SDS-PAGE Seed Protein Profiles as Distinguishing Features in Certain Varieties of Field Crops

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A total of 74 released varieties of field crops (15 in wheat, 9 in maize, 19 in soybean, 6 in mung, 7 in urd, 8 in lentil and 10 in gram) at Pantnagar, Uttarakhand were characterized using SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) of seed protein extracted in Tris-HCl buffer with the objective of finding certain critical bands which could be used as distinguishable features. Protein profiles of different field crops showed the number of bands ranging from 11 (urd, lentil and gram) to 16 (maize). Wheat and soybean displayed 12 bands each and mung showed 14 bands. It was possible to distinguish UP 2565 vs C 306, PBW 343, PBW 396, PBW 262, UP 1109, UP 2338, UP 2382 and UP 2425 vs Raj 3765, HD 2687, PBW 373, PBW 502 and UP 2113 vs PBW 175 in wheat; Pant Mung 1 and Pant Mung 2 from Pant Mung 3, Pant Mung 4, Pant Mung 5 and UPM 0205 in mung; Pant U 30, Pant U 35 and UPU 0031 from Manikya, UPU 97-10, Narendra Urd-1 and Pant U 19 in urd; PL 4 and PL 406 from PL 02, PL 5, PL 018, PL 023, PL 234 and PL 639 in lentil and PG 118 from PGK 23, PG K 24, PG 033, PG 034, PG 035, PG 036, PG 037, PG 038 and Pant G 114 in gram on the basis of protein profiles. SDS-PAGE could not distinguish 19 varieties of soybean and 9 varieties of maize. These critical bands could serve as additional descriptors in DUS testing.

Key words: SDS-PAGE, Field crops

The plant cultivars usually can be distinguished morphologically; however, vegetative and floral morphology does not always provide a clear basis for varietal identification. Electrophoretic separation of seed proteins has been a widely used and valuable technique for electrophoretic characterization of a taxon, assessment of species relationship and to trace origin of cultivated plants (Ladizinsky and Hymowitz, 1979).

Seed protein profile is species specific and highly stable characteristics. Accessions among cultivated plants form different geographical areas and adapted to diverse ecological zones still possess essentially the same profile (Larsen, 1967; Johnson, 1975). Seed proteins are mainly storage proteins and are not likely to be changed in dry mature seed. Thus, mature seeds of different age still possess the same profile (Robinson and Megarrity, 1975). Intrinsic changes in the plant such as chromosomal rearrangements or even doubling of the chromosome numbers have no or very small effects on the seed protein profile (Ladizinsky and Johnson, 1972; Nakai, 1977). Thus, use of protein profiles will add to a precise description of newly bred released cultivar and that is necessary to distinguish it from other cultivars of the same kind in order to protect the rights of plant breeders and producers (Arus, 1983; Bailey, 1983; Smith and Smith, 1992).

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Numerous electrophoretic methods are available to identify cultivars by protein patterns. Of these methods, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) provides the best resolution (Smith and Simpson, 1983; Heisel *et al.*, 1986). With the enactment of Protection of Plant Varieties and Farmers' Rights Act, 2001 by Government of India, molecular characterization of released cultivars has become more relevant. Therefore, present study was undertaken to find out the varietal variation of 74 different released varieties of wheat, maize, soybean, mung, urd, lentil and gram developed at Pantnagar, Uttarakhand.

Materials and Methods

The banding pattern of sodium dodecyl sulphate extracts of seed proteins were used for SDS-PAGE following Laemmili (1970).

Extraction of Protein

0.1 g seed of different released varieties of wheat (15), maize (9), soybean (19), mung (6), urd (7) lentil (8) and gram (10) was taken in pestle and mortar adding 1 ml extraction buffer (1 M Tris-HCl-pH 8.0, 2 % SDS, 10 % glycerol, 1 mM PMSF-phenyl methyl sulfonyl fluoride and 2 % mercaptoethanol). The sample was homogenized and heated in a boiling water bath for 5 minutes at 100°C. The contents were centrifuged at 10,000 rpm for 30 minutes at 4°C and supernatant containing protein fraction was stored at -20°C till further

use. Protein samples were appropriately diluted with the sample buffer (Tris-pH 7.4, 2 % SDS, 2 % mercaptoethanol and bromophenool blue) and heated in boiling water bath for 5 minute at 65°C just before loading in the gel.

