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SDS-PAGE PROFILING MEDIATED CHARACTERIZATION OF POTATO CULTIVARS

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ABSTRACT

Separation of denaturated proteins of 42 Potato cultivars showing 29 bands ranging from 200 kDa to 6.5 kDa were resolved. The dendrogram constructed from SDS-PAGE data was more effective at identifying relationships between cultivars. The maximum (19) bands were recorded in Cv. Kufri Khyati. While the minimum, no bands (7) were observed in Kufri Ganga and Kufri Lalima. A cluster analysis using SDS-PAGE revealed 4 clusters, with most genotypes closely related and showing little genetic diversity.
Keywords **:** SDS-PAGE, Potato, Biochemical Markers, Protein, Genetic Diversity

Introduction

Potato is a crop which has always been the poor man's friend. Potato makes up a considerable proportion of the Indian diet (Kumar *et al.,* 2013). The potato is a highly nutritious, easily digestible, wholesome food which contains 79.3% water and the rest average dry matter composition is 17.5% carbohydrates including 15.4% starch and 2.2% dietary fiber; 0.85%-4.2% proteins, 0.09% lipids, 0.019% vitamins and considerable number of other vitamins. Potatoes are an excellent source of lysine and minerals like Fe, Mn, K, Cu (Miedzianka *et al.,* 2019). These compounds are having dietary importance, including phosphorous, calcium, magnesium, and this, coupled with the presence of high vitamin C (42mg/100 g), helps in its absorption. It is also a good source of chlorogenic acid (0.2-2193 mg/100 g dry matter) (Akyol *et al.,* 2016) like caffeoyl-Quinic acid (Joly *et al.,* 2020) is the main polyphenol. Solanine (0.075mg/g) and Chaconine (0.12mg/g) are two main glycoalkaloids in potatoes (Kolasa, 1993; Burlingame *et al.,* 2009).

Because of the variety of tuber macromolecules within the commercialism, tuberosum protein evaluation via a natural process may be an accessible and effective method of studying genetic variation (Andrews and Simpson, 1987). The constant method is meant to show genetic stability in gene banks within which massive quantities of plant materials are conserved. In such assessment, the check atmosphere ought to be standardized, for this reason permitting the genetic version to look clearly (Rajapakse *et al.,* 1991; Barta *et al.,* 2003). Natural action types of soluble macromolecules and isozymes are used as a robust tool for have a study of genetic variability of genus Solanum species has the advantage owing to the vegetative propagation there is also no genetic variability variety of the flora of every cultivar. In Europe, collections of potato cultivars have been discriminated within the direction of via their protein and esterase designs (Barta *et al.,* 2003).

Tubers are suitable for separating proteins, since they represent a stable source of proteins and provide a uniform material for protein extraction. The protein patterns of potatoes were found to be stable regardless of the addition of growth regulators or changes in environmental factors (Rajapakse *et al.,* 1991). For any breeding program, especially for hybridization, it is important to have an understanding of the affinity between different varieties and cultivars. Genetic fingerprinting has been accomplished traditionally through the use of isozymes, total seed proteins, tuber

storage protein (Alvarez *et al.*, 2003; Barta *et al.*, 2003).

Materials and Methods

Plant materials

The tubers of 42 potato cultivars obtained from Agriculture and Horticultural Research Station, Khambolaj, Anand Agricultural University, were harvested at maturity and used for protein extraction.

Protein extraction

1g of each potato tuber (fresh weight) was crushed in 1 ml of extraction buffer (40 mM phosphate buffer pH-7.2, 0.2% polyvinyl pyrrolidone, 250 mM sucrose, 10 mM ethylene diamine tetra acetic acid, 1% triton X-100, 2% 2-Mercapitoethanol.). The homogenate was centrifuged at 12000 rpm for 15 min, then the supernatant was added to a new tube. Supernatant was used as a protein sample (Ali and Javad, 2007).

Preparation of slab gel

The gels were prepared according to the method described by Laemmli, 1970; 9. A vertical slab 1 mm thick gel stock solutions were mixed in the following way in to these two types of gels

Separating gel (15%)

15 % gels were prepared by mixing 6.2 ml of distilled water, with 1.3 ml of 30 % Acrylamide, 2.5 ml of Separating gel buffer, 50 µL of 10 % APS, 100 µL of 10 % SDS and 10 µL of N, N, N, N-tetramethyl ethylenediamine (TEMED) are mixed well and added to glass plates. The gel was left for polymerization during 15-20 min.

