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Tuber Protein Electrophoregram as a Tool of Genetic Purity Test in Potato (*Solanum tuberosum* L.)*

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ABSTRACT In this investigation 38 potato (*Solanum tuberosum* L.) genotypes were used for morphological characterization and tuber protein electrophoresis. Results indicated that all 38 genotypes could be categorized into 14 distinct groups consisting of morphologically almost identical genotypes. The morphological ambiguity was distinguished through electrophoregrams emphasizing presence or absence and intensity of bands. For example, the electrophoregram of morphologically similar genotypes Kufri Lalima and JP-100 were quite distinct in regard to B3, B4, B6 & B7 that were additional in JP-100. Similarly, other genotypes with common morphology could be differentiated. The protein bands in molecular weight range of 68 kD-40 kD exhibited maximum variability across the genotypes. The tuber protein profiles of all randomly sampled tubers in lot of each genotype were identical. Thus, the SDS-PAGE of tuber protein appeared as a good tool for genetic analyses, breeding, establishing relationships, classification and testing of genetic purity in potato.

Keywords: Protein, Electrophoresis, Potato, variability

Uniformity and trueness to type are important aspects for genetic purity assessment and certification of crop varieties. Certification of seed and/or planting materials includes field inspections at different stages of crop at which maximum distinguishing morphological characters appear. But morphology may not reveal real genetic differences, as the former is an interaction of genotype and environment. In potato, there is lot of morphological ambiguity within *Tuberosum* released and advance breeding lines. Thus, characterization of gene pool based on only morphological descriptors is inappropriate. Therefore, descriptors based on highly reproducible entities like proteins including isozymes [1-12] and DNA markers [13, 14J are reliable tools for characterization, identification, verification of seed purity, establishing relationships and taxonomic positioning. Potato tubers are considered as good source of soluble proteins used in identification and verification of cultivars [15] but have inconsistent composition with developmental stages. Besides, micro tubers raised *in vitro* [16], field grown tubers can effectively be used in variability studies through protein electrophoresis [17-20]. Many of earlier and recent workers [16, 20] have proved that the protein subunits in physiologically mature tubers are consistent and suitable for genetic variability and genetic purity test. Therefore, present investigation was undertaken for assessing genetic purity of varieties and planting materials electrophoretically taking fully mature field grown tubers of potato.

MATERIALS AND METHODS

Thirty-eight potato genotypes representing released varieties and advance generation materials were grown during *rabi* 2000-2001 for morphological and electrophoretic characterization through tuber proteins (Table 1). For protein extraction and electrophoresis, at least two randomly sampled mature field grown tubers of 25-30 g weight (at 80 days after planting) in each genotype were collected. In many genotypes viz., JP-100, Kufri Sutlej, 85-P-718, JX-115, JX-123, and JX-249 three tubers and in Kufri Chipsona-1 four tubers were sampled.

A sample of 0.5 g of tuber from each line was crushed with 500 µl of sample buffer [21] without dye, supplemented with 50 µl of 10 mM

