Reclassifying Indian Soybean [*Glycine max* (L.) Merrill.] Varieties on the Basis of New and Improved DUS Characteristics and Categories

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ABSTRACT

Background: Soybean [*Glycine max* (L.) Merrill.] stands as the leading oilseed crop globally and in India, valued for its dual role as a vegetable oil and protein source. India has released and notified 176 soybean varieties since the 1970s. This evolution aligns with intellectual property rights, particularly the TRIPS agreement, prompting India to establish the Protection of Plant Varieties and Farmers' Rights Act in 2001, followed by guidelines for variety protection, including Distinctiveness, Uniformity and Stability (DUS) testing for soybean initiated in 2009. As soybean cultivation in India progresses from limited early introductions to a diverse range of genetically enhanced varieties, there remains potential for expanding the morphological characteristics to ensure better differentiation and distinctiveness among varieties.

Methods: The ICAR-Indian Institute of Soybean Research, Indore, studied 127 soybean varieties released for commercial cultivation in India between 2016 and 2022 during each *kharif* (rainy) season. A total of 127 released and notified soybean varieties were observed for seedling hypocotyl pigmentation, leaf shape, leaf size, inflorescence pattern, leaf blistering, pod color, seed color, seed hilum color, pod pubescence, by randomly selecting a group of 20 plants from each plot for the respective varieties following PPV and FRA guidelines.

Result: Our findings revealed discrepancies in trait identification among several varieties based on DUS guidelines. We identified opportunities to expand these guidelines by incorporating additional traits like categorizing hypocotyl anthocyanin pigmentation as high, medium, low, or absent; classifying leaf shapes into lanceolate, triangular, pointed ovate and round ovate and assessing leaf size and blistering. Inflorescence patterns, seed colour, hilum colour and pod colour should also be better defined. This finding underscores the importance of re-evaluating existing assessment criteria to include such traits, which could enhance the understanding and classification of soybean varieties.

Key words: Distinctiveness, Soybean, Stability, Trait, Uniformity.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill.] stands as the premier oilseed crop globally and in India, leading in both acreage 132.55 lakh hectare and production of 130.62 lakh tonnes with the productivity of 985 kg/ha during *Kharif* 2023-24 (DA and FW) (Anonymous, 2025). Beyond its crucial role in providing vegetable oil, soybean is also a vital source of plant-based protein, making it indispensable to food security and nutrition. As we face the pressing challenges posed by climate change, characterized by increasing biotic and abiotic stresses, the introduction of innovative soybean varieties has become paramount to enhance productivity and resilience within the soybean production system. Since the dawn of soybean cultivation in India during the early 1970s, a total of 176 varieties have been recommended for commercial cultivation, reflecting a dynamic evolution in agricultural practices.

In this era defined by intellectual property rights, India, as a signatory to the TRIPS agreement, is obligated to implement effective mechanisms for plant variety protection. Accordingly, the Protection of Plant Varieties and Farmers' Rights Act was enacted in 2001, paving the way for structured guidelines aimed at safeguarding diverse plant varieties. The advent of the guidelines for Distinctiveness, Uniformity and Stability (DUS) testing in soybean in 2009 (Anonymous, 2009) marked a significant milestone in this journey. These guidelines encompass 22 specific characteristics among these 19 pertaining to morphological features of the plant and seed, along with 3 biochemical traits ensuring rigorous assessment of new soybean varieties for their distinctiveness and marketability.

The evolution of soybean cultivation in India, from its modest beginnings in the 1960s with limited introduced

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lines to the contemporary landscape characterized by a plethora of advanced breeding techniques utilizing both exotic and indigenous genetic resources, underscores the progress achieved in this sector. However, there remains substantial potential for the inclusion of new morphological traits and the introduction of novel plant characteristics. Which is essential for improved characterization and differentiation of soybean varieties, thereby ensuring the distinctiveness and uniqueness of specific cultivars more effectively.

