DOI : 10.5958/0974-0112.2019.00109.9

Indian J. Hort. 76(4), December 2019: 683-690

SDS-PAGE based protein profiling and diversity assessment of indigenous genotypes of cucumber

Chandan Singh Ahirwar* and D. K. Singh

Department of Vegetable Science, G.B. Pant University of Agriculture and Technology, Pantnagar 263145, Uttarakhand

ABSTRACT

The present investigation was carried out to determine the relationships among genotypes categorization of seed storage proteins profiles of 44 genotypes with two checks PantKhira-1 and Pointsette of cucumber (*Cucumis sativus* L.) was performed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) during from July-October, 2014 and February-June, 2015. The entire profile comprised of 15 protein bands, distributed into three major zones A, B and C in increasing order of electrophoretic mobility i.e., Zone A was nearest and Zone C was farthest from the point of protein sample application. Among 15 bands maximum number of bands were observed in PCUC-193 (9 bands), PCPGR-6762 (8bands), PCPGR-7013 (8bands) and PCPGR-7795 (8bands) followed by PCUC-199 (7 bands), PCUC-832(7 bands), PCUC-44 (7 bands), PCUC-23 (6 bands). Minimum bands were shown by PCPGR-7795 (1 band). The un weighed pair group method using arithmetic average (UPGMA) analysis of 46 cucumber genotypes was done and two major clusters obtained through seed protein analysis expressed grouping of genotypes of cucumber. Approximately 83.339 and 83.332 per cent of variation was recorded due to first five principle components on various genotypes in first season and second season respectively. The first four principle components recorded a per cent variation of 66.617 among various genotypes in pooled analysis. Protein profiling of genotypes resolved all forty six indigenous germplasm lines into different groups on the basis of presence and absence of fifteen protein bands distributed into A, B and C zone respectively.

Key words: *Cucumis sativus*, similarity index (S.I.), UPGMA cluster analysis, divergence.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important cucurbits that grown as a salad (Arunkumar *et al*., 1). Cucumber has originated in India. It is a member of the Cucurbitaceae family (Harlan, 7). The crop is grown throughout the world and is the fourth most important vegetable crop after tomato, cabbage, and onion (Tatlioglu, 16). The problem of cultivar identification has been simplified to a great extent by the combined use of morphological, biochemical and molecular markers. Morphological markers are highly influenced by environment. The biochemical markers are proteins that can be isolated and their polymorphism identified through electrophoresis. The biochemical techniques are quicker and more reliable, since the expression of isozyme loci are codominat and not altered by environmental factors (Arus, 3; Smith and Smith, 11). Polymorphism at protein level can help to measure genetic distance or genomic similarities between pairs of parental lines and hybrids. Seed proteins have the advantage of being scorable from in viable organs or tissue and the electrophoretic protocol for bulk protein assay is generally simpler than isozymes (Cooke, 4). The profiles of the seed storage protein extracted from seed are stable, reproducible and least influence by the environment and are therefore, examined for the purpose of variety identification as well as genetic purity testing (Dadlani and Varier, 5). Numerous electrophoretic methods are available to identify cultivars by protein banding patterns. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) provides the best resolution (Smith and Simpson, 9). Therefore, SDS-PAGE was carried out with germplasms of cucumber to determine the protein-banding pattern.

MATERIALS AND METHODS

The present investigation was conducted with two season during July-October, 2014 and February-June, 2015 at Vegetable Research Centre, Department of Vegetable Science in G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. Pantnagar is situated in the foot hills of Himalayan region (Shivalik hills) and falls under humid subtropical climate zone in narrow belt called Tarai. Geographically, Vegetable Research Centre is situated at the latitude of 29.5°N, longitude 79.3°E and at an altitude of 243.84 meters above the mean sea level. Total 46 genotypes of cucumber (*Cucumis sativus* L.) were used as experimental material in

*Corresponding author's E-mail: csrahul126@gmail.com

present experiment. The genotypes were diverse with respect to morphological and important economical traits. The experiment was laid out in randomized block design with three replications. Healthy and uniform sowing of seeds was main field in plots with a spacing of 3 meters × 0.60 cm during the evening hours of during July-October, 2014 and February-June, 2015.

The crops were grown with standard package of practices. The observations on various growth, yield and qualitative characters viz. observed highly significant differences for all the traits under study. Loading samples were prepared by appropriately diluting the extracted protein with sample buffer (Tris pH-7.4, 2% SDS, 2% mercaptoethanol, bromophenol blue) and further placed in hot water for 5 minutes at 65°C before loading in the gel. Medium range (14 to 100 kDa) molecular weight marker was used along with the sample for determining the molecular weight of separating fractions. Five µl of prepared sample was loaded to each well along with 2µl marker protein in one well of gel. The SDS solubilized protein samples were then subjected to vertical SDS-PAGE with 12% separating and 5% stacking gels using Tris-glycine electrode buffer (Tris-glycine and SDS, pH-8.6). The samples were electrophoresed at 80V initially and increased by 100V and current 500mA, when the tracking dye passed from the stacking gel. The run was stopped when the dye was approximately 0.5 cm from the bottom of the gel, which took around 4 to 5 hours. The gel was removed with the help of spatula and dipped for 12 hours in staining solution (0.25 g Coomassie Brilliant Blue R-250, 60 g TCA, 180 ml methanol; and 60 ml glacial acetic acid). The staining solution was then replaced the next day with destaining solution (3 % NaCl). The gel was intermittently and carefully shaken and destaining solution was changed till the blue colour of the background of the bands disappears. The position and intensity of the bands were visualized in the gels on a Syngen Gel Documentation system for documentation and photography. The clustering was performed with Jaccard'scoefficient of similarity and UPGMA clustering using NTSYS PC (version 2.0 i).