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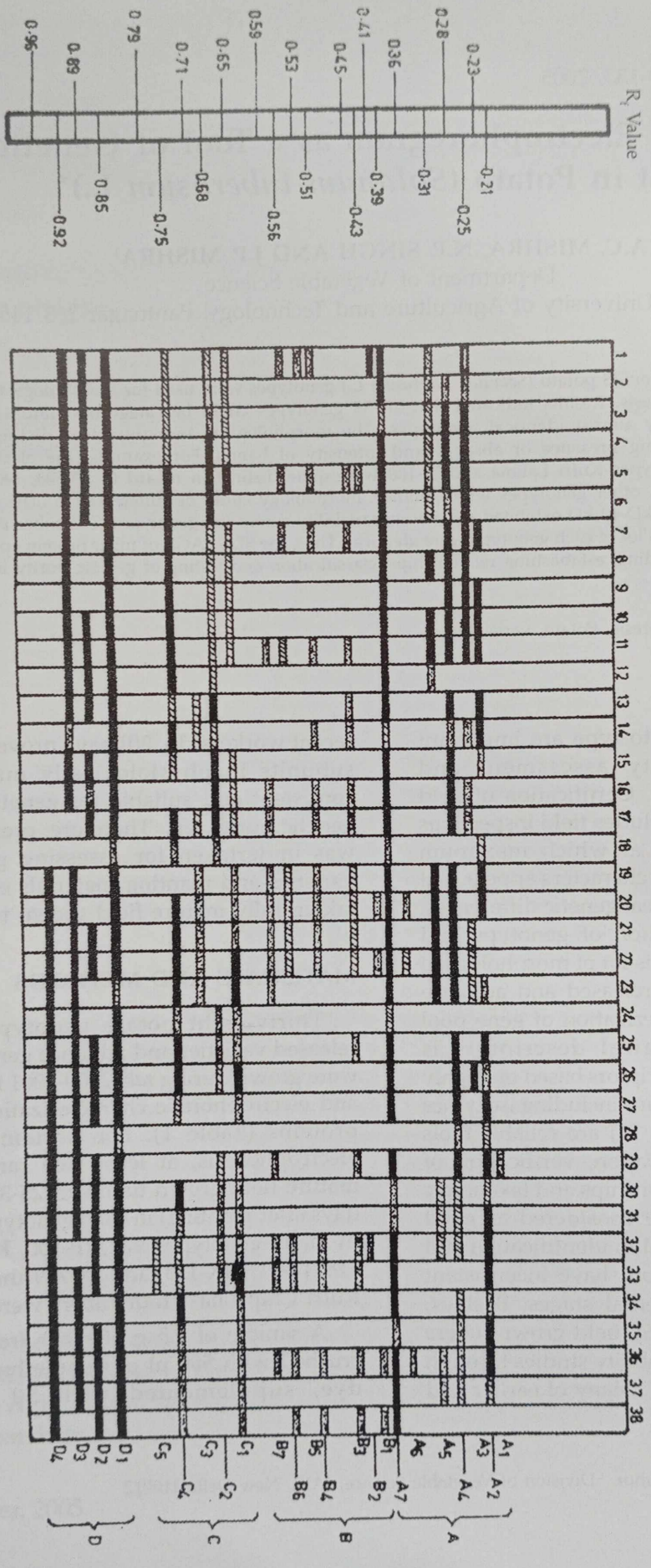

Fig. 1 Diagramatic SDS—PAGE electrophoregram of 38 potato (*Solanum tuberosum* L.) genotypes

Table 1. List of potato genotypes serially shown in diagrammatic sketch of tuber protein profile

Genotype		Parentage
1.	Kufri Pukhraj	Craig's Defiance X JEX/B-687
2.	Kufri Badshah	Kufri Jyoti X Kufri Alankar
3.	Kufri Ashoka	EM/C-1020 X Allerfriitheste Gelbe
4.	Kufri Anand	PJ-376 X PH/F-1430
5.	Kufri Jawahar	Kufri Neelamani X Kufri Jyoti
6.	Kufri Bahar	Kufri Red X Gineke
7.	Kufri Chipsona-1	MEX-750826 X MS/78-79
8.	Kufri Chipsona-2	F-6 X QB/B 92-4
9.	Kufri Jyoti	3069d(4) X 2814a(1)
10.	Kufri Lalima	Kufri Red X AG 149 (Wis X 37)
11.	JP-100	Kufri Alankar X CP-1406
12.	Kufri Sutlej	Kufri Bahar X Kufri Alankar
13.	JW-23	-
14.	JTH/C-107	Selection from collected material
15.	AB-667	Kufri Jyoti X SS-1603
16.	JV-67	JF-4700 X JL-5857
17.	JW-96	Kufri Jyoti X CP-1362
18.	85-P-127	-
19.	MF-1	Local selection
20.	JX-1	Kufri Jyoti X CP-1481
21.	85-P-11	-
22.	85-P-670	Kufri Bahar X PS-4904
23.	85-P-718	Kufri Bahar X PS-4904
24.	JX-23	Kufri Jyoti X CP-1481
25.	JX-371	JE 812 X Kufri Jyoti
26.	JX-576	JE 812 X Kufri Jyoti
27.	JX-235	JE 812 X Kufri Jyoti
28.	JX-216	JE 812 X Kufri Jyoti
29.	JX-115	CP 1346 X MS/78-62
30.	TPS I/7	-
31.	TPS C-3	JTH/C-107 X EX/A-680-16
32.	JX-44	CP1546 X SLB/X-23
33.	JX-123	JE812 X Kufri Jyoti
34.	JX-249	JE-812 X Kufri Jyoti
35.	TPS I/13	MF-1 X TPS-13
36.	MS/92-3128	MS/82-638 X MS/80-758
37.	MS/92-1090	Kufri Jyoti X PH/F-1545
38.	MS/91-1325	Ma/83-27 X CP-1673

