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Yield stability analysis of pearl millet genotypes in arid region of India using AMMI and GGE biplot

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Abstract

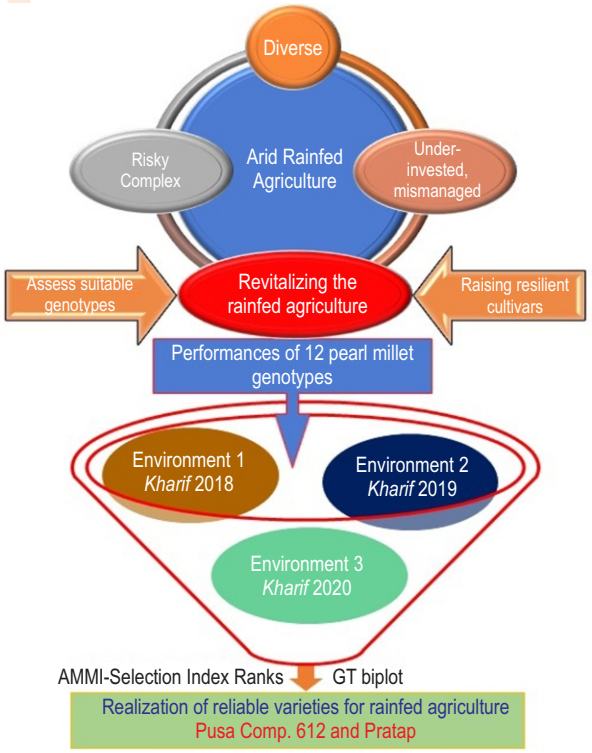
Aim: To assess genotypes with ability to sustain yields under arid region and to understand the significance of interactive effects of genotype × environment to develop resilient cultivars for rainfed farming.

Methodology: Performances of 12 pearl millet varieties and hybrids released for commercial cultivation in India, were estimated using RBD with three replications during rainy seasons of 2018, 2019 and 2020. The trials were conducted at the Agricultural Research Station, ANGRAU, Ananthapuramu, located in the Scarce Rainfall Zone of Andhra Pradesh, India, with an average annual rainfall of nearly 553 mm and 300 mm to 400 mm during rainy season.

Results: The environment and genotype effects were highly significant in the Additive Main Effect and Multiplicative Interaction (AMMI) model, implying that environments are varied and genotypes performed differently in each environment offering a great scope for selecting better adaptive genotypes. Apart from moisture stress, the amount of rainfall received during both the anthesis and grain maturation stages were influenced grain yield through plant height, 1000-grain weight and dry fodder yield. Environment 3 was the best discriminating environment and the hybrids Pratap, 86M86 and NBH 5767 had outperformed the popular open pollinated varieties in ideal conditions.

Interpretation: In terms of grain yield,Pusa Comp. 612 and Pratap have proved to be reliable and had a mean higher than the general mean over environments. As a result, Pusa Comp. 612 and Pratap are suggested for large-scale assessment in a wide range of locations in rainfed situations of arid regions.

Key words: GT-biplot, Grain yield, Pearl millet, Stability, Trait associations



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Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important food crop of arid and semi-arid regions of the world and is mainly grown under rainfed conditions where yields are commonly affected by drought and drought induced heat stress. In India, pearl millet is the fourth most widely cultivated food crop, after rice, wheat and maize. It occupies 7.41 million ha area, producing 10.3 million tonnes and has a productivity of 1391 kg ha^{-1}. The arid tracts are grown with the landraces/OPVs (open pollinated varieties) that are poor yielders. The low production of pearl millet in India necessitates the development of stable, high-yielding varieties and hybrids that are more adaptable. Cytoplasmic male-sterility (CMS) system has significantly increased the yield of pearl millet by allowing commercial hybrid seed production (Kelley *et al.,* 1996). Increased research efforts aimed at diversifying parental lines have resulted in hybrids outperforming varieties in terms of yield and comparable tolerance to biotic stresses. On the other hand, few OPVs are popularly grown owing to their resilience to marginal and harsh conditions, good grain quality and reasonable yield levels. It is critical to select genotypes, which have the ability to buffer crop production against these multiple stresses linked with climate change and produce sustainable yield in order to maximize profitability.

