Biological Forum – An International Journal **14(1): 627-631(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Study of Seed Protein Profiling of Cucumber (*Cucumis sativus* L.) Genotype using SDS-PAGE for Determination of Genetic Relationship

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(Received 25 October 2021, Accepted 27 December, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: A quantitative categorization of seed storage protein profile of 25 genotypes of *Cucumis sativus* L. was performed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The banding patterns were characterized by 3 clear district zones *i.e.* Zone A, Zone B and Zone C. Zone A has 9 bands, zone B has 10 bands and zone C included 8 bands. Maximum number of bands were observed in NDCUC-106 (9 bands) followed by NDCUC-160 (8 bands) whereas, minimum bands were shown by PPC-2, PPC-3, Pant Khira-1 and NDCUC-73 i.e. 1bands. The unweight pair group method using arithmetic average (UPGMA) analysis of 25 cucumber genotype was done and two major cluster obtained through seed protein analysis expressed better grouping of genotypes.

Keywords: Cluster analysis, Germplasm, SDS-PAGE, *Cucumis sativus* L., Cucumber.

INTRODUCTION

A cucumber (*Cucumis sativus* L., 2n=2x=14) belongs to the family Cucurbitaceae grown commercially throughout the country and is a most important summer vegetable crop of tropical and subtropical region. The fruits are rich in nutrients and vitamins and mainly consumed as salad, pickles, pudding etc. (Dhiman and Parkash, 2005). The centre of origin is of Asian region. *Cucumis sativus* var. *hardwickii* may be probable progenitor of cucumber, first found in the Himalayan Mountains and used by native peoples of Northern India as a laxative (Deakin *et al.*, 1971). In India, cucumber is cultivated in an area of 0.108 million ha with annual production of 1.663680 million tonnes (NHB, 2021). The cucumber was mainly grown in state of Madhya Pradesh, Haryana, Bihar, Orissa, Punjab, Rajasthan, Uttar Pradesh and West Bengal. Genetic diversity is extremely necessary for the effective breeding program and to conservation, maintenance, evaluation and utilization of germplasm because it is the only source to be exploited for the development of new varieties during breeding programs. The problem of cultivar identification has been solved to a great extent by the combined use of morphological, biochemical and molecular markers. Morphological markers are highly influenced by environment (Goodrich *et al.*, 1985). The biochemical markers are proteins that can be isolated and their polymorphism identified through electrophoresis. Biochemical markers such as proteins and isozymes have served as important tools to detect genetic relationships in plants (Mukhlesur *et al.*, 2004; Erum *et al.*, 2011). The genetic variation in plant varieties can be classified on the basis of electrophoresis of proteins (Isemura *et al.*, 2001). The banding pattern through electrophoresis of proteins is very stable and used for cultivars identification purpose in crop varieties. It has been widely accepted that such banding patterns could be important supplemental method for cultivars identification (Tanksley and Jones, 1981; Thanh and Hirata, 2002). Various electrophoresis methods are available which are used to identify cultivars by protein banding patterns. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) provides the best resolution image of banding pattern. Analysis of SDS-PAGE is simple and inexpensive, which is an added advantage for use in practical plant breeding. Therefore, present study was undertaken to characterize 25 genotypes of Cucumber through seed protein banding pattern.

MATERIAL AND METHOD

The experiment was conducted in biotechnology laboratory of the A.N.D.U. & T., Kumarganj, Ayodhya in 25 genotype of cucumber collected from different part of India. The SDS-PAGE is used for seed protein profiling as described by Laemmli, (1970) with slight modification. Each sample having 0.1g seed were crushed in 1 ml extraction buffer (1 M Tris-HCl pH 8.0, 2% SDS, 10% glycerol, 1mM PMSF- phenyl methyl sulphonyl fluoride and 2% mercaptoethanol). The mixture was heated at 65°C for 20 minute and centrifuged for 20 minute at 10,000 rpm at room temperature. The supernatant was collected and stored at 4°C for further use. Equal volume (20 µl) of supernatant *i.e.* protein sample and sample buffer were mixed, incubated at 60°C in water bath for 5 min and after cooling samples were loaded to each well along with marker protein in one well with help of micro syringe. The SDS solubilised protein samples were then subjected to vertical slab SDS-PAGE with 12.5% separating gel and 5% stacking gel using Tris-glycine electrode buffer. The samples were electrophoresed at 80 V initially and increased upto 100 V with current 500 mA, when the tracking dye passed from the stacking gel. The run was stopped when the dye was approximately 0.5 cm away from the bottom of the gel, which took around 6 to 7 hours. The gel was removed with the help of spatula and dipped for overnight in staining solution (0.2% coomassie brilliant Blue R 250, 60 g TCA,180 ml methanol and 60 ml glacial acetic acid). Next day, destaining was performed; the gel was observed on Syngene Gel Documentation system and photographed. Protein bands were scored for their presence as 1 and absence as 0 from the de-stained gel. Presence and absence of the bands were entered in a binary data matrix. Based on results of electrophoretic band spectra, similarity index was calculated for all possible pairs of electrophoregrams. Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by UPGMA (unweighed pair group method using arithmetic average) method by SAHN clustering function of NTSYS-PC (Numerical taxonomy and Multivariate analysis System programme) version 2.0 (Rohlf, 1987).

