



Genetic Divergence and Multivariate Analysis of Isabgol Genotypes for Yield-Related Traits in Arid Environments

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Abstract: Isabgol is a commercially significant medicinal crop cultivated for its valuable seed husk, but its productivity in arid environments remains constrained by limited genetic diversity. To cope up with this limitation, 25 genotypes were examined with the objective of assessing genetic variability and identifying high-performing genotypes for yield enhancement in the present study under arid conditions, including two checks (GI-4 and VI-3). A broad spectrum of genetic variability was observed among the isabgol genotypes for yield and related traits. Genotype CZI-24-1 recorded higher seed yield (23.72 g), whereas CZI-24-20 demonstrated superior performance for key yield-contributing traits, including the number of seeds per spike (134.17), number of spikes per plant (127.78), and spike length (8.19 cm). Multivariate analyses using Principal Component Analysis (PCA) and cluster analysis identified genotypes CZI-24-6 and CZI-24-16 as potential candidates for yield enhancement, whereas CZI-24-20, CZI-24-23, and CZI-24-21 demonstrated superior performance for key yield-contributing traits. The genotype CZI-24-16 displayed a unique expression of white-color spikes, representing the first documented occurrence of this trait in isabgol. PCA and correlation analyses indicated that spike morphology traits such as spike length, spikes per plant, and seeds per spike, were the major determinants of yield variability; whereas seed yield per plant contributed independently with limited association to other yield traits. These findings revealed the significant genetic diversity, offering a foundation for selecting elite genotypes of *isabgol*. However, multilocation and multiyear testing is needed to confirm their stability and adaptability.

Key words: Isabgol, yield related components, multivariate analysis, genotype selection, trait association.

Isabgol (*Plantago ovata* Forsk.), an annual herb of the Plantaginaceae family, is a major medicinal crop cultivated in India for its seed husk, the primary economic product. India produces nearly 90% of the world's isabgol and holds a monopoly in its export, earning about US\$ 0.5 million annually from seed husk and related products (Basak, 2017; Basak and Saha, 2022). The crop is predominantly cultivated during the *rabi* season across Rajasthan, Gujarat, Madhya Pradesh,

and parts of Haryana. Beyond India, isabgol is also grown to a limited extent in Pakistan, Iran, Afghanistan, and Egypt (Meena *et al.*, 2024). Arid regions provide the most favorable conditions for the commercial cultivation of isabgol, as the crop thrives in environments with minimal or no rainfall following anthesis (Patel *et al.*, 2014). The growing global demand for isabgol products has steadily expanded its cultivation area in India (Meena *et al.*, 2024). In these dry zones, isabgol serves as a profitable crop option for farmers, offering high economic returns even under low-input and minimal fertilizer conditions.

Despite its economic significance, isabgol in India exhibits low and inconsistent yields, mainly attributed to narrow genetic variability among cultivars, vulnerability to environmental and biological stresses, and inadequate agronomic practices (Tyagi *et al.*, 2016; Kaswan *et al.*, 2018). Enhancing productivity and yield stability in isabgol necessitates a systematic assessment of genetic resources to identify high-yielding and regionally adapted genotypes tailored to specific agro-climatic conditions.

The yield potential of isabgol is governed by complex polygenic interactions involving key morphological and yield related attributes such as spike length, spikes per plant, seed weight, and seeds per spike (Amanullah *et al.*, 2016; Islam *et al.*, 2020). Employing multivariate analytical approaches to elucidate the interrelationships among these traits offers critical insights for efficient selection and genetic improvement programs (Leite *et al.*, 2018).

PCA and cluster analysis serve as robust multivariate tools for dissecting trait variability, identifying major contributors to yield performance, and classifying genotypes based on their similarity in phenotypic expression and overall performance (Ramteke *et al.*, 2024). These multivariate techniques have been effectively applied across various crops to unravel complex trait interrelationships, facilitating the identification of superior genotypes and guiding targeted selection for yield enhancement and breeding advancement (Leite *et al.*, 2018; Sabaghnia *et al.*, 2024). Despite their proven utility in other crops, extensive multivariate evaluations of trait associations in isabgol remain limited, particularly within the arid environments where it is primarily grown.

Several studies have assessed isabgol genotypes in semi-arid regions, revealing substantial genetic variability for yield and its components under moisture-limited conditions (Tyagi *et al.*, 2016; Kaswan *et al.*, 2018; Arya *et al.*, 2021). However, studies focusing on the assessment of genetic variability for yield and associated components in arid environments remain scarce.

Therefore, the present investigation was conducted to assess 25 isabgol genotypes, including two checks (GI-4 and VI-3), for yield and its component traits under arid conditions. The study aimed to quantify genetic variability, explore inter-trait relationships, and identify high-performing genotypes through multivariate approaches such as PCA and cluster analysis. The outcomes are anticipated to facilitate the selection of elite, high-yielding, and well-adapted genotypes, thereby supporting the development of improved isabgol cultivars suited to dry land agro ecosystems.

