Principal components of genetic diversity and association studies for yield related traits in pearl millet
\textit{[Pennisetum glaucum (L.) R. Br.]}  

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Abstract  
One sixty-eight pearl millet germplasm accessions were evaluated using multivariate techniques, principal component and cluster analysis to explore the extent of genetic diversity and association among the yield and yield contributing traits. The first three principal components PC1, PC2 and PC3 with eigen values more than one explains 25.27, 22.15 and 13.38 per cent, respectively, with a cumulative effect of 60.80 per cent of the total variation. Based on factor loading of three PC's and association analyses explained that the traits \textit{viz.}, number of productive tillers per plant, 1000-seed weight, panicle diameter and plant height function as excellent selection indicators that exhibit positive loading toward genetic diversity. In cluster analysis all the germplasm accessions under the study were sorted into seven distinct clusters. Cluster I having the highest number of lines followed by cluster IV due to similar ancestry of parental lines. The germplasm accessions 4129, 4163, 4157, 5007, 5058, 4104, 4105, 4146, 4150, 4140, 4123 and ICMV 221 exhibit diversity in both cluster and PCA analyses, indicating that utilizing these lines for hybridization procedure to harness heterosis and to select superior segregants in pearl millet.  

Keywords: Pearl Millet, PCA, Biplot, Trait Association and Grain Yield  

INTRODUCTION  
Pearl millet (\textit{Pennisetum glaucum} (L.) R. Br.) is a highly cross-pollinated, diploid (2n=14) annual C\textsubscript{4} crop native to central tropical Africa. It is widespread across Africa and India’s arid and semi-arid areas, where it was traditionally cultivated. Pearl millet exhibits a tremendous amount of diversity at both phenotypic and genotypic levels and it has protogyne and anemophily mechanisms, which meet the biological criteria for hybridization. In the process of genetic improvement of the crop, genetic diversity among germplasm based on the contribution of various qualitative and quantitative traits plays a key role, as it allows for the identification of the most divergent parents for further utilization breeding programme. Therefore, there is a need to evaluate available germplasm for their genetic diversity. Apart from genetic divergence, correlation coefficient indicates which traits have a direct impact on seed yield and provides background for selection and development of new genetically diverse inbred.  

Multivariate statistical techniques like cluster analysis (CA) and principal component analysis (PCA) based on morphological or molecular markers assists the breeders in selecting appropriate genotypes that fulfil the breeding programme objectives (Mohammadi and
Prasanna, 2003). PCA has an edge over the other as it eliminates multicollinearity among the independent variables and shows the relevance of the largest contributor to the total variance at each differentiation axis. Previously, multivariate analysis was extensively employed for genetic diversity analysis in pearl millet (Jyoti et al., 2018; Singh et al., 2018; Kaushik et al., 2018a; Rasitha et al., 2020; Sanjana Reddy et al., 2021). The goal of this study was to use PCA to assess the relative contribution of various traits to total variability in a collection of pearl millet germplasm accessions, as well as to cluster analysis to group these accessions into distinct clusters and establish an acceptable selection criterion for selecting the best parental lines for a trait-specific line development programme.

MATERIALS AND METHODS
The experiment was conducted at the Agricultural Research Station, ANGRAU, Ananthapuramu (14°41’ N, longitude: 77°40’ E and 373 m above mean sea level) in Andhra Pradesh, India, in the scarce rainfall zone. This district has an annual rainfall average of about 553 mm and is characterized by low and erratic rainfall in terms of area, time and distribution during the season. During Kharif 2020, 168 genotype, comprising 163 germplasm accessions and five popular varieties serving as checks were planted in an Augmented design. Each accession representing one row of 4 m length spaced 50 cm apart with 15 cm between hills. The field was uniformly fertilized with a basal dose of 30 kg Nitrogen, 20 kg P₂O₅ and 20 kg K₂O per hectare just before seeding and a dose of 30 kg Nitrogen per hectare 35 days after seeding. Standard cultural and agronomic practices were adopted to achieve good crop growth. Five competitive plants per genotype were selected randomly for recording observations on different characters viz., plant height (cm), number of productive tillers per plant, panicle length (cm) and panicle diameter (cm), while observations on days to 50% flowering, days to maturity and grain yield (kg/ha) were recorded on plot basis. 1000-Seed weight, a sample of 1000 grains were counted randomly from the threshed seed and the weight is recorded in grams. R-software was used to compute Pearson’s correlation coefficients for the nine individual traits (R Development Core team 2020). Variability among the genotypes was calculated using principal component analysis (PCA) following Davis (1986) using a correlation matrix in the R program (R core team 2018). The PCs with eigen value >1.0 were considered determining the agromorphological variability in the genotypes (Kaiser, 1960). For PCA analysis data was normalized using square root transformation and PC-1 and PC-2 scores were used to create a scatter plot of traits.

