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RESEARCH ARTICLE

Multivariate analysis of soybean genotypes: Uncovering agro-morphological insights

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Abstract

Soybean (*Glycine max* (L.) Merrill) is a vital legume crop known for its high protein and oil content, playing a crucial role in global food security and various industrial applications. Its adaptability to diverse environments and nutritional importance underscores the need for improving genetic diversity to improve yield potential and stress resilience. This study analyzed the genetic diversity among fifty soybean genotypes using cluster analysis and Principal Component Analysis (PCA) based on eleven morphological and quantitative traits. The genotypes were evaluated at the Soybean Breeding Farm, Jawaharlal Nehru Krishi Vishwa Vidyalaya (JNKVV), Jabalpur, in a Randomized Complete Block Design with three replications over three seasons (*Kharif* 2019, 2020 and summer 2021) under both rainfed and irrigated conditions. Cluster analysis using the D^2 method revealed distinct genetic groupings, with genotypes forming 10 clusters in environment E1, where Cluster I comprised 24 genotypes and Cluster V included 9 genotypes; similar clustering patterns were observed in environments E2, E3 and in the pooled data across years. PCA identified the number of pods per plant, number of seeds per plant, days to flower initiation and days to maturity as key traits contributing to genetic variability. Traits such as the number of primary branches per plant, number of pods per plant and number of seeds per plant had a significant influence on genetic variability. Promising genotypes, including JS 22-78, JS 22-66, JS 22-88, AGS 31, JS 22-77, JS 22-90, JS 22-106, JS 22-69, JS 22-10, JS 22-84, JS 22-91, JS 22-92 and AGS 48, exhibited high genetic diversity, demonstrating the utility of cluster analysis and PCA in facilitating the selection of superior genotypes from diverse germplasm pools.

Keywords: cluster; environments; principal component analysis; soybean

Introduction

Soybean is often referred to as the "Miracle Crop" of the 21st century due to its high nutritional value and diverse applications (1, 2). It is a vital multi-purpose crop, serving as a key source of protein for both human consumption and animal feed (3, 4). Beyond its economic and industrial significance, soybean contributes essential raw materials for various industries, thereby playing a crucial role in the Indian economy. The crop is also rich in therapeutic and nutraceutical compounds, earning it the title of "Gold from the Soil" (4-6). Additionally, its symbiotic association with nitrogen-fixing bacteroids enhances soil fertility, making it an environmentally sustainable crop (7-9). Given its vast utility, global efforts have been directed toward improving soybean production and optimizing its agricultural potential (10).

Despite its importance, soybean cultivation in India faces several challenges. Factors such as self-pollination, a narrow genetic base and susceptibility to both biotic and abiotic stresses contribute to relatively low productivity, underscoring the importance of genetic diversity assessment essential for crop improvement. While studies employing PCA and cluster analysis have provided valuable insights into gentic diversity, many have focused on a single crop season. Expanding such analyses across multiple seasons allows for a broader and more stable assessment of trait expression and genotype performance over time. This study adopts a multi-season approach to genetic diversity analysis, offering a more comprehensive perspective on variability that can support long-term soybean breeding strategies.

Genetic diversity analysis plays a crucial role in plant breeding by facilitating the selection of genetically diverse parents to introduce new alleles into crop improvement programs (11-16). PCA is an effective tool for simplifying complex trait data, helping identify key dimensions that explain most of the observed variability, which aids in selecting promising genotypes for cultivar improvement (17). Similarly, cluster analysis is widely used to classify soybean germplasm and to understand genetic relationships among genotypes (15, 18). Although these techniques have been successfully applied in soybean research, evaluating genetic diversity across multiple crop seasons provides additional insights into trait stability and genotype performance. By incorporating this approach, the present study aims to offer a more dynamic and reliable understanding of genetic variation, thereby contributing to informed breeding decisions and the development of improved soybean cultivars.

Materials and Methods

Experimental material

The 48 soybean advanced breeding lines, developed from varied crosses is outlined in Table 1, were advanced through successive generations. These lines were evaluated during the *Kharif* seasons of 2019 and 2020, as well as the summer season of 2021, denoted as E1, E2 and E3 respectively, for each respective year.