MATERIALS AND METHODS

The study was carried out at ICAR-Indian Institute of Soybean Research, Indore field and laboratory during *kharif* (rainy) season of 2016-2022. A total of 127 released and notified soybean varieties were observed for seedling hypocotyl pigmentation**,** leaf shape, leaf size, inflorescence pattern, leaf blistering, pod color, seed color, seed hilum color, pod pubescence, by randomly selecting a group of 20 plants from each plot for the respective varieties. Data recorded in accordance with the DUS testing guidelines established by the Protection of Plant Varieties and Farmers' Rights Authority (PPV and FRA), New Delhi. Leaf area measurements were conducted using a LI-COR leaf area meter (Model LI-3100C, USA). Leaf size was determined by measuring the length and breadth, followed by the calculation of the length-to-breadth ratio (Jeong *et al.,* 2011). The ratios of leaf length to width and leaf area were statistically analyzed for Least Significant Difference (LSD) or Critical Difference (CD) at a 5% significance level, based on triplicate measurements, using SAS software.

RESULTS AND DISCUSSION

There is ample scope of rectification of different categories or states of soybean plant and seed morphological characteristics and inclusion of new characters for classification of soybean varieties and future testing of new varieties.

Our findings indicated that several varieties exhibited discrepancies in trait identification as per DUS guidelines. Moreover, our analysis suggested opportunities to expand the DUS test guidelines (2009) by incorporating additional traits and exploring a broader range of existing plant or seed characteristics.

Seedling hypocotyl pigmentation

Anthocyanin pigmentation in the hypocotyl region of soybean seedlings is a critical morphological trait for differentiating various cultivars. The existing guidelines for Distinctness, Uniformity and Stability (DUS) testing classify soybean hypocotyl anthocyanin pigmentation into two categories: present and absent. Our research identified significant variations in the intensity of this pigmentation among the surveyed soybean varieties. In the analyzed collection of notified varieties, the intensity of hypocotyl anthocyanin pigmentation ranged from very high to very low. Based on these observations, soybean varieties can be classified into four distinct categories: (i) High, (ii) Medium, (iii) Low and (iv) Absent (Plate 1).

In contrast, under the previous classification, 48.3% of varieties were considered absent, with 51.7% classified as present. The varieties exhibiting high pigmentation included NRC 130, NRC 131, MAUS 158, JS 335 and Punjab Soya 1. Conversely, varieties such as JS 93-05 and NRC 7 demonstrated medium pigmentation, while JS 20-69, NRC 138, PK 416, RKS 18, SL 295 and SL 525 had low pigmentation. Varieties characterized by the total absence of pigmentation were JS 20-116, JS 20-98, JS 20-29 and JS 20-34, among others (Table 1). The coloration of hypocotyls is correlated with flower color; specifically, the presence of hypocotyl pigmentation is associated with purple flowers, while its absence correlates with white flowers. Palmer and Payne (1979) reported that flower color is determined by the dominant/recessive alleles of the W1 locus, which pleiotropically regulates hypocotyl color. Our observations suggest that even minimal anthocyanin presence in the seedling hypocotyl relates to the occurrence of white flowers. The hypocotyl anthocyanin color is governed by the T and W1 loci. The W1 gene encodes a flavonoid 3′, 5′ -hydroxylase (F3′ 5′ H), which is crucial for the synthesis of flavonoids exhibiting the 3′, 4′, 5′ B-ring hydroxylation pattern (Buzzell *et al.,* 1987; Zabala and

Variation in hypocotyl anthocyanin pigment concentration at seedling stage in different varieties and per cent of varieties in that category

High	Medium	Low	Absent
(26.8%)	(24.8%)	(15%)	33%



Plate 1: Pigment concentration at the hypocotyl region.

Vodkin, 2007). Furthermore, Murai *et al.* (2016) highlighted that soybean plants with the dominant W1 allele exhibit purple hypocotyls regardless of the T locus, while recessive w1 and t alleles lead to green hypocotyls and recessive w1 paired with dominant T results in light anthocyanin or bronze hypocotyls. In our study, it was apparent that varieties demonstrating low anthocyanin pigmentation in hypocotyls were also closely linked to the presence of white flowers.

This investigation underscores the importance of hypocotyl pigmentation as a morphological marker in soybean breeding, seed production and classification, contributing to the advanced understanding of genetic traits associated with flower color and overall plant characterization.

