AMMI stability model and cluster analysis, thus these hybrids will be promoted into advanced hybrid trials for national level testing and adoptive trials.

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