Experimental site and design

The experiment was carried out at the Seed Breeding Farm, Department of Plant Breeding and Genetics, JNKVV, Jabalpur. The geographical coordinates of the farm are approximately between 22°49′ N to 20°80′ N and 78°21′ E to 80°58′ E , at an elevation of 488 m above sea level. The study included 50 genotypes, including two national checks, arranged in a randomized complete block design with three replications. Each plot measured 1.2 m x 3.0 m, with a row spacing of 40 cm and plant spacing of 7 cm, across all three environments.

The soil of the experimental area was medium-deep black with good drainage. The soil has neutral pH (7.5) and is mainly constituted of sand (25.3), silt (18.9) and clay (55.8). It has a high cation exchange capacity (30.24 Cmol kg^{-1}), low salt content (EC = 21 dSm^{-1}) and also low organic carbon (0.52 g kg^{-1}).

Phenotypic characterization

The study involved fifty soybean genotypes, accompanied by two checks, which were subjected to phenotypic characterization. Data was collected for eleven traits from five randomly selected plants in each plot. These traits included days to flower initiation (DTFI), days to 50 % flowering (DTFF), days to maturity (DTM), plant height (PH), number of primary branches per plant (PBPP), number of pods per plant (NPPP), number of seeds per plant (NSPP), biological yield per plant (BYPP), 100 seed weight (HSW), harvest index (HI) and seed yield per plant (SYPP). The data collection for these traits was done manually.

Statistical analysis

Mahalanobis generalized distance D^2 was employed to assess the genetic variation among populations based on various traits (19). To group the populations into clusters, Tochers' method was used (20). Wards' method (1963), employed to construct tree diagrams using Euclidian distances and clustering was performed accordingly. PCA was conducted using R software to extract components. Descriptive statistics and PCA were employed for data analysis.

Results and Discussion

Cluster analysis

Table 2 presents the clustering of 50 soybean genotypes using Tochers' method, based on Mahalanobis D^2 values to assess genetic divergence. In E1, 10 clusters were formed, with Cluster I comprising 24 genotypes, followed by Cluster V with 9, Cluster II with 6 and Cluster VI with 5 while the remaining clusters each contained a single genotypes. In E2, three clusters were observed, with Cluster I containing 48 genotypes and the other two clusters each having one genotype. In E3, six clusters were formed, with Cluster I including 39 genotypes, Cluster II comprising 7 and the remaining clusters represented by single genotypes. These results are consistent with previous studies (15, 18, 21-27).

Analysis of pooled multi-season data revealed six clusters, confirming stable genetic divergence. Cluster I contained 32 genotypes, while smaller clusters emphasizing genotype uniqueness with distinct adaptive traits. The clustering pattern in pooled data further corroborates findings from previous research (15, 24, 28, 29). D^2 analysis is crucial for selecting diverse parents in hybridization programs to enhance genetic variation and improve soybean yield and resilience (27, 30). Larger clusters suggest the presence of adaptability, while