Leaf shape

In accordance with established testing guidelines, three leaf shape categories have been recognized: (a) Round ovate, (b) Pointed ovate and (c) Lanceolate. Notably, the distinction between pointed ovate and round ovate leaf shapes is minimal, with the primary differentiating factor being the leaf tip morphology. The width-to-length ratios for these two categories often overlap across different varieties, making differentiation challenging. Certain varieties, such as JS 90-41, exhibit characteristics that deviate significantly from the pointed ovate classification, displaying neither a perfect pointed ovate nor lanceolate form; these are more accurately described as triangular. Therefore, it is advisable to expand the classification of leaf shapes to include: (i) Lanceolate, (ii) Triangular, (iii) Pointed Ovate and (iv) Round ovate (Plate 2) (Table 1). According to Bernand and Weiss (1973), the leaf shape in question is governed by a single gene, where the homozygous dominant state (LnLn) results in broader leaves, while the recessive homozygous state (lnln) produces narrower leaves. The UPOV (2022) has also provided a similar classification for this trait.

Leaf size

The ratio of leaf length to leaf width L:W has been found to be a significant factor in classifying soybean leaf shapes. This ratio ranges from 1.11 to 2.71 and can be divided into two main categories. The first category, with an L: W ratio ranging from 1.11 to 1.87, encompasses pointed ovate and round ovate leaf types. The second category, with ratios between 2.50 and 2.71, includes only lanceolate leaf types. Notably, the L: W ratio for lanceolate leaves was significantly higher (LSD 0.1903) than that of the other groups. Higher L: W ratios indicate a more pointed leaf shape, with ratios of 1.87 (JS 90-41), 1.84 (JS 2) and 1.83 (JS 20-34) reflecting a transition towards triangular shapes (Fig 1). According to Sawada (1991), a Leaf Shape Index (LSI) value exceeding

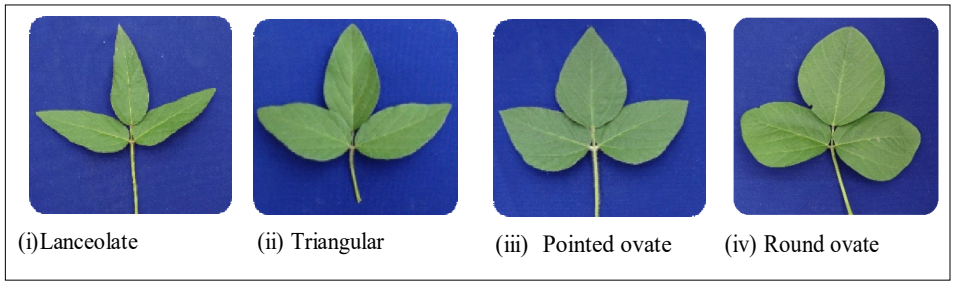


Plate 2: The different shapes of soybean leaves (i) Lanceolate (PS 1347) (ii) Triangular (JS 90-41) (iii) Pointed ovate (PK 472) (iv) Round ovate (JS 335).

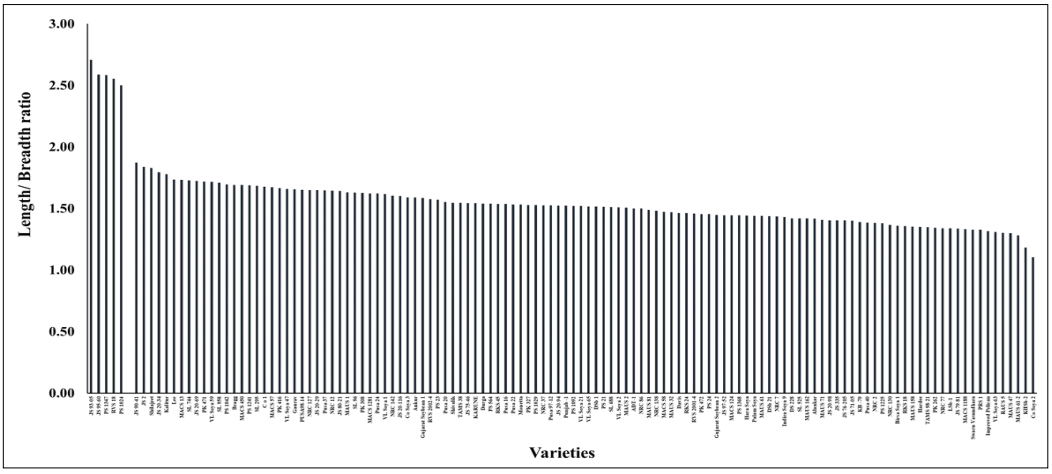


Fig 1: The distribution of leaf shape as expressed by length to breadth ratio among soybean varieties.

