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PHYLOGENETIC RELATIONSHIPS WITHIN SELECTED INDIAN SOYBEAN (*GLYCINE MAX* (L.) MERR.) VARIETIES BASED ON SDS-PAGE OF SEED PROTEINS

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Abstract: SDS-PAGE of seed protein was used to assess genetic diversity of fourteen soybean varieties being cultivated in India. Total protein content was estimated and compared with 100 seed weight of each variety. A total of 29 bands were recorded and their presence or absence in different varieties was noted. Based on the polymorphism generated by the presence or absence of protein bands Jaccard's similarity matrix was obtained and subjected to UPGMA cluster analysis. The dendrogram thus generated revealed four major groups which are comparable to an earlier study on polymorphism of Indian varieties using AFLP markers. Very little correspondence between the clustering pattern and the pedigree, place of release or target area of the variety could be observed in the present study. However, a moderately high genetic diversity could be observed within the fourteen varieties tested. The results indicate that a more diverse genetic base should be used in soybean breeding programme.

Keywords: *Soybean; Seed protein; SDS-PAGE; Cluster analysis; Dendrogram.*

I. Introduction

Soybean grains are nutritionally a rich source for high quality protein and oil. It has a much higher content of protein (40%) compared to rice (7%), wheat (12%), maize (10%) and other pulses (20-25%). Besides, soybean protein is rich in lysine (5%) which is absent in most of the cereals (Smith and Circle, 1976; Aslam *et al.*, 1995; Malik *et al.*, 2009). With respect to area under cultivation and grain production India ranks fifth in the world and within India soybean is the third most important oilseed crop after groundnut, rapeseed and mustard (Rai, 1999; Satyavathi *et al.*, 2006; FAO, 2008). Importance of soybean could be gauged from the fact that about two-thirds of protein and one-fourth of edible oil requirement of the world is met by this crop (Golbitz, 2001; Hongxia and Burton, 2002).

Assessment of genetic variation in the gene pool of a species provides the basis for tailoring desirable genotypes and for initiation of effective breeding programmes (Tiwari *et al.*, 1999). A low genetic diversity of soybean genetic base has been reported by Bharadwaj *et al.*, (2002). An investigation of genetic diversity in soybean germplasm is necessary to broaden genetic variation in future soybean breeding.

Morphological traits can be used for assessing genetic diversity but are often influenced by the environment. Seed storage proteins are unique for any given species and cultivar. The quality and composition of the proteins is a product of genes and completely independent of environmental effect (Chanyou *et al.*, 2006). Storage proteins are under the control of polymorphic multigenic families. Any variation in the composition of the seed storage protein directly reflects on numerous variations at the genetic level (Doll and Brown, 1979; Perry and McIntosh, 1991; Masood *et al.*, 2004; Yu-Xia *et al.*, 2008). This holds the key for exploiting the variations in the seed protein composition for breeding purposes. Such biochemical markers can be effectively used to

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screen a large number of germplasm in a very short time. The electrophoretic patterns of total seed proteins and seed storage protein subfractions (albumins, globulins, prolamins, glutelins) as revealed by SDS-PAGE have provided valid evidence for addressing taxonomic and evolutionary problems (Ladizinsky and Hymowitz, 1979; Kamel *et al.*, 2003; Freitas *et al.*, 2004; Ribeiro *et al.*, 2004; Fukuda *et al.*, 2005; Emre *et al.*, 2007; Yüzbaşıoğlu *et al.*, 2009; Haider *et al.*, 2010) and this technique is widely used for its simplicity and effectiveness for describing the genetic structure of crop germplasm (Murphy *et al.*, 1990; Anwar *et al.*, 2003; Javaid *et al.*, 2004; Oppong-Konadu *et al.*, 2005).

Soybean proteins have been extensively studied primarily because of their nutritional value (Wolf and Sly, 1967; Catsimpoolas *et al.*, 1968; Wolf, 1970; Hill and Briedenbach, 1974; Liu *et al.*, 2006). The sensitive of the SDS-PAGE was amply demonstrated by Liu *et al.* (2007) in separating 10 µl extracts for identifying soybean mutant variants differing in α and β subunits of the 7S protein constituent. The present study has been undertaken to separate total soluble protein through electrophoresis using SDS-PAGE in fourteen cultivars of soybean recommended for growing at different regions of India. Based on the bands obtained through SDS-PAGE a dendrogram was developed to elucidate the relation between the various cultivars.

II. Materials and Methods

Fourteen accessions of soybean (Table I) growing at different geographical locations in India were obtained from National Research Centre for Soybean (NRCS), Indore. The germplasm were maintained by cultivating them at the Botanical Garden of the Institute. After seed set the fruits were harvested allowed to dry in shade and seeds collected and stored at 4 °C until use.

