Identification of Soybean Cultivars Using Biochemical and Morphological Characteristics at Seed and Seedling Stage

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Seventy five cultivars of soybean were characterized for seed storage proteins, amylase isozymes, seed coat peroxidase, hypocotyl pigmentation. All the cultivars were first classified into twelve major groups based on visual characteristics *viz.* seed coat colour, hilum colour and seed coat lustre. These groups were further classified based on seed coat peroxidase reaction, hypocotyl pigmentation, amylase isozymes and seed storage protein, generating 73 groups. Thus, most of the cultivars could be uniquely identified based upon seed and seedling characteristics. Only four cultivars *viz.* JS2, Punjab1, MACS13 and MAUS71 fell into two groups each consisting of two cultivars.

Key Words: Amylase, Cultivar, Identification, Peroxidase, Soybean, SDS-PAGE

Varietal identification is essential for describing a new cultivar, testing genotypic purity and expediting DUS (distinctness, uniformity and stability) test for candidate cultivar. Various laboratory techniques employed for varietal identification range from simple methods of examining seed and seedling morphology to the more advanced procedure of analyzing DNA polymorphism (Smith and Smith, 1992; Cooke, 1995; Chen *et al.*, 1994). Seed morphology, studied by visual examination can provide useful information for differentiating genotypes (Wiesner *et al.*, 1993; Aggarwal and Powar, 1990). Examination of seedling morphology under controlled conditions of growth can be useful and cost-effective for describing and distinguishing cultivars (ISTA, 1992). SDS - PAGE and isozyme polymorphism of seed storage proteins have been found to be very useful tools in varietal identification in soybean (Abdel–Tawab *et al.*, 1993; Doong and Kiang, 1987; Cardy and Beversdorf, 1984). For rapid identification of varieties, the distinction should be made at seed and seedling stage by scoring maximum number of discriminating biochemical and morphological characteristics. Therefore, with a view to score maximum characteristics at seed and seedling stages, 75 cultivars of soybean were studied for seed coat color, hilum color, seed coat lustre, seed coat peroxidase reaction, hypocotyl coloration, SDS–PAGE of seed proteins and amylase isozymes.

Materials and Methods

Single plant progenies of 75 soybean cultivars were grown in the field at National Research Centre for Soybean, Indore. Seeds from a single plant of each of the varieties were hand harvested and studied for the following parameters.

Seed Colour

A minimum of 20 seeds from each cultivar were examined for seed coat colour, hilum color and seed coat lustre. Four seed coat colour classes *viz.* green, greenish yellow, yellow and black, four hilum categories *viz.* black, brown, imperfect black (black hilum bordered by a brown line) and gray and three seed coat lustre categories *viz.* shiny, intermediate and dull were clearly distinguishable among cultivars. Each variety was assigned to one of the classes for each of the traits.

Seed Coat Peroxidase Reaction

The method of Buttery and Buzzell (1968) was used to analyse seed coat peroxidase reaction. Cultivars were placed into one of the two groups based upon appearance (positive) or absence (negative) of reddish brown color after reacting seed coat with guiacol and hydrogen peroxide.

Anthocyanin Pigmentation on Hypocotyl

Twenty seeds of all the cultivars were sown in the germination tray under constant light. The pigmentation on hypocotyls was observed on 4th day and the cultivars were placed into one of the three groups *viz.* green, bronze or purple based upon appearance of color.

Sample Preparation for SDS-PAGE and Amylase Isozyme Analyses

Ten seeds of each cultivar were ground using pestle and mortar. The flour so obtained was defatted using petroleum ether. Hundred mg defatted flour was extracted in 1 ml of 0.05M Tris-Cl buffer (pH 6.8) and centrifuged at 10000 rpm. The supernatant was decanted and used for SDS-PAGE and Amylase analysis.