2.6 suggests narrow leaves, while values below this threshold indicate broad leaves Plate 3.

In the current DUS Test guidelines, leaf size has not been included as a criterion, despite being a prominent morphological characteristic for differentiating plant varieties. Leaf size has been assessed in terms of length, width and area. Significant variation in leaf length was observed among varieties, ranging from 7.7 cm in Davis to 16.2 cm in PS 1347 (Plate 3). The leaf width varied from 4.67 cm in PS 1024 to 11.17 cm in Swarn Vasundhara. Lanceolate varieties such as PS 1024, JS 93-05, RVS 18, PS 1347 and JS 95-60 exhibited relatively narrow leaves, measuring between 4.67 cm to 5.67 cm. In contrast, pointed and round ovate leaves ranged from 4.67 cm to 11.17 cm. Notably, certain varieties such as PS 1024 (4.67 cm), Davis (5.17 cm), Gaurav (5.33 cm) and JS 80-21 (5.67 cm) displayed widths smaller than that of the lanceolate type JS 95-60 (5.67 cm), indicating a narrower leaf shape within their respective Similar categories had been adopted by UPOV (2022).

Furthermore, significant differences in leaf area were noted among the various soybean varieties, with areas ranging from 30.9 cm^2 to 116.8 cm^2 (LSD at 10.96). Generally, varieties with larger leaf areas tended to belong to the pointed or round ovate categories, in comparison to some lanceolate varieties, such as PS 1347, RVS 18, JS 95-60, JS 93-05 and PS 1024, which had leaf areas of 59.0 cm^2, 58.0 cm^2, 53.4 cm^2, 43.3 cm^2 and 42.2 cm^2, respectively. Among the lanceolate types, PS 1347, RVS 18 and JS 95-60 exhibited significantly larger areas than JS 93-05 and PS 1024 (Fig 2). For pointed ovate and round ovate leaf types, the distribution of leaf area among varieties was quite broad.

Additionally, smaller seed size varieties such as Davis, Gaurav, SL 96 and JS 20-34 had leaf areas ranging from 30 cm^2 to 50 cm^2 Varieties with medium leaf sizes, measuring between 51 cm^2 and 70 cm^2, included Lee, JS 2, MACS 450 and JS 97-52. Lastly, those with larger leaf sizes (greater than 70 cm^2) included RKS 24, MAUS 158, NRC 86, PS 1029, Karune, NRC 130 and Swarn Vasundhara (Plate 3) and (Fig 2).



Plate 3: The different sizes of soybean leaves.

Leaf blistering

Leaf blistering refers to the degree of roughness on the surface of leaves. This characteristic can be categorized into two distinct types: smooth and rough. Previously, leaf blistering was not included in DUS (Distinctness, Uniformity and Stability) testing guidelines (Anonymous, 2009) due to a lack of sufficient reference varieties. However, with the recent introduction of new varieties exhibiting this trait, there is an opportunity to add this characteristic to the test guidelines for improved variety classification.

Example varieties that display leaf blistering include NRC 130, NRC 131 and Karune. In alignment with UPOV (Union for the Protection of New Varieties of Plants) guidelines, which classify this characteristic into five categories (i) absent, (ii) very weak, (iii) weak, (iv) medium, (v) strong and (vi) very strong (UPOV, 2022) the incorporation of leaf blistering into DUS testing protocols could be beneficial. Incorporating these available reference varieties, the characteristic of leaf blistering could be effectively integrated into the DUS testing guidelines of the PPV and FRA (Protection of Plant Varieties and Farmers' Rights