Electrophoresis

The SDS solubilized protein samples were analyzed by one dimensional, discontinuous vertical SDS-PAGE with 12.5 % separating and 4 % stacking gels using Tris-glycine electrode buffer (Tris-glycine and SDS pH 8.6). Medium range molecular weight marker (Bangalore Genei, India) protein sample was used along with the samples for determining the molecular weight of separating fractions. Electrophoresis was carried out on a vertical slab gel (Atto. Corp., Japan). The samples were electrophoresed at a constant voltage of 100 V. The run was stopped when the dye was approximately 0.5 cm from the bottom of the slab gel after about three and a half hours.

Staining and Destaining of the Gel

The gels were dipped overnight in staining solution (0.25 g Comassie Brilliant Blue R-250, 60 g TCA, 180 ml methanol and 60 ml glacial acetic acid). The staining solution was replaced the next day with destaining solutions (3 % NaCl). The gels were intermittently shaken and destaining solution was changed till the blue colour of the background of the bands disappeared. The gels were visualized on a Syngene Gel Documentation System for documentation and photography.

Results and Discussion

The protein banding patterns of different released varieties of wheat, maize, soybean, mung, urd, lentil and gram are given in Table 1.

Wheat (*Triticum aestivum*)

The protein banding pattern were divided into few distinct zones like A, B and C by bands intensity/width/ distinctness and within zone, the individual bands were numbered. There were 3 distinct zones i.e. A, B and C with a total of 12 bands.

Zone A consisted of 4 thin bands (A_1, A_2, A_3 and A_4) however, band A_1 was absent in UP 2565 and A_2 was absent in Raj 3765, HD 2687, PBW 373, PBW 502, UP 2113 and UP 2565. The zone B consisted of five dark bands (B_1, B_2, B_3, B_4 and B_5) in all the varieties except PBW 175 which lacked band B_2. The C zone had three bands C_1, C_2 and C_3 in all the released varieties of wheat (Table 1). Thus, UP 2565 was distinguishable from the other 14 varieties. There were four distinguishable groups i.e. UP 2565 in first group, C 306, PBW 343, PBW 396, PBW 262, UP 1109, UP 2338, UP 2382 and UP 2425 in the second group, Raj 3765, HD 2687, PBW 373, PBW 502 and UP 2113 in third group and PBW 175 in the fourth group.

Maize (Zea mays)

The protein banding pattern of maize showed a total of 16 bands divided into 4 distinct zones i.e. A, B, C and D. All the varieties remained indistinguishable.

Soybean (Glycine max)

Nineteen varieties showed similar 12 electrophoretic bands distributed into 4 zones.

Mung (Vigna radiata)

Seed protein profiles were characterized by 14 bands spread over 4 zones. The 6 varieties of mung were classified into 2 distinct electrophoretic groups where Pant Mung 1 and Pant Mung 2 had 3 faint bands in A zone which were missing in the remaining 4 varieties. Further, a prominent band B_3 was present in Pant Mung 1 and Pant Mung 2 and altogether lacking in the other 4 varieties. Thus, Pant Mung 1 and Pant Mung 2 were distinguishable from the other four varieties.

Urd (Vigna mungo)

A total of 11 bands were present in the released varieties of urd. The distinguishable zone was A. There was no difference in zone B and C. A_1, A_2 and A_3 bands were almost absent in Pant U 30 and Pant U 35. A_2 and A_3 bands were absent in UPU 0031.

Lentil (Lens culinaris)

Eight varieties of lentil (Table 1) were electrophoresed. All the varieties of lentil were alike in protein banding pattern except PL 4 and PL 406, in which C_2, C_3 and C_3 bands respectively were absent. The zone D consisted of only one dark band in all the released varieties of lentil. Thus, on the basis of seed protein profile, PL 4 and PL 406 were distinguishable from the other varieties. Incidentally, PL 4 and PL 406 both were small seeded and the rest mostly bold seeded. Thus, the difference between small seeded and bold seeded lentil cultivars seems to be linked with certain qualitative genes having direct bearing on seed size.