SDS-PAGE of Seed Storage Proteins

The polypeptide patterns of seed storage proteins were resolved using discontinuous SDS-PAGE system as given by Laemmli (1970). The stacking gel consisted of 5% acrylamide-bis acrylamide (29:1), pH 6.8 and separating gel consisted of 12% acrylamide-bisacrylamide (29:1), pH 8.9. The protein bands were numbered from loading well to the end of the gel. The varieties were categorized based upon presence or absence of specific bands. Rm value of each polypeptide was calculated as follows:

$$Rm = \frac{\text{Distance travelled by particular polypeptide}}{\text{Distance travelled by tracking dye}}$$

Amylase Isozymes

Amylase isozymes were resolved on native PAGE consisting of 5% stacking gel and 7% separating gel containing 0.5% starch. Isozymes were visualized by negative staining by the method given by Stoddart (1971). The varieties were categorized based on presence or absence of a particular band. Rm value of each isozyme was calculated as follows:

$$Rm = \frac{\text{Distance travelled by particular isozyme}}{\text{Distance travelled by tracking dye}}$$

Results and Discussion

Seed Colour

Based on seed coat color 75 cultivars were separated into four groups. The largest group with yellow seed coat consisted of 69 cultivars followed by black seed group consisting of four varieties and green and greenish yellow group each consisting of one variety only. For the hilum color, the largest group with brown hilum contained 42 varieties followed by black hilum group with 27 cultivars and gray hilum group consisting of 5 cultivars and imperfect black hilum group having one cultivar only. Maximum cultivars (46) displayed intermediate seed coat lustre while the group with shiny seed coat lustre comprised of 25 varieties. Only four cultivars were classified under dull seed coat luster.

Seed Coat Peroxidase Reaction

Seed coat peroxidase reaction differentiated 75 cultivars into two groups. Thirty one cultivars exhibited positive reaction and forty four cultivars exhibited negative reaction for seed coat peroxidase.

Hypocotyl Pigmentation

Among three groups, the largest group with purple pigmentation consisted of 45 cultivars followed by the group with bronze pigmentation consisting of 22 cultivars and the group with green pigmentation consisting of eight cultivars.

SDS-PAGE of Seed Storage Proteins

A total number of 35 polypeptide bands were resolved on gel with molecular weight ranging from 17 kDa to 122 kDa (Figs. 1-6). Maximum number of bands (29) were observed in Bragg and T49 while minimum number of bands (22) were observed in Hardee. Out of 35 bands, 10 bands were found to be common to all the cultivars. The common bands with their Rm values are: 3rd band (0.12), 5th band (0.14), 6th band (0.16), 7th band (0.17), 10th band (0.22), 12th band (0.24), 17th band (0.31), 19th band (0.36), 29th band (0.61), 35th band (0.82). There were some unique bands present in only one cultivar.

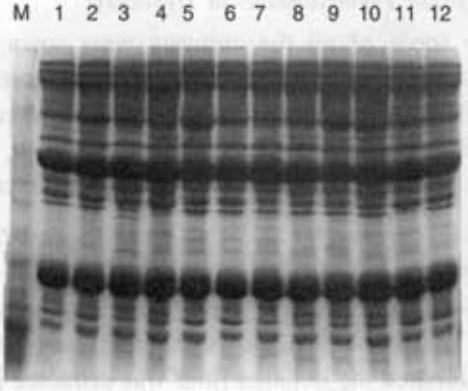

Fig.1. SDS-Page profile of soybean cultivars. Lane 1-12 represents the cultivars Lee, MACS 450, VLS 47, Pusa 24, JS 72-44, GS1, Punjabi1, PK. 327, NRC 7, VLS1, JS 72-280 and RAUS5. M represents molecular weight markers.

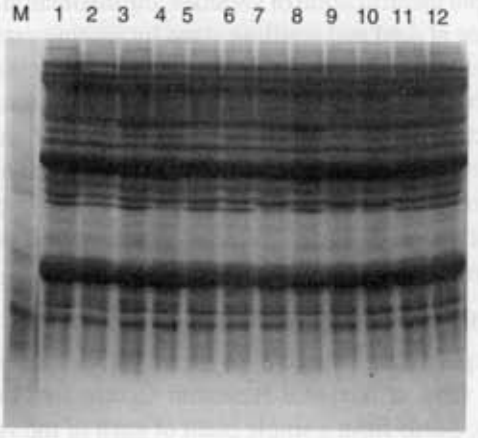

Fig.2. SDS-Page profile of soybean cultivars. Lane 1-12 represents the cultivars JS 75-MACS 124, PK 1024, JS 79-81, PK 472, ADT-1, MAUS 32, MACS13, Alankar, JS 80 MAUS 1 and NRC 37. M represents molecular weight markers.

