



Characterisation of soybean varieties of Himachal Pradesh using morphometric descriptors

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Abstract

Characterization plays a significant role in plant science. It enables easy discrimination between varieties and helps in defining a variety. In this study, seven released and/in release pipeline soybean varieties of Himachal Pradesh were morphologically characterised using DUS descriptors. Each variety was tagged to the set of morphological characteristics. Further, the characterization revealed that 11 characters out of 21 namely seed shape, seed colour, seed cotyledon colour, seed hilum colour, seed size, plant growth habit, leaf shape, leaf colour, flower colour, pod pubescence colour and pod colour were very informative in providing a sound identification of seven soybean varieties of Himachal Pradesh. The information generated could be a ready reckoner for all the actors involved in undertaking activities related to maintenance breeding during seed multiplication and for genetic purity assessment using the Grow Out Test.

Key words: Characterization, DUS descriptors, Genetic identity, Soybean.

Soybean (*Glycine max* L. Merrill) also called as the 'Golden Bean' or 'Miracle crop' of the 21st century, is one of the principle leguminous crop of the world contributing significantly as an oilseed in terms of total production and international trade. Since 1970s, the world area under soybean production has the highest per cent increase among all the major crops (Hartman *et al.* 2011). With modern tools in hand, a number of soybean varieties have come up in the seed multiplication chain during the last 2-3 decades. The seed production areas are specific and limited, handling multiple varieties at a single time. A thorough and keen understanding of defining characteristics of a variety is important for maintaining the genetic identity throughout the supply chain and securing quality assurance through Grow Out Test. Varietal maintenance during seed multiplication and genetic purity assessment using Grow Out Test have close linkage to varietal traits. It is of utmost importance that the detailed information on characteristics is effectively circulated among all the actors involved in seed multiplication and genetic purity testing.

Characterization enables easy discrimination between varieties and helps in defining a variety which is important to grant legal justice to the breeder by

means of plant variety protection to encourage research and innovation. The traits incorporated in the variety have a market value. The seeds reaching the farmers should be genetically pure in order to realise the full potential of a variety. The indispensable association of characters to a variety makes it important on part of seed scientists to have a thorough knowledge of characters in order to maintain the traceability and originality. Nowadays, many tools are available to characterise the cultivars, however, morphological characterisation offers an easy applicability and wide acceptance in seed production units. In the present study, an attempt was made to formulate an identification key for seven soybean varieties grown in Himachal Pradesh using 21 DUS enlisted descriptors to avoid any biasness while observing the qualitative and quantitative variables and formulating the results. The process done would provide a ready reckoner information for rest of the seed production units in the state for maintaining and testing the genetic identity of the varieties under the production program.

Materials and Methods

The seeds of composite varieties of seven released

and/in release pipeline soybean varieties of Himachal Pradesh namely, Hara Soya, Palam Early Soya 1, Shivalik, Palam Soya, PS 1556, Him Soya and Him Palam Hara Soya 1 were sown at experimental farm of the Department of Seed Science and Technology CSK HPKV Palampur in the Kharif season 2018. The design adopted was Randomized Block Design (RBD) with three replications per variety with row to row and plant to plant distance of 45 cm and 15 cm, respectively. The field consisted of 21 plots each of 5.4 m² (3 m x 1.8 m) area. The size of the plot was adequate for taking measurement and observations of the plants or plant parts without prejudicing the other observations of the standing plant until the end of growing season.

A set of 21 DUS descriptors (Table 1) including both qualitative and quantitative traits were used for characterisation of soybean varieties. The field observation on qualitative traits were collected on ten randomly selected plants per replication at designated stages of crop growth as per the national DUS testing guidelines on soybean (Anonymous 2009). Characteristics involving the colour were observed using the Horticulture Colour Chart to avoid any human error and biasness. The environmental conditions were conducive for normal growth and expression of all the qualitative characteristics. The data on quantitative traits *viz.*, 100 seed weight, seed length, seed width and plant height was recorded in the following manner. A homogeneous representative seed sample of each variety was drawn from each replication. One hundred seeds were then counted from each extracted sample and their average weight was recorded in grams. Seed length and seed width was recorded on ten randomly selected seeds extracted from each sample per replication using the vernier calliper. The average length and width was recorded in millimetre. Further, the plant height was recorded at physiological maturity on ten randomly selected plants per variety per replication from the base above the soil surface up to the tip of the main stem using a measuring scale and the mean height was computed and reported in centimetre. The data recorded was statistically analysed using OPSTAT software.

