Genetic Variation Among Potato Genotypes for Tuber Protein Subunits

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Tuber protein subunit based genetic variation was analysed among 38 potato genotypes using similarity index value. Results indicated that Kinship did not correspond to similarity in banding patterns of the cultivars. Cultivar combinations *viz.*, Kufri Pukhraj and JX-216, JW-96 and JX-216, MS/91-1325 and JX-216, 85-P-127 and 85-P-670, 85-P-127 and 85-P-11 etc. with low SI values were considered to be divergent parents and recommended for hybrid breeding progarammes.

Plant morphology has been an important criterion for identification, classification and documentation of species and cultivars (Pauksens, 1975). It plays an important role in study of genetics and breeding behaviour of plants. But, since morphology reflects interaction of genotype with its environment, it is inappropriate to compare morphological data for cultivars that have been collected across different years and/or locations. Therefore, descriptors based on proteins (including isozymes) and deoxyribonucleic acid (DNA) are more reliable (Tanksley and Orton, 1983; Ryskov *et al.*, 1988). Isozyme and protein profiles by electrophoresis have been used to characterize potato cultivars (Desborough and Peloquin, 1968; Stegemann and Loeschcke, 1976; Oliver and Martinez-Zapater, 1985) and to investigate species relationships (Hosaka and Matsubayashi, 1983; Oliver and Martinez-Zapater, 1984). Due to the diversity of tuber proteins within the Group *Tuberosum,* protein analysis by electrophoresis is a convenient and effective method of studying the genetic variation. Tubers are suitable for separating proteins, since they represent a stable source of proteins and provide a uniform material for protein extraction (Rajapakse *et al.*, 1991). According to Stegemann *et al.* (1973), mature potato tubers can be used for variety identification by the Tris (hydroxymethyl) aminomethane (Tris) and borate buffer system of polyacrylamide gel electrophoresis. *Tuberosum* is known to have a narrow genetic base (Plaisted, 1972) and a little improvement is observed in tuber yield and its components (Howard, 1963; Mendoza and Haynes, 1974). In present study, an attempt has been made to establish the relationship among *Tuberosum* genotypes and identify the divergent parents through tuber protein profiles.

Materials and Methods

Thirty-eight potato genotypes (Table 1) representing advance generation hybrids and released Indian cultivars were evaluated in a randomized block design with three replications.

About 0.5 g of tuber (80 DAP, 25-30 g weight) from each line was crushed in 500 µl of sample buffer (Laemmli, 1970) without dye, supplemented with 0.1 volumes (50 µl) of 10 mM phenylmethyl sulphonyl fluoride (dissolved in 50% ethanol) at 4°C. The supernatant was separated by centrifugation at 10,000 rpm for 10 min at 4°C. The supernatant was kept for 1 h at room temperature and used directly for sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

The gels were prepared according to the method of Laemmli (1970) and Rajapakse *et al.*, (1991). Protein samples of 25 µl in each genotype was loaded in duplicate 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 (1976) 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 containing, 0.025 M Tris, 0.192 M glycine and 1 g l^{-1} SDS, with pH 8.4. Gels were stained with 0.2% coomassie 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.

The level of similarity among genotypes was determined in terms of similarity index (SI = number of matching bands between two genotypes/total number of bands x 100) by positioning the individual bands in each gel through relative front mobility value

Table 1. List of potato genotypes and their parentage

Genotypes		Parentage
1.	Kufri Pukhraj	Craigs 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	3069 d(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 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 1/7	–
31.	TPS C-3	JTH/C-107 x EX/A-680-16
32.	JX-44	CP 1546 x SLB/X-23
33.	JX-123	JE812 x Kufri Jyoti
34.	JX-249	JE-812 x Kufri Jyoti
35.	TPS 1/13	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

(Rf value = distance moved by particular protein band/ total run in a gel). Based on SI values, a dendrogram was constructed using UPGMA (unweighted pair group method using arithmetic average) cluster analysis.

Results and Discussion

Similarity index (SI) indicates the degree of closeness among genotypes (Huang *et al.*, 1997; Shamina *et al.*, 1998). SI values ranged from 22.22% (Kufri Pukhraj and JX-216) to 100% (Kufri Badshah and Kufri Anand, Kufri Badshah and Kufri Ashoka, Kufri Anand and Kufri Ashoka, JX-235 and TPS 1/13 and TPS 1/7 and TPS C-3) (Table 2 & Fig. 2). High SI values indicated close relationship between genotype pairs whereas low values indicated high divergence. Closeness may be due to common parentage or confluence of similar genes from different parents in development of varieties (Bassiri and Adams, 1978; Hosaka *et al.*, 1994). In this investigation, genotype combinations involving common parents did not have identical banding patterns and showed varying degree of SI values. For example, genotypes 85-P-670 and 85-P-718 (Kufri Bahar x PS-4904), JX-1 and JX-23 (Kufri Jyoti x CP – 1481) and JX-123, JX-371, JX-576, JX-216, JX-249, and JX-235 (JE-812 x Kufri Jyoti) exhibited SI values in a range of 31.58% to 94.12%. Similarly, Kufri Badshah, Kufri Jawahar and AB-667, Kufri Lalima and Kufri Bahar, Kufri Sutlej and Kufri Badshah having one common parent exhibited SI values in the range of 69.57% to 90.0%. It was also observed that some genotype combinations like Kufri Badshah and Kufri Ashoka, Kufri Badshah and Kufri Anand,

