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CHARACTERIZATION OF SOYBEAN (*GLYCINE MAX* L. MERRILL) GERMPLASM BASED ON DUS DESCRIPTORS

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ABSTRACT

Soybean is a self-pollinated annual legume. To maintain and improve the crop productivity, exploring and utilizing the genetic variation within the gene pool is a critical step. Current experiment is focused on characterizing of 5000 germplasm accessions based on twelve DUS (Distinctness, Uniformity, Stability) traits. The experimental material was sourced from Indian Institute of Soybean Research, Indore, Madhya Pradesh. The present investigation was carried out during summer and *Kharif* of 2023 at ZARS (Zonal Agricultural Research Station), UAS, GKVK, Bengaluru. The germplasm accessions (5000) along with three checks were sown in an augmented design with 49 blocks. Morphological characterization based on twelve qualitative traits revealed that evident diversity was observed for growth habit, pubescence colour, seed coat colour, pubescence density, pod colour and hilum colour. Cluster analysis was carried out for 5000 accessions resulting in ten major clusters with similarity coefficient ranging from 0.70 to 0.89. Maximum number of germplasm accessions *viz.*, 941 were grouped under cluster VII and minimum number of accessions under cluster X. Cluster I and VII showed maximum number of sub-groups *viz.*, 8 and 7 respectively, with minimum similarity coefficient indicating the maximum diversity. The characterization trait-specific accessions could be utilized for identification of the target germplasm accessions, maintenance of genetic purity and for utilization in future breeding programmes.

***Keywords*: ** DUS, Morphological characterization, Clustering, Similarity Coefficient, Qualitative.

Introduction

Soybean [*Glycine max* (L.) Merrill] is an economically important leguminous crop, as it provides vegetable protein to more than one-third of the world's population. Soybean is an important crop worldwide due to its nutritious seed composition, consistent genetic gain and multiple industrial end-uses. Constant genetic gain in soybean has led to its widespread adaptation globally. Soybean seeds contain about 34 *per cent* carbohydrates, 20.9 *per cent* oil, and 41.70 *per cent* protein (Zhang *et al.* 2001). Soybean has a genome size of 1.1 to 1.5 GB with 40 (2n=40) chromosomes. The nine standard essential amino acids required for humans are present in soybean *viz.*, methionine, histidine, leucine, phenylamine, lysine, threonine, isoleucine, tryptophan and valine. Soybean seeds are rich source of fiber, iron, calcium, zinc and B vitamins (Hassan 2013).

USA stands first in both productivity and production with 3258 kg/ha and 97.65 million tons respectively from 311.03 lakh hectares of area. India is the fifth largest producer of soybean covering 112.5 lakh ha area with production of 9.00 million tones and productivity of 814 kg/ha (USDA foreign agricultural services 2022). Overall, India's share in the world soybean production is only 4.04 *per cent* despite the area contribution of 10% representing the poor productivity of the crop in India. Therefore, it invariably urges the broadening of the narrow genetic base by means of enhanced utilization of diverse germplasm accessions (Annon 2023-24).

Despite the numerous varietal improvement programmes devoted to the development of varieties and hybrids in soybean, the productivity of the crop has remained stagnant throughout time. Any crop breeding programme begins with identification of diverse genetic resources. Development of core and composite collections from the available germplasm facilitates their utilization in crop improvement programmes. Core collections developed from the thousands of soybean accessions around the world based on the DUS traits have aided their utilization in the breeding programme (Zhang *et al*. 2022).

The DUS traits are quite stable and they could be used as molecular markers in characterization and evaluation of genotypes (Gireesh *et al*. 2017). Characterization of germplasm accessions is a potential approach for assessing and capturing the genetic diversity and it serves as a powerful tool for identification of beneficial trait-specific accessions in germplasm and also for evaluation of germplasm (Shruthi *et al*. 2022).

The current study was focused on assessing the variability of morphological (DUS) traits among the germplasm accessions at the experimental plots of University of Agricultural Sciences (UAS), Bengaluru, which helps the researchers in understanding the traits that can be potentially utilized in identification and grouping of genotypes for utilization in future breeding programmes.

Material and Methods

The experiment consisted of 5000 germplasm accessions sourced from Indian Institute of Soybean Research, Indore, Madhya Pradesh and three standard checks *viz.,* KBS-23, JS335 and Karune. The 5000 germplasm accessions along with the three checks were raised in 49 blocks of an augmented design (Federer 1957) during summer and *kharif* of 2023 in the ZARS (Zonal Agricultural Research Station), UAS, GKVK, Bengaluru (77ºC 36' east longitude, 12ºC 59' north latitude and 932m above the mean sea level). 47 blocks consisted of 102 accessions each and two blocks with 103 germplasm accessions along with three standard checks (replicated twice) in all the 49 blocks. Each entry was of 1.50 m row length with a spacing of 45×10 cm. Recommended cultural, plant protection and agronomic practices were followed to maintain the crop stand healthier.