Gram (Cicer arietinum)

All the 10 varieties of gram showed similar seed protein

Table 1. Seed protein profiles as resolved through SDS-PAGE in released varieties of field crops

Wheat

Varieties	A_1	A_2	A_3	A_4	B_1	B_2	B_3	B_4	B_5	C_1	C_2	C_3
C 306	+	+	+	+	+	+	+	+	+	+	+	+
Raj 3765	+	–	+	+	+	+	+	+	+	+	+	+
HD 2687	+	–	+	+	+	+	+	+	+	+	+	+
PBW 175	+	+	+	+	+	–	+	+	+	+	+	+
PBW 343	+	+	+	+	+	+	+	+	+	+	+	+
PBW 373	+	–	+	+	+	+	+	+	+	+	+	+
PBW 396	+	+	+	+	+	+	+	+	+	+	+	+
PBW 502	+	–	+	+	+	+	+	+	+	+	+	+
PBW 262	+	+	+	+	+	+	+	+	+	+	+	+
UP 1109	+	+	+	+	+	+	+	+	+	+	+	+
UP 2113	+	–	+	+	+	+	+	+	+	+	+	+
UP 2338	+	+	+	+	+	+	+	+	+	+	+	+
UP 2382	+	+	+	+	+	+	+	+	+	+	+	+
UP 2425	+	+	+	+	+	+	+	+	+	+	+	+
UP 2565	–	–	+	+	+	+	+	+	+	+	+	+

Maize

Varieties	A_1	A_2	A_3	A_4	B_1	B_2	B_3	B_4	B_5	B_6	C_1	C_2	C_3	D_1	D_2	D_3
Surya	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Kanchan	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gaurav	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Shweta	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Naveen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D 765	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pragati	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tarun	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Soybean

Varieties	A_1	B_1	B_2	B_3	B_4	B_5	B_6	C_1	C_2	D_1	D_2	D_3
Shilajeet	+	+	+	+	+	+	+	+	+	+	+	+
PK 472	+	+	+	+	+	+	+	+	+	+	+	+
PS 1347	+	+	+	+	+	+	+	+	+	+	+	+
Bragg	+	+	+	+	+	+	+	+	+	+	+	+
PK 327	+	+	+	+	+	+	+	+	+	+	+	+
PS 1241	+	+	+	+	+	+	+	+	+	+	+	+
Pb 1	+	+	+	+	+	+	+	+	+	+	+	+
PK 1225	+	+	+	+	+	+	+	+	+	+	+	+
PS 1092	+	+	+	+	+	+	+	+	+	+	+	+
PK 416	+	+	+	+	+	+	+	+	+	+	+	+
PK 564	+	+	+	+	+	+	+	+	+	+	+	+
Ankur	+	+	+	+	+	+	+	+	+	+	+	+
PS 1042	+	+	+	+	+	+	+	+	+	+	+	+
VLS 47	+	+	+	+	+	+	+	+	+	+	+	+
PK 262	+	+	+	+	+	+	+	+	+	+	+	+
PK1029	+	+	+	+	+	+	+	+	+	+	+	+
PKB 75	+	+	+	+	+	+	+	+	+	+	+	+
JS 335	+	+	+	+	+	+	+	+	+	+	+	+
PS 1024	+	+	+	+	+	+	+	+	+	+	+	+

Mung

Varieties	A_1	A_2	A_3	B_1	B_2	B_3	B_4	B_5	C_1	C_2	C_3	D_1	D_2	D_3
Pant Mung 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pant Mung 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pant Mung 3	–	–	–	+	+	–	+	+	+	+	+	+	+	+
Pant Mung 4	–	–	–	+	+	–	+	+	+	+	+	+	+	+
Pant Mung 5	–	–	–	+	+	–	+	+	+	–	+	+	+	+
UPM 02–05	–	–	–	+	+	–	+	+	+	–	+	+	+	+

Urd

Varieties	A_1	A_2	A_3	B_1	B_2	B_3	B_4	C_1	C_2	C_3	C_4
Pant U 30	–	–	–	+	+	+	+	+	+	+	+
Manikya	+	+	+	+	+	+	+	+	+	+	+
Pant U 35	–	–	–	+	+	+	+	+	+	+	+
UPU 97 10	+	+	+	+	+	+	+	+	+	+	+
Narendra Urd 1	+	+	+	+	+	+	+	+	+	+	+
UPU 0031	+	–	–	+	+	+	+	+	+	+	+
Pant U 19	+	+	+	+	+	+	+	+	+	+	+

