



Research Article

Improving the process of identification of superior pearl millet populations using Genotype by Yield×Trait (GYT) biplot

R. Papanna^{1*}, V. K. Koraboyana¹, R. Kalakanti¹, P. Hemanth², B. N. Keerthi³ and S. K. Gupta¹

¹ICRISAT, Patancheru, Hyderabad-502324, Telangana, India

²KVAFSU, Bidar- 585403, Karnataka, India

³BCU, Bangalore-560034, Karnataka, India

*E-Mail: raxit4444@gmail.com

Abstract

Utilizing a trait-based breeding approach is crucial for developing diverse materials with various agronomic traits, emphasizing a common focus on achieving high grain yield, as seen in many breeding programs. Genotype evaluation based on yield × trait combinations is essential, with yield as the primary variable. Genotype by yield × traits biplot analysis was used in pearl millet to assess associations among yield-trait combinations, trait profiles, and superiority rankings. The Biplot analysis reveals prevalent positive associations among yield-trait combinations, implying that selecting multiple traits can augment grain yield productivity. Notably, ICMV 221 and Dhanashakti exhibit elevated levels of YLD×Fe and YLD×Zn, while SOSAT C88, EC C6, Raj 171, and CZIC 618 excel in combining grain yield with traits such as plant height (PH), panicle length (PL), and days to maturity (DM). Based on overall superiority in yield-trait combinations, populations were ranked as follows: AIMP 92901 > ICMV 221 > SOSAT C88 > CZIC 618 > Raj 171 > ICMV 87307 > EC C6. This study demonstrates the practical utility of the genotype by yield × traits biplot approach for selecting pearl millet germplasm with diverse trait combinations, enhancing the breeding program's effectiveness.

Keywords: Trait Association, Trait Profile, Selection Index, Multiple Trait-Based Selection, GYT-Biplot

INTRODUCTION

Cultivar development in agriculture depends on selecting genotypes with optimal combinations of target traits, striking a balance between positive traits and mitigating negative trait associations. In the past, approaches like independent culling, tandem selection, and index selection have been applied to reach this goal. However, these methods are susceptible to bias, as breeder-determined weights and thresholds may vary, yielding different selection outcomes. In order to tackle this issue, Yan and Fregeau-Reid (2018) presented an innovative Genotype by Yield × Trait (GYT) biplot, building upon the Genotype by Trait (GT) biplot, aiming to enhance the selection process. The GT biplot visualizes the relationships between genotypes and traits, helping to

identify associations and evaluate genotype performance across multiple traits. The GT biplot method has been applied across various crops to explore the relationships between different traits and genotypes (Paramesh *et al.*, 2016; Shafi *et al.*, 2020; Zulfiquar *et al.*, 2021; Santana *et al.*, 2021). GYT biplot method assesses genotypes not just on the performance of individual traits but also on their capacity to efficiently integrate yield with other sought-after traits. Given that yield is often the paramount trait, GYT biplot analysis provides a unique perspective, emphasizing the importance of achieving a high yield while considering other relevant traits. Furthermore, it introduces a Superiority Index (SI) feature for genotype selection, distinguishing it from traditional GT biplot

analysis. Notably, the GYT biplot technique has been effectively employed in different of crop studies, including oat (Yan and Fregeau Reid 2018), wheat (Merrick *et al.*, 2020 and Mohammadi, 2019), Cotton (Peixoto *et al.*, 2022), Jatropha (Purwati *et al.*, 2022), and Sunflower (Gholizadeh and Ghaffari, 2023) *etc.* These studies have utilized the innovative GYT-biplot approach to aid in the selection of optimal genotypes based on their associations with yield traits.