Materials and Methods

Experimental site and biological materials

The study utilized 23 *isabgol* genotypes developed through single-plant selection based on plant traits such as spike length, compactness, shape, and color, using germplasm lines obtained from the Agricultural University, Jodhpur, and the ICAR-National Bureau of Plant Genetic Resources, New Delhi. Two standard breeder checks, GI-4 and VI-3, were included for comparison. The field evaluation was conducted during the rabi season of 2024 using a randomized complete block design with three replications at ICAR-Central Arid Zone Research Institute, Jodhpur (26°15'02.9"N 72°59'44.5"E). The experimental field is characterized by sandy loam texture of soil. The site lies in an arid climatic zone, receiving an average annual rainfall of about 300 mm, mainly during the southwest monsoon period (July-September). Each genotype was planted in paired rows measuring 3 m in length, with a spacing of 30 cm between rows and 10 cm between plants. Recommended agronomic practices were followed to ensure uniform crop establishment and minimize environmental variability (Das and Trivedi, 2023). At the harvesting stage, data were collected from five randomly selected plants per replication to evaluate yield-contributing

traits. Observations included the number of spikes per plant, spike length (cm), number of seeds per spike, test weight (g), and seed yield per plant (g). These measurements were used to assess genotypic variation and identify promising isabgol lines exhibiting superior yield potential and associated morphological characteristics. To validate and distinguish between white and light brown spike color, colorimetric parameters were determined using a portable colorimeter (Konica Minolta, Japan). Measurements were taken under standardized conditions to ensure reliable and reproducible color evaluation (Rampackova *et al.*, 2021).

Statistics analysis

Analysis of variance (ANOVA) was conducted using the “agricolae” package in R (Mendiburu, 2021) to evaluate the magnitude and significance of variation among isabgol genotypes. The least significant difference (LSD) test was performed with the “doebioresearch” package (Popat and Banakara, 2020) to compare mean differences and identify statistically distinct genotypic performances. The interrelationships among yield-associated traits were examined through correlation analysis using the “PerformanceAnalytics” package in R (Peterson and Carl, 2020), while Principal Component Analysis (PCA) was implemented via the “Factoextra” package (Kassambara and Mundt, 2020). These multivariate approaches facilitated the identification of key trait associations and enabled the selection of superior isabgol genotypes exhibiting desirable combinations of yield contributing attributes. Cluster analysis was carried out using the “pheatmap” package in R (Kolde, 2019), applying Ward’s minimum variance method with Euclidean distance to classify the isabgol genotypes into distinct groups based on their similarity in yield-related trait performance and overall phenotypic expression.

Results and Discussion

Spike characteristics of isabgol genotypes

Analysis of variance indicated highly significant differences ($p < 0.001$) among isabgol genotypes for spikes per plant, spike length, 100-seed weight, and seed yield per plant, while the number of seeds per spike varied significantly ($p < 0.01$), indicates a wide range of genetic diversity within the evaluated

genotypes for yield related traits (Arya *et al.*, 2021). Such diversity is essential for crop improvement, enabling the identification and selection of superior genotypes with desirable agronomic traits suited to arid environments (Pal and Raychaudhuri, 2003).

Seed yield per plant varied widely among the genotypes, ranging from 9.42 g to 23.72 g (Table 1). The wide range of variation observed among genotypes highlights the existence of substantial genetic diversity for yield potential within the evaluated population (Kaswan *et al.*, 2018). Such diversity reflects the involvement of multiple genetic factors controlling yield and offers scope for selecting high-yielding parental lines through direct identification of superior genotypes of isabgol.

Among the evaluated isabgol genotypes, CZI-24-1 produced the highest seed yield per plant (23.72 g), significantly outperformed most other genotypes, including the breeder checks GI-4 (10.31 g) and VI-3 (12.04 g). However, no statistically significant differences were observed in seed yield per plant between CZI-24-1 (23.72 g) and CZI-24-6 (23.20 g), CZI-24-16 (21.43 g), CZI-24-8 (20.43 g) and CZI-24-22 (20.00 g), indicates their potential for use as promising parental lines in breeding programs aimed at improving yield (Amanullah *et al.*, 2016). Similar findings of significant genotypic variability for seed yield in *Plantago ovata* have been reported earlier (Beniwal *et al.*, 2007; Tyagi *et al.*, 2016; Islam *et al.*, 2020; Arya *et al.*, 2021).

The number of seeds per spike ranged from 107.89 to 134.17 (Table 1). The considerable variation among genotypes for this trait indicates a wide genetic base that affects seed set efficiency and reproductive capacity (Saroj *et al.*, 2021). Genotype CZI-24-20 recorded the highest number of seeds per spike (134.17), significantly exceeding most other genotypes, including the breeder checks GI-4 (121.39) and VI-3 (117.56). However, no statistically significant differences were observed in seed numbers per spike between CZI-24-20 and genotypes CZI-24-21 (132.94), CZI-24-23 (132.17), CZI-24-15 (126.06), and CZI-24-22 (123.94). This highlights their potential as promising parental lines for breeding programs focused on enhancing reproductive efficiency and yield-related traits. Similar findings of significant genotypic differences for seed

Table 1. Spike morphology and yield-related traits of different isabgol genotypes