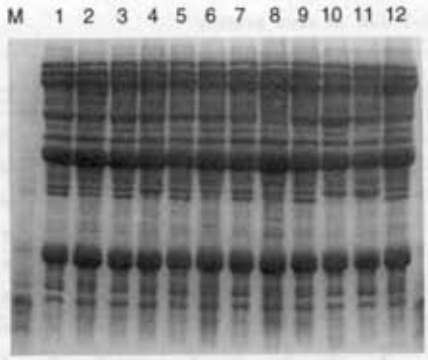

Fig.3. SDS-Page profile of soybean cultivars. Lane 1-12 represents the cultivars JS90-41, Shilajeet, Shivalik, GS2, VLS 21, Pusa37, MAUS71, MACS 58, PK 262, Improved pelican, NRC2 and JS 75-46. M represents molecular weight markers.

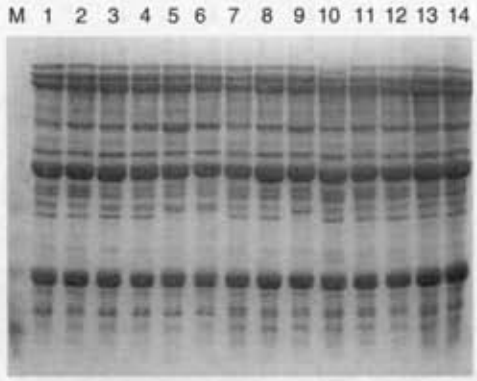

Fig.5. SDS-Page profile of soybean cultivars. Lane 1-14 represents the cultivars JS 2, PK416, MACS57, Co1, KB 79, IS 9, Harit Soya, VLS, MAUS2, JS71-05, NRC12, Pusa 16, Palam soya, PK 308. M represents molecular weight markers.

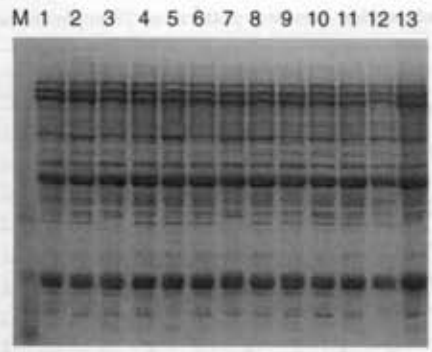

Fig.4. SDS-Page profile of soybean cultivars. Lane 1-14 represents the cultivars Pusa 40, JS 76-205, T49, PK 1042, Pusa 22, SL 295, MAUS 61-2, Pusa 20, KHSb2, An, Bragg, Kalitur, MAUS 61, PK 1092. M represents molecular weight markers.

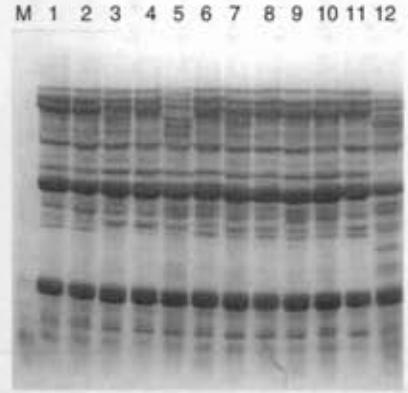

Fig.6. SDS-Page profile of soybean cultivars. Lane 1-12 represents the cultivars Birsa soya 1, PK 564, Monetta, MAUS 47, JS 335, PS1029, CO2, PK 471, SL 96, SL 459, JS 93-05, Hardee. M represents molecular weight markers.