RESULTS AND DISCUSSION

The protein profile and zymogram of banding pattern are given in respective plate 1, 2, 3, 4 and 5. The protein was divided into three zones A, B, C and each zone was allocated with a number of subzones. Zone A was nearest to origin and comprises protein bands of high molecular weight while C was the farthest from origin and thus had protein bands of low molecular weight. A standard medium range protein molecular weight marker of known molecular weight (5 to 80 KDa) was used along with the samples. For genotype discrimination the presence and absence of protein bands, their thickness, width (Dark, Medium and Light) was the criteria for characterization of germplasm differentiation.

The entire profile comprised of 15 protein bands, distributed into three major zones. The high molecular weight proteins were located into upper region and low molecular weight protein in the lower region of gel. Each zone was further divided into a number of bands. Zone A representing the heaviest molecular weight protein was subdivided into 5 distinct bands, while zone B having subdivided into 5 subzones, zone C was subdivided into 5 bands. Some having thick bands while sub have medium thick bands and some have thin bands. Thus a total of 15 bands could be resolved in protein. The protein banding pattern of 46 germplasm lines with 2 checks i.e. Pointsette and Pant Khira-1 in terms of presence and absence

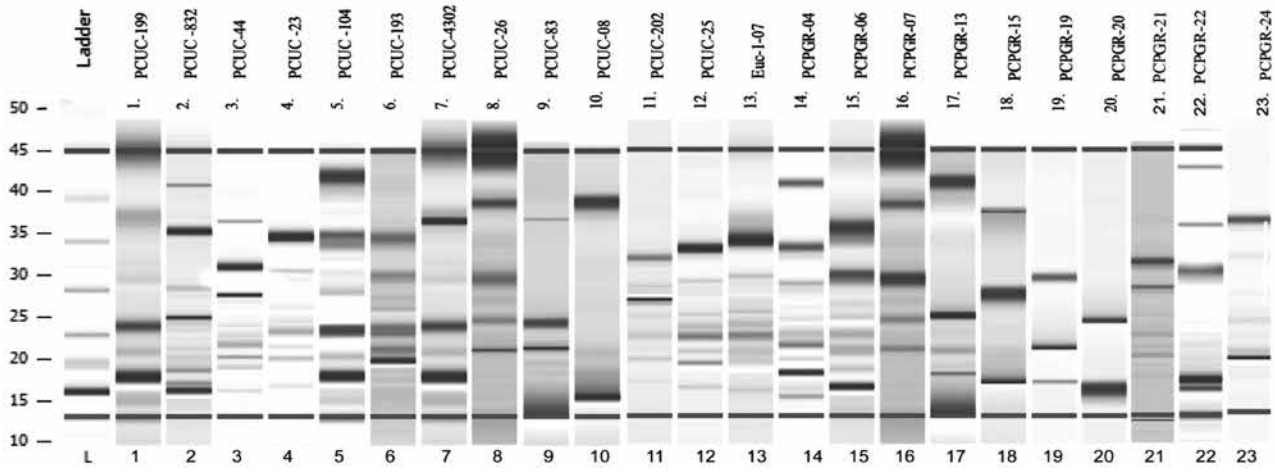


Plate 1. Protein profiling through SDS-PAGE.

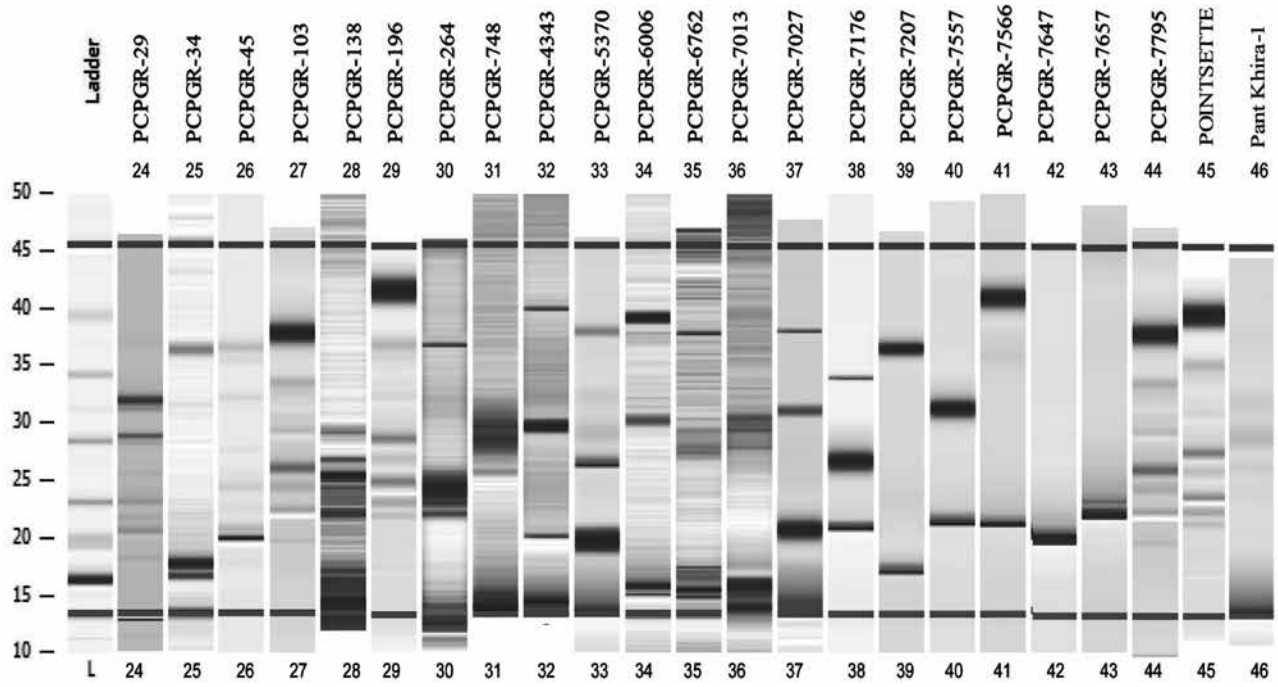


Plate 2. Protein profiling through SDS-PAGE.