Table 1. Parental details of the experimental material

Genotypes	Pedigree	Genotypes	Pedigree
JS 22-63	JS 20-30 X JS 95-60	JS 22-88	JS 20-63 X JS 20-35
JS 22-64	JS 97-52 X JS 95-60-5-12-1	JS 22-89	JS 20-29X JS 93-05
JS 22-65	JS 20-53 X JS 20-34	JS 22-90	JS 20-82 X JS 95-60
JS 22-66	JS 20-71 X JS 20-22	JS 22-91	JS 20-74 X JS 20-22
JS 22-67	JS 20-53 X JS 20-34	JS 22-92	JS 20-88 X JSM 196
JS 22-68	JS 20-71 X JS 20-22	JS 22-93	JS 20-63 X JS 95-60
JS 22-69	JS 20-88 X JS 20-34	JS 22-94	JS 20-53 X JS 20-34
JS 22-70	SL 738 X JS 95-60	JS 22-95	JS 20-98 X JS 20-34
JS 22-71	JS 20-53 X 20-34	JS 22-96	JS 20-53 X JS 20-34
JS 22-72	JS 20-98 X JS 20-34	JS 22-97	JS 20-29 X JSM 275
JS 22-73	JS 20-29 X JSM 275	JS 22-98	JSM 226 X 20-34
JS 22-74	JS 20-63 X JS 20-35	JS 22-99	JS 97-52 X JS (IS) 90-5-12-1
JS 22-75	JS 20-75 X JS 20-14	JS 22-100	JS 20-29 X JS 93-05
JS 22-76	JS 20-79 X JS 335	JS 22-101	SL 738 X JS 95-60
JS 22-77	JS 20-53 X JS 20-34	JS 22-102	JS 20-69 X JS 335
JS 22-78	JS 20-63 X 20-35	JS 22-103	JS 20-88 X JSM196
JS 22-79	20-09 X PS1475	JS 22-104	JS 20-53 X JS 20-34
JS 22-80	20-82 X 95-60	JS 22-105	JS 20-79 X JS 335
JS 22-81	20-29 X 20-22	JS 22-106	JS 20-71 X JS 20-22
JS 22-82	20-63 X 96-60	JS 22-107	JS 20-29 X JS 20-22
JS 22-83	20-29 X 20-22	JS 22-108	JS 20-09 X JSM 258
JS 22-84	NRC 86 X 20-34	JS 22- 109	JS 20-29 X JSM 275
JS 22-85	JS 20-71 X JS 20-22	JS 22- 110	SL 738 X JS 20-88
JS 22-86	JS 20-63 X JS 20-35	JS 20-98	JS 97-52 X SL 710
JS 22-87	JS 20-29 X JS 93-05	JS 20-34	JS 98-63 X PK 768

Table 2. Distribution of fifty soybean genotypes in different clusters across all environments

E1		
Cluster	**Number of genotypes**	**Genotypes**
I	24	JS 22-85, JS 22-86, JS 22-95, JS 22-71, JS 22-104, JS 22-105 , AGS-31, JS 22-70, JS 22-97, JS 22-75, JS 22-106, JS 22-83, JS 22-64, JS 22-96, JS 22-89, JS 22-101, AGS48, JS 22-98, JS 22-67, JS 22-72, JS 22-93, JS 22-87, JS 22-103, JS 22 -107
II	6	JS 22-80, JS 22-92, JS 22-81, JS 22-100, JS 22-94, JS 22-79
III	1	JS 22-73
IV	1	JS 22-76
V	9	JS 22-63, JS 20-98, JS 22-108, JS 22-90, JS 22-108, JS 20-34, JS 22-99, JS 22-65, JS 22-102
VI	5	JS 22-77, JS 22-88, JS 22-78, JS 22-84, JS 22-91
VII	1	JS 22-68
VIII	1	JS 22-66
IX	1	JS 22-82
X	1	JS 22-69
E2		
Cluster	**Number of genotypes**	**Genotypes**
I	48	JS 22-76,JS 22-77, JS 22-78, JS 22-79, JS 22-80, JS 22-81, JS 22-82, JS 22-83, JS 22-84, JS 22- 109, JS 22-85, JS 22-86, JS 22-88, JS 22-89, JS 22-91, JS 22-92, JS 22-93, JS 22-94, JS 22-95, JS 22-96, JS 22-97, JS 22-98, JS 22-99, JS 22-100, JS 22-101, JS 22-102, JS 22-103, JS 22-104, JS 22-105, JS 22-106, , JS 22- 110, JS 22-107, JS 22-73, JS 22-72, JS 22-65, JS 22-70, JS 22-66, JS 22-69, JS 22-68, JS 22-67, JS 22-63, JS 22-64, JS 22-71 JS 22-108, JS 22-74, JS 22-75, JS 20-98, JS 20-34
II	1	JS 22-90
III	1	JS 22-87
E3		
Cluster	**Number of genotypes**	**Genotypes**
I	39	JS 22-76, JS 22-77, JS 22-79, JS 22-80, JS 22-81, JS 22-82, JS 22-83, JS 22-84, JS 22- 107, JS 22-86, JS 22-87, JS 22-88, JS 22-89, JS 22-90, JS 22-91, JS 22-93, JS 22, -94, JS 22-95, JS 22-96, JS 22-97, JS 22-99, JS 22-100, JS 22-101, JS 22-102, JS 22-103, JS 22-104, JS 22-105, JS 22-107, JS 22-73, JS 22-72, JS 22-70, JS 22-66, JS 22-67, JS 22-63, JS 22-64, JS 22-71, JS 22-108, JS 22-74, JS 22-75
II	7	JS 22-85, JS 22-69, JS 22-65, JS 20-98, JS 22-106, JS 22-68, JS 20-34,
III	1	JS 22-78
IV	1	JS 22-98
V	1	JS 22-92
VI	1	JS 22- 110
Pooled		
Cluster	**Number of genotypes**	**Genotypes**
I	32	JS 22-76, JS 22-79, JS 22-80, JS 22-83, JS 22- 109, JS 22-85, JS 22-86, JS 22-87, JS 22-93, JS 22-94, JS 22-95, JS 22-96, JS 22-97, JS 22-98, JS 22-99, JS 22-100, JS 22-101, JS 22-102, JS 22-103, JS 22-104, JS 22-105, JS 22-106, JS 22-107, JS 22-73, JS 22-72, JS 22-69, JS 22-63, JS 22-64, JS 22-71, JS 22-108, JS 22-74, JS 22-75
II	7	JS 22-65, JS 20-34, JS 20-98, JS 22-90, JS 22-68, JS 22-70, JS 22-89
III	1	JS 22-67
IV	5	JS 22-82, JS 22- 110, JS 22-88, JS 22-92, JS 22-81
V	3	JS 22-77, JS 22-78, JS 22-84
VI	2	JS 22-91, JS 22-66

