

## **Genotype by Trait Biplot Studies on Yield, Physiological and Nutritional Traits in Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

A field experiment was conducted at Dry land farm, S.V. Agricultural College, Tirupati in *Kharif* 2018 to assess the visual comparison among genotypes on the basis of multiple traits and also to graphically describe the interrelationships among all measured traits on the basis of overall pattern of the data for the 42 genotypes of pearl millet that were evaluated for 22 physiological, nutritional, and yield related traits in a randomized complete block design, using GT biplot analysis through which, the principal components PC1 and PC2 explained 45.63% of the total variation based on all the traits and the genotypes. The GT biplot analysis is a powerful statistical tool for studying relationship among traits, evaluating cultivars based on multiple traits and for identifying those that are superior in certain traits. Few yield related traits, SPAD Chlorophyll Meter Reading (SCMR), harvest index, Leaf Area Index (LAI), Leaf Area Duration (LAD) and protein content had a positive relationship with yield which were recognized as crucial traits for boosting grain yield and nutritional quality. The genotypes ATP-15, ATP-13, ATP-10, ATP-16, ATP-5, ATP-7, ATP-11, ATP-18, ATP-14 and ATP-12 showed superior performance for grain yield as well as other yield contributing traits. Whereas, the genotypes ICHiFe-18, ICHiFe-12, ATP-3, ICHiFe-21, ATP-2,

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ICHiFe-20, ATP-4, ATP-8, ATP-15, ICHiFe-14, ICHiFe-4, ICHiFe-2 and ICHiFe-5 showed superior performance for nutritional traits. By considering all the studied traits, the genotype ATP-15 was selected as the ideal and most desirable cultivar.

**Keywords:** Principal components; variation; traits; genotypes; quality.

## 1. INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a member of poaceae (graminae) family and the genus *Pennisetum*. The genetic constitution is diploid ( $2n = 2x = 14$ ) in nature and is usually known as cattail millet or bulrush millet in English [1] After rice and wheat, it is India's third most valuable cereal crop. For millions of people living in harsh conditions with irregular rainfall and nutrient-poor soils, it provides nutritionally superior and staple food [2].

It is rightly termed as “nutricereal” because of high protein content (12-15%) with balanced amino acids, carbohydrates (60-70%), calcium, phosphorus, iron, zinc and fats (5-10%) [3-5]. It is rated as good source of fodder owing to its prolific regeneration capacity, good growth, heavy tillering, leafiness, succulent stem and has capacity to provide nutrients. In areas growing pearl millet, 35% of total consumption of energy, protein, iron and zinc is from this millet. It is found to be the economical source for rural residents to get micronutrients [6]. However, the stable expression of these nutrients is required for the benefit of mankind in terms of nutritional security.

In order to meet this, developing stable pearl millet cultivars with high yield and improved nutritional quality is a top priority. Because it is endowed with immense amount of variability for micronutrients particularly for grain Fe and Zn content. Identifying stable genotypes for high amounts of micronutrients and using them in breeding programmes allows for micronutrient enrichment in pearl millet.

To achieve these goals, an effective breeding program helps to keep track of the system's vital aspects and their interrelationships. Though there are many techniques for determining the relationship between characters, such as correlation and regression, Genotype by Trait (GT) biplot analysis outperforms simplistic correlation coefficients and is getting prominence among breeders. Although basic correlation coefficients only represent the association between two traits, it graphically portrays the

interrelationships between all assessed traits depending on the aggregate pattern of the results [7].

Moreover, the GT biplot analysis is a visual comparative platform for genotypes focused up on several traits that can be used in independent culling and comparing selection strategies, making it useful for cultivar evaluation as well as parental selection [7-8]. The current research used genotype by trait biplot analysis to investigate the extent of association between various yield, physiological, and nutritional traits in pearl millet, recognizing the need for such a systematic study. It's an excellent method for visualizing genotypes based on trait data. As a result, the research was carried out in order to identify ideal cultivars and traits that combine better nutritional quality with increased yield using GT biplot approach.