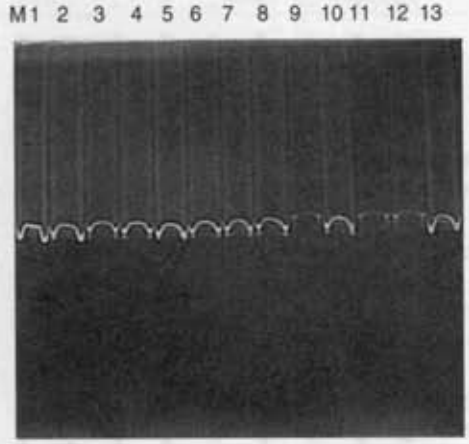

Fig.7. Electrophoretic pattern of amylase isozyme of soybean cultivars. Lanes 1-13 represent the cultivars PK 1092, MAUS71, JS79-81, Pb1, VLS2, Bragg, Lee, PK 309, Hara Soya, MAUS61, Lsb1, SL96 and JS80-21

For example, the fourth band (Rm 0.13) was present only in JS 335, 14th band (Rm 0.26) was present only in MAUS 61-2 and 21st band (Rm 0.40) was present only in MAUS 32. Rest of the cultivars were categorized based on presence or absence of any of the 25 polymorphic bands (Table 1).

Amylase Isozyme Variability

A total of four bands with Rm values 0.031, 0.45, 0.48 and 0.50 are observed. Three bands with Rm values 0.031, 0.45 and 0.50 were present in all the cultivars. The third band with Rm value 0.48 was absent in three varieties *viz.* LSb1, SL96 and Hara Soya (Fig. 7).

Varietal Identification Key

A varietal identification key was framed based on all the differentiating characters studied (Table 2). The cultivars were first classified based upon most obvious character, seed coat colour. Two cultivars, Hara Soya and JS90-41, were uniquely identified based on their seed coat colour. Remaining 73 cultivars were further sub-classified based on hilum colour. The variety Pusa

Table 1. Polypeptide banding pattern of 75 soybean cultivars derived from SDS–PAGE of seed protein

