Indian J. Genet., **66**(1): 79-81 (2006)

Short Communication

Protein electrophoregrams use in soybean [*Glycine max* (L.) Merrill] cultivar identification

S. C. Sharma and S. R. Maloo

Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, MPUAT, Udaipur 313 001

(Received: February 2005; Revised: November 2005; Accepted: March 2006)

Cultivar identification with respect to its genetic purity is important in national and international seed and breeding programmes. Different cultivars are most commonly identified on the basis of taxonomic differences of seed, seedling and mature plants. These parameters are highly susceptible to environmental, physiological and ecological conditions. Therefore, precise characterization of varieties should be done at both levels, *viz.* morphological as well as biochemical levels [1]. Proteins being the direct gene products reflect the genomic composition of varieties accurately to some extent and therefore, are ideal for genotypic distinctness. Biochemical approach like gel electrophoresis of proteins is a powerful tool to distinguish varieties. In the present investigation genetic diversity among soybean [*Glycine max* (L.) Merrill] varieties was determined on the basis of their electrophoregrams through Discontinuous Polyacrylamide Slab Gel Electrophoresis (DPAGE) method [2].

Healthy seeds of 14 soybean varieties of different geographical origin namely JS 335, MACS 57, Monetta, NRC12, PK 416, PK 564, Pusa 20, Pusa 24, PK 327, JS 79-81, RAUS 97-1, Pusa 22, Pusa 20 and PK 471 were procured from Soybean Breeder, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur. The experiment for identification of soybean varieties on the basis of electrophoregrams of soluble seed protein was carried out at Biochemistry and Plant Physiology Laboratory, Seed Technology Research, Rajasthan Agricultural University, Agricultural Research Station, Durgapura, Jaipur. Seeds of each variety were crushed and powdered using pestle and mortar. Seed powder (0.5 g) was vigorously mixed in ethylacetate (5 ml) for 20 minutes with the simultaneous addition of sodium sulphite (1mg) and sodium metabisulphite (1 mg) and centrifuged at 5000 RPM for 10 minutes. The ethylacetate is decanted-off and residue was treated with CMA solution (Chloroform : Methanol: Acetone, 2:1:1, v/v/v, respectively) for 20 minutes with vortexing and then solution was decanted off. The treatment was repeated one more time as above and centrifuged at 5000 RPM for 10 minutes. The final residue was kept in suitable volume (1 ml) of extraction buffer (0.1 M Tris-HCl buffer pH 7.5) for 18 hours in cold. Contents were then centrifuged at 10,000 RPM for 15 minutes. The clear supernatant was used as protein sample for electrophoretic analysis. During protein extraction, treatments with ethylacetate and CMA solution removed most of the lipids, as presence of lipids interferes during isolation of proteins [3]. To prevent auto-oxidation of phenols, sodium sulphite and sodium metabisulphite were added. The loss of proteins by above treatment was negligible. Discontinuous Polyacrylamide Slab Gel Electrophoresis (DPAGE) was carried out according to standard method [1] using 12% acrylamide separating gel (0.5 M Tris-HCl buffer pH 8.8) with a top layer of 6% acrylamide stacking gel (0.5 M Tris-HCl buffer pH 6.8). The standardized volume of protein sample (40 µl) was loded on slab gel wells. Electrophoresis was conducted at a constant current of 36 mA for 45 minutes and then raised to 58 mA until the tracking dye (bromophenol blue) migrated to the anode end of the slab gel. The staining was done for 2 hours in coomassie brilliant blue R-250 (CBBR) solution (0.5 g CBBR dye was dissolved in 250 ml methanol, 10 ml acetic acid and 240 ml water). The gels were destained by repeated washing with methanol : acetic acid : water (50:70:880, v/v/v, respectively). The protein bands were numbered from cathode end and their relative mobilities (Rm) were calculated.

Of the techniques available, analysis of seed protein using electrophoresis is widely used because of their reliability, rapidity and cost effectiveness. Gel electrophoresis of seed protein act as a function of "genotypic fingerprinting" for distinguishing plant varieties and allied aspects. This approach may offer a useful and rapidly performed adjunct to the more traditional methods of varietal identification in soybean.

Analysis of seed proteins using electrophoresis (PAGE) has been employed for identification of soybean varieties [2, 4, 5, 6]. The protein banding pattern of all 14 soybean varieties were compared and results are presented in Table 1. The inter-varietal differences were clearly expressed in these patterns. Rm values ranged from 0.021 to 0.920 and indicated different mobility pattern thereby suggesting wider range of variability in protein band expression. In all 20 protein bands of different Rm values were identified on the basis of electrophoretic mobility among 14 soybean varieties being maximum (17) in PK 416 and minimum (7) in PK 327 and JS 79-81. Similarly 15 bands in 8 soybean varieties were also reported [5]. The similarities in certain protein bands among varieties as observed from the Table 1 were noted and this might be due to genetic relationship among them. On the basis of protein electrophoregram, protein band No. 2, 3, 4, 5, 6, 8, 9, 10, 13, 14, 15 and 18 were useful for discriminating varieties. On the other hand protein band No. 16 was distinctly present in only 2 varieties, namely, RAUS 97-1 and PK 471, while band No. 10 was found in Monetta, PK 416 and Pusa 24 (Table 1). It was noticed that protein band No.l, 7, 11, 12 and 17 were observed to be universally present in all 14 varieties studied, indicated the genes controlling the expression of these protein bands appeared to behave a single block as also revealed in rice [7]. The protein band No. 19 and 20 were only present in 3 varieties, *viz.*, PK 416, PK 564 and Pusa 24 with low intensity.