Genotypes	Seed yield (g) / plant	Number of seeds / spike	Test weight (g)	Number of spikes/plant	Spike length (cm)	Spike shape	Spike colour
CZI-24-1	23.72 ^a ± 4.34	112.83 ^{e-i} ± 6.71	0.20 ^{a-d} ± 0.01	75.67 ^h ± 3.71	6.08 ^{d-g} ± 0.53	Cylindrical	Light brown
CZI-24-2	15.25 ^{e-g} ± 1.86	107.89 ^j ± 8.11	0.20 ^{b-d} ± 0.01	90.33 ^{f-h} ± 16.37	5.19 ^g ± 0.50	Cylindrical	Light brown
CZI-24-3	15.60 ^{d-f} ± 4.16	121.11 ^{c-f} ± 6.57	0.20 ^{a-c} ± 0.02	103.22 ^{b-f} ± 7.71	6.32 ^{b-f} ± 0.04	Cylindrical	Light brown
CZI-24-4	14.40 ^{f-i} ± 4.67	118.28 ^{d-i} ± 5.78	0.18 ^{c-f} ± 0.01	93.11 ^{c-h} ± 14.37	5.8 ^{e-g} ± 0.67	Cylindrical	Light brown
CZI-24-5	14.20 ^{f-i} ± 2.66	117.44 ^{d-i} ± 4.00	0.18 ^{c-f} ± 0.02	101.56 ^{b-f} ± 13.66	5.86 ^{e-g} ± 0.79	Cylindrical	Light brown
CZI-24-6	23.20 ^{ab} ± 4.76	120.17 ^{d-g} ± 7.31	0.17 ^{d-f} ± 0.01	88.11 ^{f-h} ± 6.62	6.14 ^{c-g} ± 1.22	Cylindrical	Light brown
CZI-24-7	9.42 ^k ± 1.51	119.67 ^{d-h} ± 6.37	0.20 ^{a-c} ± 0.01	79.11 ^{gh} ± 5.17	7.21 ^{a-d} ± 0.60	Cylindrical	Light brown
CZI-24-8	20.43 ^{a-c} ± 1.75	119.50 ^{d-i} ± 9.01	0.20 ^{a-d} ± 0.02	99.22 ^{b-g} ± 16.55	5.84 ^{e-g} ± 0.26	Cylindrical	Light brown
CZI-24-9	9.75 ^{jk} ± 1.78	111.11 ^{f-i} ± 5.44	0.20 ^{b-d} ± 0.03	90.22 ^{f-h} ± 7.32	5.52 ^g ± 0.45	Cylindrical	Light brown
CZI-24-10	15.88 ^{d-f} ± 2.76	108.11 ^{hi} ± 5.88	0.17 ^{d-f} ± 0.01	103.33 ^{b-f} ± 11.14	5.91 ^{e-g} ± 0.12	Cylindrical	Light brown
CZI-24-11	16.09 ^{c-f} ± 1.37	120.94 ^{c-f} ± 5.98	0.21 ^{a-c} ± 0.01	112.56 ^{a-d} ± 13.18	7.42 ^{ab} ± 0.43	Cylindrical	Light brown
CZI-24-12	15.13 ^{e-h} ± 1.39	117.72 ^{d-i} ± 4.93	0.19 ^{b-e} ± 0.02	92.33 ^{d-h} ± 11.86	5.26 ^g ± 0.35	Cylindrical	Light brown
CZI-24-13	19.10 ^{b-e} ± 1.86	118.94 ^{d-i} ± 4.23	0.20 ^{b-d} ± 0.02	99.44 ^{b-g} ± 14.00	7.43 ^{ab} ± 1.11	Club	Light brown
CZI-24-14	16.01 ^{d-f} ± 2.05	109.1 ^{g-i} ± 5.86	0.20 ^{a-c} ± 0.01	86.22 ^{f-h} ± 17.18	5.84 ^{e-g} ± 0.93	Cylindrical	Light brown
CZI-24-15	10.86 ^{g-k} ± 1.45	126.06 ^{a-d} ± 10.20	0.21 ^{ab} ± 0.02	99.89 ^{b-f} ± 15.12	5.2 ^g ± 0.39	Cylindrical	Light brown
CZI-24-16	21.43 ^{ab} ± 0.65	121.83 ^{b-f} ± 7.36	0.16 ^f ± 0.00	96.67 ^{c-g} ± 16.17	6.28 ^{b-g} ± 1.12	Cylindrical	White
CZI-24-17	10.80 ^{h-k} ± 2.27	121.06 ^{c-f} ± 7.78	0.18 ^{c-f} ± 0.03	111.89 ^{a-e} ± 3.53	6.22 ^{b-g} ± 0.78	Cylindrical	Light brown
CZI-24-18	13.29 ^{f-k} ± 3.32	122.33 ^{b-f} ± 6.55	0.19 ^{b-e} ± 0.01	91.56 ^{e-h} ± 20.07	5.62 ^{e-g} ± 0.87	Cylindrical	Light brown
CZI-24-19	13.98 ^{f-j} ± 2.64	113.83 ^{e-i} ± 6.41	0.20 ^{b-d} ± 0.01	96.22 ^{c-h} ± 10.06	5.08 ^g ± 0.73	Cylindrical	Light brown
CZI-24-20	11.11 ^{g-k} ± 1.84	134.17 ^a ± 6.71	0.20 ^{a-d} ± 0.02	127.78 ^a ± 9.71	8.19 ^a ± 1.02	Cylindrical	Light brown
CZI-24-21	12.57 ^{i-k} ± 3.84	132.94 ^{ab} ± 8.84	0.19 ^{b-e} ± 0.02	113.22 ^{a-c} ± 17.35	7.30 ^{a-c} ± 0.92	Cylindrical	Light brown
CZI-24-22	20.00 ^{a-d} ± 2.84	123.94 ^{a-e} ± 8.47	0.18 ^{c-f} ± 0.02	103.1 ^{b-f} ± 20.72	5.22 ^g ± 0.20	Cylindrical	Light brown
CZI-24-23	14.55 ^{f-i} ± 3.19	132.17 ^{a-c} ± 10.27	0.22 ^a ± 0.02	119.11 ^{ab} ± 17.42	8.11 ^a ± 0.29	Cylindrical	Light brown
GI-4	10.31 ^{i-k} ± 1.19	121.39 ^{b-f} ± 6.94	0.19 ^{b-e} ± 0.02	98.33 ^{c-g} ± 12.99	6.82 ^{b-e} ± 1.40	Cylindrical	Light brown
VI-3	12.04 ^{f-k} ± 3.61	117.56 ^{d-i} ± 7.60	0.17 ^{e-f} ± 0.02	89.33 ^{f-h} ± 20.58	6.10 ^{c-g} ± 0.58	Cylindrical	Light brown