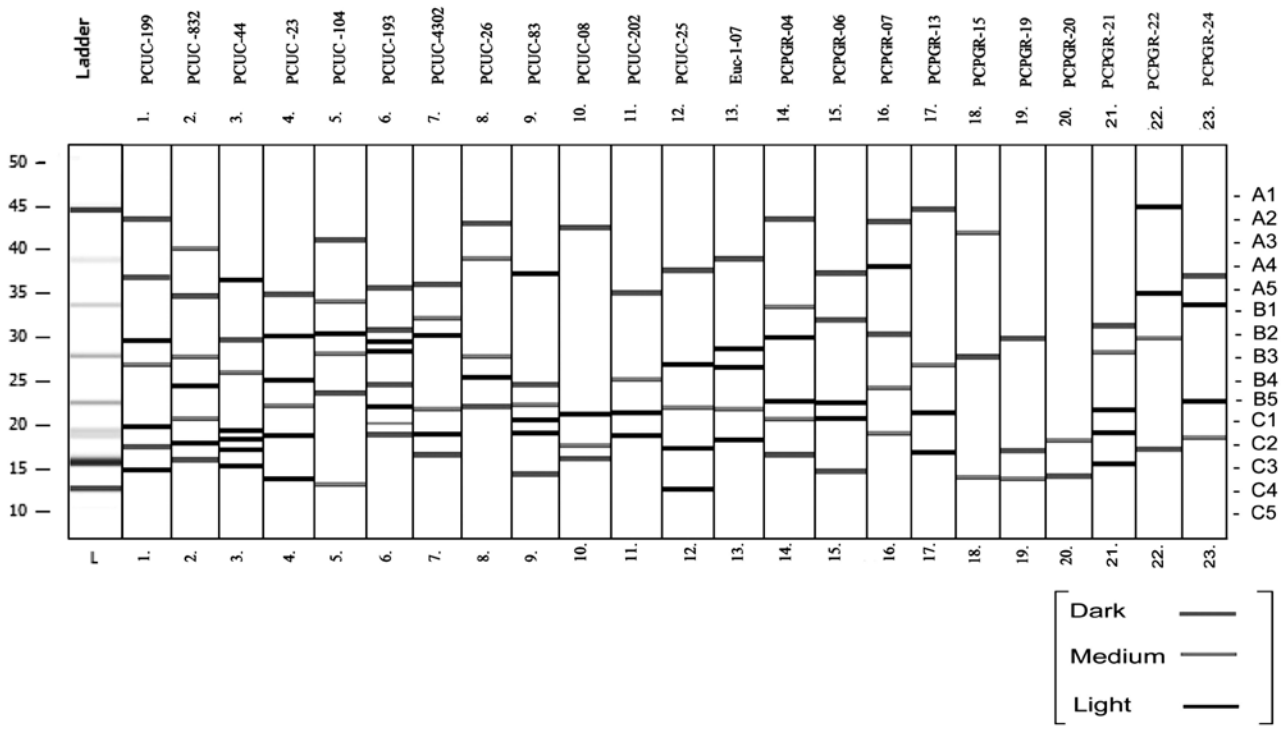


Plate 3. Protein profiling through SDS-PAGE.