Stacking gel (4.5%)

4.5 % gels were prepared by mixing 2.4 ml of distilled water, with 5 ml of 30 % Acrylamide, 2.5 ml of Separating gel buffer, 50 µL of 10 % APS, 100 µL of 10 % SDS 10 µL of TEMED The solution was immediately poured in the chamber containing polymerized running gel. The comb was placed in the stacking gel and allowed to set for 20-30 minutes. The comb was removed after complete polymerization without distorting the shape of the wells.

Sample preparation, loading and separation

Protein extracts (10 µL) were mixed with 10 µL bromophenol blue dye and loaded into well. Protein molecular weight markers (5 µL) were also run along with sample. Electrophoresis was carried out on Cleaver Scientific omni-PAGE mini vertical electrophoresis unit using 1 mm gel. Electrophoresis was performed at 30 mA/gel until gel loading dye moved at bottom of the stacking gel, followed by changing the 45 mA/gel until loading dye reached bottom of running gel.

Staining and Destaining of the gel

When the blue coloured tracking dye reached the bottom of the running gel, power supply was turned off. The gel was gently removed from the space between the plates and immersed in the staining solution (40 %(V/V) Methanol, 10 %(V/V) Acetic Acid, 0.1 % Coomassie brilliant blue R-250) contained tray. The tray was shaken for 6 h on Gel Rocker for uniform staining. The gel was transferred in destaining solution (40 % (V/V) Methanol, 10 % (V/V) Acetic Acid) and destained until bands were clearly seen on clear gel.

Gel analysis

The destained gel was photo documented using the gel imaging equipment and the relative mobility of the different protein bands were analysed using Image Lab™ Software version 5.0 (Bio-Rad Laboratories). Band positions for each extract were scored as either present (+) or absent (−), the molecular weight (M.Wt.) and the relative mobility (Rm) of each band was calculated automatically, and the banding pattern tables were generated.

Results and Discussion

Potato tuber protein has been analyzed using 15 % resolving gel through Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Analysis of total protein profile by SDS-PAGE revealed the presence of 29 bands of diverse molecular weight ranging from 6.5 to 200 kDa (Figure no: 3). The maximum no bands (19) were recorded in Cv. Kufri Khyati. While the minimum no bands (7) were observed in Kufri Ganga and Kufri Lalima. Cultivars were identified on the basis of presence and absence of bands (Table 1).

Bands with M. Wt 200 kDa were seen in Kufri Kesar and Kufri Mohan and Band with M. Wt 6 kDa is seen in 3 cultivars Kufri Himalini, Kufri Bahar, Kufri Khyati.

Binary data matrix was generated taking 1 as presence and 0 as absence. The statistical calculation was using NTSYSpc version 2.0 program. The similarity matrix showed maximum similarity with Ali and Javad (2007); 2. They were clustered in between 0.52 to 0.97 with a mean value of 0.61 showing the similarity is greater than dissimilarity between potato cultivars. Dendrogram (Figure. 1) was obtained by Unweighted Pair Group Method of Arithmetic Mean (UPGMA)clustering of the similarity matrix data showed in Table 2.

The minimum similarity index 0.0 was observed between Kufri Ganga and 14-(P-30-600); 0.05 was observed in between Kufri Chandramukhi and 7-(P-14-800). Maximum similarity coefficient 0.92 was observed in between Kufri Lima and 4-(C-20-200).

In this we obtained 4 major bands with molecular weight ranging from 22 to 45. Two bands are patatin (40-45 kDa) according to Racusen and weller. (2007) and remaining 3 (22-31 kDa) bands are isomers of sporamin according to the Ali and Javed. (2007) they could be seen as 2 major bands in middle portion of PAGE gel, are the major proteins in protein extracted from the tuber flesh.

Ali and Javad (2007) have compared 13 varieties of potato by SDS-PAGE and reported that certain bands could be easily useful for researchers to identify varieties. Skyorova and Matejova (2006) have used for the characterisation of 25 potato varieties by PAGE and concluded that electrophoretic profiles of soluble tuber proteins, which are highly polymorphic and stable, can be considered as valuable for variety identification.