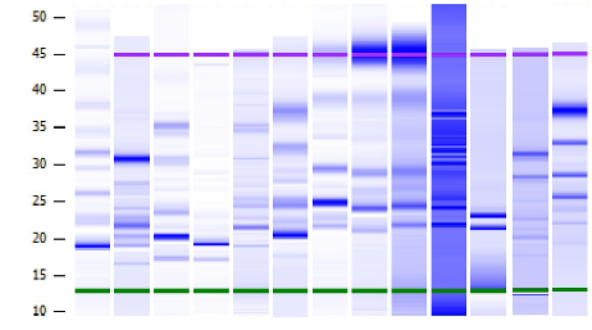

Plate 1. Protein profiling of cucumber through SDS-PAGE.

RESULT AND DISCUSSION

Seed protein and isozyme variants that move at different rates have been popularly used as a marker for characterization and classification of cucurbits (Dane, 1983; Knerr *et al.*, 1995). Variation is shown in the form of presence or absence of bands and the relative mobility of some electrophoretic bands were observed among the cucumber genotypes on the basis of seed proteins. The degree of variation in the bands is representing as a measure of genetic divergence among genotypes (Siddiqui and Naz, 2009). The seed protein fragments (Plate 1 and 2) exhibited detectable polymorphism amongst the 25 genotypes used in present study and the diagrammatic representation has been presented in Zymogram (Plate 3 and 4). A total of 27 protein bands were formed which were further classified under 3 distinct Zones A, Zone B and Zone C depending on their decreasing molecular weight and increasing Rf values (Plate 1 and Plate 2). The high molecular weight proteins were located in upper region and low molecular weight protein in the lower region of the gel. A standard medium range protein marker of known molecular weight (5 to 50 kDa) was used along with samples. Singh *et al*. (2015) have also reported 25 and 35 band while analyzing genotypes of *Cucurbits* and *C. grandis* respectively. The protein bands were stacked according to their molecular weight *i.e.* high molecular weight proteins were located in upper region and low molecular weight proteins in the middle and lower region of the gel, respectively. For genotype discrimination, the presence and absence of protein bands was the criteria for characterization of germplasm differentiation. Mann *et al*. (2005) reported that the number of bands give an account of polypeptides present in a protein. As equal amount of proteins is loaded in all, thus banding pattern shows range of proteins present in the respective varieties.

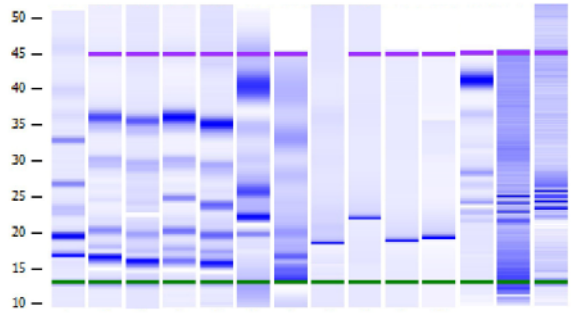

Plate 2. Protein profiling of cucumber through SDS-PAGE.

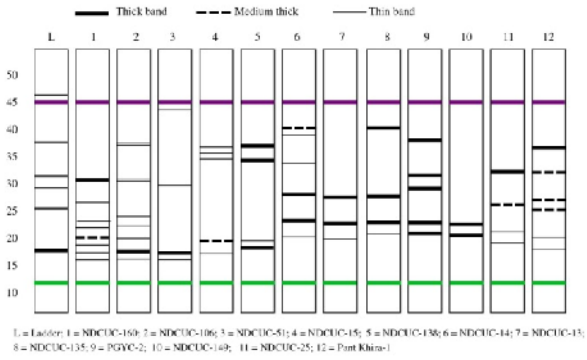

Plate 3. Protein profiling of cucumber through SDS-PAGE.