Soybean accessions were subjected to DUS (Distinctness, Uniformity and Stability) characterization based on 12 qualitative traits *viz.,* hypocotyl colour, early plant vigour, leaf shape, plant pubescence, plant pubescence colour, plant pubescence density, hilum colour, growth habit, seed coat colour, flower colour, pod colour and seed coat lustre. The germplasm accessions were scored for each trait according to the standard scores of the DUS characters mentioned by PPV&FR (Protection of Plant Varieties and Farmers Right, 2007). The morphological data was subjected to cluster analysis and similarity index was calculated using R software.

Results and Discussion

The germplasm accessions diversity can be estimated based on multiple approaches *viz.,* qualitative traits, quantitative or agronomical traits and molecular marker. The classification of genotypes based on qualitative traits is the reliable and also the simplest method. The expression of morphological traits is unaffected by dynamic environmental factors and could be used as morphological markers in trait-specific accession identification.

In the current study, an attempt was made to analyse the variability in germplasm accessions of soybean by utilizing twelve DUS traits (Fig. 1). Evident diversity was exhibited by all the twelve qualitative traits and scoring was carried done according to the PPV&FR guidelines (Table 1).

Germplasm collection was dominated by determinate types (79.26%) followed by semi-determinate (13.22%) and indeterminate types (7.56%). Indeterminate type of growth habit is more beneficial to develop high-yielding soybean hybrids and varieties with a greater number of secondary branches and pods per plant. Meanwhile, lodging remains a persistent problem in indeterminate type of varieties in India. Indeterminate types have longer main stem length compared to determinate and semi-determinate accessions (Tian *et al*. 2010), this character could be effectively exploited for the development of improved varieties with low lodging risk.

Table 1 : Variability of DUS characteristics, their frequency and relative percentage in germplasm accessions of soybean

Trait	Score	Class	Frequency	Percentage
Hypocotyl colour	1	Green	1335	26.70
	2	Purple	3665	73.30
Early plant vigour	1	Poor	85	1.70
	2	Good	4402	88.04
	3	Very good	513	10.26
Leaf shape	1	Broad	3264	65.28
	2	Intermediate	425	8.50
	3	Narrow	1311	26.22
Plant pubescence	0	Absent	180	3.60
	1	Present	4820	96.40
Plant pubescence colour	0	Absent	180	3.60
	1	Grey	490	9.80
	2	Light tawny	1676	33.52
	3	Tawny	2654	53.08
Plant pubescence density	0	Absent	180	3.60
	1	Sparse	830	16.60
	3	Semi-sparse	1792	35.84
	5	Normal	1992	39.84
	7	Dense	206	4.12
Growth habit	1	Determinate	3963	79.26
	2	Semi-determinate	661	13.22
	3	Indeterminate	376	7.52
Flower colour	1	White	1397	27.94
	2	Purple	3603	72.06
Pod colour	1	Light brown	2106	42.12
	2	Brown	1682	33.64
	3	Dark brown	1212	24.24
Hilum colour	1	Buff	1770	35.40
	2	Brown	2065	41.30
	3	Black	1165	23.30
Seed coat colour	1	Yellow	3725	74.50
	2	Green	201	4.02
	3	Reddish brown	229	4.58
	4	Black	845	16.90
Seed coat lustre	1	Dull	2972	59.44
	2	Shine	2028	40.56

Determinate types are popular because of their reduced branching and above ground biomass, shorter internodes, synchronized flowering, compact growth habit, more uniform pod maturity and stabilized harvest index (Qiu *et al.* 2017). Hence, development of soybean varieties and hybrids with determinate type of growth habit to optimize the allocation of resources between reproductive and vegetative phases is a major objective in soybean breeding programmes. Nachilima *et al.* (2021) have also recorded lodging resistance and more grain yield in determinate type of soybean lines.

