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RESEARCH ARTICLE

Unlocking qualitative traits diversity in soybean [*Glycine max* (L.) Merrill] genotypes

Riya Mishra[1], Yamini Gautam[1], Goutam Mohbe[1], Sanjeev Sharma[1], Jagendra Singh[2], Niraj Tripathi[3], D K Payasi[4], Sandeep Singh Tomar[2], M K Tripathi[1,2*] & Sharad Tiwari[5]

[1]Department of Genetics & Plant Breeding, Rajmata Vijayaraje Scindia Agriculture University, Gwalior 474 002, Madhya Pradesh, India
[2]Zonal Agricultural Research Station, Rajmata Vijayaraje Scindia Agriculture University, Morena 476 001, Madhya Pradesh, India
[3]Directorate of Research Services, Jawaharlal Nehru Agriculture University, Jabalpur 482 004, Madhya Pradesh, India
[4]Regional Agricultural Research Station, Sagar, Jawaharlal Nehru Agriculture University, Jabalpur 482 004, Madhya Pradesh, India
[5]Faculty of Agriculture, Mangalayatan University, Jabalpur 483 001, Madhya Pradesh, India

*Correspondence email - drmanojtripathi64@gmail.com

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Abstract

Glycine max (L.) Merrill is a vital leguminous oilseed crop valued for its high protein and oil content, contributing significantly to human and animal nutrition. Understanding genetic diversity exist within soybean germplasm lines is essential for breeding, conservation and crop improvement. This study aimed to assess the qualitative trait variability present among 153 soybean genotypes to aid in genotype classification and conservation strategies in future crop improvement programmes. The experiment was conducted at Zonal Agricultural Research station ZARS, Morena, RVSKVV, Gwalior, Madhya Pradesh, India during the *Kharif* 2024, using an augmented block design. Ten morphological traits *viz.*, hypocotyl colour, leaf shape, leaf colour, plant growth habit, flower colour, pod pubescence, seed shape, seed colour, seed lustre and hilum colour were evaluated following the Protection of Plant Varieties and Farmers' Rights Authority (PPVFRA) and Distinctiveness, Uniformity and Stability (DUS) testing criteria. The results indicated existence of considerable phenotypic variation among the genotypes. For instance, the semi-erect growth habit, which was predominant and observed in 122 genotypes and dark green leaf colour, which was the most common and recorded in 102 genotypes. The Shannon diversity index (H') identified seed hilum colour as the most variable trait, suggesting its potential utility in genetic improvement. Cluster analysis (UPGMA) classified the genotypes into two major clusters, providing insights into genetic relationships and aiding in parental selection for hybridization. These findings underscore the importance of morphological characterization in genotypic classification, germplasm conservation and utilization in breeding programmes. This study provides a scientific foundation for soybean genetic resource management, supporting breeding efforts for improved yield, adaptability and sustainability.

Keywords: genetic diversity; morphological variation; phenotyping; qualitative traits; soybean