Abbreviations: E1= *kharif* -2019, E2= *kharif* -2020, E3= summer-2021

smaller clusters indicate unique genotypes with specialized traits like drought tolerance or disease resistance. This multi-season approach strengthens the identification of stable genotypes, thereby reducing environmental bias in selection. The presence of single-genotypes clusters highlights unique breeding potential. Overall, these findings provide valuable insights for breeders aiming to enhance soybean genetic diversity and develop climate-resilient, high-yielding cultivars.

Furthermore, analyzing cluster stability across multiple years provides insights into the consistency of genotype performance under varying environmental conditions. Genotypes that consistently cluster together across different crop seasons, it indicates genetic stability, making them ideal candidates for breeding programs focused on yield stability and stress adaptation. The presence of genotypes in different clusters across environments suggests strong environmental influences on trait expression. Selecting genotypes from different clusters could optimize breeding strategies, maximizing heterotic potential. Future research should explore genotype-by-environment interactions further to refine selection criteria and enhance soybean improvement efforts.

Principal Component Analysis

Principal Component Analysis (PCA) of yield and yield-related traits in advanced soyabean breeding lines of identified five principal components (PCs) with eigenvalues exceeding than one, explaining 81.85 % of variation in E1, 83.40 % in E2, 90.61 % in E3 and 85.77 % in pooled data (Table 3). This indicates that these components captured the majority of genetic variability across environments. In E1, PC1 accounted for 31.3 % of the variation, while PC2 explained 20.02 %. In E2, PC1 and PC2 were explained 31.32 % and 18.11 % respectively. In E3, PC1 accounted for 38.09 %, while PC2 explained 19.97 % of the variation. In pooled data, PC1 contributed 37.13 % of the variance, while PC2 explained 17.75 %.