Cultivar	Band no 1	2	4	8	9	11	13	14	15	16	18	20	21	22	23	24	25	26	27	28	30	31	32	33	34
Rm	.07	.08	.12	.18	.19	.23	.25	.26	.28	.29	.33	.39	.40	.42	.45	.48	.50	.53	.54	.56	.64	.67	.70	.73	.76
Lee	–	+	–	+	–	+	+	–	+	+	–	–	+	+	+	–	–	–	–	–	+	+	+	+	+
MACS450	–	+	–	+	–	+	+	–	+	+	+	–	+	+	+	–	–	–	+	–	+	+	+	+	+
VLS47	–	+	–	+	–	+	+	–	+	+	+	+	+	+	–	+	–	–	+	–	+	+	+	+	+
Pusa24	–	+	–	+	–	+	+	–	+	+	+	+	+	+	–	–	–	–	+	–	+	+	+	+	+
JS7244	–	+	–	+	–	+	+	–	+	+	+	+	+	+	+	–	–	–	+	–	+	+	+	+	+
GS1	–	+	–	–	–	+	+	–	+	+	–	+	+	+	+	–	–	–	+	–	+	+	+	+	+
Pb1	–	+	–	+	–	+	+	–	+	+	–	+	+	+	+	–	–	–	+	–	+	+	+	+	+
PK 327	–	+	–	+	–	+	+	–	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+
NRC7	–	+	–	+	–	+	+	–	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+
VLS1	+	+	–	+	–	+	+	–	+	+	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+
JS72–280	–	+	–	+	–	+	+	–	+	+	+	–	+	+	–	–	+	+	+	–	+	+	+	+	+
RAUS5	–	+	–	–	–	+	+	–	+	+	+	–	+	+	+	–	+	+	+	–	+	+	+	+	+
JS75–46	–	+	–	+	–	–	+	+	+	+	–	+	+	+	+	–	–	+	+	–	+	–	+	+	+
MACS124	–	–	–	+	–	+	+	–	+	+	–	+	+	+	+	–	–	+	+	–	+	–	+	+	+
PK 1024	–	+	–	+	–	+	+	–	+	+	–	+	+	+	+	–	–	+	+	–	+	+	+	+	+
JS 79–81	–	–	–	+	–	+	+	–	+	+	–	+	+	+	–	–	–	+	+	–	+	+	+	+	+
PK472	–	+	–	+	–	+	+	–	+	+	–	+	+	+	+	–	–	+	+	–	+	+	+	+	+
ADT1	–	+	–	+	–	+	+	–	+	+	+	+	+	+	+	–	–	+	+	–	+	–	+	+	+
MAUS32	–	+	–	+	–	+	+	–	+	+	+	+	+	+	–	–	–	+	+	–	+	–	+	+	+
MACS13	–	+	–	+	–	+	+	–	+	+	–	+	+	+	+	–	–	+	+	–	+	+	+	+	+
Alankar	–	+	–	+	–	+	+	–	+	+	+	+	+	+	–	–	–	+	+	–	+	+	+	+	+
JS80–21	–	+	–	+	–	+	+	–	+	+	–	+	+	+	–	–	–	+	+	–	+	+	–	+	+
MAUS1	+	+	–	+	–	–	+	+	+	+	–	+	+	+	+	–	–	+	+	–	+	–	–	+	+
NRC37	–	+	–	+	–	+	+	–	+	+	–	+	+	+	+	–	–	+	+	–	+	–	–	+	+
JS 90–41	–	+	–	+	–	+	+	–	+	+	–	+	+	+	+	–	–	+	+	–	+	+	+	+	+
Shilajeet	–	–	–	+	–	+	+	–	+	+	–	+	+	+	+	+	+	+	+	–	+	–	+	+	+
Shivalik	–	+	–	+	–	+	+	–	+	+	–	+	+	+	+	–	–	+	+	–	+	+	–	+	+
GS2	–	+	–	+	–	+	+	–	+	+	–	+	+	+	–	–	–	+	+	–	+	+	+	+	+
VLS21	–	+	–	+	–	+	–	–	+	+	–	+	+	+	+	–	–	+	+	–	+	+	+	+	+
Pusa37	–	–	–	+	–	+	+	–	+	+	–	+	+	+	–	–	–	+	+	–	+	–	+	+	+
MAUS71	–	+	–	+	–	+	+	–	+	+	+	+	+	+	+	–	–	+	+	–	+	+	+	+	+
MACS58	–	–	–	+	–	+	+	–	+	+	+	+	+	+	+	+	–	+	+	–	+	–	+	+	+
PK262	–	+	–	+	–	+	+	–	+	+	+	+	+	+	+	–	–	+	+	–	+	+	+	+	+
Imp pelican	–	+	–	+	–	+	+	–	+	+	–	+	+	+	–	–	–	+	+	–	+	+	–	+	+
NRC2	–	+	–	+	–	+	+	–	+	+	–	+	–	+	–	–	–	+	+	–	–	+	+	+	+
Pusa 40	–	+	–	+	–	+	+	–	+	+	–	+	+	–	+	+	–	–	+	–	+	+	+	+	+
JS 76–205	+	+	–	+	–	+	+	–	+	+	+	+	+	–	+	+	–	–	+	–	+	+	+	+	+
T49	–	+	–	+	–	+	+	–	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	+	+
PK1042	–	+	–	+	–	+	+	–	+	–	–	+	+	–	+	+	+	+	+	–	+	+	+	+	+
Pusa 22	–	+	–	+	–	+	+	–	+	+	–	+	+	–	+	+	+	+	+	–	+	+	+	–	+
SI295	–	+	–	+	–	+	+	–	+	+	–	+	+	–	+	+	+	+	+	–	+	+	+	+	+
MAUS61–2	–	+	–	+	–	+	+	+	+	–	–	+	+	+	–	+	+	+	+	–	+	+	+	+	+
Pusa20	–	+	–	+	–	+	+	–	+	+	–	+	+	–	+	+	+	+	+	–	+	+	+	+	+
KHSb2	–	+	–	+	–	+	+	–	+	+	–	+	+	–	+	+	–	–	+	–	+	+	+	+	+
Ankur	–	+	–	+	–	+	–	–	+	+	+	+	+	+	+	+	–	–	+	–	+	+	+	+	+
Bragg	–	+	–	+	–	+	+	–	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Kalitur	+	+	–	+	–	+	+	–	+	+	+	+	+	–	+	+	+	–	+	–	+	+	+	–	–
MAUS61	+	+	–	+	–	+	+	–	+	+	–	+	+	+	–	+	+	+	+	–	+	+	+	+	+
Pk1092	+	+	–	+	–	+	+	–	+	+	–	+	+	+	+	–	+	+	+	–	+	+	+	+	+