Ananthapuramu is a drought-prone district in the rain shadow area of Andhra Pradesh, India. The annual rainfall is low (553 mm), highly variable and erratic with prolonged dry spells of 45-50 days. Within and between years, the start and cessation of rains vary significantly throughout pearl millet growing regions. Under different stages of crop growth, soil-moisture stress would result in inadequate plant population and poor seed setting and thereby have implications for crop yields. In addition to drought and terminal heat stress, low soil fertility, strong wind speeds, shallow rooting depth, moderate slopes and salinity are the major constraints for pearl millet productivity. Therefore, the varieties or hybrids evaluated at this location are considered ideal for cultivation across the regions and seasons as reported earlier by Shanthi *et al.* (2016) and Sanjana Reddy *et al.* (2021). Evaluation of various pearl millet genotypes under diverse environments and identifying environmentally stable and productive genotypes, is crucial and one of the prime research requirements.

Yield is a dependent attribute that arises from the interplay of many related traits. The influence of environment usually results in low heritability more so in locations prone to abiotic stresses. As a result, direct selection for yield is not sufficient in increasing crop productivity. Hence, understanding the degree of correlation that exists between yield and its yield components is crucial for selection. Stability refers to a variety's or hybrid's ability to adapt to a wide range of growing conditions and maintaining the same level of production efficiency as predicted. Understanding the trait's stability over environments is a vital prerequisite for attaining sustainability in crop production and productivity. The estimates of genetic parameters obtained in one environment are biased due to the confounding of the G x E interaction effect with the genotype effects. It is therefore, necessary to take into account the G x E interaction while determining the estimates of various genetic parameters to have realistic estimates. Presence of considerable genotype and genotype by environment interactions (GEI) complicate the selection process and warrant the use of multi-environment trials (METs) to evaluate the relative performance of genotypes over the environments. Stability analysis like most popular Additive Main Effects and Multiplicative Interaction (AMMI) and GGE biplot analysis is commonly used to analyze MET data. GGE biplot is a tested tool for analyzing and interpreting complex GEI data across environments (Yan, 2001; Yan and Tinker, 2006). It can be used effectively to discriminate high yielding and stable cultivars, to determine the relationship between environments and identify ideal environments (Yan *et al.,* 2007; Ajay *et al.,* 2021; Chuni Lal *et al.,* 2021). In order to find and suggest suitable hybrids/varieties for the rainfed situation of dry regions, this study used AMMI and GGE biplot models to analyze the responses of 12 improved pearl millet genotypes to changing rainfall patterns over a period of three years in Ananthapuramu.

Materials and Methods

Experimental location and details: The experimental material involved 12 pearl millet genotypes consisting of eight released hybrids (HHB 299, NBH 5767, Pratap, GHB 558, NBH 5061, 86M86, Kaveri Super Boss and 86M64) and four popular open-pollinated varieties (Dhanshakti, ICMV 221, ICMV 155 and Pusa Comp. 612).The material was evaluated during rainy season of three consecutive years, 2018, 2019 and 2020 at the Agricultural Research Station, ANGRAU, Ananthapuramu, located in the Scarce Rainfall Zone of Andhra Pradesh, India. The district of Ananthapuramu receives an average annual low rainfall of around 553 mm and a rainy season rainfall of 300 mm to 400 mm. The region also witnesses frequent prolonged dry spells during the season due to scanty and erratic distribution of rainfall in terms of location and time during the season.

Soils are light textured, gravel, shallow Alfisols, low in nutrients. In each environment, the trial was planted immediately after first good rain (*i.e.,*> 20 mm) in a Randomized Block Design with three replications. Each entry was sown in a six rows plot of 4.0 m length keeping row-to-row and plant-to-plant distance of 50 cm and 15 cm, respectively. Two weeks after sowing, the plants were thinned to one plant per hill. The field was uniformly fertilized with recommended dose of 30 kg nitrogen, 20 kg P_2O_5 and 20 kg K_2O per hectare as basal, *i.e.,* just before seeding and 30 kg nitrogen per hectare at 35 days after seeding. To achieve good crop growth, standard cultural and agronomic practices were followed.Five competitive plants per genotype in each replication in each environment 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, dry fodder yield (kg ha^{-1}) and grain yield were recorded on plot basis. 1000-grain weight, a sample of

1000 seeds were counted randomly from the threshed seed, and the weight was recorded in grams.