Seed Evaluation

Seeds of the fourteen accessions were studied for variation in their size and weight. Hundred seed weight for the accessions was recorded for latter comparison with total soluble protein.

Table I
Soybean Accessions, their Parentage, Area of Adaptation and Duration of Growth that were used in Present Study

S. No.	*Accession No.*	*Parentage*	*Area of adaptation**	*Duration (days)*
1.	JS335	JS 78-77 x JS 75-1	CZ	95–100
2.	JS71-05	Selection from Lee type exotic material	CZ	90-95
3.	JS93-05	Secondary selection from PS 73-22	CZ	90-95
4.	MACS450	Bragg x MACS 111	CZ, SZ	90–95
5.	MAUS81 (Shakti)	KB-74 x JS 335	CZ	93-97
6.	MAUS47	MAUS 47 P 73-7 x Hark	CZ, SZ	90–95
7.	MAUS61 (Pratikar)	JS 71-1 x PK 73-94	SZ	90-100
8.	NRC12 (Ahilya-2)	Mutant of Bragg	CZ	96–99
9.	NRC37 (Ahilya-4)	JS 72-44 x Punjab 1	CZ	96–101
10.	NRC7 (Ahilya-3)	Selection from S 69-96	CZ	90–99
11.	PS1024	PK 308 x PK 317	NPZ	115
12.	PS1029	PK 262 x PK 317	SZ	90-95
13.	PS1042	Bragg x PK 416	NZ	110-119
14.	PUSA16	CNS x Lee	NPZ, NHZ, NEZ	105-115

*CZ= Central Zone; SZ= South Zone; NPZ= North Plain Zone; NHZ= North Hill Zone; NEZ= North East Zone

Source: http://www.nrcsoya.nic.in/varietiesinfo.htm

Table II
The Fourteen Varieties of Soybean fall in four Groups based on their Jaccard's Similarity Matrix. The Zones of Distribution and the Parental Lineages of these Varieties have been given for Comparison

	Variety	*Zone*	*Parentage*
Group I	Pusa16	NP, NH, NE	CNS x Lee
	NRC37	CZ	JS 72-44 x Punjab 1
	PS1029	SZ	PK 262 x PK 317
	JS9305	CZ	Secondary selection from PS 73-22
	JS7105	CZ	Selection from Lee type exotic material
Group II	NRC12	CZ	Mutant of Bragg
	MAUS61	SZ	JS 71-1 x PK 73-94
	MAUS81	CZ	KB-74 x JS 335
Group III	PS1024	NPZ	PK 308 x PK 317
	PS1042	NZ	Bragg x PK 416
	MACS450	CZ, SZ	Bragg x MACS 111
	MAUS47	CZ, SZ	MAUS 47 P 73-7 x Hark
Group IV	NRC7	CZ	Selection from S 69-96
	JS335	CZ	JS 78-77 x JS 75-1

Protein Extraction

Seeds were ground to a fine powder in mortar and pestle. The powder was then soaked in n-Hexane for 24 hrs for defattening. This defatted powder was kept at 4 °C until required for protein extraction. Total soluble proteins were extracted by incubating 100 mg seed powder overnight at room temperature with 1000 µl of extraction buffer consisting of 0.1M Tris with 5% NaCl at pH 8.0. The following day the incubated sample was centrifuged at 12000 rpm for 20 min and the supernatant collected. Protein in the supernatant was quantified using Lowry's method (Lowry *et al.*, 1951).

SDS-PAGE

Extracted soluble proteins were fractionated by one dimensional discontinuous SDS-PAGE by using 15% polyacrylamide gel according to the modified method of Laemmli (1970). Prior to loading the samples were boiled for 5 min with 2% SDS, 1% Dithiothreitol, 10% glycerol and 0.1% Bromophenol Blue. Gels were stained with 0.2% Coomassie Brilliant Blue (CBB) in methanol:acetic acid:distilled water (45:10:45 v/v) for 2 hours followed by destaining in methanol:acetic acid:distilled water (30:10:60) with occasional shaking till the protein bands were distinctly visible on a clear gel.

Data Analysis

Polymorphism was scored for the presence (1) or absence (0) of bands and entered in a binary data matrix. A dendrogram based on the genetic distance matrix was constructed by the unweighted pair group method with arithmetic averages (UPGMA) cluster analysis (Rohlf, 1998). The distance matrix and dendrogram were both constructed using the NTSYS-pc version 2.02 (Exeter Software, New York, USA).

III. Results

The results of seed weight and total protein content in all the cultivars of soybean studied are represented in Figure 1. There was wide variation in seed weights of the cultivars. Seeds of MACS450 weighed the least (97 g per100 seeds) and those of NRC12 were the heaviest (183 g per 100 seeds). Protein content in the fourteen cultivars tested varied from as high as 42 µg/µl in PS1029 to as low as 14 µg/µl in JS335.