Conclusion

The protein profile of potato tuber by SDS-PAGE revealed the presence of 29 bands of diverse molecular weight ranging from 6.5 to 200 kDa. The maximum no bands (19) were recorded in Cv. Kufri Khyati. While the minimum no bands (7) were observed in Kufri Ganga and Kufri Lalima. The minimum similarity index 0.0 was observed between Kufri Ganga and 14-(P-30-600); 0.05 was observed in between Kufri Chandramukhi and 7-(P-14-800). Maximum similarity coefficient 0.92 was observed in between Kufri Lima and 4- (C-20-200). The four major bands (Mol. wt. 22 to 45 kDa) were recorded among all the genotypes/cultivars of potato. From that the 2 bands of protein patatin (Mol. Wt. 40-45KDa) as well as 2 bands of sporamin (22- 31 kDa) were recorded in each potato genotypes/cultivars. Over all from the present experiment data it can be concluded that the 3 cultivars 7-(P-14-800), 14-(P-30-600), and 5-(C-28-200) cultivars were clearly genetically differ as compared to other 39 potato cultivars.

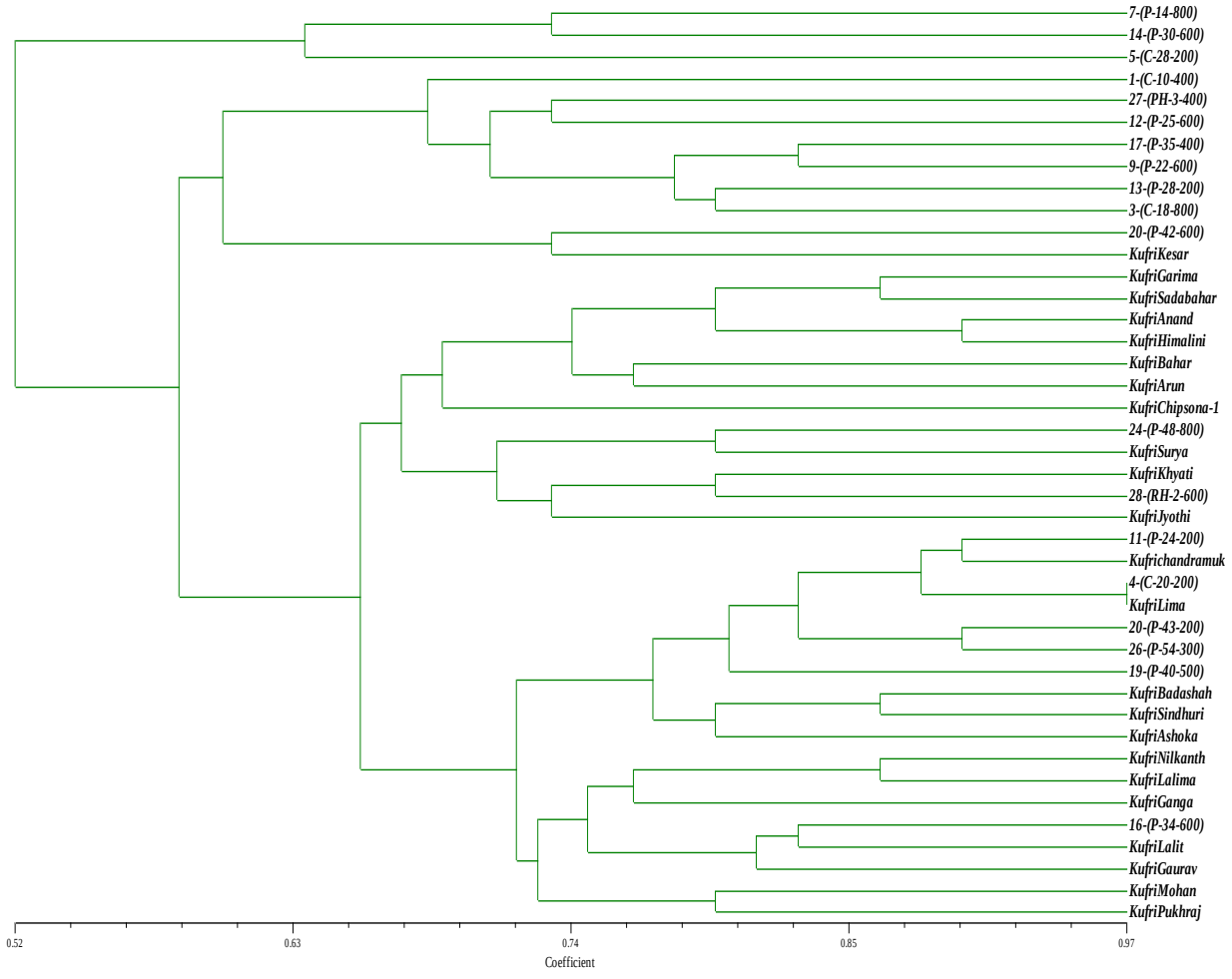

Fig. 1: Dendrogram based on total protein profile in 42 potato cultivars

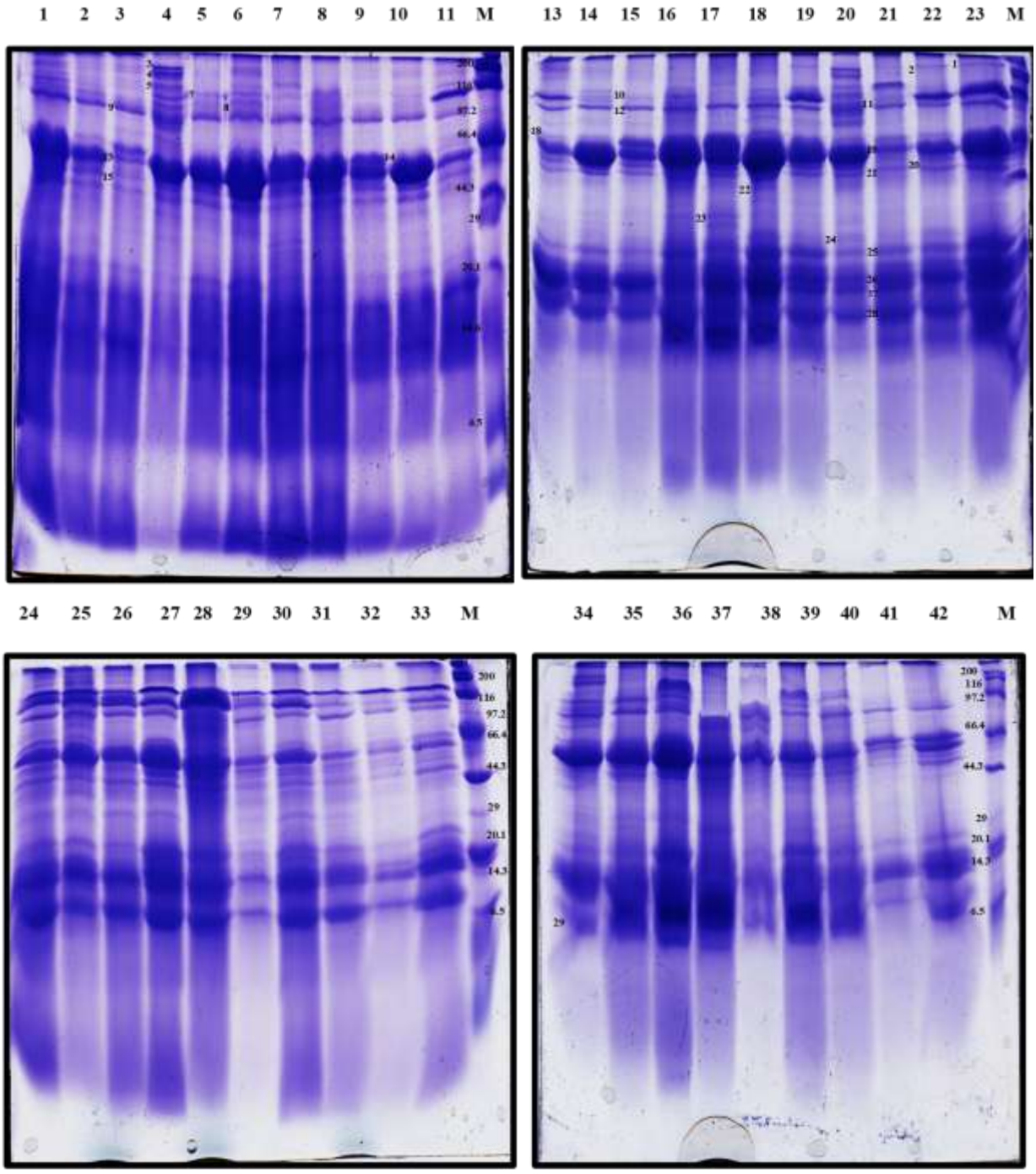

Fig. 3: SDS-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of proteins in potato tubers.