Pearl millet (*Pennisetum glaucum*) is a hardy cereal crop widely cultivated in arid and semi-arid regions of Africa and Asia, known for its resilience to drought and poor soil conditions. It is a staple food for millions, providing essential nutrients such as iron and zinc, and is vital for food security in these regions. Understanding the relationships between yield and its component traits, as well as the interactions among these traits, can enhance the effectiveness of selection in pearl millet breeding (Vetriventhan and Niramalakumari, 2007; Patil *et al.*, 2021). In cultivar diversification, the strategic utilization of diverse germplasm and breeding materials is crucial. This study, conducted in the context of pearl millet breeding, recognizes the significance of employing germplasm from various geographical regions in the development of better cultivars, capitalizing on their inherent morphological diversity. With a focus on achieving high grain yield, the trait based breeding approach has been adopted to generate a wide array of pearl millet materials tailored to meet diverse agronomic demands. This study represents the application of the GYT biplot, a multivariate approach, to evaluate pearl millet populations based on multiple traits. Traditional breeding methods often focus on individual traits, but this study emphasizes the importance of integrating yield with other desirable traits such as plant height, panicle length, and micronutrient content (iron and zinc). The GYT biplot approach allows breeders to visualize and rank genotypes based on multiple traits simultaneously, improving the efficiency of selection and to identify genotypes with balanced trait profiles. The primary objective is the selection of superior pearl millet populations exhibiting desirable traits, with potential applications as commercial cultivars or as valuable parents in future breeding programs.

MATERIALS AND METHODS

Evaluation of pearl millet populations : Fourteen pearl millet populations (P-1 to P-14) involving African and Asian genetic materials (**Table 1**) developed at ICRISAT or in collaboration with partners from National Agricultural Research System (NARS) were evaluated in Randomized Complete Block design (RCBD) with two replications during the rainy season of 2020 at four different locations, namely, Dehgam (Gujrat); Jamnagar (Gujrat); Gurugram (Haryana), Palem (Telangana). Standard agronomic management practices were used at all locations to ensure good crop growth.

Observations recorded: In each replication, twenty plants within every plot were randomly tagged for the

assessment of traits such as Plant height (PH) (cm), Number of productive tillers per plant (NPT), Panicle length (PL) (cm), and Panicle girth (PG) (cm). Meanwhile, traits including Days to 50% flowering (DFF) (days), Days to maturity (DM) (days), 1000-grain weight (TGW) (g), Grain Iron (Fe) and Zinc (Zn) density (mg kg⁻¹), and Grain yield (GY) (kg ha⁻¹) were recorded on a plot basis. Harvesting involved collecting all panicles for each entry at maturity, which were then sundried for 10 to 15 days and subsequently threshed for grain yield. After threshing, the grain from all plants in a plot was weighed, and the plot yield was converted to kg ha⁻¹.

Statistical analyses: Bartlett's test (Bartlett, 1937) was employed to check the homogeneity of residual variances. A combined analysis of variance, based on a randomized block design for the recorded data, was performed using SAS (ver. 9.4) (SAS, 2017). Initially, mean data across locations were calculated for each trait, including the grain yield of each pearl millet population (**Table 2**). This dataset was utilized to compute the overall mean and standard deviation for all parameters. The GYT table (**Table 3**) was generated by multiplying or dividing the grain yield of each genotype by the respective trait value, depending on the breeding objectives (Yan and Fregeau-Reid, 2018). For traits such as PH, NPT, PL, PG, TGW, Fe, and Zn, the GY trait combination values were obtained by multiplying the GY with the trait value of each genotype. Conversely, for DFF and DM traits, where a lower value was desirable, the yield trait combinations were computed by dividing the grain yield by these traits. To address discrepancies due to varying measurement units of traits, the values of traits or combinations of yield×traits in the GYT table were standardized according to the formula proposed by Yan and Fregeau-Reid (2018).

$$P_{ij} = \frac{T_{ij} - T_j}{S_j}$$

In the formula, P_{ij} denotes the normalized value of the i^{th} genotype for the specific yield-trait combination j within the standardized dataset. T_{ij} signifies the original value of genotype i for the same yield-trait combination j in the GYT dataset. T_j represents the mean value across all genotypes for the given yield-trait combination j , while S_j is the standard deviation for that particular yield-trait combination. To assess superiority across multiple traits, the standardized GYT data from **Table 4** were employed to calculate SI and mean SI values, facilitating the identification of superior genotypes. The SI is determined by calculating the mean across the standardized values for yield×traits for each genotype. This index serves as a criterion for ranking the genotypes. Furthermore, the dataset underwent GYT biplot analysis, enabling the generation of graphical representations such as the tester vector biplot, which-won-where biplot, and Average Tester Coordinate (ATC) biplot. These graphical analyses were performed using the GGE-biplot tool within the Genstat software program.