Seed protein separated as distinct bands in all the cultivars. A total of 29 distinct bands of seed proteins could be observed in the electrophoregrams (Fig. 2&3). PS1042 was characterised by the presence of two distinct bands of relatively heavy molecular weight. All the bands were scored to study genetic diversity amongst the fourteen cultivars studied. Genetic

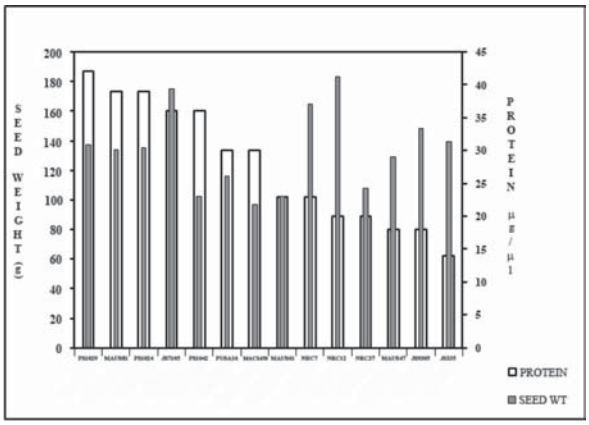

Figure 1: Seed weight vs protein in the fourteen varieties of soybean used in the present study

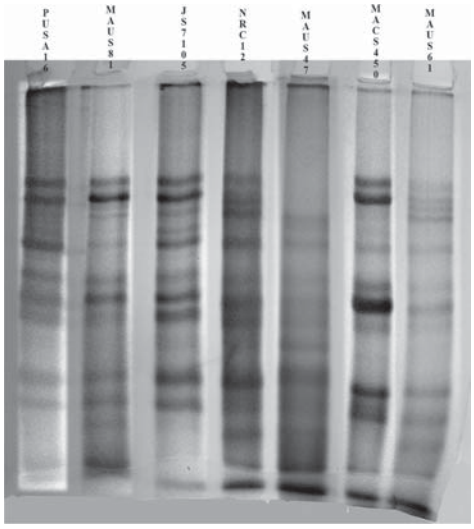

Figure 2: SDS-PAGE of seed protein of seven soybean varieties (PUSA16, MAUS81, JS71-05, NRC12, MAUS47, MACS450, MAUS61) used in the present study

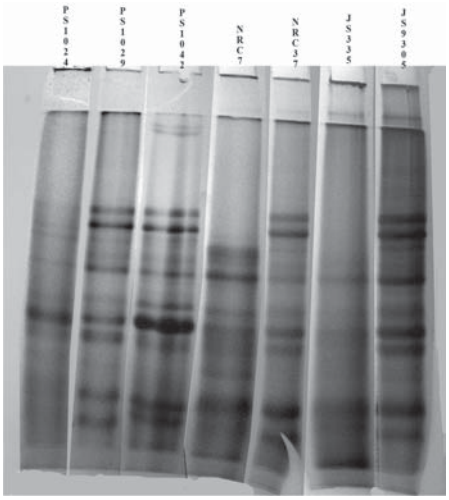

Figure 3: SDS-PAGE of seed protein of seven soybean varieties (PS1024, PS1029, PS1042, NRC7, NRC37, JS335, JS93-05) used in the present study

proximity of various accessions in the present study was assessed through Jaccard's similarity coefficient matrix obtained from the NTSYS-pc software version 2.02 (Rohlf, 1998). The similarity coefficients were used for UPGMA cluster analysis and to develop the dendrogram (Fig. 4). The varieties PUSA16 and NRC37 (0.90) exhibited least diversity while JS335 and NRC12 (0.16) were most diverse based on their protein profile. Four distinct groups, Group I, Group II, Group III and Group IV could be identified from the dendrogram. These groups are presented in Table II along with their parentage and zone of cultivation. Group I was the biggest with five accessions which had close genetic similarity. Group II had three accessions, NRC12, MAUS61and MAUS81 with similarity coefficient of 0.75 to 0.81. Group III comprising four accessions appears to be a more artificial grouping. PS1024 and PS1042 of this group are much closer (coefficient of similarity: 0.70) to each other than to either MACS450 or MAUS47, which show a similarity coefficient of 0.55 to 0.57 amongst themselves and the other two accessions of this group. The Group IV was very distinct with two accessions, NRC7 and JS335 that are very close (coefficient of similarity: 0.88) and very distant to all other accession. Incidentally, JS335 has the lowest protein content (Fig. 1). Though, total protein and the groupings have shown no