RESULTS AND DISCUSSION
The degree of divergence between the selected parents determines the effectiveness of producing highly heterotic hybrids and provides ample scope for selection of beneficial recombinants among the segregants. Cluster analysis is a method to classify the total entities into a number of different groups based on a set of deliberately chosen criteria, while keeping related accessions together in the same group. Based on different traits, all 163 germplasm accessions and five checks were classified into seven main groups (Table 1) using cluster analysis. These seven primary clusters further divided into sub clusters Fig. 1.

Table 1. Grouping of pearl millet germplasm accessions into different clusters

| S. No | Cluster number | Number of genotypes | Genotypes |
|-------|----------------|---------------------|-----------|
| 1     | I              | 139                 | 5012, 5016, 5007, 4096, 4183, 5014, 5034, 4082, 4181, 4155, 5020, 4089, 5029, 5010, 4084, 4112, 4085, 5044, 4167, 4178, 4111, 5042, 5057, 4107, 4081, 4141, 4116, 5041, 4160, 5072, 5049, 5004, 5030, 5021, 5035, 4054, 4166, 5005, 5024, 4156, 4110, 5058, 5022, 5053, 4164, 5069, 4169, 5063, 5003, 4037, 5064, 5040, 5091, 4115, 5011, 5017, 5070, 4171, 5066, 4170, 5051, 5026, 5052, 5076, 5054, 5069, 5095, 4083, 4109, 4130, 4088, 5074, 5025, 4148, 4146, 4180, 5059, 4165, 4151, 4113, 4135, 4131, 4168, 4120, 5037, 5039, 5094, 5100, 4121, 4176, 4108, 5065, 4175, 4163, 4149, 4132, 4172, 4126, 4087, 4177, 5068, 5066, 4127, 5008, 5060, 5038, 5061, 4106, 4103, 4179, 4114, 4138, 5046, 5001, 5043, 4157, 5094, 4129, 4124, 4090, 5087, 4128, 4143, 4119, 5002, 4138, 4161, 5105, 4142, 5045, 4157, 5050, 5013, 4123, 5015, 4134, 5033, 5102 |
| 2     | II             | 7                   | 4098, 4101, 4075, 4058, 4103, 4014, ABV 04, Pusa Comp. 612 |
| 3     | III            | 3                   | 4052, 4053, ICMV 155 |
| 4     | IV             | 11                  | 4140, 4043, 4100, 4102, 4079, 4047,4086, 4139, 4173, 4122, 4147 |
| 5     | V              | 2                   | 4104, 4105 |
| 6     | VI             | 2                   | 4146, 4150 |
| 7     | VII            | 4                   | 4158, Dhanshakti, 5036, ICMV 221 |

Note: Bolded accessions showed diverse nature in PCA scatter plot also.

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Out of seven major clusters, cluster I was the largest, with 139 accessions, followed by cluster IV with eleven accessions, cluster II with five accessions along with two recently released varieties ABV 04 and Pusa Comp. 612; cluster VII with two accessions along with two popular varieties Dhanshakti and ICMV 221 and cluster III with two accessions with one popular variety ICMV 155. Clusters V and VI, on the other hand, each included two accessions, suggesting a significant degree of variability among the accessions. In pearl millet, Ramya et al. (2017), Singh et al. (2017), Kaushik et al. (2018b), and Kumar et al. (2020) previously described clustering of germplasm accessions based on quantitative data to identify prospective parents for heterotic expression of yield components. Selection of parents from these cluster I, V and VI will serve as a potential breeding material for improving the yield attributing traits in hybridization programme. Similar results were reported by Singh et al. (2017) and Kumari et al. (2016). Early flowering genotypes mostly suitable for cultivation in drought prone area Sankar et al. (2014). Considering this point, individuals belonging to cluster I used for developing early flowering lines with higher yield.

Yield amelioration is the ultimate aim of any plant breeder in crop improvement programme which can be achieved through gaining insight knowledge on association of component traits with grain yield. The correlation matrix is a technique for concurrently analysing the interdependence of several variables simultaneously and these associated traits enables the selection of superior genotypes via phenotypic selection of easily heritable traits. In correlation matrix (Fig. 2) on the lower half, the vicariate schematic model plots with a line of best fit can be seen and upper half represents, the values of the correlation coefficients indicated with significance level as stars. Each traits distribution is represented on the diagonal whether it is distributed normally or not.

Fig. 1. Dendrogram depicting clustering pattern of 168 germplasm accessions obtained by cluster analysis in Pearl millet.