Table 1: Band scoring of SDS-PAGE

No of band	MW of band	Rm value	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1	200	0.014	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	180	0.018	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	170	0.022	1	0	1	1	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
4	160	0.045	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0
5	150	0.057	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
6	140	0.077	0	1	0	1	1	1	1	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
7	130	0.091	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	1	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0
8	120	0.106	0	0	1	1	1	0	0	1	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	1	1	1	0	0	1	0	1	1	1
9	110	0.131	0	0	0	1	0	1	1	1	1	0	0	1	0	0	0	1	1	0	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0
10	100	0.142	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	1	0	1	1	0	0	0	1	0	1	0	0	1	1	0	0	1	0	0	1	1	1
11	90	0.173	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	1	1	0	0	1	0	1	0	0	1	0	0	1	1	0	1	1	1	1	1	1	0	0	0
12	85	0.191	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	1	1	1	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1	1	1
13	80	0.208	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	1	0	0	0	0
14	75	0.218	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
15	70	0235	0	1	1	1	1	0	1	1	1	1	0	1	1	0	1	1	1	0	0	0	0	0	1	1	0	1	1	0	1	0	0	1	0	1	0	1	1	0	0	0	0	0
16	65	0.260	0	0	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0
17	60	0.283	1	1	1	0	1	0	1	1	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
18	55	0.297	0	0	0	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
19	50	0.314	1	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	1	1
20	45	0.350	0	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	1
21	40	0.373	0	0	0	1	0	0	1	1	0	0	0	1	1	1	1	1	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	0	0
22	35	0.422	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	1	1	1	0	0	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
23	30	0.459	0	1	1	1	1	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
24	25	0.525	1	0	1	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1
25	20	0.608	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1
26	17	0.696	0	1	0	0	0	1	0	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
27	15	0.760	0	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
28	10	0.988	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	1	1	1	0	0	0	0	1	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1	1	0	1
29	6.5	0.998	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0