All type of protein band intensity i.e. low, medium and high were observed in various soybean varieties. However, in some varieties the quantitatively similar numbers of total protein bands were noted, but differences in presence and/or absence of particular band at particular position and their Rm values, as well as different protein band intensity for common bands showed diverse nature of these varieties to each other. Table 1 revealed total 12 protein bands in electrophoregram of variety JS 335 and NRC 12, but band position was found variable. The band No.6 was characteristically present in JS 335 while missing in NRC 12. Similarly band No. 13 was present in NRC 12 and missing in JS 335. Electrophoregrams of variety MACS 57, Pusa 22 and PK 471 showed quantitatively equal 11 protein bands, but diverse banding pattern revealed clear-cut differences among them. For example, band No. 14 and 18 were present in MACS 57 and absent in Pusa 22, whereas band No. 13 present in Pusa 22 and missing in MACS 57. Similarly, presence of band No. 2, 3, 14 and 18 in MACS 57 and band No. 8, 13 and 16 in PK 471 made great variation between them. Total numbers of protein bands of PK 564 were equivalent to that of Pusa 24 but difference in the presence and/or absence of band No. 10 in Pusa 24 and 13 in PK 564 discriminated them (Table 1).

Table 1. Protein banding patterns of soybean varieties

Protein band No.	Rm value	Varieties (Presence/absence of protein bands and their intensity)													
		JS 335 (12)	MACS 57 (11)	Monetta (15)	NRC 12 (12)	PK 416 (17)	PK 564 (16)	Pusa 20 (13)	Pusa 24 (16)	PK 327 (7)	JS 79-81 (7)	RAUS 97-1 (9)	Pusa 22 (11)	Pusa 40 (8)	PK 471 (11)
1.	0.021	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+++	+++	+
2.	0.085	+	+	+	+	+	+	+	+	-	-	-	+	-	-
3.	0.122	+	+	+	+	+	+	+	+	-	-	-	+	-	-
4.	0.146	-	+	+	-	-	-	+	-	+	+	+++	+++	+	+
5.	0.175	+	+	+	+	+	+	+	+	-	-	-	+	+	+
6.	0.191	+	-	+	-	+	+	-	+	-	-	-	-	-	-
7.	0.252	+	+	+	+	+	+++	+	+++	+++	+	+	+	+	+
8.	0.284	+	-	+++	+	+	+++	+	+	-	-	-	-	-	+
9.	0.382	-	-	+	-	+	+	+	+	-	-	-	-	-	-
10.	0.452	-	-	+	-	+	-	-	+	-	-	-	-	-	-
11.	0.486	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+++	+++
12.	0.537	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	+++	+	+
13.	0.582	-	-	-	+	+	+	-	-	-	-	+	+	-	+
14.	0.617	+++	+++	+++	+++	+++	+++	+++	+++	-	-	-	-	-	-
15.	0.654	-	-	-	-	-	-	-	-	+++	+++	+++	+++	+++	+++
16.	0.696	-	-	-	-	-	-	-	-	-	-	+	-	-	+
17.	0.781	+	+	+	+	+	+	+	+	+++	+++	+++	+++	+++	+++
18.	0.816	+	+	+	+	+	+	+	+	-	-	-	-	-	-
19.	0.898	-	-	-	-	+	+	-	+	-	-	-	-	-	-
20.	0.920	-	-	-	-	+	+	-	+	-	-	-	-	-	-

+++ : High, ++ : Medium and + : Low intensity. The values in parenthesis indicate the total number of protein bands.

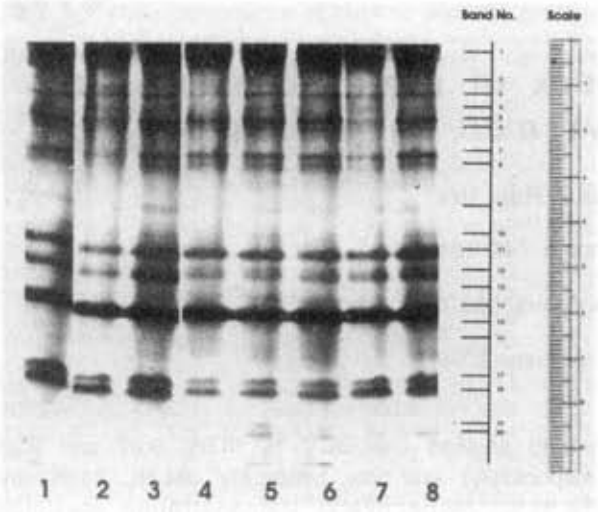

Fig. 1. Protein banding patterns of soybean varieties: 1. JS 335, 2. MACS 57, 3. Monetta, 4. NRC 12, 5. PK 416, 6. PK 564, 7. Pusa 20, 8. Pusa 24