The data represent mean value and standard error of three replicates per treatment. Different superscript letters indicate statistically significant differences at $p < 0.05$, as determined by Fisher's Protected Least Significant Difference (LSD) test.

number per spike in *Plantago ovata* have been reported earlier (Amanullah *et al.*, 2016; Kaswan *et al.*, 2018; Arya *et al.*, 2021).

The test weight showed considerable variation among the genotypes, ranging from 0.16 g to 0.22 g (Table 1), suggesting that genetic factors governing seed development and grain filling differ among genotypes (Kaswan *et al.*, 2018; Gupta *et al.*, 2018). Genotype CZI-24-23 recorded the highest test weight (0.22 g), significantly surpassing most other genotypes, including the breeder checks GI-4 (0.19 g) and VI-3 (0.17 g), while no significant differences were observed compared to CZI-24-15 (0.21 g), CZI-24-11 (0.21 g), CZI-24-7 (0.20 g), CZI-24-14 (0.20 g), CZI-24-3 (0.20 g), CZI-24-1 (0.20 g), CZI-24-8 (0.20 g) and CZI-24-20 (0.20 g). Similarly, test weight of 0.20 g was also recorded in the *Plantago ovata* genotype P-001,

supporting the present findings (Islam *et al.*, 2020).

The number of spikes per plant varied widely among the genotypes, ranging from 75.67 to 127.78 (Table 1). The marked variation among genotypes suggests substantial genetic diversity influencing reproductive development and spike formation (Bannayan *et al.*, 2008; Islam *et al.*, 2020). Genotype CZI-24-20 produced the highest number of spikes per plant (127.78), significantly surpassing most other genotypes, including the breeder checks GI-4 (98.33) and VI-3 (89.33). However, no statistically significant differences were observed in number of spikes per plant between CZI-24-20 and genotypes CZI-24-23 (119.11), CZI-24-21 (113.22), CZI-24-11 (112.56), and CZI-24-17 (111.89), indicating these genotypes possess superior tillering and inflorescence initiation capacity, which are key

determinants of yield potential (Eragegowda *et al.*, 2021). Similar findings of significant genotypic differences for number of spikes among *isabgol* genotypes have been reported earlier (Amanullah *et al.*, 2016; Islam *et al.*, 2020; Arya *et al.*, 2021).

The spike length exhibited significant variation among the genotypes, ranging from 5.08 to 8.19 cm (Table 1). Variation in spike length among genotypes indicates genetic diversity influencing inflorescence development, with longer spikes enhancing seed number and yield through improved photosynthate allocation (Bannayan *et al.*, 2008). Genotype CZI-24-20 exhibited the longest spike length (8.19 cm), significantly surpassing most other genotypes, including the breeder checks GI-4 (6.82 cm) and VI-3 (6.10 cm), while no significant differences were observed compared to CZI-24-23 (8.11 cm), CZI-24-13 (7.43 cm), CZI-24-11 (7.42 cm), CZI-24-21 (7.30 cm) and CZI-24-7 (7.21 cm). The mean performance of different *Isabgol* genotypes for spike length was in agreement with the findings of earlier studies (Islam *et al.*, 2020; Arya *et al.*, 2021).