Statistical analyses: AMMI model was applied for grain yield related characters using package 'agricolae' in the R program® Development Core Team, 2018). The Modified AMMI Stability Index (MASI) was calculated as described in the R program (R Development Core Team, 2018) using the package 'ammistability' (Ajay *et al.,* 2019).

$$MASI = \sqrt{\sum_{n=1}^{N'} PC_n^2 x \theta_n^2}$$

Where, PCn: scores of nth IPC; and θn: percentage sum of squares explained by the nth principal component interaction effect. Smaller MASI scores indicate a more stable genotype across environments. Simultaneous Selection Index (SSI) for yield and stability was calculated to select stable and high yielding genotypes. SSI incorporate both mean grain yield and stability in a single criterion. Low value of this parameter shows desirable genotypes with high grain yield and stability. GGE-biplot analysis was performed using package 'GGE Biplot GUI' in the R program (R Development Core Team, 2018). Variability among 12 genotypes was calculated using principal component analysis (PCA) using a correlation matrix in the R program (R Development Core Team, 2018). The PCs with eigen value >1.0 were considered determining the agro-morphological variability in the genotypes. 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 environments representing the rainy seasons of 2018, 2019 and 2020 were categorized as Environment-1, Environment-2 and Environment-3, respectively. Apart from rainfall intensity and distribution, there was a wide variation in temperature (19.5^{0}C - 35.4^{0}C), wind speed (4.4 kmph-19.5 kmph) and evaporation (4.1 mm per day -7.9 mm per day) over the crop periods, all of which had a significant influence on crop performance. Occurrence of prolonged dry spells and droughts are widespread in Ananthapuramu and the detrimental effects of drought are exacerbated by severe wind speeds, low soil depth and poor soil nutritional status in this region. All this contributes to make Ananthapuramu an excellent choice for evaluating pearl millet cultivars under rainfed conditions. Therefore, Ananthapuramu is an ideal test site for efficiently selecting pearl millet cultivars that are adaptable across India, since it provides the best discrimination and representative than other locations. According to Shanthi *et al*. (2016) across the three environmental studies as well as Sanjana Reddy *et al*. (2021) report over locations across India, Ananthapuramu is the best test site for selecting pearl millet cultivars that can be successfully adapted throughout India. In the Environment-1, the entire crop cycle was completed within 12 standard meteorological weeks, with a total rainfall of 210 mm received in 8 rainy days. This year was designated as drought year as it recorded a cumulative rainfall of 268 mm received in 14 rainy days, compared to a normal rainfall of 553 mm with average rainy days of 34. In this environment, crop endured two dry spells (defined as a stretch of more than 15 days without rain) affecting all three growth stages (GS) *viz.,* GS 1 (seedling stage to panicle initiation), GS 2 (panicle initiation to flowering) and GS 3 (flowering to grain maturity stage). The first was a 16 days dry spell at growth stage 1 and the second was 38 days long dry spell between GS 2 and GS 3 growth stages (panicle initiation and grain hardening stage) of the crop. In the Environment-2, crop period received a total of 528 mm rainfall in 26 rainy days. It was distinguished by slightly higher rainfall than the other two environments.