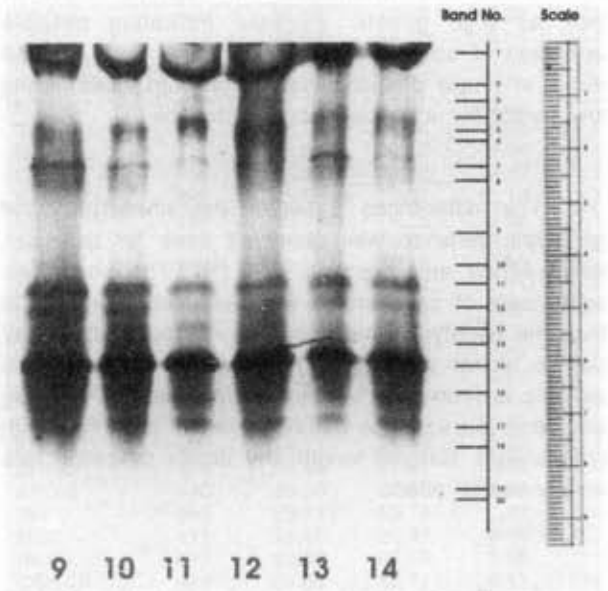

Fig. 2. Protein banding patterns of soybean varieties: 9. PK 327, 10. JS 79-81, 11. RAUS 97-1, 12. Pusa 22, 13. Pusa 40, 14. PK 471

On the other hand, soybean varieties *viz.*, PK 327 and JS 79-81 displayed a different kind of situation as they had identical banding pattern in their electrophoregrams indicating their high order of closeness in seed protein polymorphism/profile. However, differences were observed in the protein band intensity e.g., band No. 1 and 7 were more intense in PK 327, while band No. 4 was relatively less intense as compared to that of JS 79-81. Such types of close relationships and/or minor variations were also observed in grass pea [8], barley [9] and chickpea [10]. Further, if the parentage are looked into for JS79-81 (Bragg × Harosay) and PK 327 (UPSM 82 × Semmes) are showing extreme diversity and that may be reflected in their pedigrees also. Therefore, for clear discrimination between PK 327 and JS 79-81 work on Slab Gel Electrophoresis under denaturing conditions or by PCR based DNA fingerprinting is needed.

These findings could open a scope for further research in the specific area of "Varietal identification". Therefore, it is concluded that all 14 soybean varieties studied were genetically diverse and could be used in breeding programme. Hence, the DPAGE technique can be effectively used for varietal identification which is found to be quick, reliable, economical and avoiding the field work.

References

1. **McDonald M. B. Jr.** 1997. New techniques for cultivar identification of soybeans. Seed Technology, Kalyani Publishers, pp.117-130.
2. **Davis B. J.** 1964. Disc electrophoresis-II. Method of application to human serum proteins. *In*: Gel Electrophoresis. Ann. N.Y. Acad. Sci., **121**: 404-427.
3. **Stegemann H. and Pietsch G.** 1983. Methods for characterization of seed proteins in cereals and legumes. *In*: W. Gottschack and H.P. Mueller (eds.), Seed Proteins: Biochemistry, Genetics, Nutritive Value, pp. 45-75, Martinus Nijhaff, The Netherlands.
4. **Kumar H. and Ram H. H.** 1989. Identification of soybean cultivars using electrophoretic patterns of seed proteins. Crop Improv., **16**: 5-8.
5. **Goyal K. C. and Sharma S. N.** 1998. Biochemical tool (Discontinuous PAGE) for varietal identification in soybean [*Glycine max* (L.) Merrill]. Seed Res., **26**: 204-206.
6. **Anonymous.** 2003. Morphological, chemical and electrophoretic descriptors for soybean [*Glycine max* (L.) Merrill]. Technical Bulletin No. 15, National Seed Project (Crops), IARI, New Delhi.
7. **Padmavathi G., Kole C. and Siddiq E. A.** 1999. Detection of protein markers for identification of rice genotypes resistant to green leaf hopper. Indian J. Genet., **59**: 417-421.
8. **Roy M., Mandal N. and Das P. K.** 2001. Seed protein characterization and isozyme diversity for cultivar identification in grass pea (*Lathyrus sativus* L.). Indian J. Genet., **61**: 246-249.
9. **Sasek A., Bradora J. and Cerni J.** 1995. Hordein marker gene in certified Czech and Slovak varieties of barley (*Hordeum vulgare* L.). Potravinosske vedy, **13**: 57-69.
10. **Kharkwal M. C.** 1999. Seed storage proteins and interspecific relationship in *Cicer arietinum* L. Indian J. Genet., **59**: 59-64.