Results and Discussion

The seven soybean varieties were characterized using the DUS qualitative and quantitative descriptors

for adequate identification and generating classification information for further future use (Table 2 and Table 3).

Seed qualitative characters

Qualitative characters are more dependable for DUS characterization since they behave stably over environments and generations (Dhaliwal *et al.* 2020). Therefore, these traits can be employed as the most credible phenotypic markers for differentiating species and varieties (Dhaliwal *et al.* 2020). The seed colour of soybean is grouped as yellow, yellow green, green and black as per standard DUS characterization. Green seed coat was present in Hara Soya and Him Palam Hara Soya 1, whereas Palam Early Soya 1, Shivalik, Palam Soya, PS 1556 and Him Soya exhibited yellow seed coat (Fig 1). Seeds of Hara Soya and PS 1556 were spherical in shape whereas, Palam Early Soya 1, Shivalik, Palam Soya, Him Soya and Him Palam Hara Soya 1 were categorized as elliptical in shape. All the seven varieties under consideration in the present study had shiny seed coat lustre. Hara Soya and Him Palam Hara Soya 1 exhibited green coloured cotyledons, whereas Palam Early Soya 1, Shivalik, Palam Soya, PS 1556 and Him Soya manifested yellow coloured cotyledons. Seed hilum colour is a highly stable and polymorphic trait (Dhaliwal *et al.* 2020). Brown hilum coloured seeds were present in Shivalik, Palam Soya and Him Palam Hara Soya 1 while seeds were black hilum coloured in Hara Soya, Palam Early Soya 1, PS 1556 and Him Soya. On similar lines, Gupta *et al.* (2010) and Talla *et al.* (2016) used various seed morphological descriptors like hilum colour, seed shape and seed coat colour for characterizing various soybean cultivars. Pawale *et al.* (2019) took morphological observations for seed lustre and observed shiny seed lustre for all the soybean cultivars under study.

Seed morpho-metrical and seedling morphological characters

In the present study, maximum 100-seed weight (20.46 g) was recorded for Him Palam Hara Soya 1, followed by Palam Early Soya (114.06 g), Palam Soya (13.79 g), Shivalik (12.93 g), Hara Soya (11.47 g), Him Soya (11.44 g) and PS 1556 (9.73 g), respectively (Table 4). Seeds of Palam Early Soya 1, Palam Soya and Him Palam Hara Soya 1 were classified as large

Table 1. Morphometric descriptors used for characterization of seven soybean varieties

S No.	Characteristics	States	Note	Stage of observation	Type of assessment
1	2	3	4	5	6
I) Seed qualitative characters					
1.	Seed: Colour	Yellow	1	Harvested product (Seeds)	VS
		Yellow green	2		
		Green	3		
		Black	4		
2.	Seed: Hilum colour	Yellow	1	Harvested product (Seeds)	VS
		Grey	2		
		Brown	3		
		Black	4		
		Variiegated	5		
3.	Seed: Shape	Spherical	1	Harvested product (Seeds)	VG
		Elliptical	2		
4.	Seed: Lustre	Shiny	1	Harvested product (Seeds)	VG
		Dull	9		
5.	Seed: Cotyledon colour	Yellow	1	Harvested product (Seeds)	VS
		Green	2		
II) Seed morphometrical and seedling morphological characters					
6.	Seed: Size (100 seeds weight)	Small (≤ 10.0 g)	3	Harvested product (Seeds)	MG
		Medium (10.1-13.0 g)	5		
		Large(> 13.0 g)	7		
7.	Seed: Length	Short (<10mm)	3	Harvested product (Seeds)	MS
		Medium (10-12mm)	5		
		Large(>12mm)	7		
8.	Seed: Width	Short (<7mm)	3	Harvested product (Seeds)	MS
		Medium (7-8mm)	5		
		Long (>8mm)	7		
9.	Hypocotyl:Anthocyanin pigmentation	Absent	1	Cotyledons completely unfolded	VS
		Present	9		
III) Plant morphological characters					
10.	Plant: Growth type	Determinate	1	About 70 % of the pods have attained full length (30-50 mm)	VG
		Semi-determinate	2		
		Indeterminate	3		
11.	Plant: Growth habit	Erect	1	Flowering: About 50 % plants have at least one flower open	VG
		Semi-erect	2		
12.	Plant: Height	Short (≤ 40 cm)	3	Advanced ripening: About 50 % of the pods are ripe	MS
		Medium (41-60 cm)	5		
		Tall (>60 cm)	7		