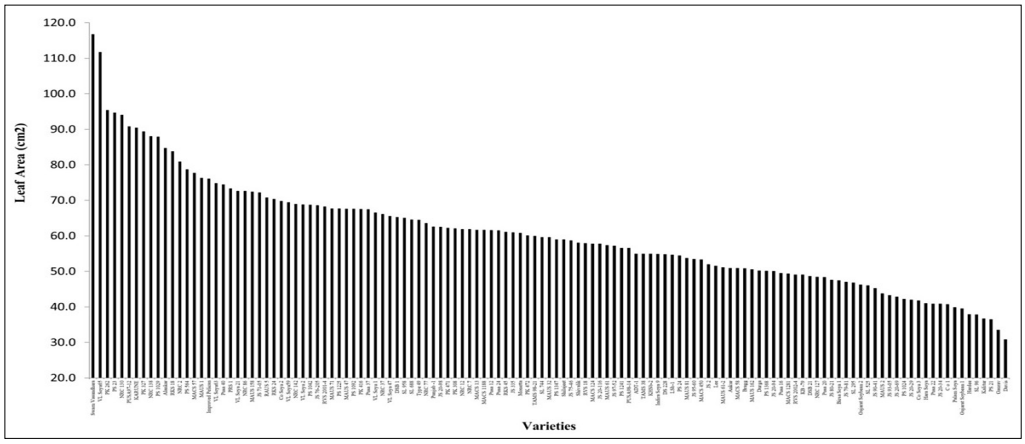


Fig 2: The distribution of leaf area among the soybean varieties.

Authority), under the following categories, Leaf Surface: (i) Smooth and (ii) Rough (Plate 4) (Table 1).

Inflorescence pattern in soybean varieties

The study of inflorescence patterns across various released and notified soybean varieties has revealed notable variation in this characteristic. The inflorescence typically presents as an axillary raceme on both the main stem and its branches. In certain varieties, flowers cluster in groups of four to five at the nodes of the main stem and branches, exhibiting a sessile arrangement. Conversely, other varieties feature flowers on elongated peduncles, where the length of the peduncle is greater compared to the shorter peduncles observed in sessile arrangements.

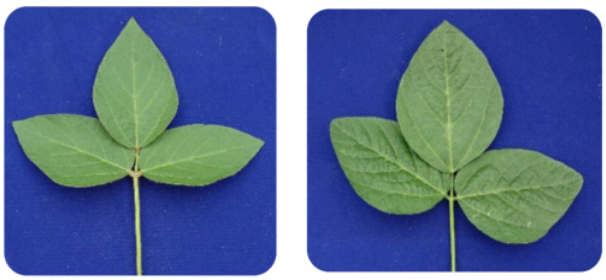


Plate 4: The leaf surface characteristics of soybean (i) Smooth surface (ii) Rough surface.

Schaik and Probst (1958) distinguished between pedunculate inflorescences-characterized by long peduncles with sessile flowers and found that the pedunculate form (Se) is dominant over the sessile form (se). In light of these findings, a new characteristic for DUS (Distinctness, Uniformity and Stability) testing is proposed, leading to the categorization of soybean inflorescences into two distinct types: (i) Pedunculate Inflorescence and (ii) Sessile Inflorescence (Plate 5). Examples of soybean varieties with Pedunculate Inflorescence include Ankur, JS 93-05, JS 76-205, JS 95-60, MAUS 158, PS 1024, JS 20-34 and MACS 1520. Conversely, varieties exhibiting Sessile Inflorescence include Hardee, Pusa 97-12, MACS NRC 1667, NRC 142 and NRC 150. Notably, some sessile varieties, such as Type 49, JS 2, KHSb 2, Pusa 24, JS 335, SL 295 and MACS 1188, display very small peduncles (Table 1). Currently, this characteristic has yet to be addressed by UPOV (International union for the protection of new varieties of plants).

Pod pubescence

Pod pubescence refers to the presence of hairs on soybean plants and their pods. This trait is influenced by specific genetic loci-Pd1, Ps and Pl-located on linkage groups D1a+Q, H and K on chromosome 9 (Cregan *et al.,* 1999). According to Bernard and Singh (1969), a sparse or semi-sparse allele is dominant at the Ps locus, yielding various types of pubescence: Glabrous (P1), curly pubescence (Pc), dense pubescence (Pd), sparse pubescence (Ps) and