The crop encountered one dry spell in this environment, which was during the crop's GS 1. In environment-3 the whole crop cycle was completed in 13 standard weeks, with overall rainfall of 475 mm evenly distributed in 34 rainy days. In this environment, the crop was affected by a rain during the grain formation to harvesting stage. In environments-1 and 2, the crop was late seeded during the second fortnight of August, however, in environment-3, the crop was seeded as normal sowing during the first fortnight of June with the commencement of monsoon. Overall, as a consequence of terminal drought and heat stress, there were substantial differences in environmental conditions resulting in varied grain yields across the genotype both within and between years. Even though there was a normal sowing date and good number of rainy days with no dry spells in the Environment-3 compared to other two environments, the grain yield was affected by more number of rainy days during flowering to grain maturation stage. On other hand, the crop in the Environment-1 experienced moisture stress in the GS 2 and GS 3 stages and endured a prolonged dry spell and frequent dry spells than the other two environments. Pearl millet's two growth stages, GS 2 and GS 3, are extremely sensitive to moisture stress, resulting in poor grain yields. Therefore, when compared to Environments-2 and 3, the grain yields of all genotypes in Environment-1 were observed to be poor. These findings are consistent with Shanthi *et al.* (2016). Our findings demonstrate that among pearl millet genotypes studied have a limitation in post-flowering drought tolerance, *i.e.,* a season with no rain after flowering results in a significant yield reduction owing to reduced plant growth, 1000-grain weight and dry fodder yield.

Similarly, Halilou *et al.* (2020) reported that terminal drought during flowering resulted in reduced pollen tube development and fertilization, leading in lower seed-set, while stress during grain filling led to decrease in grain weight. It is evident that capacity of pearl millet genotypes to withstand terminal drought is also associated with the availability of moisture throughout the grain-filling stage. During the critical period of grain filling, sufficient soil moisture supports photosynthesis, allowing for continuous carbon supply to the grain (Vadez *et al.,* 2013). Environment-1, a drought year with less crop growing period (> 10 days) when compared to other

Table 1: AMMI analysis of variance of grain yield of twelve pearl millet genotypes tested at three environments

Source	df	Sum of Squares	Mean Squares	Percentage SS
Genotype (G)	11	5768832.00	524439.00**	7.14
Environment (E)	2	60880205.00	30440103.00**	75.37
G*E interaction	22	14127133.00	642142.00**	17.49
PCA 1	12	9787254.00	815604.50**	69.30
PCA 2	10	4339879.00	433987.90**	30.70
Rep (E)	6	490018.00	81670.00	
Residuals	66	5416645.00	82070.00	

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

Table 2: Mean grain yield of twelve pearl millet genotypes tested at three environments, PCA scores for the GE interaction effects as delivered from AMMI model and selection index ranks

Genotypes	Environment-1 (kg ha^{-1})	Environment-2 (kg ha^{-1})	Environment-3 (kg ha^{-1})	Mean	PCA 1	MASI	Rank (MASI)	SSI (rank mean + rank MASI)
86M64	1005	3442	1933	2127	-9.43	7	7	11
86M86	1504	2411	2721	2212	22.91	16	12	14
Dhanshakti	1119	3390	1358	1956	-13.44	10	8	16
GHB 558	846	2521	1860	1743	6.07	5	3	14
HHB 299	914	3752	1576	2081	-19.91	14	11	16
ICMV 155	1328	1935	1080	1448	13.43	11	10	22
ICMV 221	1003	2827	1666	1832	-0.44	0	1	10
Kaveri Super Boss	1526	2723	1962	2071	9.22	7	6	12
NBH 5061	1248	3016	1223	1829	-6.52	6	5	15
NBH 5767	839	3671	2083	2198	-13.73	10	9	12
Pratap	1467	3016	2465	2316	8.32	6	4	5
Pusa Comp. 612	933	2910	2257	2033	3.51	5	2	9
Local Mean	**1144**	**2968**	**1849**	**1987**				
PCA 1	**15.85**	**-34.66**	**18.81**					

environments resulted in a substantial yield loss. Plaut *et al.* (2004) reported similar effects on grain yield due to the occurrence of combined drought and heat stress during seed filling, which reduced seed-filling duration. In drought years, apart from yield drop there was a sharp decline in the plant height, 1000-grain weight and dry fodder yield, however, all the genotypes maintained a consistency in the size and thickness of the panicle across the environments. These findings were supported by reports of Bieler *et al.* (1993) for reduction in grain yield might be driven by a reduction in assimilate supply rather than a reduction in grain storage capacity. Reduced straw yields due to moisture shortage suggest a detrimental impact on assimilate production and delivery to developing grains, resulting in reduced yields. Yadav *et al.* (2002) found a similar reaction in pearl millet under terminal drought, with a 7.5 - 55.2% drop in straw yield.