## 2. MATERIALS AND METHODS

The base material for the present study comprised of forty two diverse genotypes including two checks, which were collected from ICRIAT, Hyderabad and Agricultural Research Station, Ananthapur were grown in a randomized complete block design (RCBD) during the *kharif autumn season* 2018, with three replications at the Sri Venkateswara Agricultural College Dry Land Farm in Tirupati. Each genotype was sown in a three-meter-long row with 50-centimeters row spacing and 12-centimeters plant spacing with a total of 386 m<sup>2</sup> land area. Five competitive plants from each replication were used to collect the data for plant height, no. of productive tillers plant<sup>-1</sup>, panicle length, panicle girth, 1000 - grain weight, grain yield plant<sup>-1</sup>, green fodder yield plant<sup>-1</sup>, specific leaf area at 40 DAS, SPAD chlorophyll meter reading at 40 DAS, leaf area index at 40 DAS, leaf area duration, harvest index, iron content, zinc content and protein content.

The characters *viz.*, days to 50% flowering and days to maturity were recorded on per plot basis. Subsequently, the data was tested for analysis of variance (ANOVA) using the Randomized Complete Block Design. To show the genotype

by trait two-way results, the GT biplot method was employed. The biplot was generated using the trait's standardized mean values.

The following is the model equation for genotype by trait interaction biplot analysis:

$$\frac{\alpha_{ij} - \beta_j}{\sigma_j} = \sum_{n=1}^2 \lambda_n \xi_{in} \eta_{jn} + \varepsilon_{ij} = \sum_{n=1}^2 \xi_{in}^* \eta_{jn}^* + \varepsilon_{ij}$$

Where,

$\alpha_{ij}$  = The mean value of genotype i for trait j

$\beta_j$  = The mean value of all genotypes for trait j

$\sigma_j$  = The standard deviation of trait j among genotype means

$\lambda_n$  = The singular value for Principal Component ( $PC_n$ )

$\xi_{in}$  = The  $PC_n$  score for genotype i

$\eta_{jn}$  = The  $PC_n$  score for trait j

$\varepsilon_{ij}$  = The residual effect associated with genotype i in trait j

The singular value  $\lambda_n$  must be absorbed by the singular vector for genotype  $\xi_{in}$  and for traits  $\eta_{jn}$ , in order to achieve trait-focused scaling between genotype and trait scores. That is,  $\xi_{in}^* = \xi_{in} \lambda_n^0 = \xi_{in}$  and  $\eta_{jn}^* = \eta_{jn} \lambda_n^1 = \eta_{jn} \lambda_n$ . Only PC1 and PC2 are kept in the model since  $n=2$  in a biplot, and this is the ideal model for extracting patterns and reducing noise from the data. By plotting PC1 scores against PC2 scores for each genotype and trait, the "R" package (version 3.1.1) is adopted to construct a Genotype by Trait (GT) biplot.

### 3. RESULTS AND DISCUSSION

The analysis of variance revealed extremely significant differences between genotypes for all 17 characters examined in this study, suggesting a great deal of variation among the 42 genotypes of pearl millet, and the results were then subjected to GT biplot analysis.

#### Performance of pearl millet genotypes based on multiple traits:

In Fig. 1, genotype-by-trait (GT) biplot having polygon view depicts the data of 42 pearl millet genotypes for 17 characters. Higher values were desirable for all of the studied traits, except for the trait-specific leaf area, for which a low value was desirable. The traits were considered as the tester and the cultivars as the entries. For the measured traits, the biplot accounted for around 45.63 percent of the total variation among the varieties.

Considering the farthest genotypes from the biplot origin, a polygon was first drawn on genotypes such that all other genotypes are within the polygon (Fig. 1). Later, perpendicular lines on either side of the polygon were drawn beginning from the biplot origin. The perpendicular lines on the polygon are equality lines for neighboring genotypes, allowing for easier visual comparison [8]. The biplot was divided into sectors by the equality lines, with the winning genotype for each sector being the one on the respective vertex. Vertex genotypes are the genotypes that held the vertex position in the biplot and were considered to have the highest trait value within the sector. As a result, they could be exploited as potential parents in hybridization programmes to develop varieties, hybrids, and populations that excel in certain traits.