Introduction

Glycine max (L.) Merrill is a self-pollinated, annual leguminous and oilseed crop of global significance, domesticated from *Glycine soja* in eastern China over 3000 years ago. It later spread to Japan, Korea and Southeast Asia before reaching America and Brazil, where it now achieves high yields (1). Soybean was introduced to India from China in the 10th century AD *via* the Himalayan routes and through Burma by Indonesian traders. It has also been traditionally cultivated on a small scale in regions like Himachal Pradesh, Uttarakhand, Eastern Bengal and parts of Central India. India is considered a secondary centre of soybean domestication after China (2). It is a globally significant legume, contributing 25 % of the world's edible oil and two thirds of the protein concentrates for livestock nutrition. It has been a dietary staple in China, Japan, Korea and Southeast Asia for centuries, offering meat and dairy alternatives. Its diverse uses have earned it moniker like "Cow of the field" and "Gold from soil" (3). It is a highly nutritious crop, rich in protein, healthy fats, dietary fibre and bioactive compounds like isoflavones and tocopherols. Traditionally, it has been a staple in Asian diets, contributing to foods such as tofu, soy sauce, miso and tempeh, while also serving as a key plant-based protein source. Beyond its dietary significance, it is widely utilized in industrial applications, including biofuel production and the development of biodegradable materials (4). As of 2024, global soybean production reached approximately 420.76 million metric tons, reflecting its agricultural dominance, with Brazil (39 %), United States (29 %) and Argentina (12 %) being the top producers. China (5 %) and India (3 %) contributed smaller yet significant shares, with South and North America dominating global production (5). In India, soybean cultivation is concentrated in mainly Madhya Pradesh (44 %) followed by Maharashtra (39 %) and Rajasthan (8 %), where favourable agro-climatic conditions advanced agricultural practices supported high productivity. These key regions play a vital role in sustaining domestic demand and contributing to export markets (6).

Genetic diversity is a fundamental requirement for conducting breeding programmes as it enables the identification and selection of superior genotypes for crop improvement. Maximising the extent of genetic variability among genotypes enhances the prospects of breeding for desirable traits (7). Phenotyping characterization serves as a primary tool for assessing genetic variability and plays a critical role in the optimal utilization of gene bank resources. Diversity studies typically rely on evaluation of both qualitative and quantitative traits, with quantitative traits exhibiting continuous variation. This approach aids in the effective utilization of genetic resources for breeding and conservation (8). In the case of soybean (*Glycine max*), domestication and the intentional selection of novel genetic variants by ancient farmers have resulted in greater morphological diversity compared to its wild progenitor (*Glycine soja*) (9). Although soybean is extensively cultivated worldwide, its growth and productivity are significantly influenced by unfavourable environmental factors (10). Consequently, the morphological characterization of soybean genotypes plays a crucial role in enhancing soybean breeding programmes by facilitating genetic improvement and trait selection (11). Such characterization not only aids in understanding genetic diversity but also informs strategies for germplasm conservation (12). Evaluating genetic variability is crucial for the advancement of high yielding and nutritionally enhanced soybean cultivars (13). Furthermore, soybean's extensive genetic variability and agronomic traits make it valuable for genetic mapping, marker development and genome wide association studies. These approaches aid in identifying key genes, driving advancements in soybean breeding (14). However, systematic studies are lacking on morphological characterization of a large set of soybean genotypes. Therefore, the present study aimed to identify genetic variability exist among 153 soybean genotypes using key morphological traits, that may provide a foundation for further genetic improvement.

Materials and Methods

Characteristics of experimental site

Morena district, located in northern Madhya Pradesh, borders Rajasthan (west), Uttar Pradesh (north) and the districts of Gwalior, Bhind and Sheopur Kalan (south), providing a strategic agro-climatic location. Geographically, it is positioned between latitudes 26°05′-26°42′N and longitudes 77°05′00′′–78°30′00′′E, encompassing a total area of 4988 km^2. The district is represented on the survey of India Toposheets 54 F and J. During the crop season from July to October 2024, as per the agrometrology observatory RVSKVV-ZARS, Morena had the mean weekly temperatures ranged between 29.9 °C to 34.7 °C (max) and 19.9 °C to 27.9 °C (min). The site received about 752.3 mm rainfall over 34 rainy days, with relative humidity arrayed between 66.3 % to 94.2 % (morning) and 51.2 % to 81.0 % (afternoon). Pan evaporation increased towards October, while wind speeds remained moderate between 3.8 and 5.1 km/hr. Overall, the climatic conditions were favourable for *Kharif* crop growth under semi-arid conditions.