The ultimate seed weight, a key component of total grain yield, is also affected by the rate and duration of seed filling (Yang and Zhang, 2006). Genotype × Environment Interactions (GEI) continue to be challenging among researchers to predict the performance of genotypes across different environments. The AMMI analysis of variance (Table 1) revealed highly significant differences among genotypes, environments and GEI. This indicated that genotypic and environments have a broad range of diversity and genotypes performance differed across environments, which will aid breeders in selecting genotypes for specific environments. The proportion of variance due to environmental variations was largest (75.37%) followed by the variance due to GEI (17.49%) and eventually variance due to genotypes (7.14%). Thus, ANOVA model only accounted for 82.51% of the overall treatment variation (Trial SS) attributed to genotypes and environment effects. In this study, GEI explained more than double the variation than genotypes alone. This explains the varied performances among the genotype with change in environment, providing great scope for selecting better adaptive genotypes.

Joint consideration of mean performance of genotypes pooled over three environments and stability parameters, revealed that hybrids Pratap, 86M86, NBH 5767, 86M64 and Kaveri Super Boss have specific adaptation, and OPVs ICMV 221 was highly stable followed by Pusa Comp. 612 with moderate stable yield (Table 2). The highly significant G x E interactions were further partitioned into two PCA axes, out of which the first

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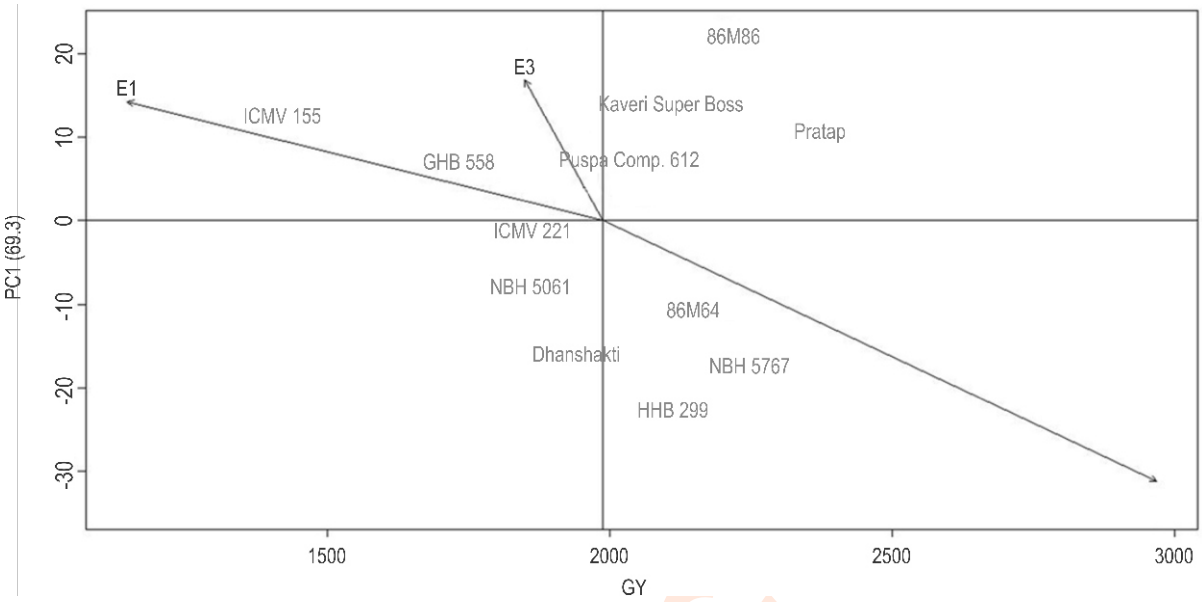

Fig. 1: AMMI biplot of twelve pearl millet genotypes across three environments for grain yield.