Most genotypes exhibited cylindrical spikes, whereas CZI-24-13 displayed a club-shaped spike (Table 1). Comparable variations in spike shape, such as club-shaped and cylindrical forms, have been observed previously in *Plantago ovata* germplasm (Singh *et al.*, 2009 and Singh and Lal, 2009). Similar morphological variability in spike traits has been noted earlier (Singh *et al.*, 2009; Singh and Lal, 2009). Additionally, the majority of genotypes had light brown spikes, while CZI-24-16 produced white-color spikes, which appears to be the first documented report of such variation in *Plantago ovata*. The white mutant exhibited significantly higher lightness ($L^* = 57.83$) compared to the normal spike ($L^* = 48.00$), indicating a markedly brighter and whiter appearance. Furthermore, the mutant showed substantially lower redness ($a^* = 0.50$ vs 2.23) and reduced yellowness ($b^* = 15.65$ vs 17.95). These findings confirm a considerable reduction in pigment accumulation in the white mutant, suggesting impairment in pigment biosynthesis pathways (Dai *et al.*, 2025).

This novel observation highlights potential underlying genetic diversity influencing spike pigmentation in *isabgol* germplasm. This color mutant line could be effectively utilized as a

phenotypic marker in *isabgol* hybridization programmes, especially in the *isabgol* crop where genetic variation for spike quality-related traits is limited. Similar applications of color-based morphological mutants as visible phenotypic markers have been documented across several crops (Nadeem *et al.*, 2018).

Identification of superior genotypes for yield related traits

PCA was applied to explore trait relationships and distinguish *isabgol* genotypes based on their yield related traits. The first two principal components (PC1 and PC2) explained 67.6% of the total phenotypic variation, contributing 45.5% and 22.1%, respectively (Fig. 1). The high percentage of explained variance indicates strong and complex interrelationships among the evaluated traits across the genotypes (Sabaghnia *et al.*, 2024). The first two principal components effectively captured most of the total variability, making them adequate for reliable prediction and clear visualization in two-dimensional biplot analysis, consistent with previous research findings (Porkabiri *et al.*, 2019).

The PC1 exhibited high positive loadings for the number of spikes per plant (0.57), spike length (0.51), and seeds per spike (0.52), indicating that these traits were the major contributors to the overall variability. The clustering of these traits along PC1 implies a close positive association among spike morphology traits, consistent with previous findings where spike and spike-related attributes were identified as key yield determinants in *Plantago ovata* (Singh *et al.*, 2009). In alignment with these observations, correlation analysis further demonstrated significant positive relationships among key yield-contributing traits. Notably, a strong association was observed between the number of seeds per spike and the number of spikes per plant ($r = 0.34$, $p < 0.001$), as well as between spike length and the number of spikes per plant ($r = 0.36$, $p < 0.001$) (Fig. 2).

Additionally, a highly significant positive correlation was recorded between the number of seeds per spike and spike length ($r = 0.39$, $p < 0.0001$). Similarly significant positive association was recorded in yield related traits in *isabgol* genotypes (Sharma and Garg, 2002). In contrast, a significant negative association was observed between the number of spikes