Fig. 2. Correlation for yield and yield contributing traits in Pearl millet.
In the present study, the number of productive tillers per plant had a significant positive association with grain yield (0.15*), while the 1000-grain weight (0.014) had a non-significant positive relationship. Previously, Annamalai et al. (2020) and Narasimhulu et al. (2021a) found a favourable connection between grain yield and productive tillers and 1000-grain weight. Plant height (-0.25**), panicle length (-0.13*) and panicle diameter (-0.141*), on the other hand, were shown to have a significant and negative relationship with grain yield, implying that greater scope to select high yielders with short stature, which may be useful for developing the cultivars for lodging tolerance and suitable for mechanical harvest. Days to 50% flowering and days to maturity had a non-significant negative association with grain yield, which is desirable for development of early maturity hybrids with high grain yield.

From the inter-association studies, it was observed that days to 50% flowering exhibited significant and positive relationship with days to maturity (0.74**) which was also confirmed by Narasimhulu et al. (2021b). Early flowering/maturation accessions exhibited fewer productive tillers per plant (-0.12) and larger panicle diameters (0.19*). Rasitha et al. (2019) and Narasimhulu et al. (2021a) found similar findings for the number of productive tillers per plant and panicle diameters. The length of a panicle, on the other hand, is proportionate to the plant’s height (0.49**) and panicle diameter (0.38**). These findings agree with the results of the Narasimhulu et al. (2021b). In a nutshell, the majority of the germplasm accessions were found to have small stature with a higher number of productive tillers in the early maturing type background, suggesting that there is greater potential for developing trait specific parental lines for future breeding programmes.

Principal Component Analysis was used as a reductionist approach of the multivariate data, to assess the relevance and contribution of each component to total variance. The significance of traits towards the principal components can be realized from the respective eigen values presented in Table 2. In practise, the PCA approach is used for genetic diversity and genotype grouping using biplot diagrams, with applications in crop breeding for selection of superior genotypes (Singh et al., 2018; Kaushik et al., 2018a; Rasitha et al., 2020). The results of PCA analysis indicated that the first three components had eigen values more than one and contributed around 60.80 per cent of total accumulated variability for the eight different morphological traits investigated among the 168 germplasm accessions.

The first principal component (PC1) accounted for 25.27 per cent of the total variation, largely due to grain yield (0.270), number of productive tillers (0.209) and 1000-grain weight (0.068) indicating that these traits were important contributors to variation among the accessions and high yielding lines could be distinguished based on these traits (Table 3). The existence of both positive and negative loading in a single component indicates the presence of
showed that variables and genotypes contributed maximally towards variability in germplasm. The findings of this study revealed that there is a lot of genetic diversity among the germplasm accession and the possibility of developing potential early maturing high yielder with larger seed size and more productive tillers per plant with shorter plant stature and larger panicle. A PC biplot Fig. 3 showed that variables and genotypes are superimposed on the plot as vectors. The contribution of these variables in the variation of the genotypes is represented by the distance of each variable with respect to PC1 and PC2. The biplot diagram shows that genotypes are equally scattered around the vectors, resulting in different distinct groups. The accessions 4129, 4163, 4157, 5007, 5058, 4104, 4105, 4140, 4123 and ICMV 221 were placed in different quadrants at extreme ends of the plot. This suggests that these genotypes highly varied from the rest and they are diverse from each other for all the characters. Hence, these accessions were identified as most divergent and distinct and could be used as candidate parents in crop improvement programme to develop promising hybrids. Another consideration of the biplot diagram is the angles of vectors. Kroonenberg (1985) concluded that the angle of vectors shows correlations among the traits. The biplot showed that grain yield and no. of productive tillers; day to 50 % flowering and day to maturity vectors were in overlapping to each other with a small angle, indicates that they had positive associations. Day to 50 % flowering, day to maturity, grain yield, panicle diameter, panicle length and plant height contributed maximum towards variability in germplasm.

The findings of this study revealed that there is a lot of genetic diversity among the germplasm accession and the possibility of developing potential early maturing high yielder with larger seed size and more productive tillers per plant with shorter plant stature and larger panicle.
This plant type is critical in the current changing climatic situation for developing robust lines with the capacity to sustain terminal drought or water scarcity, as well as being suited for mechanical harvest. Based on both cluster and PCA analyses, the germplasm accessions 4129, 4163, 4157, 5007, 5058, 4104, 4105, 4146, 4150, 4140, 4123, and ICMV 221 were identified as prospective parents for heterotic expression and to generate potential breeding material for improving trait specific materials to enhance productivity in the pearl millet.

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Fig. 3. Biplot indicating the best genotype for the target traits

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