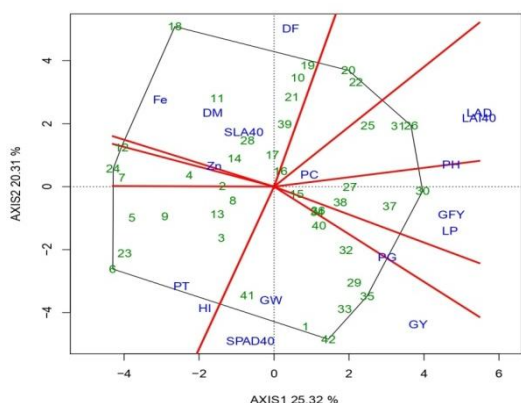
On the basis of biplot presented in Fig. 1, it was represented that the genotypes ICHiFe-6, ATP-2, ICHiFe-18, ATP-13, ICHiFe-20, ATP-4, ATP-8 and ABV-04 were regarded as vertex genotypes that exhibits superior performance for the traits allocated within the sector. The genotype ICHiFe-6 showed better performance for the characters number of productive tillers plant<sup>-1</sup> and harvest index suggesting that it might be exploited in future breeding programmes. The genotype ICHiFe-18 exhibited better performance for the traits days to 50% flowering, days to maturity, specific leaf area at 40 DAS, Fe content and Zn content. The genotype ATP-4, showed better performance, for the characters plant height, LAI at 40 DAS, leaf area duration and protein content, whereas, for the traits panicle length, panicle girth and green fodder yield plant<sup>-1</sup>, the genotype ATP-8 exhibited superior performance. Even though the genotypes ICHiFe-6, ICHiFe-18, ICHiFe-20, ATP-4 and ATP-8 performed better for yield contributing, physiological and nutritional traits, they don't perform well for grain yield. Check variety ABV-04 showed better performance for the traits 1000 - grain weight, grain yield plant<sup>-1</sup> and SPAD chlorophyll meter reading at 40 DAS. Whereas, for the trait specific leaf area the genotypes: ICHiFe-6, ATP-8 and ABV-04 showed lower value indicating that these can be chosen for the development of specific leaf area because for this trait lesser value is most preferable. From Fig. 1 it was also identified that the vertex genotypes ICHiFe-6, ATP-8 and ABV-04 showed better performance for grain yield and other morphological characters, ICHiFe-18 and ATP-4, other vertex genotypes showed higher

performance for nutritional traits. Thus, the following crosses viz., ICHiFe-6 x ICHiFe-18, ICHiFe-6 x ATP-4, ATP-8 x ICHiFe-18, ATP-8 x ATP-4, ATP-2 x ATP-13, ABV-04 x ICHiFe-18 and ABV-04 x ATP-4 might be promoted to integrate grain yield and nutritional traits in to one genotype.

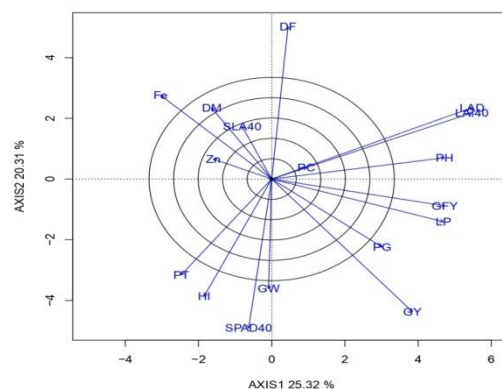
**Inter-relationship among traits:** The interrelationship among all the measured traits was given by a vector view of GT biplot (Fig. 2) which include nine morphological and eight physiological and three nutritional traits. Trait vectors are the lines that link each trait marker to the biplot's origin, and the length of each trait vector roughly corresponds to the trait's standard deviation. The correlation coefficient (degree of association) between two traits is approximated by the cosine of the angle between their vectors. Two traits are positively correlated if the angle between their vectors is an acute angle ( $< 90^\circ$ ), and negatively correlated if the angle is an

obtuse angle ( $> 90^\circ$ ). Trait vectors that are nearly at right angles ( $= 90^\circ$ ) are not closely related, i.e. independent, while traits that are at  $180^\circ$  (directly opposite) are strongly negatively correlated [9].