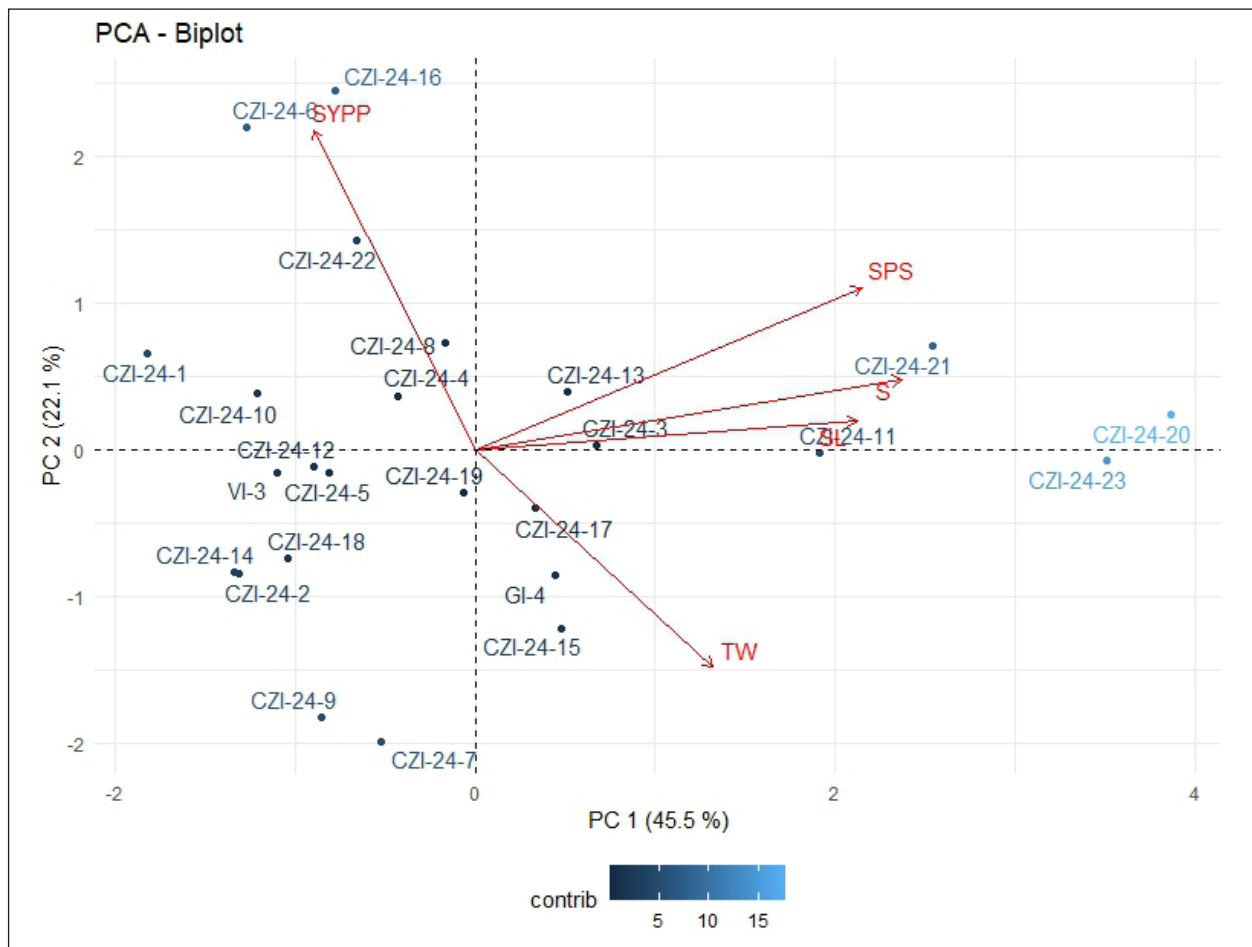


Fig. 1. PCA biplot showing distribution of isabgol genotypes based on yield and related traits.

plant⁻¹ and spike length (Kumar *et al.*, 2013). The results of both PCA and correlation analyses showed that test weight had a weak and non-significant relationship with yield and associated traits. Similarly weak association was observed with test weight and yield and associated traits (Singh *et al.*, 1995). In contrast, a significant positive association was observed between test weight and seed yield per plant (Sharma and Garg, 2002; Kumar *et al.*, 2013).

The PC2 was primarily influenced by seed yield per plant (0.75), suggesting that this trait independently contributed to genotype differentiation. These results align with previous studies suggesting that yield potential in isabgol is largely determined by the combined influence of multiple yield-related traits rather than any single morphological characteristic (Kumar *et al.*, 2013; Islam *et al.*, 2020; Arya *et al.*, 2021). In alignment with these observations, correlation analysis further demonstrated that seed yield per plant showed

a negative and non-significant association with all yield related traits. Similarly, seed yield per plant was observed non-significant association with yield related traits (Kumar *et al.*, 2013).

Genotypes located on the positive axis of PC1 were characterized by elevated values for spikes per plant, spike length, and seeds per spike (Fig. 1). Among them, CZI-24-20 (PC1 = 3.87), CZI-24-23 (PC1 = 3.51), CZI-24-21 (PC1 = 2.54), and CZI-24-11 (PC1 = 1.91) showed strong associations with these traits, suggesting their potential as promising donors for improvement of spike-related attributes. Additionally, cluster analysis revealed that genotypes CZI-24-23, CZI-24-20, and CZI-24-21 were grouped together within Cluster I, This indicated their close genetic similarity based on key yield-related attributes, including number of seeds per spike, spikes per plant, spike length, and test weight (Fig. 3). Both PCA and cluster analysis effectively separated the genotypes according to yield-related