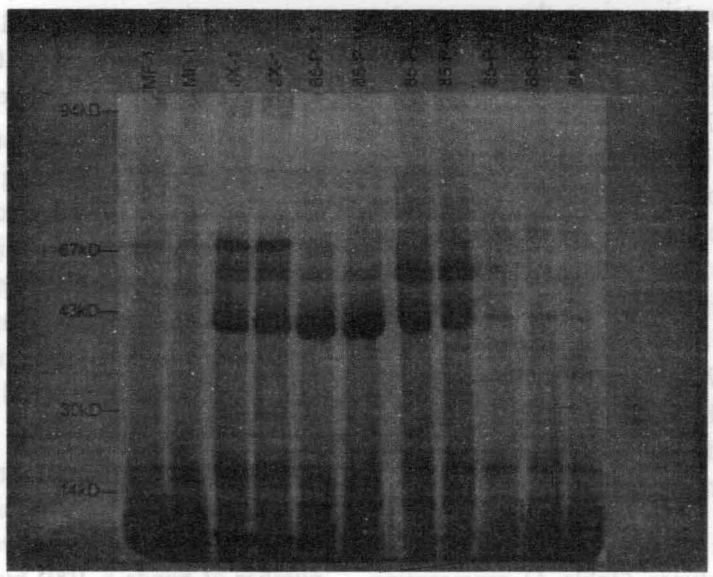

Fig. 1. SDS-PAGE patterns of tuber protein in potato genotypes

Table 2. Similarity index (SI) among potato (*Solamum tuberosum* L.) genotypes

Genotypes*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1.	-	78.26	78.26	78.26	81.48	72.72	72.0	72.72	63.64	75.00	78.58	70.00	52.18	58.34	63.64	72.00	64.00	44.44	53.84
2.		-	100.00	100.00	83.33	94.74	72.73	94.74	84.21	95.24	80.00	82.35	70.00	66.67	84.21	81.82	72.73	53.33	52.17
3.			-	100.00	83.33	94.74	72.73	94.74	84.21	95.24	80.00	82.35	70.00	66.67	84.21	81.82	72.73	53.33	52.17
4.				-	83.33	94.74	72.73	94.74	84.21	95.24	80.00	82.35	70.00	66.67	84.21	81.82	72.73	53.33	52.17
5.					-	78.26	84.62	78.26	69.57	80.00	89.66	66.67	66.67	72.00	69.57	84.62	76.92	42.11	66.67
6.						-	66.67	88.89	77.78	90.00	75.00	87.50	89.47	70.00	77.78	76.19	66.67	57.14	71.43
7.							-	76.19	85.71	78.26	88.89	52.63	72.73	86.96	76.19	83.33	75.00	58.82	72.00
8.								-	88.89	90.00	75.00	75.00	63.16	70.00	77.78	76.19	66.67	57.14	54.55
9.									-	90.00	75.00	62.50	73.68	80.00	88.89	76.19	66.67	71.43	54.55
10.										-	84.62	77.78	76.19	72.73	90.00	78.26	69.57	62.50	58.33
11.											-	63.64	72.00	76.92	72.00	88.89	81.48	50.00	78.57
12.												-	70.59	55.56	75.00	63.16	52.53	66.67	60.87
13.													-	85.71	84.21	72.73	73.73	66.67	60.87
14.														-	70.00	78.26	78.26	62.50	66.67
15.															-	76.19	66.67	71.43	54.55
16.																-	91.67	47.06	64.00
17.																	-	47.06	64.00
18.																		-	33.33
19.																			-
20.																			
21.																			
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*See Table 1

Table2 (contd. row-wise)