IV) Leaf and flower morphological characters					
13.	Leaf: Shape	Lanceolate	1	Flowering: About 50 % plants have at least one flower open	VG
		Pointed ovate	2		
		Rounded ovate	3		
14.	Leaf : Colour	Green	1	Flowering: About 50 % plants have at least one flower open	VG
15.	Plant: Days to 50 % Flowering	Early (≤ 35 days)	3	Flowering: About 50 % plants have at least one flower open	VG
		Medium (36 to 45 days)	5		
		Late (> 45 days)	7		
16.	Flower: Colour	White	1	Flowering	VG
		Purple	2		
V) Pod morphological characters					
17.	Pod: Pubescence	Absent	1	About 70 % of the pods have attained full length (30-50 mm)	VG
		Present	9		
18.	Pod: Pubescence Colour	Grey	1	About 70 % of the pods have attained full length (30-50 mm)	VS
19.	Pod: Colour	Tawny (Brown)	2	Advanced ripening: About 50 % of the pods are ripe	VS
		Yellow	1		
		Brown	2		
20.	Pod: Shattering	Black	3	Full maturity: About 95 % of the pods are ripe	VG
		Shattering	1		
		Non shattering (upto 10-days)	9		
21.	Plant: Days to Maturity	Early (≤ 95 days)	3	Full maturity: About 95 % of the pods are ripe	VG
		Medium (96-105 days)	5		
		Late (>105 days)	7		

MG	Measurement by a single observation of a group of plants or parts of plants
MS	Measurement of a number of individual plants or parts of plants
VG	Visual assessment by a single observation of a group of plants or parts of plants
VS	Visual assessment by observations of individual plants or parts of plants

sized, seeds of Hara Soya, Shivalik and Him Soya were classified as medium sized, whereas seeds of PS 1556 were classified as small sized seeds. Dhaliwal *et al.* (2020), in their findings also characterized soybean varieties on the basis of 100 seed weight. Accordingly, majority of the grain type soybean seeds were classified as small and medium-sized seeds. Large seeds were observed in vegetable type soybean. In the present study also, the largest seeds were reported in Him Palam Hara Soya 1 which is also a culinary purpose variety. Briefly, the variety Him Palam Hara Soya 1 exhibited highest value for 100-seed weight (20.46 g), seed length (7.4 mm) and seed width (6.93 mm), whereas PS 1556 manifested lowest values for all these traits i.e. 100-seed weight (9.73 g), seed length (6.3 mm) and seed width (5.3 mm).

However, based on seed length and width (Table 4), all the seven varieties were categorized under short seed length (<10 mm) and width (<7 mm). Therefore these two traits could not distinguish the varieties from each other. Although, Morris and Payne (1977) had suggested that seed traits such as seed size, length and width can be used to distinguish different soybean varieties.

Plant morphological characters

Hara Soya, Palam Soya and Him Palam Hara Soya 1 had semi erect growth habit whereas Palam Early Soya 1, Shivalik, PS 1556 and Him Soya had erect growth habit. In the present study, all the seven varieties under consideration recorded determinate growth type. Similarly, Pawale *et al.* (2019) observed the growth habit in crosses between trypsin inhibitor free and expressing soybean genotypes and recorded determinate growth type in all the cultivars. Dhaliwal *et al.* (2020) worked with 22 soybean lines and recorded monomorphic plant growth type. Further, anthocyanin pigmentation was absent in all the varieties. The presence or absence of anthocyanin pigmentation is considered as a distinct character as it is a monogenically controlled trait (Ladizinsky 1979). Him Palam Hara Soya 1 (95.8 cm) and Shivalik (95.4 cm) recorded the tallest plants. The minimum plant height was recorded for Hara Soya (71.7 cm). However, observation on plant height grouped all the varieties under tall plant height (> 60 cm) category (Table 4). Descriptors like plant growth habit, plant growth type and plant height were also used by Gupta

et al. (2010) and Dhaliwal *et al.* (2020) for the classification of soybean genotypes.