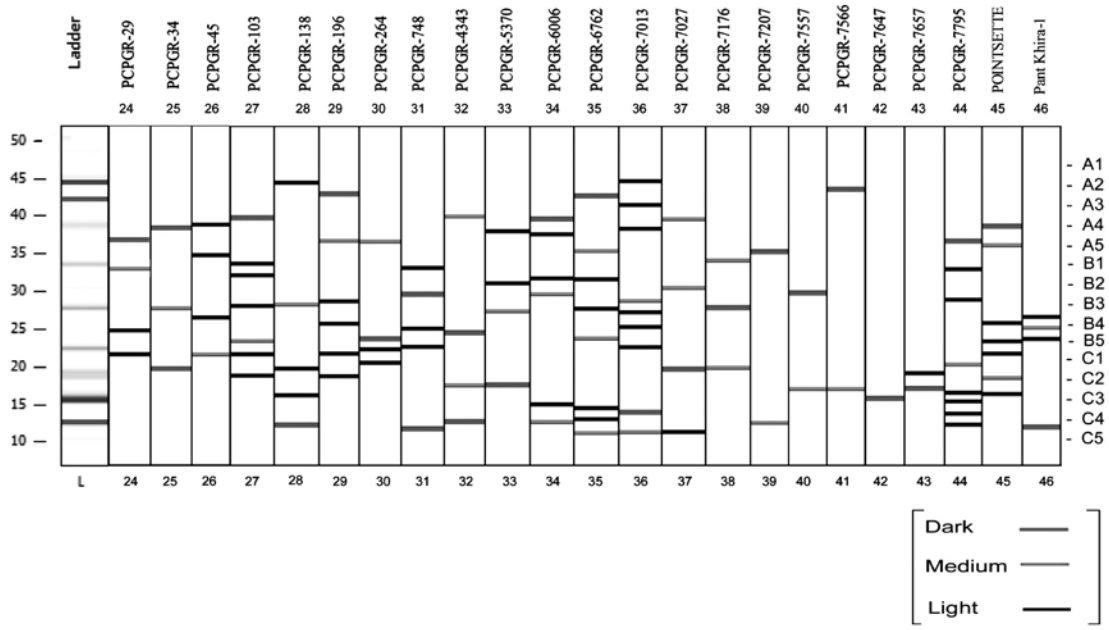
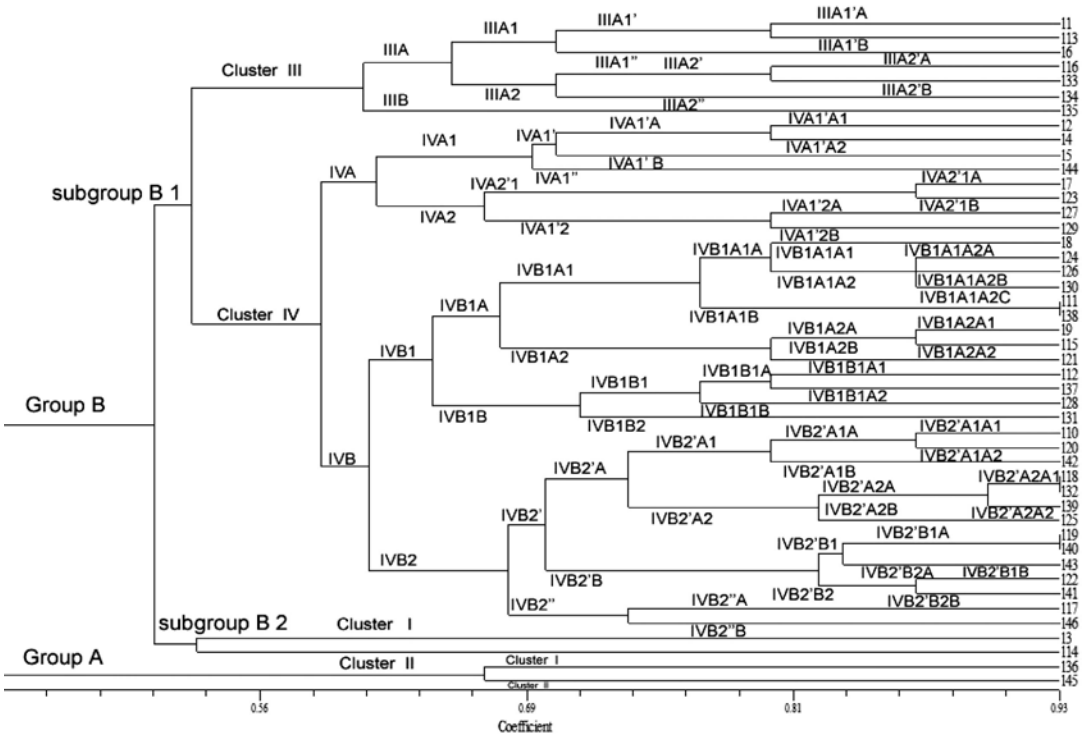


Plate 4. Protein profiling through SDS-PAGE.



L1=PCUC-199, L2=PCUC -832, L3=PCUC-44, L4=PCUC -23, L5=PCUC -104, L6=PCUC-193, L7=PCUC-4302, L8=PCUC-26, L9=PCUC-83, L10=PCUC-08, L11=PCUC-202, L12=PCUC-25, L13=Euc-1-07, L14=PCPGR-04, L15=PCPGR-06, L16=PCPGR-07, L17=PCPGR-13, L18=PCPGR-15, L19=PCPGR-19, L20=PCPGR-20, L21=PCPGR-21, L22=PCPGR-22, L23=PCPGR-24, L24=PCPGR-29, L25=PCPGR-34, L26=PCPGR-45, L27=PCPGR-103, L28=PCPGR-138, L29=PCPGR-196, L30=PCPGR-264, L31=PCPGR-748, L32=PCPGR-4343, L33=PCPGR-5370, L34=PCPGR-6006, L35=PCPGR-6762, L36=PCPGR-7013, L37=PCPGR-7027, L38=PCPGR-7176, L39=PCPGR-7207, L40=PCPGR-7557, L41=PCPGR-7566, L42=PCPGR-7647, L43=PCPGR-7657, L44=PCPGR-7795, 45=POINTSETTE and L46=Pant Khira-1

Plate 5. Dendrogram of 46 different genotypes of cucumber.

of different bands are presented in this table 4.9.1, 4.9.2, 4.9.3 and 4.9.4. Among 15 bands maximum number of bands were observed in PCUC-193 (9 bands), PCPGR-6762 (8bands), PCPGR-7013 (8bands) and PCPGR-7795 (8bands) followed by PCUC-199 (7 bands), PCUC-832(7 bands), PCUC-44 (7 bands), PCUC-23 (6 bands). Minimum bands were shown by PCPGR-7795 (1 band).