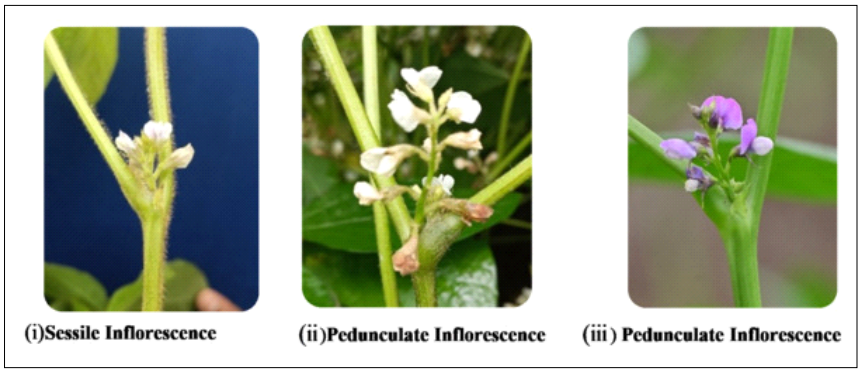


Plate 5: The pattern of inflorescence in different soybean varieties (i) Sessile inflorescence (ii and iii) Pedunculate inflorescence.

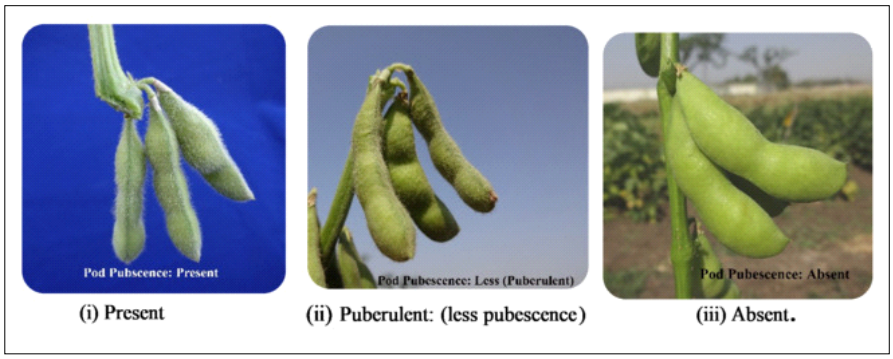


Plate 6: The pod pubescence characteristics of soybean. (i) Present (ii) Puberulent (iii) Absent.

puberulent (p2). The presence or absence of hairs on the plants and pods serves as a distinct identification marker for soybean varieties. In accordance with DUS (Distinctness, Uniformity, Stability) guidelines, pubescence is classified simply as either present or absent. However, our research has revealed that some varieties classified as pubescence absent display a low intensity of pubescence, resulting in potential confusion. Notably, certain plant types exhibit nearly non-existent pubescence. One such variety, Karune, was recently released and is characterized by its minimal pubescence on both plants and pods. To enhance clarity in classification, there is need to revise the categories of pubescence into three distinct states: (i) presence of pubescence, (ii) puberulent (sparsely present) and (iii) absence of pubescence (Plate 6) (Table 1).

Seed color

According to the DUS guidelines, soybean seed colors are classified into four categories: yellow, greenish-yellow, green and black. When yellow and black-seeded lines are crossed, they segregate into yellow, brown and black seeded progenies. Notably, while brown-seeded lines are not represented among currently released and notified varieties, sources are available within germplasm collections.

The color of the soybean seed coat is a polygenic trait influenced by multiple loci, specifically I, R, T, W1 and O (Senda *et.al.,* 2002). The homozygous recessive genotype results in a self-colored seed coat. The R and T loci determine specific seed coat colour such as black (iRT), brown (irT), or buff (irt) by regulating anthocyanin and proanthocyanin pigments. In the DUS Test guidelines, seed coat colors are currently categorized into four groups: 1. Yellow 2. Greenish-yellow 3. Green 4. Black. To enhance this classification, it is recommended to introduce an additional category for brown, leading to a total of five categories for soybean seed coat color *i.e.* 1. Yellow 2. Greenish-yellow 3. Green 4. Brown 5. Black (Plate 7) (Table 1).

Pod color

The DUS testing guidelines set forth by the PPV andFRA recognize three pod color categories: Yellow, brown and black. However, this study reveals that the brown coloration observed across various years and soybean varieties could be refined into a more detailed classification. We propose the introduction of five distinct categories: (i) Yellow, (ii) Light brown, (iii) brown, (iv) Dark brown and (v) Black (Plate 8) (Table 1). This fine categorization would enhance the differentiation of soybean varieties. Additionally, it is noteworthy that the UPOV guidelines encompass a total of seven categories based on available variability (UPOV, 2022).