axes accounted for 69.30% of the variation while the second axes accounted for 30.70% of variation. In the current analysis, the two PCA axes were found to be highly significant, implying that genotype-by-environment data contains a dynamic, multidimensional variance. AMMI models with multiple axes are expected to emit more noise due to the highly complex interactions between genotypes and environments. Furthermore, if the AMMI model has more than one PCA axis, assessing and presenting genetic stability is more difficult (Nitch *et al.,* 1992; Crossa *et al.,* 1991; Sharma *et al.,* 1998; Shinde *et al.,* 2002). Graphical representation of biplot can assist in visual interpretation of GE interactions and identify stable genotypes and ideal environments with low, medium or high levels of interaction effects. The mean performance and IPCA1 score for both the genotypes and environments of the AMMI analysis (Table 2) were used to construct the biplot (Fig. 1). The abscissa (X-axis) represents the main effects (genotype and environment means), while the ordinate (Y-axis) represents the first IPCA score. The figure clearly depicts the key consequences of both the main effects and the interaction aspect.

The standard interpretation of a biplot assay is that if a genotype or an environment has a IPCA score of approximately '0', it has minimal interaction effects and when the genotype and environment have same sign on the PCA axis, their interaction is positive; if they have opposite signs, their interaction is negative (Kempton, 1984; Shinde *et al.,* 2002). As anticipated in this analysis, the Environment-2 has the highest mean grain yield, while the Environment-1, drought year, has the lowest. Genotypes with a mean greater than the grand mean and IPCA scores near zero are considered to be relatively adaptable to all environments. On the other hand, genotypes with a high mean performance and a high value of IPCA1 scores are presumed to have specific adaptability. All OPV genotypes have lower mean values than the grand mean, except Pusa Comp. 612 and most of the hybrids, high heterosis, resulting in higher means over the grand mean. It is evident from Fig. 1 that the highest grain yielders (Pratap, 86M86 and NBH 5767) were moderately stable, while ICMV 221 and Pusa Comp. 612 were relatively good yielder with high stability. Whereas, in terms of the Modified AMMI Stability Index (MASI) score, ICMV 221 was the most stable variety followed by Pusa Comp. 612, GHB 558 and Pratap. However, the Simultaneous Selection Index, which is based on yield and stability, showed that Pratap and Pusa Comp. 612 performed well in all circumstances (Table 2).

Based on overall performance and requirement of the arid region, the genotype Pusa Comp. 612 was considered stable for grain yield, as it had the least MASI score and a mean that was greater than the general mean (1987 kg ha^{-1}). As a result, Pusa Comp. 612 is suggested for cultivation in a wide range of conditions and is considered one of the best genotypes for commercial cultivation on a large scale in rainfed situations of arid regions. It is well recognized that OPVs in pearl millet produce more consistent yields, are more widely adapted than hybrids and are less susceptible to pests and diseases (Sanjana Reddy *et al.,* 2021). Therefore, improving OPVs for grain yield has a positive impact on income sustainability and economic potential of farmers' living on the margins. The hybrids Pratap, 86M86 and NBH 5767, on the other hand, were better for intense cultivation under favorable conditions. Discrimination potential and representation of an environment can be viewed conveniently from the biplot. A small circle reflects the average environment on the average environment axis (AEA). The environment with the