The Zone A comprised of protein bands of high molecular weight. These were designated as subzones A1, A2, A3, A4 and A5. The subzone A1 of light intensity was present in PCUC-08, PCPGR-38 and PCPGR-7013 whereas dark intensity was present only in PCUC-199, PCUC-26, PCPGR-138, PCPGR-7013 and PCPGR-13.The subzone A2 of dark intensity was present only in PCPGR-04, PCPGR-07 and PCPGR-196 light intensity was present only in PCPGR-22, PCPGR-7013. Whereas subzone A3 was present in PCUC-832, PCUC-104, PCUC-4302, PCUC-26, PCUC-25, PCUC-15, PCUC-24, PCPGR-29, PCPGR-34, PCPGR-45, PCPGR-4343, PCPGR-6006, PCPGR-7013, PCPGR-7027, PCPGR-7207, PCPGR-7795 and Pointsette. The subzone A4 of light intensity was present only in PCPGR-07, PCPGR-45, PCPGR-5370, and PCPGR-7013. Whereas subzone A5 of dark intensity was present only in PCUC-23, PCUC-4302, PCUC-202, PCPGR-24, PCPGR-7207 and PCPGR-7595. The zone B1 of light intensity was present in genotype i.e. PCPGR-103, PCPGR-748 and PCPGR-7795. The subzone B2 was present in fives genotypes, namely, PCUC-199, PCUC-193, Uuc-107, PCPGR-04, and PCPGR-5370.The subzone B3 of light intensity was present in genotype i.e. PCUC-23, PCUC-26, PCPGR-103, PCPGR-196, PCPGR-264, PCPGR-7013 and Pant Khira-1.Subzone B4 was present in PCUC-832, PCUC-23, PCUC-83, PCUC-202, PCPGR-06, PCPGR-13, PCPGR-21, and PCPGR-7657.Subzone B5 was present in PCUC-44, PCUC-4302, PCUC-83, PCUC-202, PCUC-25, PCPGR-06, PCPGR-21, PCPGR-138 and PCPGR-7657. The Zone C comprised of protein bands of low molecular weight. Subzone C1 was present of light intensity PCUC-832, PCUC-23, PCUC-193, PCPGR-13, PCPGR-7795 and Pointsette. In subzone C2 was present light band PCUC-44, PCUC-25, Euc-1-07, PCPGR-21,PCPGR-138, PCPGR-6006, PCPGR-7795 and Pointsette. In subzone C3 was present PCUC-44, PCUC-23 and PCPGR-6762. Insubzone C4 was present at PCUC-44, PCUC-6762, PCPGR-138, PCPGR-6762, PCPGR-7795 and subzone C5 was present in medium band at genotype PCUC-44, PCPGR-6762 and PCPGR-7013.

Similarity index was calculated to study the genetic/evolutionary relationship among 46 genotypes of cucumber. The value of similarity index (SI) is presented in table 1. A perusal of SI values revealed that the similarity index ranged from 20% to 100%. Most of genotypes showed a high degree of similarity on the basis of similarity index based on presence or absence of protein bands. Hundred per cent similarity was observed for genotypes PCUC-202 with PCPGR-7176; PCPGR-15 with PCPGR-4343; PCPGR-19 with PCPGR-7557, while, 93 % similarity was observed for genotypes PCPGR-7207 with PCPGR-15 While PCPGR-06 with PCUC-83, PCPGR-21 with PCPGR-06, PCPGR-24 with PCUC-7302, 86% similarity was observed for genotypes. Some of the genotypes showed very less amount of similarity among themselves. Lowest value of similarity index (20%) was shown by PCPGR-6762 with PCPGR-4302, PCPGR-24, PCPGR-29 and PCPGR- 7013 with PCUC 202 depicting that this was the most diverse group in evolutionary study.On the basis of protein profile of twenty five cucumber genotypes, the unweighted pair group method using arithmetic averages (UPGMA) analysis was done. The dendrogram was presented in plate 5.The 46 genotypes were clustered into two man groups or groups (A and B) with 44 per cent similarity among them. Group A comprised of cluster I and II at 66 % similarity. Cluster I comprised of genotype PCPGR-7013 whereas cluster II contains only one genotype i.e., Pointsette. Group B was bifurcated into 2 subgroups, Subgroup B1 and Subgroup B2. Subgroup B2 comprised of cluster I and II at 48 % similarity. Cluster I comprised of genotype Euc-1-07 whereas cluster II contains only one genotype i.e. PCPGR-04. Subgroup B1 consisted of Cluster III and Cluster IV at similarity of 48%. Cluster III was again forked into cluster III A & cluster III B with 57% similarity. Cluster III A was divided into cluster IIIA1 and cluster III A2 with 60% similarity. Cluster III A1 was again bifurcated into cluster III A1' and cluster III A1'' with 72% similarity. Cluster III A1' was divided into cluster III A1'A and cluster III A1'B with 78 % similarity. Cluster III A1' A contains the genotype PCUC-199 whereas cluster III A1'B contains the genotype Euc-1-07. Cluster III A1'' contains the genotype and PCUC-193.Cluster IIIA2 was divided into cluster III2' and IIIA2''with 72% similarity. Cluster III A2' was divided into cluster III A1''A and cluster III A2'A and IIIA2B with 78 % similarity whereas cluster A2'A contains the genotype PCPGR-07. IIIA2B contains the genotype PCPGR-5370 and III A2 contains the genotype PCPGR-6006. Cluster IV was again forked into cluster IV A & cluster IV B with 58 % similarity.

Cluster IV B1 was again divided into cluster IV B1A and cluster IV B1B with 60% similarity. Cluster

Table 1. Similarity coefficient among 46 genotypes of cucumber.