Seed hilum color in soybean

According to the DUS test guidelines, the seed hilum color is classified into five distinct categories: yellow, grey, brown, black and variegated. Within the brown category, there exists a variation in the intensity of the hue among different soybean varieties, ranging from dark brown to light brown (Table 1). The color of the hilum in soybeans is controlled by multiple genes, notably the I locus, which comprises four alleles (I, ii, ik and i) responsible for flavonoid pigmentation. The presence of the I allele leads to a

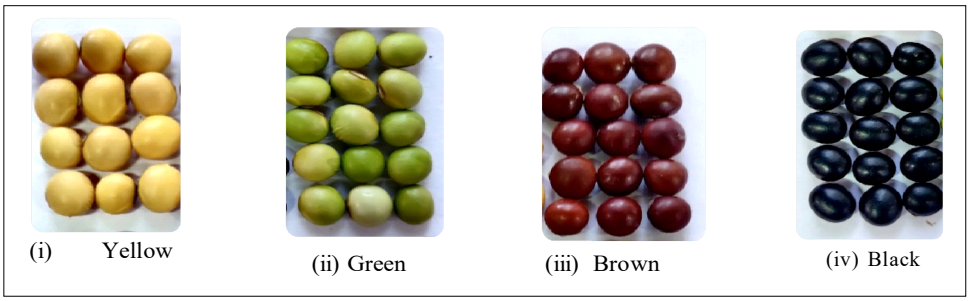


Plate 7: Seed colour variation among the varieties (i) Yellow (ii) Green (iii) Brown (iv) Black.

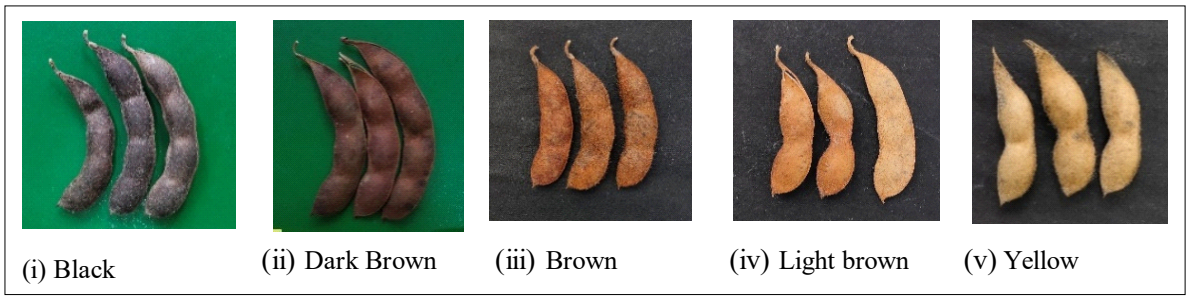


Plate 8: Different pod colour in soybean varieties (i) Black (ii) Dark Brown (iii) Brown (iv) Light brown (v) Yellow.

complete inhibition of pigmentation. Other genes, including T, R and O, also influence hilum color, particularly in varieties exhibiting the recessive i allele. For genotypes ii and ik, additional interactions with genes such as TR, TRO, Tro, tRW1, tRw1 and tr can result in various colors, including black, brown, red brown, imperfect black, buff and yellow. Notably, when the I allele is present, the TR and tRW1 genes will yield a grey hilum, while the other genes will



Plate 9: The variation in seed hilum colour within brown category (i) Light brown (ii) Brown (iii) and (iv) Dark brown.

Table 1: The new categories of different DUS traits and the example varieties.