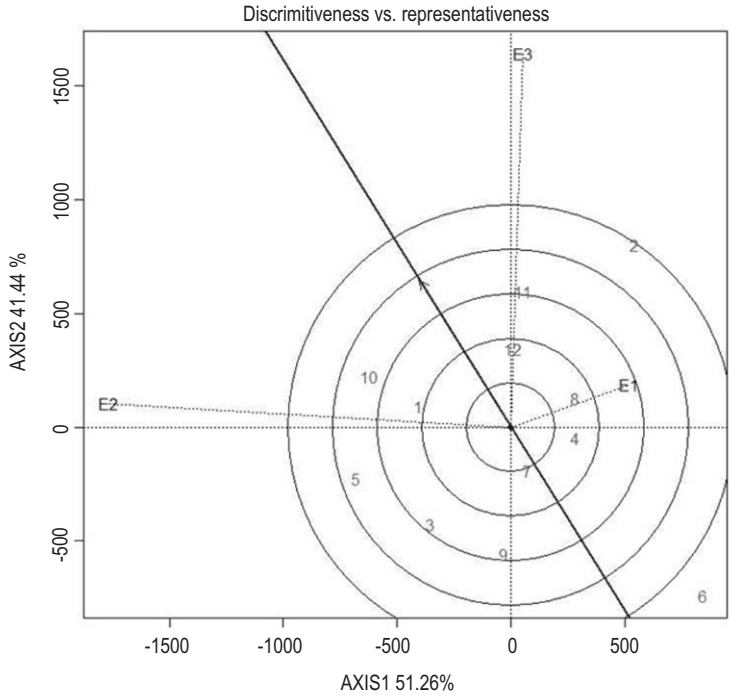

Fig. 2: Ranking of environments for grain yield based on discriminating ability and representativeness. (1) 86M64, (2) 86M86, (3) Dhanshakti, (4) GHB 558, (5) HHB 299, (6) ICMV 155, (7) ICMV 221, (8) Kaveri Super Boss, (9) NBH 5061, (10) NBH 5767, (11) Pratap, (12) Puspa Comp. 612.

longest vector and the smallest angle with an optimal environment is defined as a perfect one in terms of being more discriminating and most representative of overall environments (Pan-pan *et al.,* 2016). In the current study, environment E-3 was the most representative or discriminating environment, since it was closest to the average environment with more vector length (Fig. 2). In a near-average environment E-3, the genotypes 86M86, Pratap and Pusa Comp. 612 had higher grain yield, while genotypes NBH 5767 and Kaveri Super Boss had moderate yield and genotypes ICMV 155, NBH 5061, Dhanshakti and HHB 299 had lower than average yield. Variety ICMV 221 was highly stable, followed by Pusa Comp. 612. Broad obtuse angles between environment E-1 and E-2 vectors (Fig. 2) indicate negative correlations among the test environments, implying a heavy crossover GEI across environments for grain yield. This indicated that genotypes performing better in an environment may not be performing in the same direction in another environment. The genotype-by-trait (GT-biplot) was used to investigate the association among multiple traits and to compare and find genotypes with desirable traits.

Traits with longer vectors account for a greater proportion of detectable variation, while traits with shorter vectors explain limited variation. On the other hand, the cosine of the angle between their vectors approximates the coefficient of correlation between any two traits. GT-biplot over pooled environments was generated using PC 1 and PC 2 scores, which explained 72.37% of the overall discrepancy (85.12%) explained by three PC axes of eigen values greater than one (Fig. 3). According to the length of the vectors in GT-biplot, traits like day to 50% flowering and panicle length explained the most variation across genotypes, followed by dry fodder yield, day to maturity and plant height. These findings are in agreement with the results of Narasimhulu *et al.* (2022). The biplot also revealed a positive relationship between traits grain yield, panicle diameter and dry fodder yield with day to flowering, day to maturity, panicle length and plant height as indicated by the acute angle between them. An obtuse angle between the vector of number of productive tillers with the rest of traits in the study suggested a negative association among them. These trait association findings agree with the results of the Narasimhulu *et al*. (2021a,b). A strong positive and substantial relationship between fodder yield and grain yield meant that dual-purpose genotypes had a greater chance of being selected. Dual-purpose cultivars were identified as Kaveri Super Boss, 86M64 and an OPV Pusa Comp. 612.

This further indicates that hybrids generally having monoculm were superior and achieved higher yields. Analyzing the best performing hybrids Pratap, 86M86, NBH 5767, 86M64 and Kaveri Super Boss under arid region and their trait related responses suggested that high grain yield in pearl millet is accomplished by increasing panicle length and density in a medium to late maturity background. Apart from yield, pearl millet cultivars grown in arid regions should have early flowering and