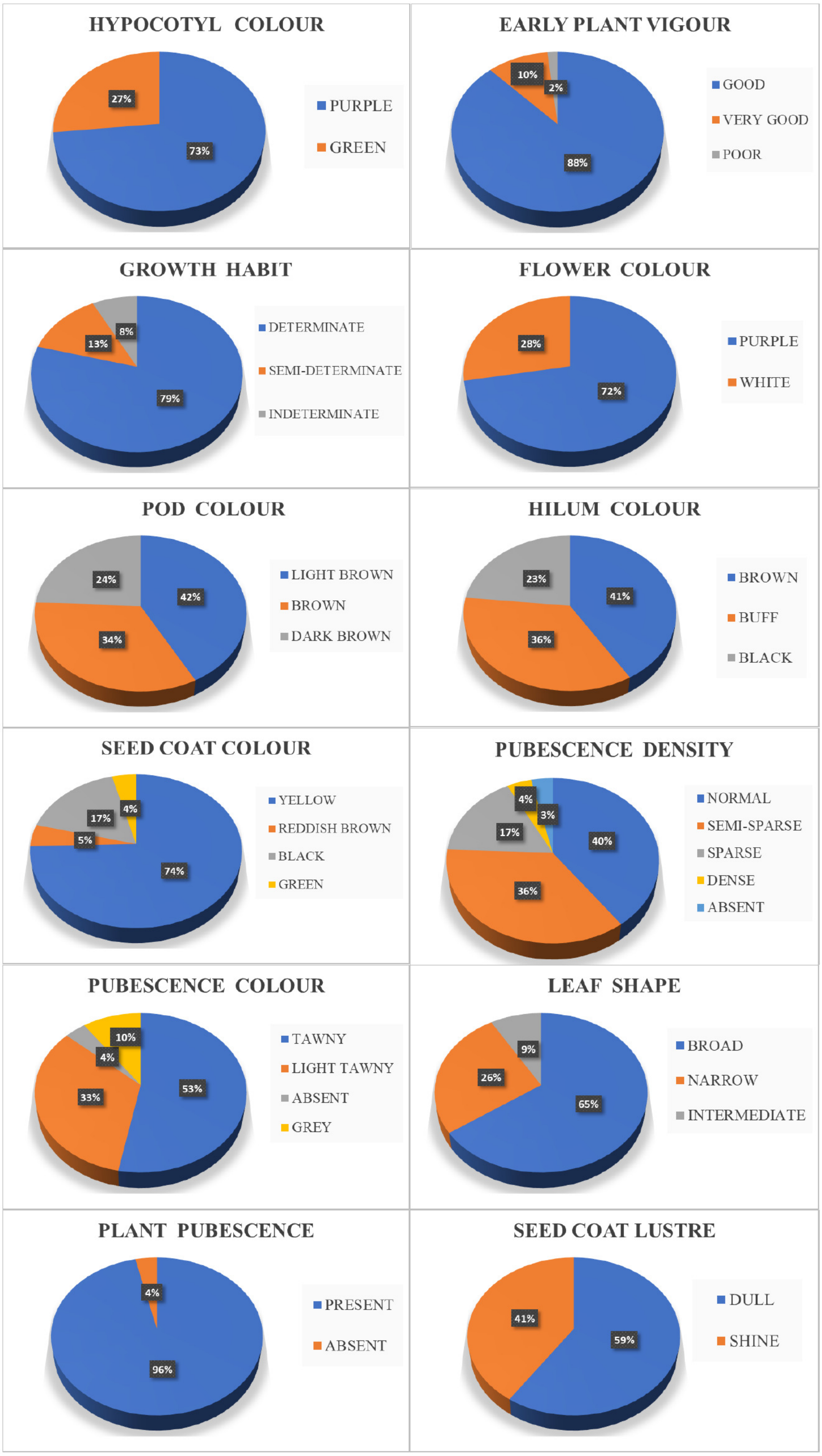

Fig. 1 : Pie charts representing the variability for qualitative traits in germplasm

Germplasm accessions with purple hypocotyl colour (73.30%) were higher in the collection followed by green hypocotyl colour (26.70%). Accessions with purple flower colour (72.06%) were highest compared to white coloured flower (27.94%) (Fig. 3). Both hypocotyl colour and flower colour can serve as an identification mark for a target germplasm accession (Malik *et al.* 2006).

Accessions with good early plant vigour (88.04%) were higher in the collection followed by accessions with very good early plant vigour (10.26%) and poor early plant vigour (1.70%) types. Good plant vigour acts as a decisive factor to consider a crop as successful in most of the pulses and cereals, as the good seedling vigour contribute to the uniform plant growth, maturity high productivity and better competition with weeds (Bewley *et al.* 2013).

Among 5000 germplasm accessions, 65.28% accessions reported broad shaped leaves followed by accessions with narrow leaves (26.22%) and intermediate type (8.50%) (Fig. 2). Plant pubescence was absent in 3.60% of the total accessions thereby, accessions with pubescence (96.40%) dominated the base collection (Fig. 4). Accessions with tawny pubescence (53.04%) with normal pubescence density (39.84%) were higher compared to accessions with light tawny (33.52%) and grey coloured (9.80%) pubescence.