Experimental details

The present investigation was conducted employing an augmented block design (Table 1) during *Kharif*, 2024 at the Zonal Agricultural Research Station (ZARS), Morena under Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya (RVSKVV), Gwalior, Madhya Pradesh (Fig. 1). The experimental field was prepared through deep tillage using a tractor mounted cultivator, followed by two passes with a disc harrow to achieve a fine tilth. Final levelling was completed using a tractor-drawn leveller. Soybean seeds were sown manually using the hand-dribbling method, ensuring uniform spacing (Fig. 2). A basal fertilizer dose of 20:60:20:20 kg/ha (N:P:K:S) was uniformly applied across all treatments. Observations were recorded from five randomly selected plants under the guidelines established by the Protection of Plant Varieties and Farmers' Rights Authority (PPVFRA) and the Distinctiveness, Uniformity and Stability (DUS) testing criteria for soybean. Qualitative traits were described using International Union for the Protection of New Varieties of Plants (UPOV) descriptors (1998) (15). Genetic relationships and variation among genotypes were assessed through qualitative data analysis. A dendrogram was constructed to elucidate the clustering pattern of genotypes, employing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm. The analysis was performed using NTSYSpc software to confirm genotype groupings and assess genetic diversity (16). Additionally, the Shannon weaver diversity index was calculated using the MS-office Excel package.

Table 1. List of genotypes and their source/origin

S. No	Germplasm	Source/origin
1.	JS-26, JS-93-05, JS-97-52, JS-20-29, JS-20-34, JS-20-53, JS-20-69, JS-20-79, JS-20-89, JS-20-94, JS-20-98, JS-20-116, JS-21-05, JS-21-07, JS-21-17, JS-21-72, JS-21-76, JS-21-77, JS-22-01, JS-22-08, JS-22-12, JS-22-16, JS-23-03, JS-23-05, JS-23-09, JS-24-26, JS-24-30, JS-24-34, JS-24-31, JS-25-03, JS-25-05, JS-25-06, JS-25-08, JS-25-02, JS-24-33, JS-25-50, JS-25-52, JS-25-53, JS-25-55	JNKVV, Jabalpur
2.	RVS-76, RVS-2001-4, RVS-2011-10, RVS-12-8, RVS-13-20, RVS-15-1, RVS-23-2, RVS-23-5, RVS-23-7, RVS-23-8, RVS-23-9, RVS-23-10, RVS-23-11, RVS-23-12, RVS-23-13, RVS-23-14, RVS-23-15, RVS-23-16, RVS-23-17, RVS-23-19, RVS-23-20, RVS-23-21, RVS-23-22, RVS-23-23, RVS-23-25, RVS-23-26	RVSKVV, Gwalior
3.	NRC-130, NRC-138, NRC-142, NRC-150, NRC-152, NRC-166, NRC-192, NRC-201, NRC-254, NRC-255, NRC-258, NRC-259, NRC-264, NRC-265, NRC-266, NRC-267, NRC-269, NRC-271, NRC-859	NSRI, Indore
4.	NRCSL-4, NRCSL-7, NRCSL-8, ASB-85, ASB-93, ASB-101, DLSB-5, DLSB-40, PS-1569, PS-1696, Himso 1696, Himso 1695, RVSM-35, RVSM-12-21, RVSM-2011-35, RVSM-2012-4, KSS-213, KSS-225, MAUS-124, MAUS-824, MAUS-791, MAUS-787, Rajsoya-24, CAUMS-3, MACS-1810, MACS-1834, MACS-824, TS-208, TS-156, TS-101, AUKS-212, AUKS-21-5, AS-26, AS-34, AS-55, Cat 492A, Cat 87, KDS-1188, KDS-1193, KDS-1201, KDS-1203, KDSIS-1394, KBSL-23-36, VLS-104, VLS-106, THPS-6, Lok soya 03, Pusa Sipani BS 8, Pusa Sipani BS 33, Pusa Sipani SPS 433	RAK, Sehore
5.	RSC-10-46, RSC-10-52, RSC-11-95, RSC-1165	Raipur
6.	DS 1510, DS 1547, DS-1589, DS-1480	Delhi
7.	AMS-2021-3, AMS-2021-4, AMS-100-39, AMS-264, AMS-22-16, AMS-2022-1	PDKV, Akola
8.	Jhimka	Landrace
9.	EC109563	Exotic collection
10.	SL-311, SL-1315	PAU, Ludhiana
11.	BAUS(M)-6	Ranchi