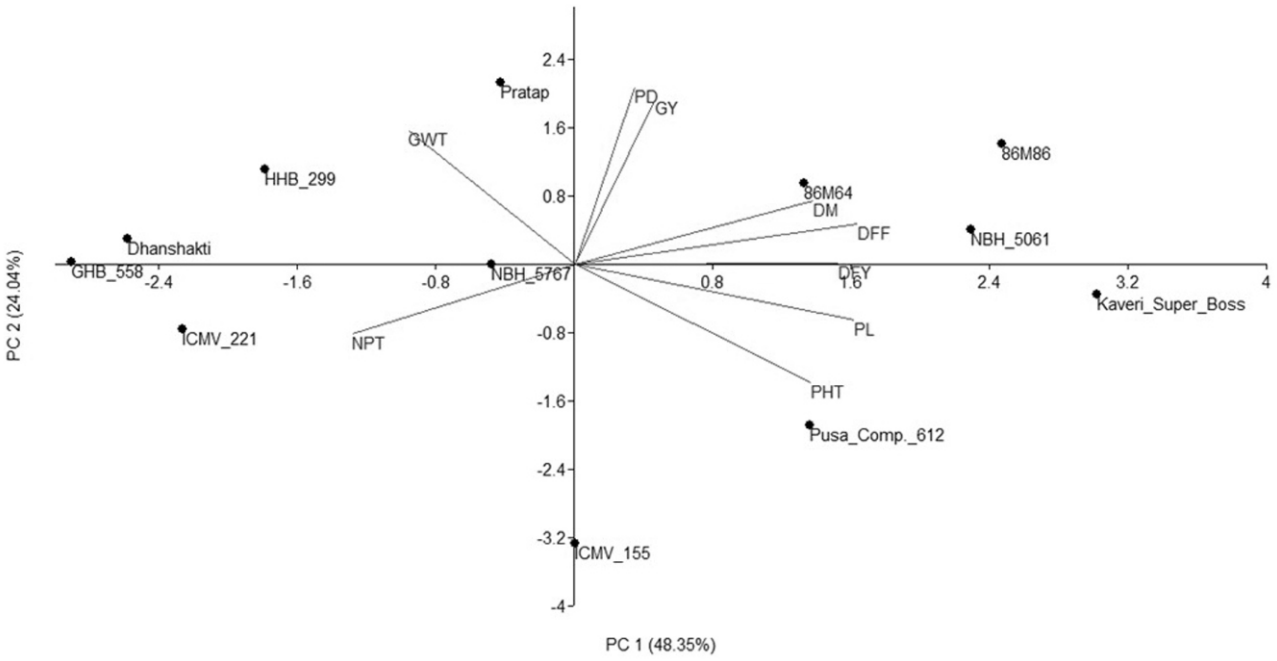

Fig. 3: GT biplot indicating which is the best genotype for the target traits. DFF: Daysto 50% flowering, DM: Days to maturity, PH: Plant Height (cm), NPT: Productive tillers (no. per plant), PL: Panicle Length (cm), PD: Panicle Diameter (cm), GWT: 1000 - grainweight (g), DFY: Dry Fodder Yield (kg ha^{-1}), GY: Grain Yield (kg ha^{-1}).

high tillering to withstand harsh climates. According to Rai *et al.* (1999), changes in sources and sink size were mostly responsible for yield improvements linked with changing yield potential traits. Hence, correlated response selection is necessary to develop a variety with better yield in the desired flowering and tillering background, which none of the varieties studied possessed. Current research has revealed a wider scope to develop promising OPVs of pearl millet with breeding strategies based on present outputs by designing an ideotype to make cultivars more adaptable to varied circumstances in arid and semi-arid tropical regions of Andhra Pradesh. Sanjana Reddy *et al.* (2021) shared the same perspective on the development of potential OPVs for arid regions in order to attain sustainable yields.

Generally, high yielding, medium to late duration hybrids and populations maturing in 80 days or more with resistance to moisture stress are primarily targeted for semi-arid regions. The results of this investigation revealed that environment E-3 was the most representative or discriminating and that yield loss in drought-stricken years was mostly due to drop in plant height, 1000-grain weight and dry fodder yield. Among the pearl millet genotypes hybrids have a higher economic potential than OPVs. However, the relevance of OPVs cannot be discounted owing to better adaptability, cheaper input costs and timely seed supply to marginal farmers. Given these facts, cultivars that give constant yield from year to year under varying moisture stress conditions must be developed. Currently, Pusa Comp. 612, a well-established OPV and a hybrid Pratap are suggested for large-scale evaluation and in-depth research with evolved genotypes, as well as other recently developed cultivars, is suggested to identify suitable genotypes for a range of locations in arid regions with diverse climatic conditions.

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