phenylmethyl sulphony fluoride (dissolved in 50% ethanol) at 4°C. The supernatant was separated by centrifugation at 10,000 rpm for 10 minute at 4°C. The supernatant was kept for 1 hour at room temperature for stagnation and used directly for sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The gels were prepared according to method described by Laemmli [19] and Rajapakse *et al.* [16]. Protein samples of 25 µl from two randomly selected tubers in lot of each genotype were loaded in consecutive wells along with molecular weight marker protein consisting of phosphorylase b (94 kD), bovine serum albumin (67 kD), egg albumin (43 kD), carbonic anhydrase (30 kD) and ribonuclease a (14.0 kD) subunits. Total protein concentration was estimated for each line by the method of Bradford [22] using bovine serum albumin (E-Merk (India) Ltd.) as standard. The quantity of protein was adjusted to 10 mg for one lane to obtain a clear separation for coomassie blue staining. Electrophoresis was done at a constant current of 8 mA per plate for 6-7 h using electrode buffer (0.025 M Tris, 0.192 M glycine and 1 g l^{-1} SDS, with pH 8.4). Gels were stained with 0.2% Commassie Brilliant Blue R solution (dissolved in ethanol: acetic acid: water = 45: 10: 45) at room temperature for 2 h. The first destaining was done using methanol: acetic acid: water (25: 10: 65) and the second with 7 (v/v) acetic acid, both at room temperature. Individual band was positioned in each gel through relative front mobility value (R_f value = distance moved by particular protein band/total run in a gel). The intensity of colour of the bands was measured in terms of optical density (OD) at 660 nm by spectrophotometer.

RESULTS AND DISCUSSION

All 38 genotypes could be categorized into 14 groups on the basis of contrasting characters of plant growth, shoot, leaf and tuber (Table 2). The genotypes Kufri Lalima and JP-100 included in group VII had erect growth, compact plant architecture, solid and purple red stem, dark green foliage, red and round tubers with deep eyes. Similarly, Kufri Badshah, Kufri Pukhraj, Kufri Chipsona-1, Kufri jyoti, JX-44 and Kufri Sutlej were included in group XII due to common characters viz., erect growth, compact architecture, solid stem and white oval tubers with fleet eyes. In this way, genetically related or unrelated genotypes fell in common clusters of identical morphology. It might be because of confluence of similar genes for morphological features within *Tuberosum* potatoes. Therefore, sole morphology is not efficient to

Table 2. Grouping of potato (*Solanum tuberosum* L.) genotypes based on morphological features