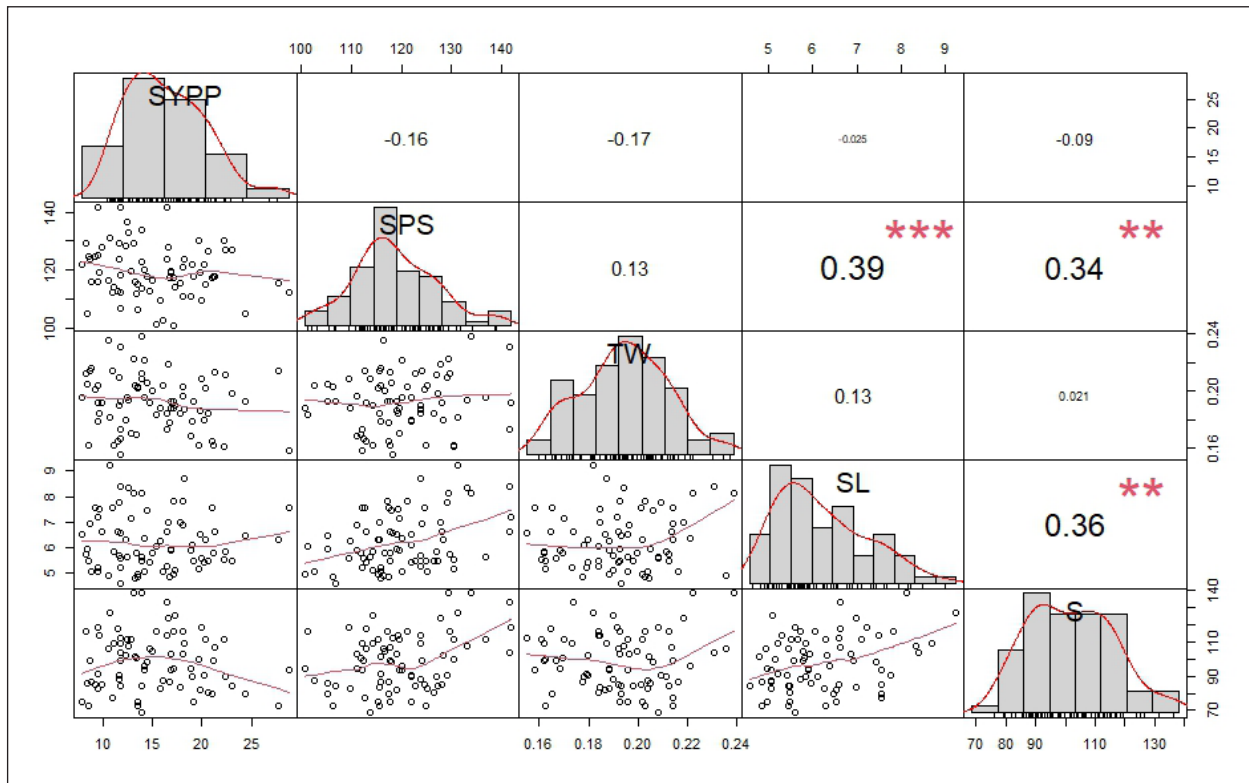


Fig. 2. Pairwise Pearson correlation plot depicting associations among yield-contributing traits among Isabgol genotypes. SYPP: seed yield per plant, SPS: number of seeds per spike, TW: test weight, SL: spike length, and S: number of spikes per plant.

characteristics, with CZI-24-20, CZI-24-23, and CZI-24-21 demonstrated better performance for other traits contributing to yield improvement. Additionally, these genotypes exhibited lower seed yield, reinforcing the notion that yield in Isabgol is a complex trait influenced by the collective contribution of multiple yield-related attributes rather than by any single morphological trait (Kumar *et al.*, 2013; Islam *et al.*, 2020; Arya *et al.*, 2021).

Genotypes positioned on the positive axis of PC2 exhibited higher seed yield per plant, highlighting their importance for direct yield enhancement. Among these, CZI-24-16 (PC2 = 2.44), CZI-24-6 (PC2 = 2.19), and CZI-24-22 (PC2 = 1.42) were strongly associated with seed yield compared to other accessions. Genotypes CZI-24-1, CZI-24-6, and CZI-24-16, characterized by higher seed yield per plant, were grouped together within Cluster II. Both PCA and cluster analysis consistently identified genotypes CZI-24-6 and CZI-24-16 as superior performers, characterized by high seed yield potential and favorable yield-contributing traits. These genotypes can serve as promising parental lines in future *isabgol* breeding programs aimed at yield enhancement and

genetic improvement. Similarly, multivariate analyses, including cluster analysis and PCA, were employed to identify superior and trait-specific genotypes based on their distinct trait combinations and performance patterns (Leite *et al.*, 2018; Ramteke *et al.*, 2024).

The genotypes positioned near the origin of the PCA biplot exhibited moderate performance across all yield-contributing traits, suggesting a balanced but non-specialized expression of phenotypic characteristics. In contrast, those located on the negative sides of PC1 and PC2 displayed reduced expression of key yield components, reflecting limited genetic potential for yield improvement. The alignment between the PCA grouping and hierarchical clustering results, where Cluster II encompassed these genotypes, reinforces the robustness of multivariate analysis in distinguishing performance groups. Such concordance between analytical approaches has also been reported in other crop species, emphasizing the reliability of PCA-cluster integration for identifying superior and inferior genotype clusters (Leite *et al.*, 2018).

Overall, the present study revealed substantial genetic variability among isabgol genotypes for key yield and spike-related traits, underscoring the availability of exploitable diversity for genetic improvement under arid environments. The combined use of analysis of variance, correlation analysis, principal component analysis, and cluster analysis provided a robust framework for dissecting trait interrelationships and identifying trait-specific as well as yield-oriented genotypes. Multivariate analyses clearly demonstrated that seed yield in isabgol is governed by the collective contribution of multiple spike-related traits rather than by any single component, highlighting the complex genetic architecture of yield determination. Genotypes such as CZI-24-6 and CZI-24-16 emerged as promising candidates for direct yield improvement, while CZI-24-20, CZI-24-23, and CZI-24-21 were identified as valuable donors for spike

morphology and reproductive efficiency traits. The strong concordance between PCA and cluster analysis further strengthened the reliability of genotype classification and selection. Notably, the identification of a white-color spike genotype (CZI-24-16) represents a novel and potentially valuable phenotypic marker for isabgol improvement. Given the limited availability of contrasting qualitative markers associated with spike traits in isabgol, this distinct spike color variation could serve as a useful morphological marker in hybridization and selection programmes, facilitating the tracking of recombination and genetic purity in segregating generations. Collectively, these findings provide a strong empirical basis for targeted parent selection and trait-based breeding strategies in isabgol and form a foundation for subsequent evaluation of quality traits, including husk recovery and mucilage