	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	43	44	45	46
1	1.00																																													
2	0.53	1.00																																													
3	0.53	0.46	1.00																																												
4	0.46	0.80	0.53	1.00																																											
5	0.53	0.73	0.60	0.66	1.00																																									
6	0.66	0.60	0.60	0.66	0.46	1.00																																								
7	0.33	0.66	0.53	0.60	0.66	0.53	1.00																																								
8	0.53	0.60	0.46	0.66	0.60	0.33	0.53	1.00																																							
9	0.53	0.60	0.46	0.66	0.33	0.60	0.66	0.60	1.00																																						
10	0.73	0.53	0.66	0.60	0.53	0.53	0.60	0.66	0.66	1.00																																					
11	0.60	0.53	0.53	0.73	0.53	0.53	0.46	0.80	0.80	0.60	1.00																																				
12	0.53	0.60	0.73	0.53	0.60	0.46	0.66	0.73	0.60	0.66	0.66	1.00																																			
13	0.80	0.60	0.46	0.53	0.46	0.73	0.53	0.46	0.73	0.53	0.66	0.60	1.00																																		
14	0.60	0.40	0.53	0.33	0.40	0.40	0.60	0.40	0.53	0.60	0.33	0.53	0.60	1.00																																	
15	0.53	0.60	0.46	0.66	0.46	0.60	0.66	0.60	0.86	0.66	0.80	0.60	0.60	0.40	1.00																																
16	0.66	0.33	0.46	0.53	0.46	0.60	0.40	0.46	0.60	0.66	0.66	0.46	0.60	0.40	0.60	1.00																															
17	0.60	0.66	0.40	0.73	0.53	0.40	0.46	0.66	0.66	0.73	0.60	0.40	0.53	0.60	0.53	0.53	1.00																															
18	0.66	0.60	0.46	0.53	0.73	0.46	0.53	0.60	0.46	0.66	0.53	0.60	0.60	0.53	0.46	0.73	0.66	1.00																													
19	0.53	0.73	0.46	0.80	0.73	0.60	0.66	0.46	0.60	0.66	0.53	0.46	0.60	0.53	0.60	0.60	0.80	0.73	1.00																												
20	0.60	0.53	0.66	0.73	0.66	0.53	0.60	0.66	0.66	0.86	0.73	0.66	0.53	0.46	0.66	0.80	0.73	0.80	0.80	1.00																											
21	0.40	0.60	0.60	0.66	0.60	0.46	0.80	0.60	0.73	0.66	0.66	0.60	0.46	0.53	0.86	0.46	0.53	0.46	0.60	0.66	1.00																										
22	0.46	0.53	0.26	0.60	0.53	0.53	0.60	0.40	0.66	0.46	0.60	0.40	0.66	0.46	0.66	0.66	0.60	0.53	0.80	0.60	0.53	1.00																									
23	0.33	0.53	0.53	0.60	0.66	0.40	0.86	0.66	0.66	0.60	0.60	0.66	0.53	0.60	0.53	0.53	0.60	0.66	0.66	0.73	0.66	0.60	1.00																								
24	0.33	0.53	0.53	0.60	0.66	0.26	0.73	0.80	0.66	0.60	0.73	0.66	0.40	0.46	0.66	0.53	0.60	0.66	0.53	0.73	0.80	0.46	0.86	1.00																							
25	0.53	0.60	0.33	0.53	0.60	0.46	0.66	0.60	0.60	0.53	0.53	0.60	0.73	0.66	0.46	0.60	0.66	0.86	0.73	0.66	0.46	0.66	0.80	0.66	1.00																						
26	0.46	0.53	0.66	0.60	0.80	0.40	0.73	0.80	0.53	0.60	0.73	0.80	0.53	0.46	0.53	0.53	0.46	0.66	0.53	0.73	0.66	0.46	0.86	0.86	0.66	1.00																				
27	0.40	0.60	0.60	0.66	0.60	0.60	0.80	0.46	0.60	0.53	0.53	0.60	0.60	0.53	0.60	0.46	0.40	0.33	0.60	0.53	0.73	0.66	0.66	0.53	0.46	0.66	1.00																			
28	0.53	0.46	0.60	0.53	0.46	0.46	0.66	0.60	0.60	0.80	0.53	0.73	0.46	0.66	0.73	0.46	0.53	0.46	0.60	0.66	0.73	0.53	0.53	0.53	0.46	0.53	0.60	1.00																		
29	0.46	0.66	0.53	0.60	0.53	0.40	0.60	0.53	0.66	0.46	0.60	0.53	0.66	0.60	0.53	0.40	060	0.40	0.53	0.46	0.66	0.60	0.60	0.60	0.53	0.60	0.80	0.40	1.00																
30	0.46	0.53	0.66	0.73	0.66	0.40	0.60	0.66	0.60	0.86	0.66	0.53	0.46	0.66	0.53	0.60	0.53	0.53	0.73	0.80	0.46	0.73	0.86	0.53	0.86	0.66	0.53	0.73	0.53	1.00														
31	0.40	0.46	0.46	0.66	0.46	0.46	0.53	0.60	0.60	0.53	0.66	0.60	0.46	0.53	0.73	0.60	0.53	0.60	0.60	0.66	0.73	0.53	0.53	0.66	0.60	0.53	0.46	0.73	0.40	0.66	1.00													
32	0.60	0.53	0.53	0.60	0.66	0.53	0.60	0.66	0.53	0.73	0.60	0.66	0.53	0.46	0.53	0.80	0.60	0.93	0.66	0.86	0.53	0.46	0.73	0.73	0.80	0.73	0.40	0.53	0.33	0.60	0.66	1.00												
33	0.60	0.40	0.40	0.60	0.53	0.66	0.60	0.53	0.66	0.60	0.73	0.53	0.66	0.46	0.80	0.80	0.46	0.66	0.66	0.73	0.66	0.73	0.60	0.60	0.66	0.60	0.53	0.66	0.33	0.66	0.80	0.73	1.00											
34	0.73	0.66	0.40	0.46	0.66	0.66	0.60	0.40	0.53	0.60	0.46	0.53	0.66	0.46	0.66	0.66	0.46	0.80	0.66	0.60	0.53	0.60	0.46	0.46	0.66	0.46	0.53	0.33	0.33	0.53	0.73	73	0.53	1.00										
35	0.60	0.40	0.53	0.46	0.53	0.66	0.20	0.26	0.26	0.33	0.46	0.40	0.53	0.33	0.40	0.66	0.33	0.53	0.53	0.46	0.26	0.60	0.20	0.20	0.40	0.33	0.40	0.40	0.33	0.33	0.53	0.46	0.60	0.60	1.00									
36	0.40	0.46	0.33	0.40	0.46	0.33	0.26	0.33	0.26	0.60	0.20	0.26	0.40	0.46	0.33	0.40	0.33	0.33	0.40	0.46	0.33	0.26	0.33	0.40	0.26	0.40	0.46	0.40	0.33	0.46	0.40	0.40	0.60	0.40	0.60	1.00								
37	0.53	0.33	0.53	0.60	0.66	0.40	0.73	0.66	0.53	0.60	0.60	0.80	0.40	0.46	0.66	0.53	0.46	0.66	0.66	0.73	0.66	0.60	0.73	0.73	0.66	0.73	0.53	0.80	0.33	0.60	0.80	0.73	0.73	0.60	0.46	0.53	1.00							
38	0.66	0.46	0.60	0.66	0.60	0.60	0.53	0.73	0.73	0.66	0.93	0.73	0.73	0.40	0.73	0.73	0.53	0.60	0.60	0.80	0.60	0.66	0.66	0.66	0.60	0.80	0.60	0.60	0.53	0.80	0.60	0.66	0.80	0.53	0.53	0.33	0.66	1.00						
39	0.60	0.66	0.53	0.60	0.80	0.40	0.60	0.66	0.53	0.73	0.60	0.66	0.53	0.46	0.53	0.66	0.73	0.93	0.80	0.86	0.53	0.60	0.73	0.73	0.80	0.73	0.40	0.53	0.46	0.60	0.53	0.86	0.60	0.73	0.46	0.40	0.73	0.66	1.00					
40	0.46	0.66	0.40	0.73	0.66	0.53	0.73	0.53	0.66	0.60	0.60	0.53	0.66	0.60	0.66	0.53	0.73	0.66	0.93	0.73	0.66	0.86	0.73	0.60	0.80	0.60	0.66	0.66	0.60	0.60	0.66	0.60	0.73	0.60	0.46	0.40	0.73	0.66	0.73	1.00				
41	0.46	0.53	0.40	0.60	0.53	0.40	0.60	0.53	0.66	0.60	0.60	0.53	0.66	0.60	0.53	0.66	0.73	0.66	0.80	0.73	0.53	0.86	0.73	0.60	0.80	0.60	0.66	0.53	0.73	0.60	0.53	0.60	0.60	0.46	0.46	0.40	0.60	0.66	0.73	0.86	1.00			
42	0.66	0.60	0.60	0.53	0.60	0.46	0.66	0.60	0.73	0.80	0.66	0.73	0.73	0.66	0.73	0.60	0.66	0.73	0.73	0.80	0.73	0.66	0.66	0.66	0.73	0.66	0.60	0.73	0.66	0.66	0.60	0.66	0.66	0.66	0.40	0.33	0.66	0.73	0.80	0.80	0.80	1.00		
43	0.46	0.53	0.53	0.73	0.53	0.53	0.73	0.66	0.80	0.73	0.73	0.66	0.66	0.60	0.66	0.66	0.73	0.66	0.80	0.86	0.66	0.73	0.86	0.73	0.80	0.73	0.66	0.66	0.60	0.73	0.66	0.73	0.73	0.46	0.33	0.26	0.73	0.80	0.73	0.86	0.86	0.80	1.00	
44	0.33	0.80	0.53	0.60	0.66	0.40	0.73	0.40	0.53	0.46	0.33	0.53	0.40	0.60	0.53	0.26	0.60	0.53	0.66	0.46	0.66	0.46	0.60	0.60	0.53	0.46	0.53	0.53	0.60	0.46	0.53	0.46	0.33	0.60	0.33	0.40	0.60	0.26	0.60	0.60	0.46	0.53	0.46	1.00	
45	0.60	0.66	0.26	0.46	0.40	0.40	0.46	0.66	0.53	0.46	0.46	0.53	0.66	0.60	0.40	0.40	0.60	0.53	0.40	0.33	0.40	0.46	0.46	0.46	0.66	0.46	0.53	0.40	0.73	0.46	0.40	0.46	0.33	0.46	0.33	0.66	0.33	0.40	0.46	0.46	0.60	0.53	0.46	0.46	1.00	
46	0.73	0.66	0.53	0.73	0.66	0.53	0.33	0.66	0.53	0.60	0.73	0.53	0.66	0.46	0.53	0.66	0.73	0.80	0.66	0.73	0.53	0.46	0.46	0.60	0.66	0.60	0.40	0.40	0.60	0.73	0.66	0.73	0.60	0.60	0.60	0.53	0.46	0.66	0.73	0.60	0.60	0.66	0.60	0.46	0.60	1.00