0 = band absent ; 1 = band present

Table 2 : Jaccard similarity coefficient data based on SDS-PAGE

Cultivars	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
7-(P-14-800)	1.00																																										
1-(C-10-400)	0.20	1.00																																									
27-(PH-3-400)	0.29	0.38	1.00																																								
20-(P-42-600)	0.28	0.28	0.28	1.00																																							
17-(P-35-400)	0.20	0.38	0.50	0.35	1.00																																					
5-(C-28-200)	0.38	0.22	0.16	0.35	0.22	1.00																																				
13-(P-28-200)	0.19	0.36	0.27	0.50	0.46	0.28	1.00																																			
3-(C-18-800)	0.17	0.31	0.40	0.37	0.50	0.39	0.57	1.00																																		
9-(P-22-600)	0.31	0.31	0.42	0.29	0.55	0.24	0.50	0.54	1.00																																	
12-(P-25-600)	0.27	0.27	0.46	0.50	0.58	0.44	0.33	0.38	0.38	1.00																																
14-(P-30-600)	0.38	0.20	0.29	0.28	0.29	0.29	0.12	0.17	0.21	0.36	1.00																															
24-(P-48-800)	0.10	0.29	0.16	0.42	0.29	0.30	0.53	0.56	0.31	0.21	0.05	1.00																														
11-(P-24-200)	0.11	0.24	0.40	0.30	0.40	0.19	0.29	0.50	0.25	0.29	0.11	0.47	1.00																													
4-(C-20-200)	0.11	0.17	0.24	0.18	0.24	0.32	0.22	0.41	0.18	0.22	0.11	0.47	0.71	1.00																												
Kufri Nilkanth	0.13	0.29	0.29	0.28	0.29	0.29	0.36	0.40	0.21	0.27	0.13	0.29	0.62	0.50	1.00																												
16-(P-34-600)	0.17	0.40	0.31	0.30	0.24	0.32	0.38	0.50	0.33	0.22	0.11	0.56	0.60	0.50	0.50	1.00																											
19-(P-40-500)	0.11	0.25	0.33	0.25	0.33	0.33	0.31	0.53	0.36	0.24	0.05	0.50	0.64	0.64	0.54	0.53	1.00																										
Kufri Garima	0.24	0.40	0.40	0.24	0.24	0.32	0.29	0.50	0.25	0.22	0.24	0.47	0.41	0.50	0.31	0.50	0.35	1.00																									
Kufri Khyati	0.27	0.33	0.22	0.27	0.22	0.39	0.26	0.41	0.29	0.16	0.27	0.45	0.41	0.55	0.27	0.48	0.43	0.55	1.00																							
Kufri Badashah	0.12	0.27	0.27	0.33	0.19	0.21	0.18	0.29	0.13	0.25	0.12	0.35	0.57	0.47	0.36	0.47	0.40	0.38	0.32	1.00																						
Kufri Ashoka	0.24	0.24	0.24	0.30	0.17	0.32	0.22	0.26	0.11	0.22	0.05	0.39	0.60	0.60	0.40	0.50	0.44	0.41	0.41	0.57	1.00																					
Kufri Lalit	0.06	0.21	0.21	0.22	0.13	0.24	0.38	0.43	0.23	0.13	0.00	0.50	0.54	0.54	0.42	0.67	0.46	0.43	0.35	0.50	0.54	1.00																				
Kufri Kesar	0.19	0.36	0.19	0.50	0.19	0.15	0.43	0.29	0.29	0.18	0.06	0.44	0.29	0.22	0.27	0.47	0.31	0.22	0.32	0.25	0.29	0.29	1.00																			
Kufri Mohan	0.17	0.31	0.24	0.30	0.17	0.25	0.38	0.41	0.18	0.16	0.00	0.47	0.50	0.50	0.50	0.50	0.53	0.33	0.35	0.38	0.50	0.43	0.57	1.00																		
Kufri Gaurav	0.06	0.42	0.13	0.29	0.21	0.31	0.38	0.33	0.23	0.20	0.13	0.50	0.33	0.33	0.31	0.54	0.36	0.43	0.35	0.50	0.33	0.60	0.29	0.25	1.00																	
Kufri Ganga	0.15	0.25	0.15	0.18	0.15	0.19	0.33	0.29	0.17	0.14	0.00	0.36	0.38	0.29	0.36	0.50	0.21	0.29	0.25	0.23	0.38	0.40	0.33	0.38	0.27	1.00																