Contd.

Table 1 Contd.

Cultivar	Band no 1	2	4	8	9	11	13	14	15	16	18	20	21	22	23	24	25	26	27	28	30	31	32	33	34
JS2	–	+	–	+	–	+	+	–	+	+	–	+	+	–	+	+	–	+	+	–	+	+	+	+	+
PK416	–	+	–	+	–	+	+	–	+	+	–	+	+	–	+	+–	–	+	+	–	+	+	+	+	+
MACS57	–	+	–	+	–	–	+	–	+	+	–	+	+	+	+	+	–	+	+	–	+	+	+	+	+
Co1	–	+	–	+	–	–	+	–	+	+	–	+	+	+	+	+	–	+	+	–	+	+	+	+	+
KB79	–	–	–	+	–	+	+	–	+	+	–	+	+	+	–	–	–	+	+	–	+	+	+	+	+
IS9	+	+	–	+	–	–	+	–	+	+	+	+	+	+	–	–	–	+	+	–	+	+	+	+	+
Hara soya	–	+	–	+	–	+	+	–	+	+	–	–	+	–	+	–	–	+	+	–	+	+	+	+	+
MAUS2	–	+	–	+	–	+	+	–	+	+	–	+	+	+	–	–	–	+	+	–	+	+	+	+	+
JS 71–05	–	–	–	+	+	+	+	–	+	+	–	+	+	+	+	–	–	+	+	+	+	+	+	–	+
NRC12	–	+	–	+	–	+	+	–	+	+	–	–	+	+	+	+	–	+	+	–	+	+	+	+	+
Pusa16	–	+	–	+	–	+	+	–	+	+	–	+	+	+	+	+	–	+	+	–	+	+	+	+	+
Palam soya	+	+	–	+	–	+	+	–	+	+	–	+	+	–	+	+	–	+	+	–	+	+	+	+	+
Pk308	+	+	–	+	–	+	+	–	+	+	–	+	+	–	+	+	–	+	+	–	+	+	+	+	+
Bsoya1	+	–	–	+	–	+	+	–	–	+	–	+	+	–	+	–	–	–	+	–	+	+	+	+	+
Pk564	–	–	–	+	–	+	+	–	–	+	–	+	+	–	+	–	–	+	+	–	+	+	+	+	+
Monetta	–	–	–	+	–	+	+	–	+	+	–	+	+	+	–	+	–	+	+	–	+	+	+	+	+
MAUS 47	–	+	–	+	+	+	+	–	+	+	–	+	+	+	–	–	–	–	+	–	+	+	+	+	+
JS335	–	–	+	+	–	+	+	–	+	+	–	+	+	+	–	+	–	+	+	+	+	+	+	+	+
Co2	–	–	–	+	+	+	+	–	+	+	–	+	+	+	+	–	–	+	+	–	+	–	+	–	+
PK471	+	+	–	+	–	+	+	–	+	–	+	+	+	+	+	+	–	+	+	–	+	+	+	+	+
SI96	+	+	–	+	–	+	+	–	–	+	–	+	+	+	+	+	–	+	+	–	+	+	+	+	+
SI459	+	–	–	+	–	+	+	–	–	+	–	+	+	–	+	+	–	+	+	–	+	+	+	+	+
JS 93–05	+	+	–	+	–	+	+	–	+	+	+	+	+	–	+	+	–	–	+	–	+	+	+	+	+
Hardee	–	–	–	+	+	–	–	–	–	–	–	+	+	+	–	–	+	+	–	–	+	+	+	+	+