From the present investigation, grain yield shows high positive correlation with all the morphological traits except flowering traits and SLA at 40 DAS, also negatively correlated with nutritional traits like iron and zinc content. Grain yield was also positively, but weakly correlated with LAI at 40 DAS and LAD. This means that, higher the values of these morphological traits, the more the grain yield. As a result, they were collectively precise, and each generates unique and important information about the cultivars. This was implied by various angle sizes and vector lengths portrayed in the biplot. Reddy et al. [10] in foxtail millet. For the physiological and nutritional characters, strong positive correlation

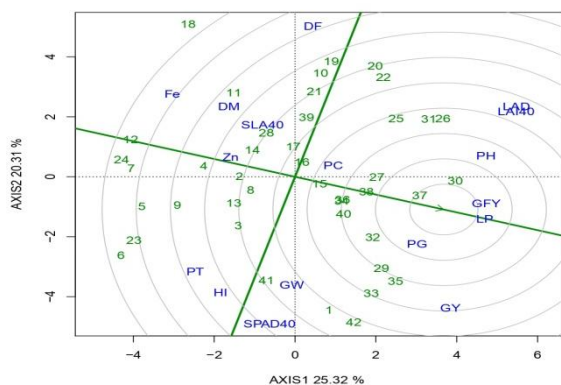


**Fig. 1. Genotype-by-trait biplot of 17 yield, physiological and nutritional traits of 42 pearl millet genotypes**

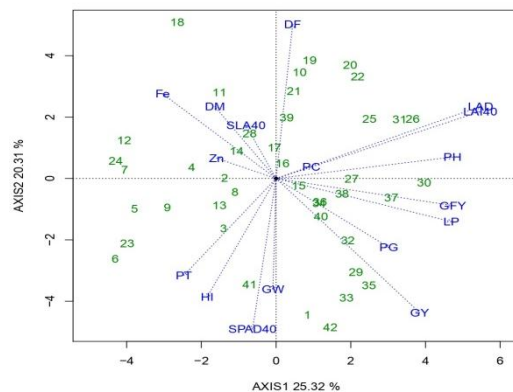


**Fig. 2. Vector view of the genotype-by-trait biplot showing inter-relationships among various yield, physiological and nutritional traits of 42 pearl millet genotypes**

<b>DF</b> = Days to 50% flowering	<b>DM</b> = Days to maturity
<b>PH</b> = Plant height	<b>PT</b> = No. of productive tillers plant <sup>-1</sup>
<b>LP</b> = Panicle length	<b>PG</b> = Panicle girth
<b>GW</b> = 1000 - grain weight	<b>GY</b> = Grain yield plant <sup>-1</sup>
<b>GFY</b> = Green fodder yield plant <sup>-1</sup>	<b>SLA40</b> = Specific Leaf Area at 40 DAS
<b>SPAD40</b> = SPAD Chlorophyll meter reading at 40 DAS	
<b>LAI40</b> = Leaf Area Index at 40 DAS	
<b>LAD</b> = Leaf Area Duration	
<b>HI</b> = Harvest index	<b>Fe</b> = Fe content
<b>Zn</b> = Zn content	<b>PC</b> = Protein content



**Fig. 3. The vector view of genotype-by-trait biplot showing the ranking of 42 pearl millet genotypes for various morpho – physiological and nutritional traits in pearl millet**



**Fig. 4. The vector view of genotype-by-trait biplot showing the ranking of 42 pearl millet genotypes for various morpho – physiological and nutritional traits in pearl millet**