Leaf and flower morphological characters

Soybean is a photosensitive crop and its growth behaviour changes with the change in altitude (Gupta *et al.* 2010). Based on 50 per cent flowering, all seven soybean varieties were grouped under late (>45 days) category (Table 3). The possible reason behind this could be that all the varieties were recommended for as well as grown at the same altitude. White coloured flowers were observed in Hara Soya, Shivalik and PS 1556 whereas Palam Early Soya 1, Palam Soya, Him Soya and Him Palam Hara Soya 1 exhibited purple coloured flowers (Fig 2). On similar lines, Ramteke and Murlidharan (2012) grouped 92 soybean varieties into two major groups with respect to flower colour *i.e.*, white flowered and violet flowered. Dark green coloured leaves were observed in Hara Soya and Shivalik while Palam Early Soya 1, Palam Soya, PS 1556, Him Soya and Him Palam Hara Soya 1 exhibited green coloured leaves (Fig 3). Dhaliwal *et al.* (2020) also concluded leaf colour as an important descriptor for the characterization of soybean. The variety PS 1556 had lanceolate leaves, Him Soya and Palam Early Soya 1 exhibited round ovate leaves and all the other varieties namely Hara Soya, Shivalik, Palam Soya and Him Palam Hara Soya 1 showed pointed ovate leaves (Fig 4). Pawale *et al.* (2019) also reported that all the three types of leaves shapes were pre dominant in soybean cultivars. However, according to Gupta *et al.* (2010) the variation in leaf shape was mainly observed between round ovate and pointed ovate leaf for 89 soybean varieties. Researchers like Gupta *et al.* (2010) and Pawale *et al.* (2019) also used descriptors like flower colour, leaf colour, leaf shape, days to flowering and days to maturity for characterising various cultivars of soybean.

Pod morphological characters

The pod pubescence has greater stability across various agro- climatic zones (Tarasatyavathi *et al.* 2004, Gupta *et al.* 2010 and Ramteke and Muralidharan 2012). Pod pubescence was present on all the seven varieties (Fig 5). Gupta *et al.* (2010) also used presence and absence of hair on pod for recording distinctness in 89 soybean varieties. Moreover,

Table 2. Scaling of soybean genotypes based on their morphological characterization

Variety	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
Hara Soya	1	3	1	4	2	5	3	3	1	2	7	2	2	7	1	9	1	3	9	7	1
Palam Early Soya 1	2	1	1	4	1	7	3	3	1	1	7	3	1	7	2	9	2	2	9	7	1
Shivalik	2	1	1	3	1	5	3	3	1	1	7	2	2	7	1	9	1	1	9	7	1
Palam Soya	2	1	1	3	1	7	3	3	1	2	7	2	1	7	2	9	2	1	9	7	1
PS 1566	1	1	1	4	1	3	3	3	1	1	7	1	1	7	1	9	2	1	9	7	1
Him Soya	2	1	1	4	1	5	3	3	1	1	7	3	1	7	2	9	2	2	9	7	1
Palam Hara Soya 1	2	3	1	3	1	7	3	3	1	2	7	2	1	7	2	9	1	3	9	7	1

A = Seed: Shape; B = Seed: Colour; C = Seed: Lustre; D = Seed: Hilum colour; E = Seed: Cotyledon colour; F = Seed: Size (100-seed weight); G = Seed: Length (mm); H = Seed: Width (mm); I = Plant: Growth type; J = Plant: Growth habit; K = plant: Height (cm); L = Leaf: Shape; M = Leaf: Colour; N = Plant: Days to 50% flowering; O = Flower: Colour; P = Pod: Pubescence; Q = Pod: Pubescence colour; R = Pod: Colour; S = Pod: Shattering; T = Plant: Days to maturity; U = Hypocotyl: Anthocyanin pigmentation