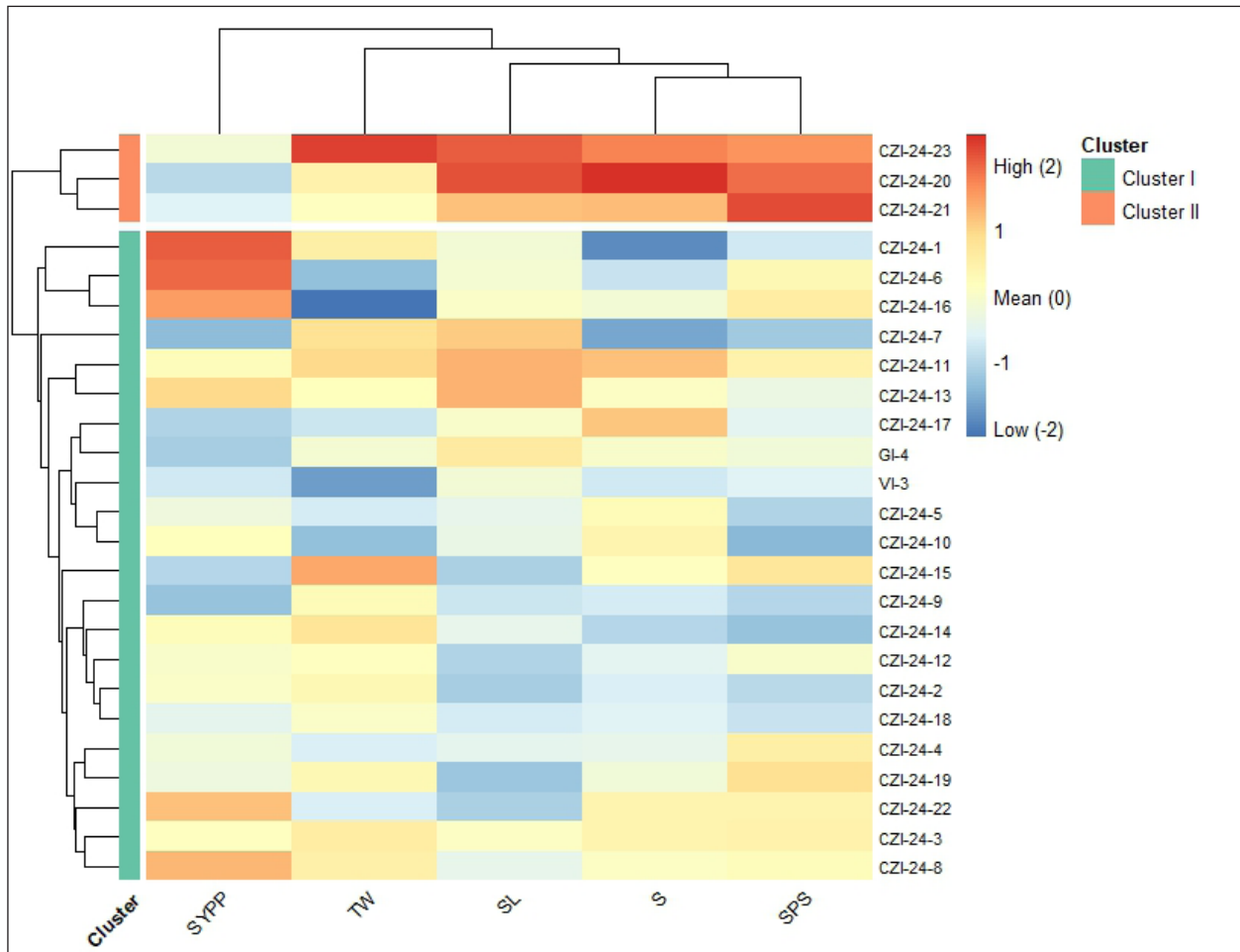


Fig. 3. Cluster heatmap showing the grouping of isabgol genotypes based on yield and related traits. SYPP: seed yield per plant, SPS: number of seeds per spike, TW: test weight, SL: spike length, and S: number of spikes per plant.

content, in advanced stages of the breeding programme.

Conclusions

A wide range of genetic variability was detected among *isabgol* (*Plantago ovata* Forsk.) genotypes for yield-associated traits. Genotypes CZI-24-1, CZI-24-6, and CZI-24-16 recorded higher seed yield, while CZI-24-20, CZI-24-23, and CZI-24-21 showed superior yield related attributes. The consistent results from PCA and cluster analysis identified CZI-24-6 and CZI-24-16 as promising candidates for yield improvement and CZI-24-20, CZI-24-23, and CZI-24-21 for yield related attributes. Spike-related traits such as spike length, spikes per plant, and seeds per spike emerged as the key determinants of yield variation in *isabgol*, while seed yield per plant contributed independently, showing limited association with other yield components. The occurrence of white spikes in CZI-24-16 suggests novel genetic variation influencing spike pigmentation within the germplasm. Overall, these findings highlight valuable genetic diversity and provide a basis for selecting elite genotypes for future breeding and improvement of *P. ovata*. However, further multilocation and multiyear evaluations are essential to validate their stability and adaptability across diverse environments before recommending them for large-scale cultivation.

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