**Genotypes**

1	ICHiFe-1	15	ICHiFe-15	29	ATP-7
2	ICHiFe-2	16	ICHiFe-16	30	ATP-8
3	ICHiFe-3	17	ICHiFe-17	31	ATP-9
4	ICHiFe-4	18	ICHiFe-18	32	ATP-10
5	ICHiFe-5	19	ICHiFe-19	33	ATP-11
6	ICHiFe-6	20	ICHiFe-20	34	ATP-12
7	ICHiFe-7	21	ICHiFe-21	35	ATP-13
8	ICHiFe-8	22	ICHiFe-22	36	ATP-14
9	ICHiFe-9	23	ATP-1	37	ATP-15
10	ICHiFe-10	24	ATP-2	38	ATP-16
11	ICHiFe-11	25	ATP-3	39	ATP-17
12	ICHiFe-12	26	ATP-4	40	ATP-18
13	ICHiFe-13	27	ATP-5	41	Dhanashakti (check)
14	ICHiFe-14	28	ATP-6	42	ABV-04 (check)

was observed between SLA at 40 DAS, iron and zinc content and between LAI at 40 DAS, Leaf Area Duration and protein content. The characters SPAD at 40 DAS, LAI at 40 DAS, LAD, harvest index and protein content showed a positive correlation with seed yield suggesting the significance of these traits in seed yield representation. Sofia [11] reported positive association in green gram for LAD and seed yield plant<sup>-1</sup>. Finally, with regard to this biplot of trait inter-relationships, it is possible to conclude that the traits plant height, number of productive tillers plant<sup>-1</sup>, panicle length, panicle girth, 1000 - grain weight, green fodder yield plant<sup>-1</sup>, SPAD at 40 DAS, harvest index and protein content would be accounted in the breeding programme targeted at increasing yield coupled with quality.

**Ranking of genotypes:** In Fig 3, the vector view of the genotype by trait biplot depicts the ranking of 42 pearl millet genotypes based on their mean performance across the selected traits. The biplot was created using trait-focused singular value partitioning (SVP = 1), making it ideal for

visualizing genotype relationships. The average tester axis, or ATC abscissa, is a horizontal line that passes through the biplot origin and the average tester, with an arrow pointing to the average tester, with its ordinate passing through the origin and perpendicular to the abscissa. PC1 and PC2 principal components explained 45.63 percent of the variation among the traits.

The ideal entry (genotype) is the one that has the longest projection onto the ATC abscissa and is the one that is nearest to the ideal entry (innermost concentric circle with an arrow in Fig. 3). ATP-15 was regarded as the desirable genotype relying on the selected traits, while ATP-8 was ranked next to it. On the basis of performance, ranking of genotypes is as follows; ATP-15 ≈ ATP-8 > ATP-16 ≈ ATP-10 ≈ ATP-5 > ATP-14 ≈ ATP-12 ≈ ATP-18 ≈ ATP-7 ≈ ATP-13 ≈ ATP-11 ≈ ICHiFe-15 ≈ ATP-3 ≈ ATP-9 ≈ ATP-4 > ICHiFe-16 > ICHiFe-17 ≈ ICHiFe-1 ≈ ABV-04 > ATP-17 > ICHiFe-8 ≈ ICHiFe-22 > ICHiFe-3 ≈ ICHiFe-13 ≈ Dhanashakti ≈ ICHiFe-14 ≈ ATP-6 ≈ ICHiFe-21 ≈ ICHiFe-20 > ICHiFe-2 > ICHiFe-

10 ≈ ICHiFe 19 > ICHiFe-4 > ICHiFe-9 ≈ ICHiFe-11 > ICHiFe-5 > ATP-1 > ICHiFe-6 ≈ ICHiFe-7 ≈ ATP-2 ≈ ICHiFe-12 > ICHiFe-18. The genotype ATP-15 was established as the ideal cultivar, followed by others. The genotypes ATP-8, ATP-16, ATP-10, and ATP-5 could also be used to initiate further breeding programmes aimed at high yield and nutritional traits, based on this biplot of all the genotypes evaluated.