Kufri Lalima	0.14	0.23	0.33	0.24	0.14	0.25	0.31	0.36	0.15	0.21	0.07	0.33	0.46	0.36	0.60	0.46	0.38	0.36	0.18	0.31	0.46	0.50	0.21	0.46	0.25	0.44	1.00														
Kufri Sindhuri	0.12	0.36	0.19	0.33	0.19	0.21	0.25	0.38	0.13	0.18	0.12	0.44	0.57	0.47	0.36	0.57	0.31	0.47	0.45	0.67	0.57	0.50	0.43	0.47	0.50	0.45	0.31	1.00													
Kufri Pukhraj	0.12	0.36	0.36	0.26	0.27	0.21	0.25	0.47	0.20	0.25	0.06	0.44	0.57	0.47	0.36	0.57	0.40	0.38	0.32	0.43	0.47	0.50	0.33	0.57	0.29	0.45	0.42	0.54	1.00												
Kufri Lima	0.11	0.18	0.25	0.19	0.25	0.26	0.24	0.35	0.19	0.24	0.11	0.41	0.77	0.92	0.54	0.53	0.57	0.44	0.50	0.50	0.64	0.58	0.24	0.44	0.36	0.31	0.38	0.50	0.40	1.00											
Kufri Anand	0.05	0.25	0.25	0.19	0.25	0.20	0.17	0.35	0.12	0.24	0.25	0.41	0.53	0.53	0.25	0.44	0.29	0.53	0.50	0.40	0.35	0.36	0.17	0.21	0.36	0.21	0.20	0.50	0.31	0.57	1.00										
Kufri Jyothi	0.28	0.35	0.21	0.33	0.28	0.50	0.26	0.44	0.29	0.26	0.15	0.59	0.44	0.44	0.28	0.63	0.56	0.44	0.57	0.41	0.44	0.38	0.33	0.37	0.47	0.33	0.24	0.41	0.41	0.39	0.39	1.00									
Kufri Chipsona-1	0.15	0.28	0.28	0.40	0.35	0.42	0.41	0.53	0.22	0.41	0.21	0.42	0.44	0.53	0.44	0.30	0.39	0.44	0.43	0.33	0.30	0.29	0.26	0.44	0.29	0.18	0.24	0.41	0.33	0.47	0.47	0.27	1.00								
Kufri Bahar	0.18	0.37	0.37	0.48	0.30	0.36	0.29	0.45	0.19	0.42	0.24	0.43	0.53	0.45	0.44	0.45	0.40	0.61	0.50	0.50	0.45	0.32	0.29	0.38	0.39	0.28	0.33	0.50	0.42	0.40	0.47	0.48	0.55	1.00							
Kufri Arun	0.28	0.44	0.35	0.33	0.35	0.35	0.33	0.44	0.22	0.33	0.28	0.42	0.37	0.37	0.28	0.37	0.32	0.63	0.43	0.50	0.30	0.29	0.20	0.30	0.47	0.18	0.17	0.41	0.33	0.32	0.47	0.47	0.56	0.63	1.00						
Kufri Sadabahar	0.12	0.36	0.27	0.20	0.27	0.21	0.33	0.57	0.29	0.18	0.19	0.53	0.47	0.47	0.36	0.57	0.40	0.69	0.45	0.33	0.29	0.38	0.25	0.38	0.38	0.33	0.31	0.43	0.43	0.40	0.50	0.50	0.33	0.50	0.50	1.00					
Kufri Surya	0.15	0.28	0.21	0.40	0.21	0.35	0.41	0.53	0.29	0.26	0.15	0.69	0.53	0.63	0.44	0.63	0.56	0.53	0.57	0.41	0.37	0.47	0.50	0.53	0.47	0.33	0.31	0.50	0.41	0.56	0.47	0.56	0.56	0.55	0.47	0.60	1.00				
Kufri Himalini	0.11	0.31	0.31	0.24	0.31	0.25	0.22	0.50	0.18	0.22	0.17	0.56	0.60	0.60	0.31	0.50	0.44	0.71	0.55	0.47	0.41	0.43	0.22	0.33	0.43	0.29	0.27	0.57	0.47	0.53	0.77	0.53	0.53	0.61	0.63	0.57	0.53	1.00			
28-(RH-2-600)	0.16	0.22	0.16	0.23	0.22	0.30	0.28	0.47	0.31	0.15	0.16	0.63	0.47	0.56	0.29	0.56	0.50	0.56	0.68	0.35	0.39	0.50	0.28	0.32	0.50	0.27	0.25	0.44	0.35	0.50	0.50	0.59	0.35	0.50	0.42	0.53	0.59	0.67	1.00		
Kufri Chandramukhi	0.05	0.18	0.25	0.25	0.25	0.20	0.24	0.44	0.12	0.17	0.05	0.50	0.77	0.77	0.43	0.44	0.57	0.44	0.43	0.62	0.64	0.58	0.24	0.53	0.36	0.31	0.38	0.62	0.62	0.69	0.47	0.39	0.47	0.47	0.39	0.40	0.47	0.64	0.50	1.00	
20-(P-43-200)	0.19	0.12	0.27	0.20	0.27	0.28	0.11	0.29	0.13	0.25	0.12	0.28	0.69	0.57	0.46	0.38	0.50	0.29	0.32	0.67	0.57	0.38	0.11	0.29	0.29	0.23	0.31	0.43	0.33	0.62	0.40	0.41	0.33	0.42	0.41	0.25	0.33	0.47	0.35	0.62	1.00
26-(P-54-300)	0.18	0.11	0.33	0.25	0.33	0.26	0.17	0.35	0.19	0.31	0.11	0.33	0.64	0.64	0.33	0.35	0.57	0.35	0.36	0.62	0.53	0.36	0.17	0.35	0.27	0.21	0.20	0.40	0.40	0.57	0.38	0.47	0.39	0.47	0.47	0.31	0.39	0.53	0.41	0.69	0.75

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