Table 2. A key to differentiate Indian soybean varieties based on seed coat color, hilum color, seed coat luster, seed coat peroxidase reaction, hypocotyl pigmentation, SDS-PAGE of seed storage proteins and amylase isozyme variability

Variety	Distinguishing character					
	Morphological/chemical					Electrophoretic variability
	SCC	HC	SCL	SCP	HP	SDS - PAGE/amylase
Hara soya	G					
JS90-41	GY					
JS76-205	B	B	S			
Kalitur	B	B	I	+		
VLS1	B	B	I	_	Bz	Protein band no. 14 present
Birsasoya1	B	B	I	_	Bz	Protein band no. 14 absent
Pusa16	Y	IB				
PS1029	Y	B	S	+	Bz	
MACS450	Y	B	S	+	P	Protein band no. 1 absent
KHSb2	Y	B	S	+	P	Protein band no. 22 absent
PS1092	Y	B	S	+	P	Protein band 1&22 present
Pusa37	Y	B	S	_	Bz	Protein band no. 2 absent
Pusa24	Y	B	S	_	Bz	Protein band no. 2 present
JS71-05	Y	B	S	_	P	Protein band no. 9 present
JS93-05	Y	B	S	_	P	Protein band no. 9 absent
LSb1	Y	B	I	_	Bz	Protein band no. 3 absent
Bragg	Y	B	I	_	Bz	Protein band no. 3 present
JS335	Y	B	I	_	P	Protein band no. 4 present
JS72-44	Y	B	I	_	P	Protein band no. 23 absent
MACS13	Y	B	I	_	P	Protein band no. 1 absent
MAUS71	Y	B	I	_	P	Protein band no. 1 absent

Contd.

Table 2 Contd.

Variety	Distinguishing character					
	Morphological/chemical					Electrophoretic variability
	SCC	HC	SCL	SCP	HP	SDS - PAGE/amylase
Indirasoya9	Y	B	I	_	P	Protein band no. 1 present
NRC2	Y	B	D	+	Bz	Protein band no. 30 absent
SL295	Y	B	D	+	Bz	Protein band no. 18 absent
JS72-280	Y	B	D	+	Bz	Protein band no. 18 present
MAUS2	Y	Br	S	+	G	
MACS124	Y	Br	S	+	P	Protein band no. 30 absent
JS79-81	Y	Br	S	+	P	Protein band no. 2 absent
Pusa40	Y	Br	S	+	P	Protein band 2 & 30 present
PK308	Y	Br	S	_	G	
NRC37	Y	Br	S	_	Bz	Protein band no. 1 absent
PK564	Y	Br	S	_	Bz	Protein band no. 2 absent
MAUS1	Y	Br	S	_	Bz	Protein band 1 & 2 present
Lee	Y	Br	S	_	P	Protein band no. 27 absent
Imp.Pelican	Y	Br	S	_	P	Protein band no. 23 absent
Shilajeet	Y	Br	S	_	P	Protein band no. 2 absent
NRC7	Y	Br	S	_	P	Protein band 2, 18 & 23 present
JS2	Y	Br	S	_	P	Protein band no. 11 present
Punjab1	Y	Br	S	_	P	Protein band no. 11 present
MACS57	Y	Br	S	_	P	Protein band no. 11 absent
Hardee	Y	Br	I	+	G	Protein band no. 9 present
VLS47	Y	Br	I	+	G	Protein band 9 & 23 absent
Shivalik	Y	Br	I	+	G	Protein band no. 18 absent
PK262	Y	Br	I	+	G	Protein band no. 18 present
PK1042	Y	Br	I	+	Bz	Protein band no. 16 absent
Alankar	Y	Br	I	+	Bz	Protein band no. 23 absent
Ankur	Y	Br	I	+	Bz	Protein band no. 18 present
PK416	Y	Br	I	+	Bz	Protein band no. 22 absent
PK1024	Y	Br	I	+	Bz	Protein band no. 22 present
GS1	Y	Br	I	+	P	Protein band no. 8 absent
JS75-46	Y	Br	I	+	P	Protein band no. 11 absent
KB79	Y	Br	I	+	P	Protein band no. 2 absent
JS80-21	Y	Br	I	+	P	Protein band no. 23 absent
Pusa22	Y	Br	I	+	P	Protein band 2 & 23 present
SL96	Y	Br	I	_	G	Protein band no. 1 present
PK472	Y	Br	I	_	G	Protein band no. 1 absent
PK471	Y	Br	I	_	Bz	Protein band no. 1 present
VLS21	Y	Br	I	_	Bz	Protein band no. 1 absent
MAUS61-2	Y	Br	I	_	P	Protein band no. 14 present
CoSoy2	Y	Br	I	_	P	Protein band no. 9 present
MAUS32	Y	Br	I	_	P	Protein band no. 21 present
NRC12	Y	Br	I	_	P	Protein band no. 20 absent
MACS58	Y	Br	I	_	P	Protein band no. 2 present
MAUS61	Y	Br	I	_	P	Protein band no. 1 present
GS2	Y	Br	I	_	P	Protein band 1 & 18 absent
ADT1	Y	Br	I	_	P	Protein band no. 31 absent
PK327	Y	Br	I	_	P	Protein band no. 24 absent
T49	Y	Br	I	_	P	Protein band 18, 24 & 31 present
Co1	Y	Br	D	+	P	
MAUS47	Y	Gr	I	+	P	Protein band no. 9 present
Monetta	Y	Gr	I	+	P	Protein band no. 9 absent
RAUS5	Y	Gr	I	_	P	Protein band no. 8 absent
VLS2	Y	Gr	I	_	P	Protein band no. 8 present