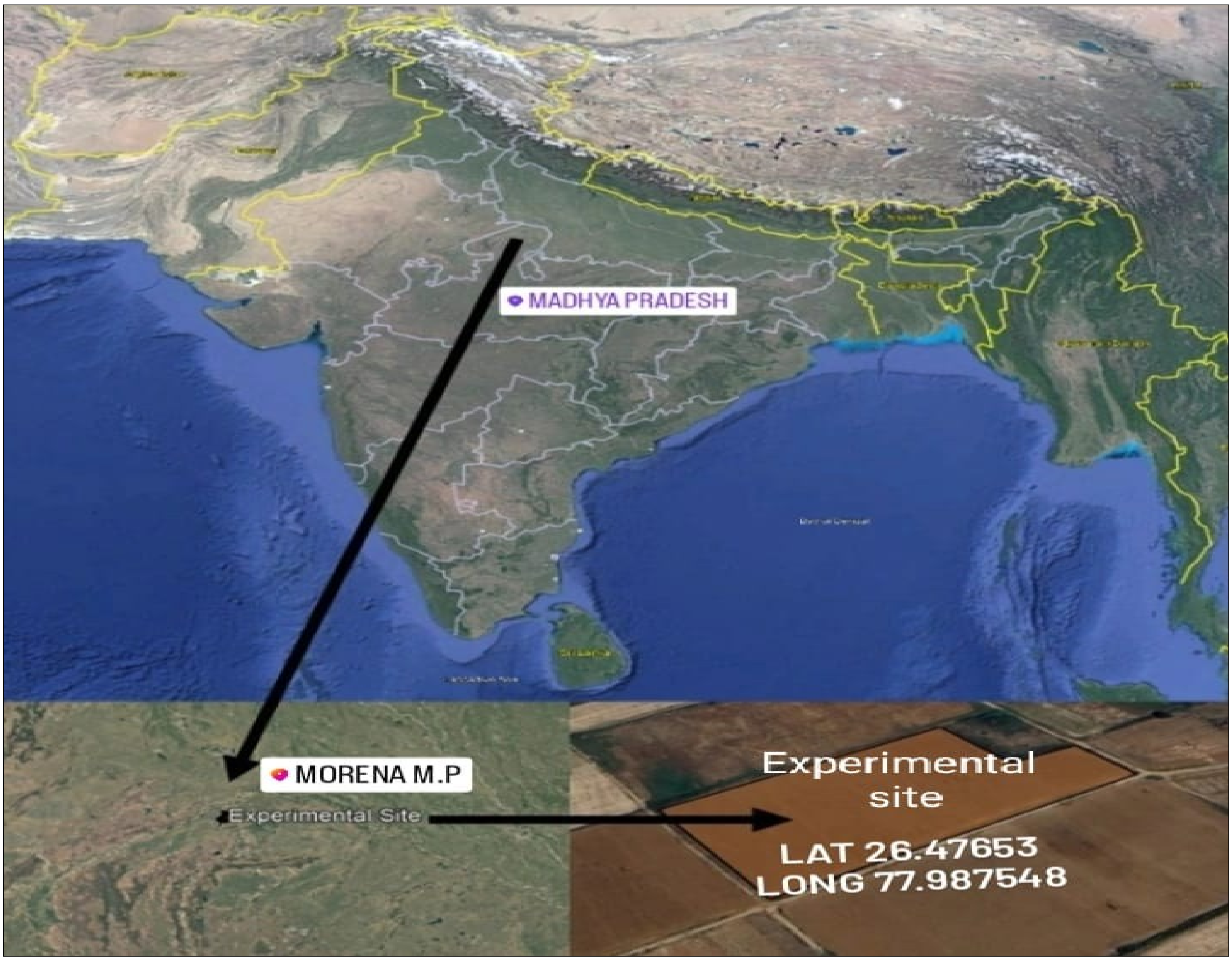


Fig. 1. Map of location of experimental field.