Table 1. Details of 14 pearl millet populations evaluated in the study

Code	Population	Origin	Bred at ecology/ Adaptation	Pedigree
P-1	AIMP 92901	Asian	Asia, Aurangabad	Bred through random mating, a total of 272 Bold Seeded Early Seeded (BSEC) S1 progenies were chosen at Aurangabad, and BSEC S1s bulk ex Pat/K91.
P-2	EC C6	Asian x African	ICRISAT, Pat	Developed in 1974 at Patancheru by random mating 153 Indian entries and 41 exotic entries.
P-3	Sudan II	African	ICRISAT, Pat	Developed at Patancheru by random mating F1s involving 8 parents - ICMV 91059, SenPop, ICMV 155, ICMP 91751, ICMV 155, AfPop 90, ICMP 87307 and Sudan Yellow selected from the late population diallel trial in 89/90 at Patancheru
P-4	SOSAT C88	African	African	An open-pollinated variety, developed by random mating 248 S1 progenies derived from the composite Souna x Sanio.
P-5	GB 8735	African	African	An open-pollinated variety was created through the random mating of four F3 lines obtained from crosses that included Iniadi and Souna.
P-6	Raj 171	Asian	ICRISAT, Pat	Bred by random mating 8 S1 progenies of IVC selected at Patancheru
P-7	ICMV 221	African	Africa	Developed through the random mating of 124 chosen S1 progenies of Bold Seeded Early Composite (BSEC) from a drought trial conducted during the summer season
P-8	ICMS 7704	Asian x African	Asia, Pakistan	Bred from 6 inbred lines derived from Indian x African crosses selected at Tandojan in Pakistan in 1977
P-9	ICMP 87307	Asian x African	ICRISAT, Pat	Developed in 1974 at Patancheru by random mating 79 visually selected superior crosses of mostly Indian x African origin
P-10	CZIC 618	Asian	Asia, Rajasthan	ICMV 95836 - Bred by rand. 13 S3 sel. ERajPop C2 at Mandor K94 for fertility restoration
P-11	ICMP 87237	African	ICRISAT, Pat	Developed at Patancheru by random mating four composites [SC1(S4), SC2(M), SC3(M) and SC4(M)] which were developed at Serere research station, Uganda.
P-12	ICTP 8203	African	Africa	The open pollinated cultivar ICTP 8203 was developed from progenies selected from Togo population. Togo population was introduced to India from the ICRISAT-Burkina Faso Cooperative Programme in 1980
P-13	GICKV 98771	Asian	ICRISAT, Pat	Bred by random-mating 212 S1 progenies from C3 cycle of the Early Smut Resistant Composite II (ESRC II) selected for downy mildew resistance in greenhouse screening at ICRISAT-Patancheru and agronomic performance at Gwalior in 1996.
P-14	Dhanashakti	African	Asia	An improved version of the high iron pearl millet variety ICTP 8203, named ICTP 8203 Fe 10-2, (Dhanashakti)

RESULTS AND DISCUSSION

The GYT biplot analysis explained 91.68% of total genotype-trait variation (**Fig. 1-3**). PC1 and PC2, accounting for over 60% of the data variation, provided a comprehensive and precise representation of the evaluated variables, efficiently revealing the nature and magnitude of the data (Yang *et al.*, 2009).