Broad Intermediate Narrow

Fig. 2 : Variations in leaf shape among the soybean germplasm accessions

Accessions with light brown coloured pod type (42.12%) were greater in number compared to brown (33.64%) and dark brown pod (24.24%) types. Accessions with brown hilum (41.30%) were prominent compared to buff (35.40%) and black (23.30%). Accessions with dull seed coat lustre (59.44%) were quite predominant compared to accessions with shiny (40.56%) seed coat lustre. Further, seeds with yellow seed coat colour (74.50%) followed by black (16.90%), reddish brown (4.58%) and green (4.02%) seed coat colour (Fig. 5). The dominance of yellow seeded accessions could be due to more directional selection of yellow seeded ones because their correlation with consumer choice and higher yield potential (Kaga *et al.* 2012).


Purple **White**

Fig. 3 : Variations in the flower colour among the germplasm accessions of soybean

Fig. 4 : Variations in plant pubescence among germplasm accessions of soybean

Fig. 5 : Variations in seed coat colour among germplasm accessions of soybean

Cluster analysis

Cluster analysis was carried out for all the 5000 accessions of soybean and were grouped into ten major clusters. Each cluster comprises of the accessions having more than 70 *per cent* similarity. The coefficient of similarity ranged from 0.70 to 0.89 (Table 2). Cluster VII consisted of maximum number of accessions (941) with 7 sub-groups and 0.70 similarity coefficient followed by cluster III with 729 accessions, 6 sub-groups and 0.78 similarity coefficient. Minimum number of accessions were grouped under cluster X with 277 accessions, 3 sub-groups and similarity coefficient of 0.82 (Fig. 6).

Table 2 : Cluster analysis and similarity coefficient of soybean germplasm accessions

Clusters	Number of accessions in each cluster	Number of sub-groups	Similarity co-efficient
I	534	8	0.71
II	349	5	0.74
III	729	6	0.78
IV	445	5	0.82
V	528	4	0.87
VI	321	3	0.81
VII	941	7	0.70
VIII	296	3	0.80
IX	580	2	0.89
X	277	3	0.82

The accessions with high similarity coefficient are believed to be descended from closely related parental lines and reported as strongly related (Adegboyegun *et al.* 2020).

Cluster I showed highest number (8) of sub-groups followed by cluster VII (7). And these two clusters showed least similarity coefficient *viz.,* 0.71 and 0.70 respectively, indicating that the germplasm accessions are more diverse compared to the accessions in the other eight clusters.

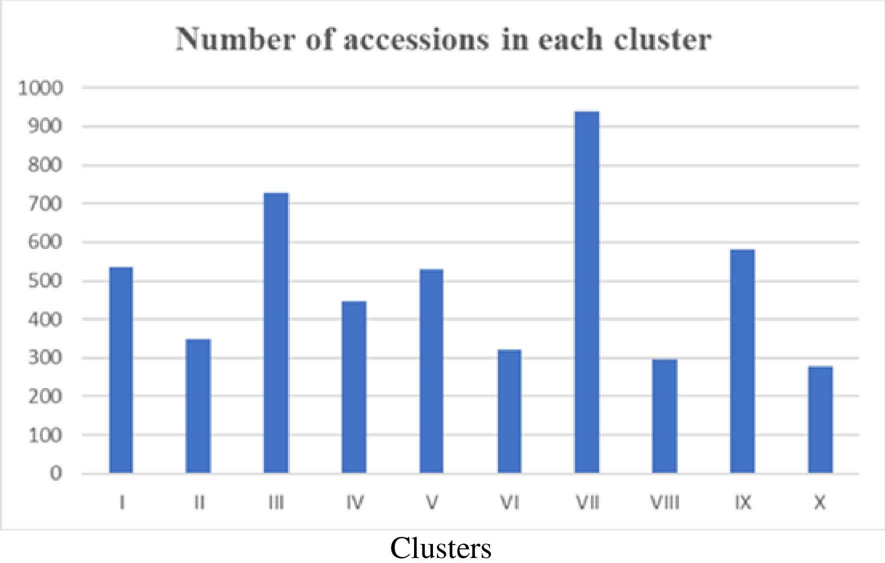

Fig. 6 : Graphical representation of variations in number of accessions in different clusters

Cluster IX reported highest similarity coefficient (0.89) followed by cluster V (0.87) and cluster IV (0.82) indicating high level of similarity within the cluster. Thus, clustering aids in selection of trait specific accessions and will reduces the level of complexity in analysing large number of germplasm accessions (Akram *et al.* 2016).

Conclusion

The DUS characterization and cluster analysis revealed that the traits *viz.,* hypocotyl colour, growth habit, early plant vigour, seed coat colour, plant pubescence, seed coat lustre and flower colour have played a significant role in the classification of accessions. Hence, these qualitative traits can be used in identification and grouping of accessions for utilization in future soybean breeding programmes. The current investigation will also provide an insight into how to maintain genetic purity of the materials under study and group them for reducing the complexity for future crop breeding programmes.

Authors' contributions

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

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