Fig. 2. Land preparation and sowing of seeds.

Result and Discussion

Characterizing crop genotypes is essential for classification and helps to avoid duplication. Qualitative traits are particularly valuable for genotype classification due to their stable inheritance across generations. These traits serve as reliable markers for genotype characterization, as they are less influenced by environmental variations (17). In this investigation, qualitative traits were recorded across 153 soybean genotypes to evaluate phenotypic diversity. The analysis of morphological traits revealed substantial variation within the population (Table 2). For hypocotyl colour, the presence was observed in 83 genotypes, slightly outnumbering the absence in 70 genotypes. Among the leaf shape variations, the pointed ovate form was predominant in 132 genotypes, while the round ovate in 6 genotypes and lanceolate in 15 genotypes forms were less frequent (Fig. 3A). Leaf colour exhibited a strong preference for dark green (102 genotypes) over green (51 genotypes) (Fig. 3B). Regarding plant growth habit, the semi-erect type was significantly more common in 122 genotypes than the erect type in 31 genotypes. Flower colour showed a slight predominance of violet in 83 genotypes compared to white in 70 genotypes (Fig. 3C). For pod pubescence, the presence (74 genotypes) and absence (79 genotypes) were nearly equally distributed (Fig. 3D). Analysis of seed morphology revealed a dominance of elliptical seed shapes in 144 genotypes (Fig. 3E), followed by yellow green in 19 genotypes and green in only one genotype, while black seed colour was found to be absent (Fig. 3F). Seed lustre was primarily shiny in 103 genotypes, with dull types being less frequent and found in 50 genotypes. Finally, hilum colour analysis indicated that black was the most frequently observed in 84 genotypes tracked by brown in 8 genotypes, gray in 19 genotypes and yellow in 12 genotypes (Fig. 3G).

In the context of the present study, a previous investigation evaluated 60 soybean genotypes for 12 qualitative characters (8). Significant variability was observed in qualitative traits such as leaf shape, colour intensity, pod characteristics, seed shape and plant growth behaviour. Leaf types varied in four categories, with 42 genotypes exhibiting pod hairiness and 43 showing pubescence. Variations were also recorded in flower colour, seed coat traits and growth habits, highlighting the genetic diversity among the genotypes. A set of 28 vegetable soybean genotypes were classified using IBPGR descriptors (7), showed variations in leaf shape and colour, flower colour, growth habit and pod traits. Leaf shapes included lanceolate, pointed ovate and round ovate with green leaf colour predominating and some genotypes exhibiting dark green shades. Furthermore, pod characteristics varied in colour, shape and pubescence presence. In a related investigation, researchers (18) assessed 53 soybean genotypes and reported variation in five qualitative traits, including leaf shape, colour intensity, flower colour, pod colour and pubescence. Leaf types varied, with pointed ovate being most common, while flower colour was either white in 25 genotypes or purple in 28 genotypes. Pod traits also exhibited diversity, with 29 genotypes having pod hairs and 24 without.