The characteristic	New states	Example varieties in new state	Percentage of varieties in new state
Hypocotyl	a. High	NRC 130, NRC 131, DSb 21, RSC 10-46	26.8%
anthocyanin	b. Medium	JS 95-60, JS 335, MACS 1520, RSC 10-52	25.5%
pigmentation	c. Low	NRC 138, RKS 18, MACS 124, JS 20-69, PS 1368,	13.4%
	d. Absent	JS 21-72, JS 20-116, NRC 150, NRC 127, Karune	34.2%
Leaf shape	a. Lanceolate	JS 95-60, JS 93-05, Raj Soya 18, PS 1347, NRC 157	4.5%
	b. Triangular	JS 90-41	0.6%
	c. Pointed ovate	PK 472, JS 20-116, JS 20-34, NRC 142, NRC 150	92.9%
	d. Round ovate	JS 335, MACS 1188	1.9%
Leaf size	a. Small	Davis, Gaurav, SL 96, JS 20-34	25.6%
	b. Medium	Lee, JS 2, MACS 450, JS 97-52	53.6%
	c. Large	RKS 24, MAUS 158, NRC 86, Karune, NRC 130 and Swarn Vasundhara	20.8%
Inflorescence pattern	a. Sessile	Hardee, Pusa 97-12, MACS NRC 1667, NRC 142, NRC 150	78.3%
	b. Pedunculate	Ankur, JS 93-05, JS 76-205, JS 95-60, MAUS 158, PS 1024, JS 20-34, MACS 1520	21.7%
Leaf: Surface texture	a. Smooth	JS 335, JS 21-72, NRC 142, NRC 150	97.4%
	b. Rough	Karune, NRC 130, NRC 131	2.6%
Pod: Pubescence	a. Absent	Karune, NRC 188	1.3%
	b. Puberulent	JS 335, JS 95-60, JS 93-05, NRC 130, NRC 131,	22.3%
	c. Present	JS 20-98, JS 20-34, JS 21-72, MACS 1520, AMS-MB 5-18	78.3%
Pod colour	a. Yellow	NRC 7, PK 472	5.8%
	b. Brown	JS 20-98, JS 335, NRC 150, NRC 142	82.6%
	c. Dark brown	MACS 1407, NRC 86, NRC 130, NRC 138	9.0%
	d. Black	JS 95-60, JS 93-05, Karune	2.6%
Seed: Colour	a. Yellow	NRC 150, NRC 142, JS 21-72	94.9%
	b. Yellow green	JS 90-41	0.6%
	c. Green	Harasoya, Karune	1.3%
	d. Brown	Available in germplams	-
	e. Black	Birsa Soya 1, VL Soya 1	3.2%
Seed hilum colour	a. Yellow	PRS 1	0.7%
	b. Light brown	MAUS 61-2, PS 1225, VL Soya 63	11.9%
	c. Brown	NRC 130, NRC 131, PS 1368, RVS 2002-4	25.9%
	d. Dark brown	NRC 128, NRC 138, NRC 86, VL Soya 59, RVS 2001-4	21.7%
	d. Grey	JS 95-60, Karune	1.4%
	f. Black	JS 20-116, JS 20-98, JS 97-52	38.5%

produce a yellow hilum (Specht and Williams, 1978; Palmer and Stanley, 1979). To enhance the classification of soybean varieties based on hilum color, it is recommended to subdivide the brown category into dark brown and light brown. Consequently, the following refined categories for hilum color are proposed: (i) yellow, (ii) grey, (iii) light brown, (iv) dark brown, (v) black and (vi) variegated (Plate 9) (Table 1). In alignment with UPOV guidelines, the brown hilum color is typically categorized into three groups: light brown, red brown and dark brown.

CONCLUSION

Plant and seed morphological characteristics had been being used for distinctiveness, uniformity and stability testing under the PPV andFR Act 2001 in different crops. Likely, there are 19 (nineteen) such characteristics among 22 in the DUS Test guidelines for soybean published in 2009, need to improve existing states or categories to evaluate the uniqueness for reducing the complexity of concluding outcomes for the DUS Characters like hypocotyl anthocyanin pigmentation, leaf shape, pod pubescence, seed colour, hilum colour and pod colour. Similarly, the new plant and seed characters may be included in the DUS testing like leaf size, inflorescence pattern, Where, the hypocotyl anthocyanin pigmentation may be categorized into (i) High (ii) Medium (iii) Low and (iv) Absent. Leaf shape may be categorized into Lanceolate, Triangular, Pointed Ovate and Round Ovate. Leaf size with caterogies of Large, Medium and Small; Leaf Blistering with caterogies of absent or present; Inflorescence pattern with categories of sessile and pedunculate may be included in varietal characterization. The seed colour should be regrouped into yellow, yellow-green, green, brown and black. The brown hilum may be re-categorized into light brown, brown and dark brown. Pod colour may be further categorized into yellow, light brown, dark brown and black.

Conflict of interest

All authors declared that there is no conflict of interest.

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