Table 3. Morphological characterisation of seven soybean varieties of Himachal Pradesh

Variety	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	
Hara Soya	Spherical	Green	Shiny	Black	Green	Medium	Small	Small	Determinate	Semi-Erect	Tall	Pointed	Ovate	Dark Green	Late	White	Present	Brown	Black	Non-Shattering	Late	Absent
Palam Early Soya 1	Elliptical	Yellow	Shiny	Black	Yellow	Large	Small	Small	Determinate	Erect	Tall	Rounded	Ovate	Green	Late	Purple	Present	Brown	Brown	Non-Shattering	Late	Absent
Shivalik	Elliptical	Yellow	Shiny	Brown	Yellow	Medium	Small	Small	Determinate	Erect	Tall	Pointed	Ovate	Dark Green	Late	White	Present	Grey	Yellow	Non-Shattering	Late	Absent
Palam Soya	Elliptical	Yellow	Shiny	Brown	Yellow	Large	Small	Small	Determinate	Semi-Erect	Tall	Pointed	Ovate	Green	Late	Purple	Present	Brown	Yellow	Non-Shattering	Late	Absent
PS 1566	Spherical	Yellow	Shiny	Black	Yellow	Small	Small	Small	Determinate	Erect	Tall	Lanceolate	Green	Late	White	Present	Brown	Yellow	Non-Shattering	Late	Absent	
Him Soya	Elliptical	Yellow	Shiny	Black	Yellow	Medium	Small	Small	Determinate	Erect	Tall	Rounded	Ovate	Green	Late	Purple	Present	Brown	Brown	Non-Shattering	Late	Absent
Palam Hara Soya 1	Spherical	Green	Shiny	Black	Green	Medium	Small	Small	Determinate	Semi-Erect	Tall	Pointed	Ovate	Dark Green	Late	White	Present	Brown	Black	Non-Shattering	Late	Absent

A = Seed: Shape; B = Seed: Colour; C = Seed: Lustre; D = Seed: Hilum colour; E = Seed: Cotyledon colour; F = Seed: Size (100-seed weight); G = Seed: Length (mm); H = Seed: Width (mm); I = Plant: Growth type; J = Plant: Growth habit; K = plant: Height (cm); L = Leaf: Shape; M = Leaf: Colour; N = Plant: Days to 50% flowering; O = Flower: Colour; P = Pod: Pubescence; Q = Pod: Shattering; T = Plant: Days to maturity; U = Hypocotyl: Anthocyanin pigmentation

Table 4. Morphometric characterisation of seven soybean varieties of Himachal Pradesh

Variety	100-seed weight (g)	Seed length (mm)	Seed width (mm)	Plant height (cm)
V1: Hara Soya	11.47	7.03	6.1	71.73
V2: Palam Early Soya 1	14.06	6.7	5.7	82.33
V3: Shivalik	12.93	6.7	5.9	95.36
V4: Palam Soya	13.79	6.6	5.56	80.63
V5: PS 1556	9.73	6.33	5.3	86.3
V6: Him Soya	11.44	6.83	6.36	74.3
V7: Palam Hara Soya 1	20.46	7.4	6.93	95.83
MEAN	15.33	6.8	5.98	65.6
SE (m)±	0.651	0.144	0.10	0.424
C.D.	0.209	0.44	0.29	1.321