A dendrogram was constructed in the present investigation based on the qualitative characteristics of 153 soybean genotypes (Fig. 4). The analysis grouped the genotypes into two primary clusters: a major and a minor cluster. The minor cluster comprised 76 genotypes, while the major cluster contained 77 genotypes. The major cluster was further subdivided into two subclusters. The minor subcluster consisted of 19 genotypes, including JS 25-06, RVS 23-13, RVS 23-7, DLSB-40, RVSM-35, RVSM 2011-35, Raj soya 24, RVS-23-17, RVS 23-5, RVS-13-20, JS 20-34, JS20-116, NRC-258, RVS-23-16, NRC-192, DS -1589, MAUS-124, KDSIS-1394 and BAUS (M)-6. Whilst the major subcluster within this group comprised 58 genotypes. Similarly, within the minor cluster, the major subcluster included 54 genotypes, whereas the minor subcluster consisted of 22 genotypes, *viz*., JS 24-30, CAUMS-3, NRC-271, RSC11-95, DS-1510, TS-208, NRC-254, AS-55, ASB-85, Cat492A, SL-1315, JS20-53, TS-156, NRC-142, KDS-1188, EC-109563, RVS-23-23, NRC-259, KSS-213, AUKS-21-5, KBSL-23-36 and SL-311.

The Shannon algorithm was employed to assess the genetic diversity index (Shannon index, H') for soybean genotypes based on ten qualitative characters. The Shannon

Table 2. Distribution of different phenotypic classes among different qualitative classes

Description	Category	Number of genotypes	Frequency (%)
Hypocotyl color	Absent	70	45.752
	Present	83	54.248
Leaf shape	Pointed ovate	132	86.275
	Round ovate	6	3.922
	Lanceolate	15	9.804
Leaf colour	Green	51	33.333
	Dark green	102	66.667
Plant growth habit	Erect	31	20.261
	Semi-erect	122	79.739
Flower colour	White	70	45.752
	Violet	83	54.248
Pod pubescence	Absent	79	51.634
	Present	74	48.366
Seed shape	Elliptical	144	94.118
	Spherical	9	5.882
Seed colour	Yellow	133	86.928
	Yellow green	19	12.418
	Green	1	0.654
	Black	0	0.000
Seed lustre	Shiny	103	67.320
	Dull	50	32.680
Seed hilum color	Black	84	54.902
	Brown	38	24.837
	Gray	19	12.418
	Yellow	12	7.843

(A)

Round Ovate

Lanceolate

Pointed Ovate

(B)

Green

Dark Green

(C)

White

Violet

(D)

	Present	**Absent**
(E)		
	Elliptical	**Spherical**

(F)			
	Green	**Yellow Green**	**Yellow**

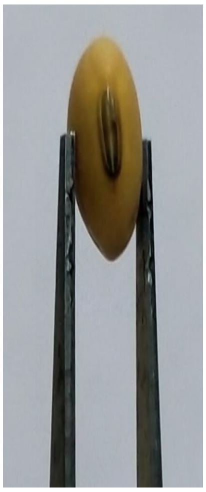
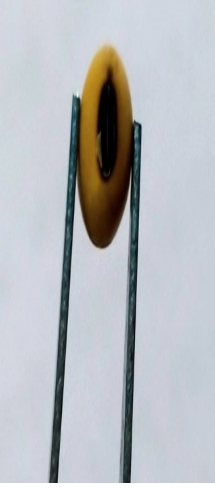
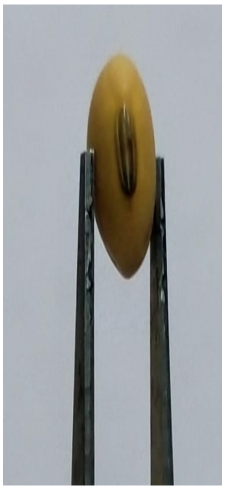
(G)				
	Brown	**Black**	**Gray**	**Yellow**

Fig. 3. Different morphological traits: (A) leaf shape; (B) leaf colour; (C) flower colour; (D) pod pubescence; (E) seed shape; (F) seed colour; (G) seed hilum colour.