Associations among various yield-trait combinations: The GYT biplot was used to visualize trait correlation in order to assess the association between yield x traits combinations (**Fig. 1**). The angles between trait vectors offer valuable insights into the relationships among traits. When these angles are small, it indicates

positive correlations between the traits. Conversely, larger angles suggest weaker correlations or even negative correlations between the traits. The cosine of the angle between two variables (yield x trait) vectors closely approximates the Pearson correlation coefficient (Yan, 2014; Yan and Frégeau-Reid, 2018). According to **Fig. 1**, all yield x agronomic trait combinations such as DFF, NPT, TGW, PG and DM, PH, and PL exhibited a highly positive correlation among themselves, implying that selection based on multiple traits could enhance grain yield; however, Fe and Zn content is an important trait in pearl millet, in which other grain yield contributing traits were not well associated. Positive correlations can guide breeders in selecting multiple traits simultaneously,

Table 2. Mean genotype by trait data of 14 pearl millet populations for ten traits evaluated across four locations

Genotype	DFF	PH	NPT	PL	PG	DM	TGW	Fe	Zn	YLD
P-1	53	201	2	25	3	81	10	45	34	3396
P-2	54	210	2	26	2	82	9	31	28	2826
P-3	55	210	2	26	2	84	10	37	35	2381
P-4	60	226	2	24	3	88	10	28	26	3406
P-5	55	219	2	24	3	82	10	64	47	1714
P-6	57	213	2	25	2	84	9	36	33	2795
P-7	54	207	2	23	3	80	10	52	40	3219
P-8	65	208	2	27	2	92	8	52	37	2333
P-9	59	208	2	26	2	85	9	52	32	2700
P-10	52	198	2	22	2	79	9	39	29	2929
P-11	51	199	2	22	3	80	10	53	40	2275
P-12	50	174	2	22	3	77	10	82	44	2108
P-13	56	204	2	28	2	84	9	36	24	2547
P-14	50	173	2	21	2	78	9	75	48	2281
Mean	55.06	203.54	2.04	24.31	2.41	82.39	9.41	48.77	35.47	2636.43
Standard Deviation	4.08	14.80	0.20	2.10	0.27	4.04	0.65	16.29	7.63	496.91

Note: The trait abbreviations are: DFF- Days to 50% Flowering, PH- Plant height (cm), NPT- Number of productive tillers per plant, PL- Panicle length (cm), PG- Panicle girth (cm), DM- Days to maturity, YLD- Grain Yield (kg ha⁻¹), TGW- 1000 grain weight (g), Fe- Grain Iron content (mg kg⁻¹) and Zn- Grain Zinc content (mg kg⁻¹)

Table 3. GYT data for 14 pearl millet populations

Genotype	YLD/DFF	YLD×PH	YLD×NPT	YLD×PL	YLD×PG	YLD/DM	YLD×TGW	YLD×Fe	YLD×Zn
P-1	64	682199	7819	84078	8523	275040	33927	152305	114041
P-2	52	594253	5155	72081	6476	231766	25320	87124	79281
P-3	43	500581	5120	62235	5621	199962	24368	88138	83972
P-4	57	768783	6484	80232	9614	298622	33064	94185	89246
P-5	31	376160	3030	40433	4459	139713	16620	110485	81256
P-6	49	594731	5316	70203	6207	235675	24089	100833	91446
P-7	60	665263	7117	73334	8372	256947	32556	167686	129305
P-8	36	485026	4056	63173	4897	213882	19028	121611	85957
P-9	46	562305	5022	71235	5895	229025	23555	140846	85823
P-10	56	580844	6302	64043	5568	230404	26262	115251	84967
P-11	45	452184	4727	51020	6430	181253	23115	120810	91631
P-12	42	366282	4728	47427	5510	161972	20775	172970	91702
P-13	45	518390	5292	70619	6130	213555	23818	90877	60564
P-14	46	394689	5223	47585	5341	177192	21012	171735	109623
Mean	55.06	203.54	2.04	24.31	2.41	82.39	9.41	48.77	35.47
Standard Deviation	4.08	14.80	0.20	2.10	0.27	4.04	0.65	16.29	7.63