The main contributing traits varied across environments. In E1, number of pods per plant (NPPP) and number of seeds per plant (NSPP) were the major contributors to PC1, while 100-seed weight (HSW), harvest index (HI) and seed yield per plant (SYPP) contributed to PC2. In E2, HI and SYPP were key traits for PC2. In E3, days to flowering initiation (DTFI), days to 50 % flowering (DTFF) and days to maturity (DTM) were the main contributors to PC1, while primary branches per plant (PBPP), NPPP and NSPP

Table 3. Parameters of principal components based on twelve agronomic traits in soybean

	Environment	PC1	PC2	PC3	PC4	PC5
Eigen value	E1	1.86	1.48	1.17	1.09	0.90
	E2	1.89	1.41	1.15	1.09	1.05
	E3	2.05	1.48	1.26	1.08	0.91
	Pooled	2.02	1.40	1.19	1.06	0.92
Proportion of variance	E1	31.29	20.02	12.40	10.74	7.40
	E2	32.32	18.11	12.12	10.81	10.04
	E3	38.09	19.97	14.34	10.63	7.59
	Pooled	37.13	17.75	12.95	10.27	7.68
Cumulative proportion	E1	31.28	51.30	63.70	74.45	81.85
	E2	32.32	50.43	62.56	73.37	83.40
	E3	38.09	58.06	72.04	83.03	90.61
	Pooled	37.13	54.88	67.83	78.10	85.77

Abbreviations: E1= *kharif* -2019, E2= *kharif* -2020, E3= summer-2021, PC= Principal components

were dominant in PC2. In pooled data, DTFI, DTFF and HI, SYPP were the primary contributors to PC1 and PC2, respectively. Certain traits showed opposite contributions across different PCs, such as NPPP in E1, which contributed positively to PC1 (0.46) but negatively to PC2 (-0.15) and DTFF in E3, which contributed positively to PC1 (0.43) but negatively to PC2 (-0.19). These findings align with previous studies (21, 24, 25, 26, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40), reinforcing the relevance of PCA in analyzing trait contributions (Fig. 1 & 2). The results suggest that the first principal component captures the most significant variation, making selection based on PC1-associated traits highly effective in breeding programs. The semi-curve line observed after the fifth PC, with only minor variations in subsequent components, supports the idea that most meaningful variation is concentrated within the first few PCs.

From a breeding perspective, PCA provides valuable insights into trait selection for improving yield stability and adaptability across different environments. The identification of NPPP, NSPP, DTFI and DTM as key yield-contributing traits suggests that these should prioritize br prioritized in breeding programmers to enhance genetic gain. Their consistent contribution to variability across environments highlights their potential for multi-environment selection strategies. Additionally, clustered traits within PCs suggest the presence of underlying genetic correlations, which can be leveraged in marker-assisted selection (MAS) and genomic selection (GS).

Selecting genotypes with higher NPPP and NSPP could indirectly improve seed yield, while focusing on DTFI and DTM may enhance crop duration management for different agroecological zones. PCA-based trait selection can also support parental selection for hybridization, ensuring that breeding crosses include genetically diverse yet complementary genotypes. By identifying the traits that drive maximum variation in soybean populations, this study provides a foundation for precision breeding efforts aimed at developing high-yielding, climate-resilient soybean cultivars. A deeper understanding of genetic variation across multiple environments will improve the efficiency of breeding programs and contribute to sustainable soybean production.

Conclusion

In conclusion, this study demonstrated the effectiveness of D^2 analysis in clustering soybean genotypes across environments, revealing significant genetic diversity both among and within clusters. Five distinct clusters were identified, while the first four PCs together accounted for 81.85-85.77 % of the total variation. Promising genotypes such asJS 22-78, JS 22-66, JS 22-88, AGS 31, JS 22-77 and others, were identified based on PCA results and