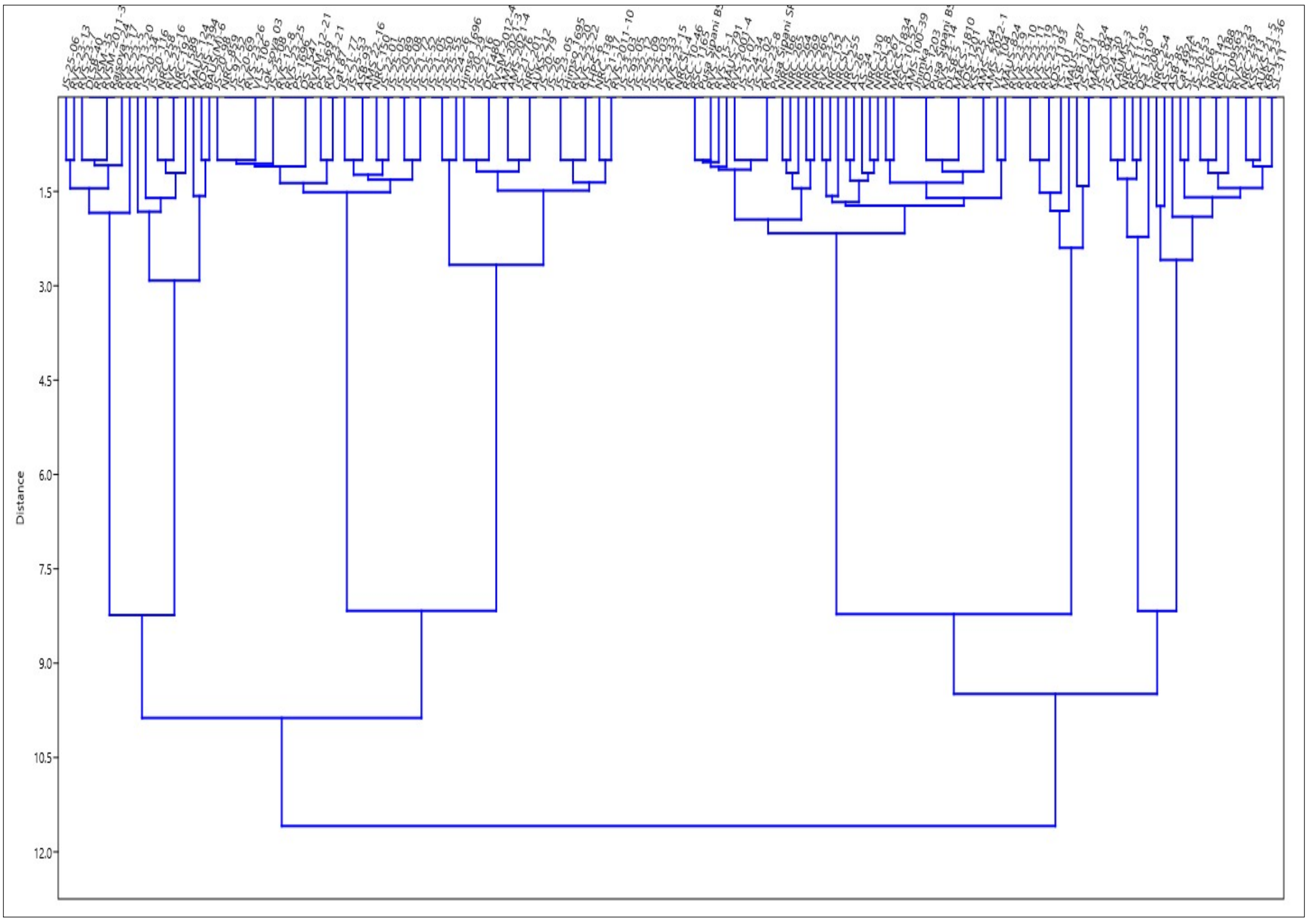


Fig. 4. Dendrogram showing relationship among different soybean genotypes based on morphological traits.