**Genotype by trait interaction biplot:** The GT biplot can be used to distinguish genotypes that perform well in specific traits based on multiple traits and thus could be chosen as parents in pearl millet breeding programmes. The biplot in Fig. 4 depicted the relationship between traits through angle form between two or more variables for 42 pearl millet genotypes. The genotypes ATP-13, ATP-7, ATP-10, ATP-18, ATP-12 and ATP-14 showed better performance for the traits panicle girth and grain yield plant<sup>-1</sup>, suggesting that these genotypes would be utilized as parents in breeding programmes. The genotype ICHiFe-11 resulted in better days to maturity, and the genotype ATP-6 performed better in terms of SLA at 40 DAS. Even though, these genotypes were identified for better SLA and days to maturity, they were not ideal for grain yield, showcasing that SLA and days to maturity were not closely related with grain yield. Dhanashakti exhibited superior performance for SPAD at 40 DAS. For the traits panicle length and green fodder yield plant<sup>-1</sup> the genotypes, ATP-15, ATP-16 and ICHiFe-15 showed superior performance. For the traits LAI at 40 DAS and LAD the genotypes, ATP-4, ATP-9 and ATP-3 showed superior performance. Hence, these genotypes were good for the traits LAI and LAD. For the trait days to 50% flowering the genotypes ATP-17, ICHiFe-16, ICHiFe-21, and ICHiFe-10 showed higher values indicating that they were not the best genotypes for days to 50% flowering (DF) because higher values for DF is undesirable.

The genotype ICHiFe-3 showed superior performance for the trait number of productive tillers plant<sup>-1</sup> and exhibited positive correlation with grain yield and green fodder yield. It usually considers that this genotype has high values for these traits implying that the above genotypes may act as effective high yielding genotypes and could be taken advantage in hybridization programmes. For the traits iron content, the genotype, ICHiFe-14 and for zinc content, the genotype, ICHiFe-4 showed better performance. Iron and zinc content are positively associated

with SLA, days to maturity and negatively associated with grain yield and 1000 - grain weight, indicating that the genotypes could be exploited as parents for the advancement of nutritionally rich cultivars.

The genotypes ATP-15, ATP-13, ATP-10, ATP-16, ATP-5, ATP-7, ATP-11, ATP-18, ATP-14 and ATP-12 showed excellent performance for grain yield as well as other yield contributing traits. The genotypes ICHiFe-18, ICHiFe-12, ATP-3, ICHiFe-21, ATP-2, ICHiFe-20, ATP-4, ATP-8, ATP-15, ICHiFe-14, ICHiFe-4, ICHiFe-2 and ICHiFe-5 showed superior performance for nutritional traits subsequently. As a result, crosses involving these two genotype categories can produce nutritionally rich genotypes combining high grain yield and yield contributing traits.

#### 4. CONCLUSION

Focusing on genotype-by-trait biplot analysis it was stated that the traits viz., plant height, panicle length, panicle girth, 1000- grain weight, grain yield plant<sup>-1</sup>, green fodder yield plant<sup>-1</sup>, LAI at 40 DAS, LAD and protein content were recognized as crucial traits for yield as well as quality enhancement and these traits could be accounted as crucial elements during the selection. The genotypes ATP-15, ATP-13, ATP-10, ATP-16, ATP-5, ATP-7, ATP-11, ATP-18, ATP-14 and ATP-12 showed superior performance for grain yield as well as other yield contributing traits. Whereas, the genotypes ICHiFe-18, ICHiFe-12, ATP-3, ICHiFe-21, ATP-2, ICHiFe-20, ATP-4, ATP-8, ATP-15, ICHiFe-14, ICHiFe-4, ICHiFe-2 and ICHiFe-5 showed superior performance for nutritional traits. The genotype ATP-15 has been described as an ideal cultivar, which is most desirable because it incorporates many useful traits in its genetic makeup and thus could act as a good genetic raw material when crossed with the other genotypes found to be better for high yield and nutritional traits, from which better cultivars for grain yield and quality might be developed.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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