IV B1A was further subdivided into cluster IV B1A 1A and cluster IV B1A1B with 77% similarity.

Cluster IVB1A1A2A contains the genotype PCPGR-24. Cluster IVB1A1A2B contains the genotype PCPGR-45. Was again subdivided into cluster was divided into cluster IVB1B1 and cluster IVB1B2 with 72% similarity. Cluster was divided into cluster IVB2' and IVB2'' with 68% similarity Cluster IVB2'A and IVB2'B contains the genotype Cluster IVB2'A contains the genotype PCPGR-13 Cluster IVB2'B contains the genotype Pant Khira-1. Sub-group IVB2'A consisted of Cluster IVB2'A1 and Cluster IVB2'A2 at similarity of 73%. In cluster IVB2'A1A divided in to two groups IVB2'A1A1 contains the genotype PCUC-08 in cluster IVB2'A1A2 contains the genotype PCPGR-20 in cluster IV2'A2 divided into two cluster IVB2'A2A and IVB2'A2B with 83% similarity. Cluster IVB2'A2A contains the genotype PCPGR-15 and PCPGR- 4343. In cluster IVB2'A2A2 contains the genotype PCPGR-7207. In cluster IVB2'B divided in two sub group IVB2'B1 and IVB2'B2 with 83% similarity in cluster IVB2'B1 divided in two sub group IVB2'B1A and IVB2'B1B with 86% similarity. Cluster IVB2'B1A contains the genotype PCPGR-24.Cluster IB2'B1A contains the genotype PCPGR-19 AND PCPGR-7557 and IVB2'B1B contains the genotype PCPGR-7657. Cluster IVB1A was again subdivided into cluster IVB1A1 and IVB1A2 with 68% similarity. Cluster IVB1A1 divided in two sub group IVB1A1A and IVB1A1B with 73% similarity. Cluster IVB1A1A divided in two sub group IVB1A1A1 and IVB1A1A2 with 73% similarity. Cluster IVB1A1A2 divided in two sub group IVB1A1A2A, IVB1A1A2B and IVB1A1A2C with 87% similarity. Subgroup IVB1B divided into two sub-group IVB1B1 and IVB1B2 with 73% similarity. Cluster IVB1B1 was again subdivided into cluster IVB1B1A and IVB1B1B with 73% similarity. Cluster IVB1B1A was again subdivided into cluster IVB1B1A1 and IVB1B1A2 with 73% similarity.