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Table 4. Standardized GYT data and superiority index for the genotypes

Genotype	YLD/DF	YLD×PH	YLD×NPT	YLD×PL	YLD×PG	YLD/DM	YLD×TGW	YLD×Fe	YLD×Zn	Mean (Superiority Index)	Ranking
P-1	1.7779	1.1958	1.9904	1.5254	1.4653	1.3203	1.7524	0.8938	1.3556	1.4752	1
P-2	0.4745	0.4630	-0.1881	0.6083	0.0784	0.3273	0.0958	-1.1585	-0.7205	-0.0022	7
P-3	-0.5303	-0.3176	-0.2171	-0.1442	-0.5010	-0.4024	-0.0873	-1.1266	-0.4403	-0.4185	10
P-4	0.9868	1.9173	0.8987	1.2314	2.2049	1.8614	1.5862	-0.9362	-0.1253	1.0695	3
P-5	-1.8720	-1.3543	-1.9255	-1.8106	-1.2882	-1.7849	-1.5786	-0.4230	-0.6025	-1.4044	14
P-6	0.1227	0.4670	-0.0566	0.4648	-0.1036	0.4170	-0.1411	-0.7269	0.0061	0.0499	5
P-7	1.2714	1.0547	1.4159	0.7042	1.3632	0.9051	1.4883	1.3780	2.2673	1.3165	2
P-8	-1.2995	-0.4472	-1.0865	-0.0725	-0.9916	-0.0830	-1.1152	-0.0726	-0.3218	-0.6100	13
P-9	-0.2585	0.1968	-0.2968	0.5437	-0.3150	0.2644	-0.2439	0.5330	-0.3298	0.0104	6
P-10	0.8821	0.3512	0.7500	-0.0060	-0.5366	0.2961	0.2771	-0.2729	-0.3809	0.1511	4
P-11	-0.3738	-0.7209	-0.5381	-1.0014	0.0473	-0.8317	-0.3285	-0.0979	0.0172	-0.4253	11
P-12	-0.6309	-1.4367	-0.5372	-1.2760	-0.5762	-1.2742	-0.7788	1.5444	0.0214	-0.5493	12
P-13	-0.2885	-0.1692	-0.0763	0.4967	-0.1564	-0.0905	-0.1933	-1.0403	-1.8384	-0.3729	9
P-14	-0.2620	-1.1999	-0.1328	-1.2639	-0.6904	-0.9249	-0.7333	1.5055	1.0918	-0.2900	8
Mean	0	0	0	0	0	0	0	0	0		
Standard Deviation	1	1	1	1	1	1	1	1	1		

Note: The trait abbreviations are: DFF- Days to 50% Flowering, PH- Plant height (cm), NPT- Number of productive tillers per plant, PL- Panicle length (cm), PG- Panicle girth (cm), DM- Days to maturity, YLD- Grain Yield (kg ha⁻¹), TGW- 1000 grain weight (g), Fe- Grain Iron content (mg kg⁻¹) and Zn- Grain Zinc content (mg kg⁻¹)

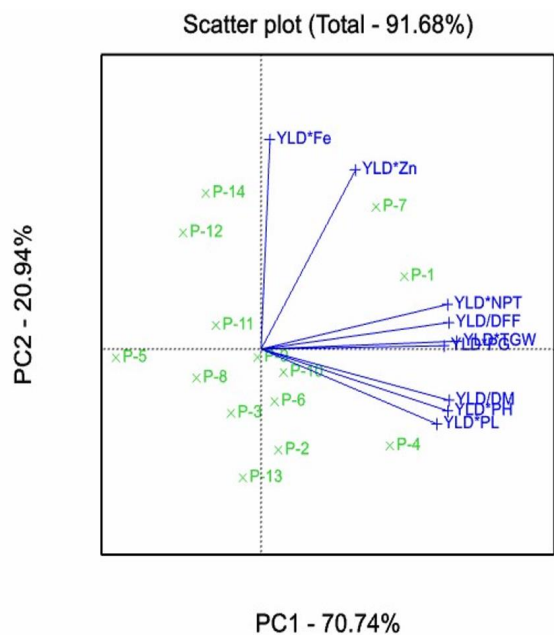


Fig. 1. Illustrates the Trait Vector view of the GYT biplot, demonstrating associations among various yield-trait combinations