Lentil

Varieties	A_1	A_2	B_1	B_2	B_3	B_4	B_5	C_1	C_2	C_3	D_1
PL 02	+	+	+	+	+	+	+	+	+	+	+
PL 4	+	+	+	+	+	+	+	+	–	–	+
PL 5	+	+	+	+	+	+	+	+	+	+	+
PL 018	+	+	+	+	+	+	+	+	+	+	+
PL 023	+	+	+	+	+	+	+	+	+	+	+
PL 234	+	+	+	+	+	+	+	+	+	+	+
PL 406	+	+	+	+	+	+	+	+	+	–	+
PL 639	+	+	+	+	+	+	+	+	+	+	+

Gram

Varieties	A_1	B_1	B_2	B_3	B_4	B_5	C_1	C_2	C_3	D_1	D_2
PGK 23	+	+	+	+	+	+	+	+	+	+	+
PGK 24	+	+	+	+	+	+	+	+	+	+	+
PG 033	+	+	+	+	+	+	+	+	+	+	+
PG 034	+	+	+	+	+	+	+	+	+	+	+
PG 035	+	+	+	+	+	+	+	+	+	+	+
PG 036	+	+	+	+	+	+	+	+	+	+	+
PG 037	+	+	+	+	+	+	+	+	+	+	+
PG 038	+	+	+	+	+	+	+	+	+	+	+
Pant G 114	+	+	+	+	+	+	+	+	+	+	+
PG 118	–	+	+	+	+	–	+	+	+	+	–

[* | In-line.WMF *]

profiles except PG 118 in which bands A_1, B_5 and D_2 were missing.

Thus, on the basis of above results, seed protein profiles as resolved through SDS-PAGE were successful in distinguishing certain varieties for example UP 2565 vs C 306, PBW 343, PBW 396, PBW 262, UP 1109, UP 2338, UP 2382 and UP 2425 vs Raj 3765, HD 2687, PBW 373, PBW 502 and UP 2113 vs. PBW 175 in wheat; Pant Mung 1 and Pant Mung 2 from Pant Mung 3, Pant Mung 4, Pant Mung 5 and UPM 0205 in mung; Pant U 30, Pant U 35 and UPU 0031 from Manikya, UPU 97-10, Narendra Urd 1 and Pant U 19 in urd; PL 4 and PL 406 from PL 02, PL 5, PL 018, PL 023, PL 234 and PL 639 in lentil and

PG 118 from PGK 23, PGK 24, PG 033, PG 034, PG 035, PG 036, PG 037, PG 038 and Pant G 114 in gram. No differences could be detected in maize and soybean. Panigrahi *et al.* (2001) and Subodh *et al.* (2001) in pigeon pea, Mallick and Sawhney (2002) in lentil and Yadav and Singh (2004) in wheat have reported varietal differences on the basis of seed protein profiles in India.

The advantages of protein data in resolving varietal differences have been reviewed by Smith and Smith (1992). The protein and DNA data are in popular use because the variation for these markers is ubiquitous and this variation is desirable in genetic terms. These characters are in routine usage and are widely accepted as source of reliable data in evolution, taxonomy and genetics. There is abundant evidence to show that protein profiles can be obtained for all crop species of major importance and that these profiles are independent of environmental and storage conditions. They are reflective of genotype. In some cases quantitative variation can occur due to environmental effects, but this contribution to variation is small and can be taken into account when considering intervarietal comparisons (Smith and Smith, 1992). Therefore, the intensity of the band has not been taken into account to evaluate their degree of significance in discriminating between qualitatively similar protein profiles in the present investigation. Protein profiles of cultivated varieties, therefore satisfy one pre-requisite of providing a fingerprint in the sense that they are specific descriptors of the genotype and the information generated in this paper adds to the list of descriptors of released crop cultivars required for facilitating the implementation of the Protection of Plant Varieties and Farmer' Right acts 2001. The fact that varieties of maize and soybean were indistinguishable from each other suggests that other electrophoretic conditions should be investigated in order to differentiate between these cultivars. In conclusion, SDS-PAGE proved to be a promising method to discriminate between certain varieties of wheat, mung, urd, lentil and gram and warrants further investigation to identify other electrophoretic conditions for identifying some critical bands in distinguishing maize and soybean varieties developed at Pantnagar.

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