Cluster IVB1B1A1contains the genotype PCUC-25 and PCPGR-7027. Cluster IVB1A2A1 contains the genotype PCPGR-19 and cluster IVB1A2A2 contains the genotype PCPGR-06.The electrophoresis of protein is a method to investigate genetic variation and to classify plant varieties (Sawant, and More, 8). Its banding pattern is very stable which advocated for cultivars identification purposes in crops. It has been widely suggested that such banding pattern could be important supplemental method for cultivar identification (Tanksley, and Jones, 17).

Analyses of SDS-PAGE are simple and inexpensive which are added advantages for use in practical plant breeding.The third is the similarity index used by Vaughan and Denford (19) which expresses the variation in the banding pattern between two gels. This similarity index was used in analysis the diallel progeny. Usefulness of seed protein profile for taxonomic and evolutionary purposes has been reviewed by several investigators (Johnson, 7). Seed protein variants have been observed to be the most widely used biochemical markers during the last century. Its reliability depends on polymorphism of seed and seedlings proteins and the fact that these proteins present primary gene products. Moreover these have advantage of being scorable from inevitable organs or tissues and the electrophoretic protocol for bulk protein assay is generally simple than that of isozyme(Cooke, 4).

Considering the magnitude of genetic divergence and magnitude of cluster means for different characters performance the genotypes may be used for developing high yielding cucumber varieties. These genotypes were found promising for most of characters. Therefore, it is recommended that such genotypes which were found promising for most of traits including yield, can be utilized as commercial cultivar of Cucumber after following the standard release procedure of variety in India. Protein profiling of genotypes resolved all forty six indigenous germplasm lines into different groups on the basis of presence and absence of fifteen protein bands distributed into A, B and C zone respectively.

REFERENCES

1. Arunkumar, K.H., Ramanjinapa, V. and Ravishankar, M. 2011. Path coefficient analysis in F2 population of cucumber (*Cucumis sativus* L.). Plant Archives, **11**: 471-74.

2. Arunkumar, K.H., Patil, M.G., Hanchinamani, C.N., Goud and I.S., Hiremath, S.V. 2011. Genetic relationship of growth and development traits with fruit yield in F2 population of BGDL × Hot season of cucumber (CucumissativusL.). Karnataka J. Agric. Sci. 24: 497-500.

3. Arus, P. 1983. Genetic purity of commercial seed lots. In. S.D. Tanksley and TJ. Orton (eds.). Isozymes on Plant Genetics and Breeding: Part A. Amsterdam, Elsevier Science Publishers. pp. 415-23.

4. Cooke, R.J. 1984. The characterization and identification of crop cultivars by electrophoresis. Electrophoresis, 5: 59-72.

5. Dadlani and Varier, A. 1993. Electrophoresis of variety identification. Technical Bulletin. Division of Seed Science and Technology, IARI, New Delhi.

6. Harlan, J. R. 1975. Crops and Man. ASA and CSSA, Madison, WI.

7. Johnson, B.L. and thein, M.M. 1969. Assessment of evolutionary affinities in gossypium by protein electrophoresis. American J. Bot. 57: 1081-92.

8. Sawant, S.V.; and More, T.A. 2002. Electrophoretic variation for seed protein of muskmelon genotypes. Acta. Hort. 588: 247-54.

9. Smith, D. B. and Simpson, P.A. 1983. Relationships of barley proteins soluble in sodium do decylsulphate to malting quality and varietal identification. J.Cereal Sci. 1: 185-97.

10. Smith, I.S.C. and Smith, A.S. 1992. Fingerprinting crop of varieties. Adv. Agron. 47: 85-140.

11. Saiki, R.K.; Gelfand, D.H.; Stofell, S.; Scharf, S.J.; Higich, R.; Horn, G.T.; Mulis, K.B. and Erlich, H.A. 1988. Primer directed enzymatic Amplification of DNA with anthermostable DNA Polymerase. Sci. 239: 487-91

12. Sambrook, T.; Fritsch, E.F. and Maniathis, T. 1989. Molecular cloning: A Laboratory manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, NewYork 2nd ed. Vol. 3.

13. Tanksley, S.D. and McCouch, S. R. 1997. Seed Banks and Molecular Maps: Unlocking Genetic Potential from the Wild. Science, 277.

14. Tatlioglu, T. 1993. Cucumber (Cucumis sativus L.). In: Kalloo, G. and Bergh, B.O. eds., Genetic improvement of vegetable crops. Pergamon Press, Ltd; Tarrytown New York, pp. 197-27.

15. Vaughan, J.G. and Denford, K.E. 1968. An acrylanmide gel electrophoretic study of the seed proteins of brassica and Sinapsis species with special reference to their taxonomic value. J. Exp. Bot. 19: 724-32

Received : August 2017; Revised : November, 2019; Accepted : November, 2019