SCC–Seed Coat Colour, HC–Hilum Colour, SCL–Seed Coat Lustre, SCP–Seed Coat Peroxidase, HP–Hypocotyl Pigmentation, G–Green, GY-Greenish Yellow, Y–Yellow, B-Black, IB–Imperfect black, Br–Brown, Gr–Grey, S–Shiny, I–Intermediate, D–Dull, Bz–Bronze, P–Purple

16 was differentiated from other varieties using seed coat colour and hilum colour. Differences in seed coat luster were used to differentiate the rest 72 varieties. The variety JS76-205 was uniquely identified based upon its seed coat colour, hilum colour and seed coat luster. Remaining 71 varieties were further sub-classified using seed coat peroxidase reaction. Two varieties Kalitur and Co1 were differentiated from other varieties using this additional character. Differences in hypocotyl pigmentation were used to differentiate the remaining 70 varieties. Five varieties *viz.* PS1029, MAUS2, PK308, SL96 and VLS21 were uniquely identified using this sub-classification. Rest 65 varieties were further sub-classified based upon presence or absence of amylase third band. Two varieties LSb1 and Bragg were uniquely identified using this sub-classification. Remaining 63 varieties were grouped into 12 broad groups based on this classification. SDS-PAGE pattern of the varieties falling under same group were compared and the varieties were differentiated based on presence or absence of different bands till the varieties were uniquely identified. Thus except four cultivars, all other 69 cultivars could be uniquely identified. The remaining four cultivars formed two groups of two each *viz.* JS2 and Punjab1 and MACS13 and MAUS 71.

Under Plant Variety Protection and Farmers Right Act 2001, varietal testing for Distinctness, Uniformity and Stability (DUS) is essential for protection of new plant varieties. This warrants comparison of all the candidate varieties with the varieties of common knowledge. The key developed in this study to differentiate Indian soybean varieties based upon seed coat color, hilum colour, seed coat luster, seed coat peroxidase reaction, hypocotyls pigmentation, electrophoretic variability of SDS-PAGE banding pattern and amylase isozymes can serve as a ready reckoner for varietal identification by plant breeders and seed certification officers. Moreover, it will work as fast method to group the candidate variety with similar varieties for DUS testing as distinguishing characters used in development of varietal identification key are scored at seed and seedling stages.

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