index values varied significantly across different qualitative traits, reflecting distinct levels of genetic diversity. The Shannon diversity index (H') for ten qualitative traits was utilized to assess the frequency distribution of diversity within the population. Among all the traits analysed, seed hilum colour exhibited the highest 'H'' value of 1.134 (Table 3), indicating the greatest genetic variability. Genotypes exhibited a narrow genetic base with H' values below 1.5 and even 1.0, highlighting the urgent need for germplasm conservation. Previous studies (18-21) have also utilized the Shannon index to assess variability in qualitative traits of soybean. Based on the phenotypic dissimilarity index, genotypes exhibiting significant divergence from others may serve as potential parental lines in future breeding programmes aimed to enhance qualitative traits through hybridization.

Phenotypic characterization of crop genetic resources plays a pivotal role not only in germplasm conservation but also in their effective utilization for breeding and genetic improvement. In the present investigation, the qualitative trait assessment across 153 soybean genotypes revealed considerable variability, although the Shannon index values indicated a relatively narrow genetic base. Such variability, particularly in traits like seed hilum colour, which showed the highest diversity (H' = 1.134), underscores its potential as a distinguishing marker to be used in future breeding programmes. The observed diversity in key morphological traits including growth habit, leaf colour, flower colour and seed morphological attributes is crucial as these traits are often linked to important agronomic characteristics such as yield stability, stress tolerance and adaptability to diverse agro-ecological conditions. Moreover, the traits like semi-erect plant growth habit and dark green leaf colour, which were predominant among the genotypes, are significant for canopy architecture and photosynthetic efficiency, directly influencing crop performance. The clustering pattern obtained through UPGMA analysis grouped the genotypes into distinct clusters, providing valuable insights into their genetic relationships. This information is vital for selecting genetically diverse parents to maximise heterosis and improve the chances of success in hybridization programmes aimed to improve qualitative as well as quantitative traits. Therefore, while this study highlights the importance of morphological diversity for germplasm conservation, it simultaneously reinforces the role of such diversity as a genetic reservoir that can be strategically exploited in breeding programmes. By identifying genotypes with unique or divergent traits, breeders can design crosses that combine complementary features, thereby advancing the development of high-yielding, nutritionally superior and stress-resilient soybean cultivars (22-27).

Table 3. Shannon weaver diversity index

Character	HC	LS	LC	PGH	FC	PP	SS	SC	SL	SHC
Shannon H' Index	0.689	0.482	0.636	0.504	0.689	0.692	0.223	0.413	0.632	1.134

Abbreviations: HC: hypocotyl colour; LS: leaf shape; LC: leaf colour; PGH: plant growth habit; FC: flower colour; PP: pod pubescence; SS: seed shape; SC: seed colour; SL: seed lustre; SHC: seed hilum colour.

Conclusion

This study underscores the qualitative trait variability among 153 soybean genotypes with seed hilum colour emerging as the most diverse trait. The morphological characterization not only facilitates the classification and conservation of soybean germplasm but also provides a critical foundation for crop improvement initiatives. The insights into genetic relationships derived from cluster analysis can guide the selection of diverse parental lines for utilization in hybridization, ultimately broadening the genetic base of breeding populations. The qualitative traits assessed here integral to plant architecture, photosynthetic efficiency and reproductive characteristics hold direct relevance for enhancing yield potential and adaptability. Consequently, integrating these morphological traits into selection strategies is essential for effective germplasm management and the formulation of breeding programmes meant to develop robust, high performing soybean cultivars.

Authors' contributions

RM, YG, GM and SS collected data and drafting and writing of original manuscript. JS, NT and DKP contributed to the conceptualization of the study and provided essential project supervision. MKT, SST and ST carried out necessary modifications and final draft preparation of this research framework. All the authors critically reviewed and approved the final version of the manuscript.

Compliance with ethical standards

conflict of interest: The authors declare no conflict of interest.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process: While preparing this work, the authors used Grammarly to reduce grammatical errors. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the publication's content.

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