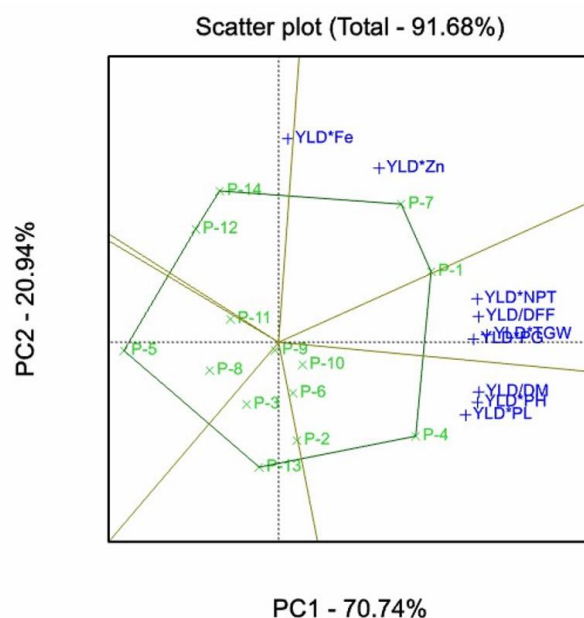


Fig. 2. The which-won-where view of the GYT biplot to highlight genotypes with outstanding profiles

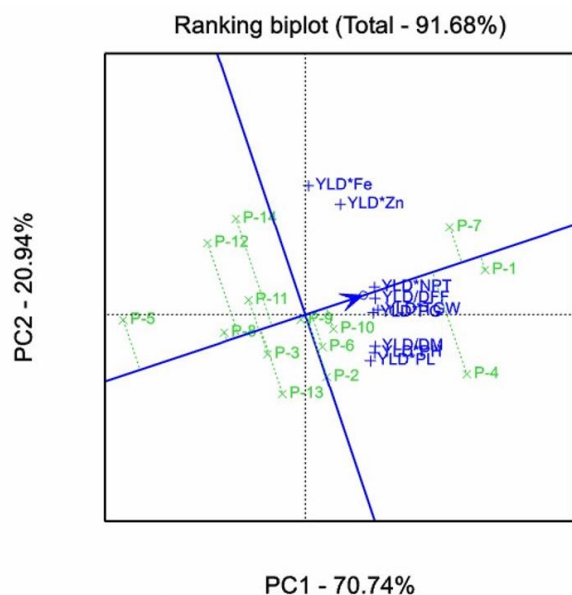


Fig. 3. ATC view of the GYT biplot, providing a ranking of genotypes based on their overall superiority as well as their strengths and weaknesses

as improvements in one might lead to improvements in the other. This can expedite the breeding process by targeting traits that have natural positive relationships. Negative correlations necessitate careful consideration of trade-offs. Breeding programs might need to balance the conflicting traits or prioritize one over the other based on specific breeding objectives.

Trait profile of the genotypes based on GYT biplot: A GYT biplot serves as a valuable tool for visualizing the intricate relationships between genotypes and traits, facilitating the interpretation of complex data patterns. It allows us to understand how genotypes perform for each trait by their positions relative to the trait vectors. Genotypes closer to a trait vector exhibit higher values for that specific trait, while those positioned farther away demonstrate lower values. In Fig. 2, the polygon view, often referred to as the “which-won-where” view, offers an effective way to visualize genotype trait profiles. Genotypes positioned at a vertex in this view showcase the highest values for yield-trait combinations within their respective sectors due to the inherent configuration of the biplot (Yan and Frégeau-Reid, 2018). As a result, ICMV 221 and Dhanashakti had the highest YLD×Fe and YLD×Zn values, suggesting that these two varieties excelled in integrating grain yield with the iron and zinc content in the grains. SOSAT C88, EC C6, Raj 171, and CZIC 618 also had the highest levels of YLD×PH, YLD×PL, and YLD/DM, indicating that these four cultivars were the best at combining grain yield with early maturity, plant height, and panicle length. Genotypes at the biplot peak can be explored to help design genotypes responsive to the traits of interest.