Seed : Colour

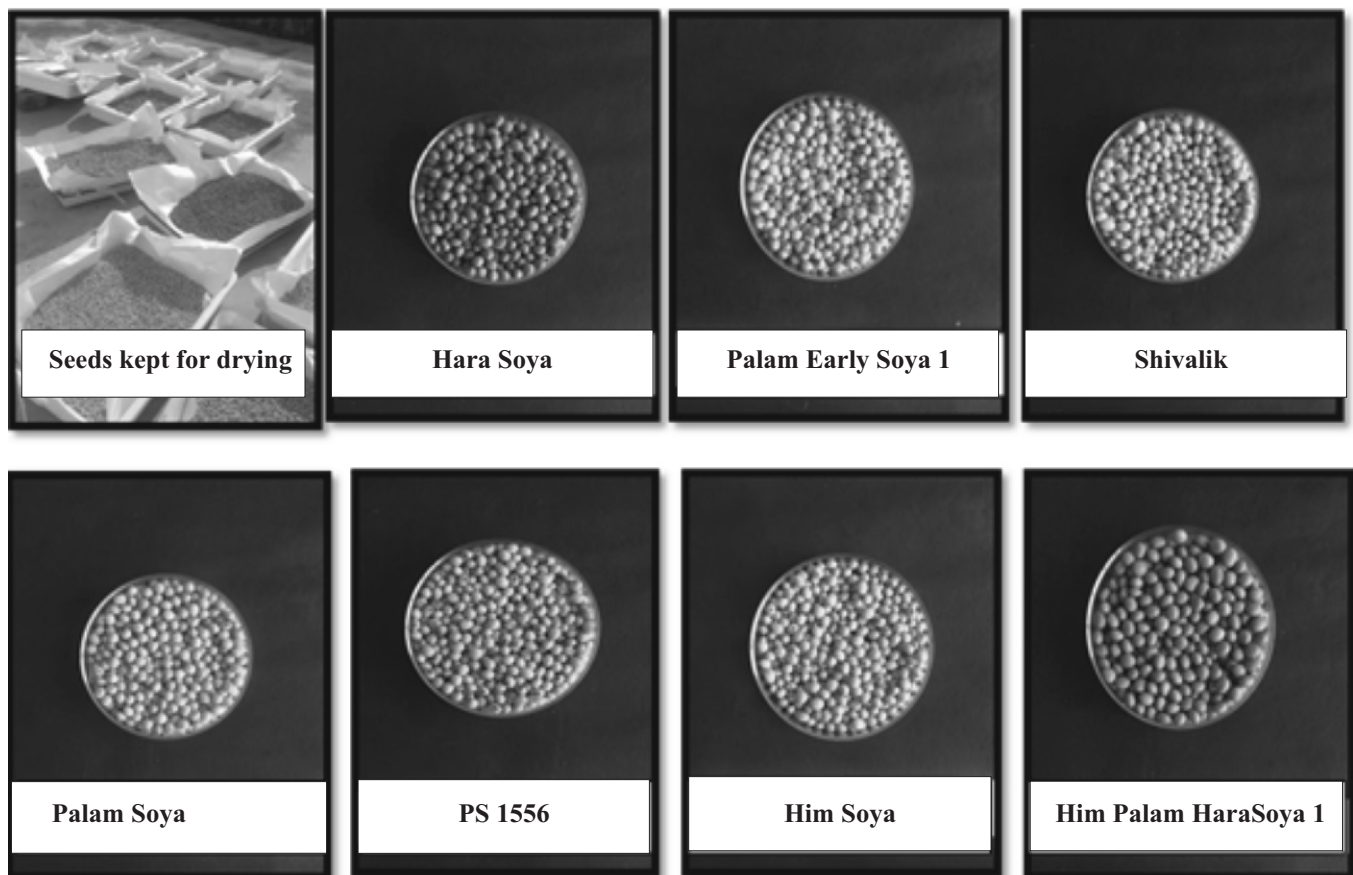
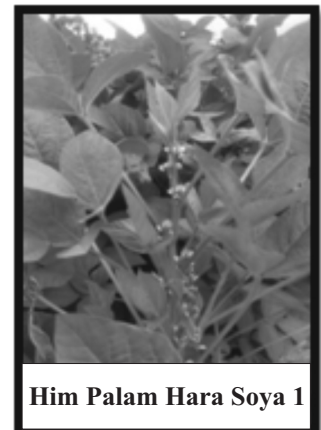