Geno-type*	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1.	62.08	64.28	73.34	56.00	60.00	58.34	66.66	54.54	22.22	48.00	66.66	66.66	62.08	66.66	47.62	54.54	68.96	60.88	69.57
2.	53.85	72.00	66.67	72.73	70.59	76.19	85.71	73.68	40.00	63.64	85.71	85.71	61.54	58.33	66.67	73.68	61.54	80.00	50.00
3.	53.85	72.00	66.67	72.73	70.59	76.19	85.71	73.68	40.00	63.64	85.71	85.71	61.54	58.33	66.67	73.68	61.54	80.00	50.00
4.	53.85	72.00	66.67	72.73	70.59	76.19	85.71	73.68	40.00	63.64	85.71	85.71	61.54	58.33	66.67	73.68	61.54	80.00	50.00
5.	66.67	82.76	77.42	61.54	57.14	72.00	72.00	60.87	31.58	61.54	72.00	72.00	80.00	71.43	54.55	60.87	73.33	66.67	66.67
6.	48.00	66.67	61.54	66.67	62.50	70.00	80.00	66.67	42.86	66.67	80.00	80.00	56.00	52.17	58.82	66.67	56.00	73.68	42.11
7.	71.43	74.07	68.97	66.67	63.16	78.26	78.26	66.67	47.06	66.67	69.57	69.57	71.43	76.92	70.00	66.67	71.43	63.64	63.64
8.	56.00	66.67	61.54	76.19	60.00	70.00	70.00	66.67	42.86	57.14	80.00	80.00	56.00	52.17	70.59	66.67	56.00	73.68	42.11
9.	56.00	58.33	53.85	76.19	75.00	80.00	90.00	77.78	57.14	66.67	80.00	80.00	56.00	60.87	82.35	77.78	56.00	73.68	42.11
10.	59.27	69.23	64.29	78.26	66.67	81.82	90.91	80.00	50.00	69.57	90.92	90.91	66.67	64.00	73.68	80.00	66.67	85.71	47.62
11.	77.42	86.67	81.25	74.07	54.55	76.92	76.92	64.00	40.00	74.07	76.92	76.92	83.87	82.76	60.87	64.00	83.87	72.00	64.00
12.	76.92	54.55	50.00	52.63	71.43	66.67	66.67	62.50	33.33	52.63	77.78	77.78	52.17	57.14	53.33	62.50	52.17	70.59	47.06
13.	61.54	64.00	59.26	63.64	58.82	85.71	76.19	73.68	53.33	72.73	76.19	76.19	61.54	66.67	66.67	73.68	61.54	70.00	50.00
14.	66.67	69.23	64.29	69.57	55.56	72.73	72.73	60.00	50.00	60.87	63.64	63.64	59.26	64.00	63.16	60.00	59.26	57.14	47.62
15.	56.00	58.33	53.85	66.67	75.00	90.00	90.00	88.89	57.14	76.19	90.00	90.00	64.00	69.57	82.35	88.89	64.00	84.21	52.63
16.	64.29	81.48	75.86	66.67	63.16	78.26	78.26	66.67	35.39	75.00	69.57	69.57	71.43	76.92	60.00	66.67	71.43	63.64	63.64
17.	64.29	81.48	75.86	66.67	52.63	69.57	69.57	57.14	23.53	66.67	60.87	60.87	71.43	69.23	50.00	57.14	78.57	54.55	63.64
18.	38.10	30.00	27.27	47.06	66.67	62.50	62.50	57.14	40.00	47.06	62.50	62.50	47.62	52.63	61.54	57.14	38.10	53.33	40.00
19.	89.66	85.71	80.00	80.00	50.00	66.67	58.33	63.64	55.56	72.00	66.67	66.67	75.86	74.07	66.67	63.64	82.76	69.57	60.87
20.	-	83.87	90.91	78.57	52.17	66.67	59.26	64.00	47.62	64.29	66.67	66.67	7.00	73.33	66.67	64.00	93.75	69.23	69.23
21		-	93.75	81.48	54.55	61.54	69.23	80.00	40.00	66.67	69.23	69.23	75.42	75.86	60.87	66.67	83.87	72.00	72.00
22.			-	75.86	50.00	57.14	64.29	61.54	36.36	62.07	64.29	64.29	72.73	70.97	56.00	61.54	90.91	66.67	74.07
23				-	63.16	69.57	78.26	76.19	58.82	66.67	78.26	78.26	64.29	61.54	80.00	76.19	71.43	81.82	45.46
24.					-	77.78	77.78	75.00	50.00	70.59	77.78	77.78	50.17	66.67	80.00	75.00	52.17	70.59	58.82
25.						-	90.91	90.00	6.250	86.96	90.91	90.91	74.07	80.00	84.21	90.00	70.07	85.71	66.67
26.							-	90.00	62.50	78.26	90.91	90.91	66.67	72.00	84.21	90.00	66.67	85.71	57.14
27.								-	71.43	76.19	90.00	90.00	64.00	69.57	94.12	100.00	72.00	94.74	63.16
28.									-	58.82	62.50	62.50	38.10	31.58	76.92	71.43	46.62	66.67	26.67
29.										-	78.26	78.26	71.43	76.92	70.00	76.19	71.43	72.43	54.55
30.											-	100.00	74.07	72.00	84.21	90.00	74.07	95.24	57.14
31.												-	74.07	72.00	84.21	90.00	74.07	95.24	57.14
32.													-	86.67	66.67	64.00	81.25	69.23	69.23
33.														-	63.64	69.57	80.00	66.67	75.00
34.															-	94.12	75.00	88.89	55.56
35.																-	72.00	94.74	63.16
36.																	-	76.92	76.92
37.																		-	60.0