Group	Growth habit	Plant architecture	Stem solidity	Tuber shape	Tuber colour	Eye depression	Genotypes
I	Erect/ Semi erect	Compact/ Semi compact	Solid/ Semi solid	Oval-long	White	Fleet	Kufri Ashoka, JW-23, 85-P-11, JX-235, Kufri Anand
II	Erect/ Semi erect	Compact/ Semi compact	Solid/ Semi solid	Oval-long	White	Deep	JX-115, MS/92-3128
III	Prostrate	Semi compact	Hollow	Oval-long	White	Fleet	JX-123, JX-249
IV	Erect/ Semi erect	Compact/ Semi compact	Solid/ Semi solid	Oval-round	White	Fleet	Kufri Jawahar, Kufri Chipsona-2, MF-1, JX-371, JX-576, JX-216, TPS I/7, TPS C-3
V	Prostrate	Semi compact	Hollow	Oval-round	White	Fleet	AB-667, JV-67
VI	Prostrate	Open	Hollow	Oval-round	White	Deep	85-P-127
VII	Erect	Compact/ Semi compact	Solid	Round	Red	Deep	Kufri Lalima, JP-100
VIII		Open	Solid	Round	White	Deep	TPS I/13, JTH/C-107, 85-P-670, MS-91-1325
IX	Erect/ Semi erect	Compact/ Semi compact	Solid/ Semi solid	Round	White	Fleet	JW-96, 85-P-718
X	Prostrate	Open	Hollow	Round	White	Fleet	JX-23
XI	Erect/ Semi erect	Compact/ Semi compact	Solid/ Semi solid	Oval	White	Fleet	Kufri Pukhraj, Kufri Badshah, Kufri Chipsona-1, Kufri Jyoti, Kufri Sutlej, JX-44
XII	Erect	Compact	Hollow	Oval	White	Fleet	JX-1
XIII	Erect	Open	Semi solid	Oval-round	White	Medium deep	MS/92-1090
XIV	Erect	Compact	Solid	Oval-round	White	Medium deep	Kufri Bahar

characterize the genetic purity of a vast gene pool [23].

The variability among the genotypes with identical morphology could be well explored with the electrophoretic patterns of their tuber proteins (Fig. 1 & 2). Protein profile of genotypes indicated the presence of 5 (JX-216) to 23 (85-P-670) scorable bands of different molecular weight (94 kD to <14 kD) and relative mobility (Rm= 0.21 to 0.96). The whole profile could be stratified in four zones viz., A, B, C & D. Protein bands in zone A were of highest molecular weight as they migrated least distance whereas those included in zone D were of lowest molecular weight. Presence or absence, thickness and intensity of these bands constituted the overall variability in genotypes. Maximum differences in thickness and presence or absence of subunits appeared between 40-68 kD molecular weight across the genotypes (zone A & B). The distinctness in tuber polypeptides in above said molecular weight range have also been explored by Rajapakse *et al.* [16] and Barta *et al.* [20].

The Electrophoregram of morphologically similar genotypes Kufri Lalima and JP-100 were quite distinct in regard to B3, B4, B6 & B7 that were additional in JP-100. Similarly, other genotypes with common morphology could be differentiated based on presence or absence and intensity of bands. The eleven released Indian potato cultivars viz., Kufri Pukhraj, Kufri Badshah, Kufri Ashoka, Kufri Anand, Kufri Jyoti, Kufri Sutlej, Kufri Chipsona-1, Kufri Chipsona-2, Kufri Bahar, Kufri Jawahar and Kufri Lalima exhibited quite distinct protein profiles. Such type polypeptide domain specificity of the genotypes can effectively be utilized in DUS (Distinctness, Uniformity & Stability) Test guidelines for Plant

Variety Protection in potato [24]. The randomly sampled tubers in each genotype exhibited similar protein subunit patterns in respect to expression and degree of expression. Such type of reproducibility of polypeptides may efficiently be used in genetic analyses, breeding, establishing relationships, classification, identification, DUS Testing for Plant Variety Protection, Genetic purity and varietal trueness in potato [4, 5, 25, 26].

Above studies indicated that tuber protein profiling through SDS-PAGE has tremendous use in studies related to genetics and breeding, varietal characterization, seed technology and taxonomy in potato.

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