Fig 1 Characterization of seven varieties of soybean based on seed colour

Flower : Colour



White coloured flowers



Purple coloured flowers

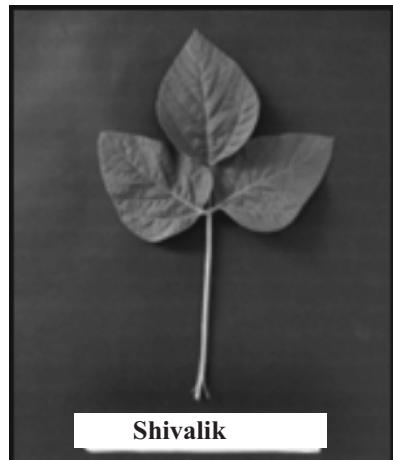
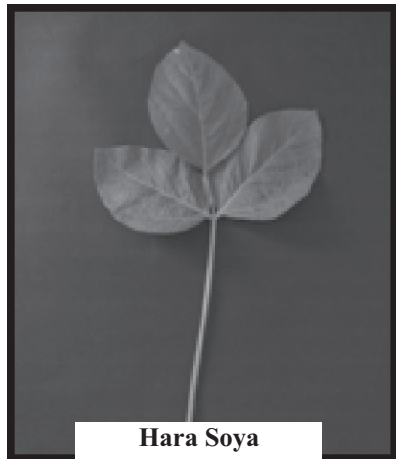
Fig 2 Characterization of seven varieties of soybean based on flower colour

Purple coloured flowers

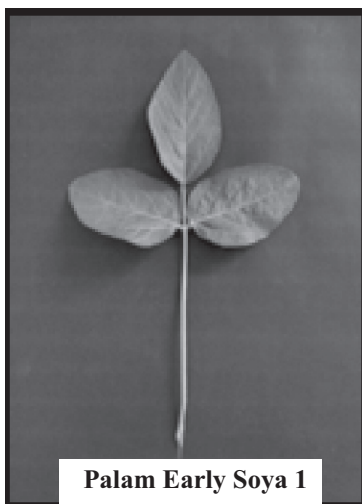


Fig 3 Characterization of seven varieties of soybean based on leaf colour

Leaf : Shape



Pointed ovate



Rounded ovate

Lanceolate

Fig 4 Characterization of seven varieties of soybean based on leaf shape



Fig 5 Presence of pod pubescence on the pods of soybean

Shivalik and Him Palam Hara Soya 1 exhibited grey pod pubescence, whereas Hara Soya, Palam Early Soya 1, Palam Soya, PS 1556 and Him Soya revealed tawny (brown) pod pubescence. Dhaliwal *et al.* (2020) also reported similar results in soybean where majority of the genotypes had tawny-coloured hair whereas some of them had grey coloured hair. Shivalik, Palam Soya and PS 1556 exhibited yellow coloured pods; Palam Early Soya 1 and Him Soya recorded brown coloured pod whereas black coloured pods were observed in Hara Soya and Him Palam Hara Soya 1. All the varieties under observation were non-shattering varieties. Similar results were reported by Pawale *et al.* (2019) during the characterisation of soybean cultivars where it was found that all the cultivars were non-shattering type. Based on days to maturity, all seven soybean varieties are categorized as late (>105 days) maturing. By comparing present work with the results published by Satyavathi *et al.* (2004), Rani *et al.* (2004), it revealed that descriptors like presence and absence of pod hair, colour of hair, days to maturity have been used before for the characterisation of soybean cultivars.

The ability to properly discriminate between varieties is important for maintaining the identity of the variety throughout the seed multiplication program. One of the most effective, quick and cheaper way of doing so is using Distinctness, Uniformity and Stability (DUS) morphological traits. Morphological traits have been used as descriptors from ancient

times. It is likely that their use will continue because they are omnipresent in agriculture (Smith and Smith 1989). Thus, a database created through morphological characterization of genotypes would greatly help the actors involved in seed multiplication and genetic purity testing to wisely establish the conscious decisiveness on variety identity.

Conclusion

It can be inferred that 11 out of 21 designated DUS characters namely seed shape, seed colour, seed cotyledon colour, seed hilum colour, seed size, plant growth habit, leaf shape, leaf colour, flower colour, pod pubescence colour and pod colour were reliable to provide a sound classification system for the identification and classification of seven soybean varieties of Himachal Pradesh. The information generated would serve a ready reckoner for all the actors involved in undertaking activities related to maintenance breeding during seed multiplication and for genetic purity assessment during the Grow Out Test.

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