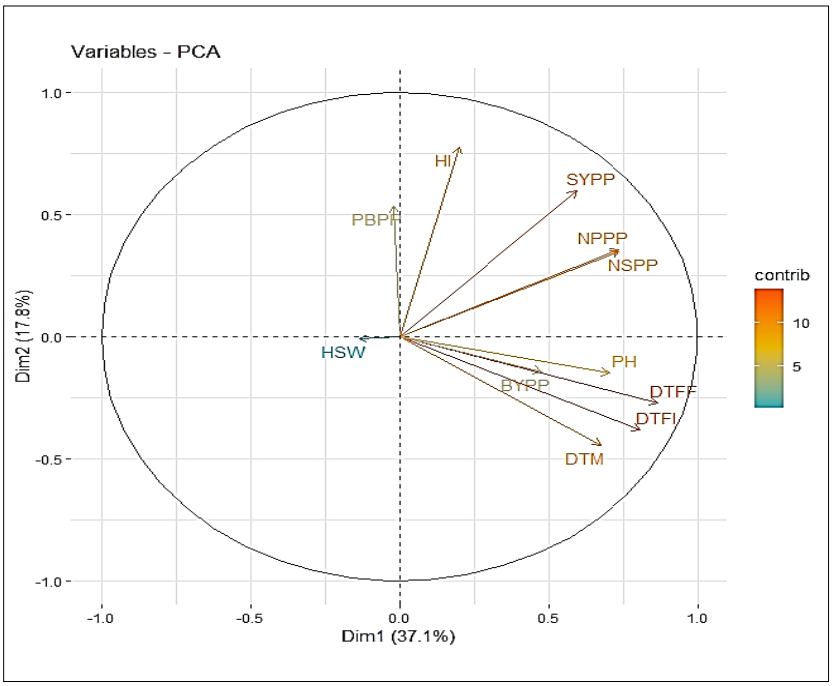

Fig. 1. Correlated response of PCs variables for pooled data of E1, E2 & E3.

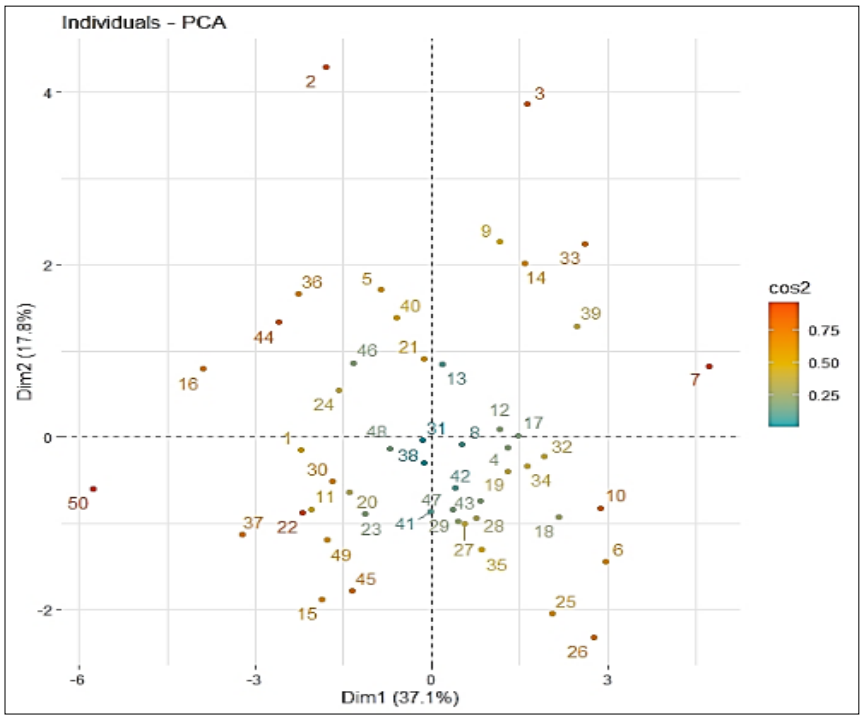

Fig. 2. PCA - biplot depicting the distribution of individual soyabean genotypes.

yield performance across multiple environments. The genotypes developed at JNKVV, Jabalpur, showed a broad genetic base owing to diversified parentage, thereby offering the potential for transgressive segregants in breeding programmes. Overall, this study highlights the combined utility of clustering and PCA in understanding genotype-environment interactions, contributing valuable insights for the development of resilient and high-performing crop varieties.

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Authors' contributions

All authors have contributed significantly to this work. MS was responsible for writing the original draft, conceptualization, software, review and editing, visualization, and methodology. MKS contributed to conceptualization, resources, investigation, validation, review, and data curation. PKA provided supervision and validation, while YS also contributed through supervision and validation. VK carried out data analysis, review, and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare

Ethical issues: None

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