* See Table 1

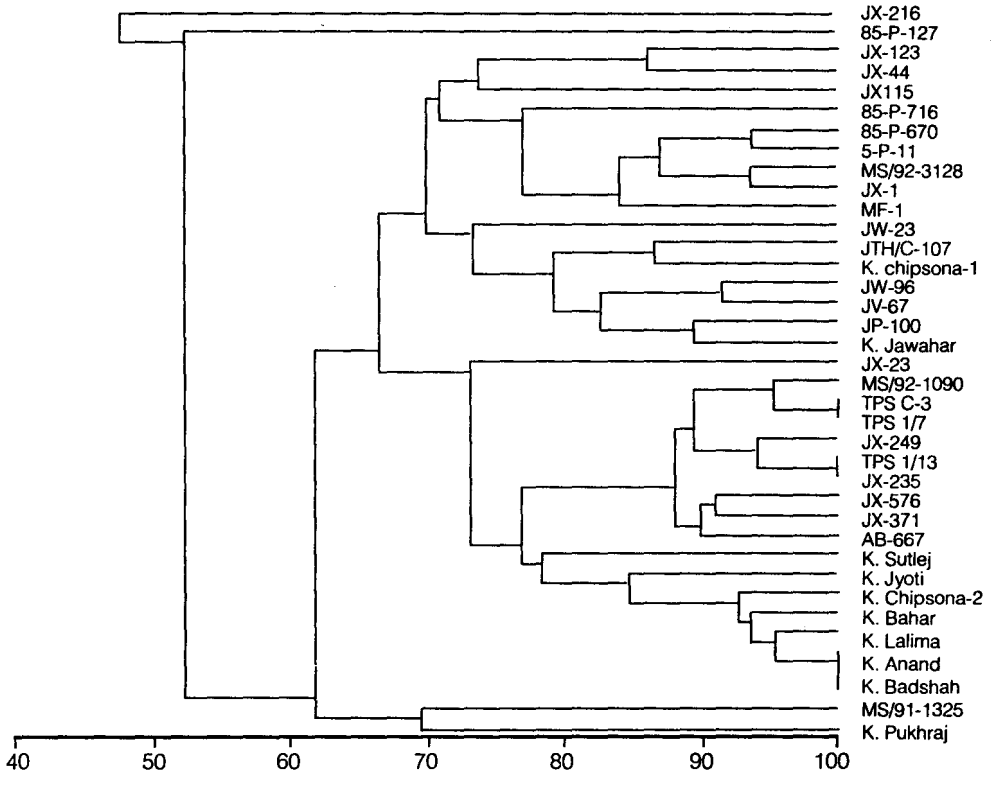

Fig. 2. Dendrogram of 38 potato (Solanum tuberosum) genotypes

Kufri Ashoka and Kufri Anand, JX-235 and TPS 1/13 and TPS 1/7 and TPS C-3 exhibiting SI value of 100% were not related by pedigree. Demeke *et al.* (1996) also studied genetic diversity of 28 North American potato cultivars and observed that cultivars with close kinship were as genetically diverse as those with no immediate relationship. The kinship could not be reflected in the similarity in banding patterns, due to highly heterozygous nature of the tetraploid genome (Cubillos and Plaisted, 1976; Tarn and Tai, 1977; Mendiburu *et al.*, 1974; Mendoza and Haynes, 1974) resulting in segregating F_1s.

Genetic divergence measured on the basis of environmentally and developmentally stable characters like tuber proteins can be reliable and reproducible (Hosaka and Hanneman, 1991; Rajapakse *et al.*, 1991). Therefore, the combinations *viz.*, Kufri Pujrak x JX-216, JX-216 x JW-96, JX-216 x MS/91-1325, 85-P-127 x 85-P-670, JX-216 x Kufri Jawahar, 85-P-127 x 85-p-11, JX-216 x Kufri Sutlej, 85-P-127 x MF-1, JX-216 x JV-67, JX-216 x 85-P-670, 85-P-127 x JX-1, JX-P-127 x JP-100, JX-216 x Kufri Anand, JX-216 x Kufri Ashoka, JX-216 x Kufri Badshah, JX-216 x 85-P-127, JX-216 x 85-P-11, 85-P-127, MS/91-1325 and Kufri Sutlej x MF-1 with low similarity index values are recommended for future hybrid breeding programmes in potato.

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