Ranking the genotypes according to their combinations of yield×traits: The GYT biplot is primarily used for genotype selection in plant breeding programs. It allows breeders to visually identify genotypes with favorable trait profiles that can lead to improved yield. Genotypes positioned closer to the favourable yield×trait vectors are considered promising candidates for further evaluation and advancement in breeding programs. In Fig. 3, the biplot features a small circle on the average tester axis (ATA), representing the location of the “average yield-trait combination” serves as a reference point for ranking genotypes in terms of their overall superiority or utility. Genotypes close to the ATA exhibit well-balanced phenotypic profiles, while those situated farther away in either direction demonstrate distinct strengths and/or weaknesses (Yan and Frégeau-Reid, 2018). Similarly, according to Fig. 3, the cultivars with the highest rankings in terms of yield-trait combinations are as follows: AIMP 92901 > ICMV 221 > SOSAT C88 > CZIC 618 > Raj 171 > ICMP 87307 > EC C6. On the far left of the biplot, the population GB 8735 holds the lowest rank. In addition to assessing genotypes based on their overall superiority, Fig. 3 also presents the trait profiles of these genotypes. Specifically, it shows that ICMV 221 and Dhanashakti are strong in grain Fe and Zn content. ICMP 87307, CZIC 618, and Raj 171 were balanced for various traits; AIMP 92901, ICMV 221, and SOSAT C88 were strong in various agronomic traits considered. Such information is necessary for deploying superior genotypes for use in breeding programs.

Some of the popular open-pollinated varieties (OPVs) from Africa, such as GB 8735 and SOSAT-C88, and popular OPVs cultivated in India, including ICTP 8203, Raj 171, ICMV 221, Dhanashakti, and AIMP 92901, were also part of the study. The study’s results align with the trait profile of these cultivars. For instance, Dhanashakti, a biofortified open-pollinated pearl millet variety, was developed through recurrent selection within the ICTP 8203 variety, harnessing intrapopulation variability. With high iron content of 71 mg/kg and a zinc content of 40 mg/kg, it was officially released in 2012 in Maharashtra and subsequently across India in 2013 (Rai *et al.*, 2014).

OPVs have proven to be valuable assets in pearl millet breeding programs in semi-arid regions of Asia and Africa, offering farmers sustainable and affordable options for improved varieties. As breeding efforts continue, the development and dissemination of well-adapted and high-performing OPVs will remain crucial to supporting smallholder agriculture and improving rural livelihoods. The GYT biplot stands as a method for evaluating genotypes across a spectrum of traits. Its effectiveness lies in its ability to visually assess and rank genotypes according to their capacity to integrate yield with other desirable characteristics. Simultaneously, it offers a clear depiction of each genotype’s merits and shortcomings. This ranking serves as an indicator of their practical value, while the insights into strengths and weaknesses

offer guidance on the appropriate utilization of these genotypes. Therefore, the selected population in pearl millet can be utilized for commercial use; the populations identified in this study were desirable in several traits, and they can be employed as base materials for the development of varieties and hybrid parents after limited selection and fine-tuning for local adaptation; and as sources of genes for yield and adaptation traits. The genetic base of hybrids and hybrid parents has narrowed considerably, the long-term success in hybrid development depends on enhancing the genetic diversity of elite hybrid parents through increased utilization of new germplasm. The insights gained from a GYT biplot guide breeders in making informed decisions about trait-based breeding *i.e.*, which genotypes to advance, which traits to prioritize, and how to develop crop varieties with improved yield potential.

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