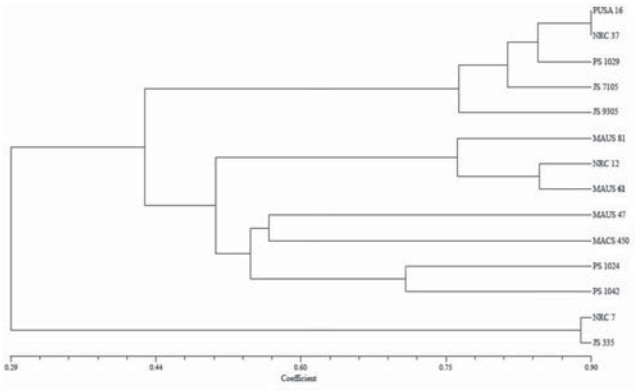

Figure 4: The UPGMA dendrogram based on the seed protein bands scored in 14 soybean varieties

correlation. In the present study no correlation between clustering groups and their pedigree could be observed. This is borne out from the clustering of apparently similar pedigree lines originating from the Bragg genotype into different

groups (NRC12 in Class II; PS1042 and MACS450 in Class III) or clustering together of very unfamiliar pedigree into the same group (MAUS61 and MAUS81). Geographical distribution also did not have any influence on the clustering pattern. As many as eight Central Zone varieties are distributed in all the four Groups.

IV. Discussion

Collectively seed storage protein profiling using SDS-PAGE has the potential to make a distinction between intrageneric species. Emre *et al.* (2007) concluded that SDS-PAGE could reveal relationship between eight species of *Lathyrus* collected from different geographical locations. Seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species (Jha and Ohri, 1996; Mennella *et al.*, 1999). However, few studies do indicate that cultivar identification was not possible with the SDS-PAGE method (Ahmad and Slinkard, 1992; De Vries, 1996). The SDS-PAGE is considered to be a practical and reliable method for species identification (Gepts, 1989). Ghafoor *et al.* (2002) demonstrated that SDS-PAGE based cluster analysis is a powerful tool for differentiating *Vigna radiata* and *Vigna mungo*. Oppong-Konodu *et al.* (2005) found genetic diversity within 60 Ghanian Cowpea (*Vigna unguiculata* L.) germplasm on the basis of seed storage proteins. SDS-PAGE has been found to be a powerful and easy tool in not only separation but also in identifying the types of seed protein and any variation within them in soybean (Liu *et al.*, 2007).

In the present study the fourteen accessions of soybean could be successfully differentiated and categorised into four distinct groups on the basis of seed protein bands obtained through SDS-PAGE. Accessions in I, II and IV groups show quite high levels of similarity. However, MACS450 and MAUS47 are the only two accessions that are almost equidistant from all other accessions. Thus members of Group III cannot be said to cluster naturally. Interestingly, the clustering pattern observed in the present study appeared to be quite independent of parental lineages in related accessions. An important aspect borne out from this study is that Group I (Pusa16, NRC37, PS1029, JS93-05 and JS71-05) and Group IV (NRC7 and JS335) are very distant from each other and can be effectively used in breeding programmes for improving protein quality.

Satyavathi *et al.* (2006) found high level of polymorphism using AFLP markers in 72 soybean collections that were put in four major clusters. Interestingly, JS335 and NRC7 have been grouped together by Satyavathi *et al.* (2006) and in the present study. This amply demonstrates that polymorphism obtained through protein profiling by SDS-PAGE and that through AFLP molecular markers do generate comparable results. The Class I created by Satyavathi *et al.* (2006) is very large with 62 accessions. Five of the accessions (MAUS47, MACS450, PUSA16, NRC37, JS71-05) used in the present study fall in this Class. Of these, PUSA16, NRC37 and JS71-05 have been grouped in Group I and MAUS47 and MACS450 have been grouped in Group III in the present study.

An interesting correlation borne out of the present study and that of Satyavathi *et al.* (2006) is that accessions grouped together have no similarity with respect to their parental lineage and those with similar parental lineage are grouped differently. This only indicates that genetic similarity or dissimilarity go much deeper than one generation of parental lineages. In this study and in the earlier reports (Kleim *et al.*, 1992; Griffin and Palmer, 1995; Brown-Guedira *et al.*, 2000; Satyavathi *et al.*, 2006) geographic distribution does not appear to have any discernable influence on variation exhibited by the soybean varieties. One reason for lack of geographical influence could be that the Indian soybean-breeding program has been utilizing the local landraces from Northern Hills, which are the traditional soybean growing regions in the country in addition to the introduced cultivars (Tiwari *et al.*, 1999).

In the present study there is appreciable variation between the accessions hence there is ample scope for genetic improvement through breeding. In conclusion, electrophoresis (SDS-PAGE) of seed storage proteins can be economically used to assess genetic variation and

relation in germplasm and also to differentiate mutants from their parent genotypes. The variation of the existing gene pool could be of enormous value to breeders for developing new cultivars. It can also help the breeders to design their hybridization program with greater probability of success.

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