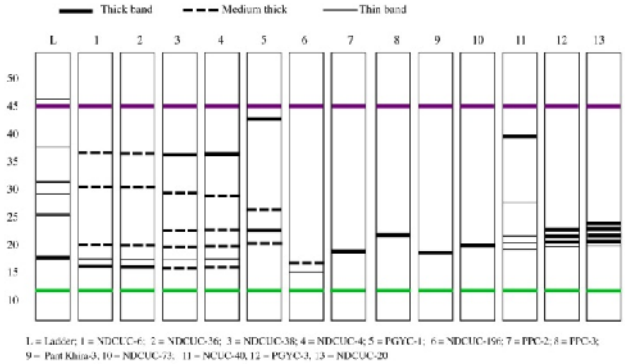

Plate 4. Protein profiling of cucumber through SDS-PAGE.

Among 27 bands, Zone A depicting the highest molecular weight protein ranges from 45-35 Kda was subdivided into 9 distinct bands (Plate 1 and 2). The subzone A1 of light intensity was present only in NDCUC-51. The subzone A2 of dark intensity was present only in PGYC-1wheras subzone A3 was present in NDCUC-14, NDCUC-135 and NDCUC- 40. The subzone A4 of light intensity was present only in NDCUC-14 whereas subzone A5 of dark intensity was present only in PGYC-2.The subzone A6 of light intensity was present only in one genotype *i.e.* NDCUC-106. The subzone A7 was present in eight genotypes, namely, NDCUC-106, NDCUC-15, NDCUC-138, Pant Khira-3, NDCUC-6, NDCUC-36, NDCUC-38 and NDCUC-4. The subzone A8 of light intensity was present only in one genotype i.e. NDCUC-15. Subzone A9 was present in NDCUC-15, NDCUC-138 and NDCUC-14. Zone B was subdivided into 10 subzones. Subzone B1 of dark intensity was present in NDCUC-25. Whereas B2 of dark intensity was present in PGYC-2. Subzone B3 was present in NDCUC-160 and NDCUC-106. Subzone B4 was present in two genotypes, namely, NDCUC-106 and PGYC-2. Subzone B5 of medium thick intensity was present in NDCUC-6, NDCUC-51 and NDCUC-36 whereas subzone B6 was present in NDCUC-38 and NDCUC-4. Subzone B7 of dark intensity was present in NDCUC-14 and NDCUC-40. In genotypes, NDCUC-13, NDCUC-135 and Pant Khira-3 subzone B8 was present. Subzone B9 was present in genotypes, namely, NDCUC-160, NDCUC-25 and PGYC-1. Subzone B10 was present in check *i.e.* Pant Khira-3. Zone C was subdivided into 8 bands where in band intensity varied from thick to thin. Subzone C1 was present in NDCUC-20 and NDCUC-106. Thus, a total of 27 bands are formed from 25 genotypes/varieties of cucumber. These results are similar with finding of (Singh and Singh, 2019).

For selection of superior genotypes based on diversity, cluster analysis help in distinguishes genotypes which could be used as basis of selection of genotypes (Punetha *et al.*, 2018). The dendrogram generated by UPGMA cluster analysis divided 25 genotypes into two major groups (Fig. 1) at about 68% similarity index. Group A comprised of cluster I and II at 74 % similarity. Cluster I consisted of genotype NDCUC-160 whereas cluster II contains only one genotype *i.e.* NDCUC-106. Group B was subdivided into 2 subgroups, Subgroup B1 and Subgroup B2. Subgroup B2 contains the genotype NDCUC-14. Subgroup B1 formed two clusters, namely, Cluster III and Cluster IV at similarity of 74%. NDCUC-38 and NDCUC-4 in one cluster had 100 % genetic similarity within group whereas in another cluster NDCUC-6 and NDCUC-36 and PPC-2 and Pant Khira-1 also expressed 100% genetic similarity. The dendrogram showed that the genotype NDCUC-14 was most dissimilar from other genotypes.

As genetic diversity and genetic variation is most important for successful breeding programmes as well as for taxonomic studies. Marker based on biochemical/banding pattern are used to distinguished differently diverse population. Different patterns of electrophoretic from seed protein profiling elucidate the different qualitative and quantitative expression of genotype. Morphological data is highly influenced by environment and based on that, characterization of genotypes/varieties is difficult because these characters may change with change with environments. Therefore, in vitro identification, selection and classification of genotypes/varieties for various qualitative and quantitative characters is of great magnitude as it helps in obtaining desirable result and hence proved a benefit to the researcher as well as farmers. Thus, the seed protein profiling emerges as a persuasive technique to generate wide diverse of polymorphism and as such it could serve valuable information for varietal identification and extent of genetic diversity. Further, an integrated approach utilizing both morphological and biochemical markers could help in proper characterization of cucumber germplasm.

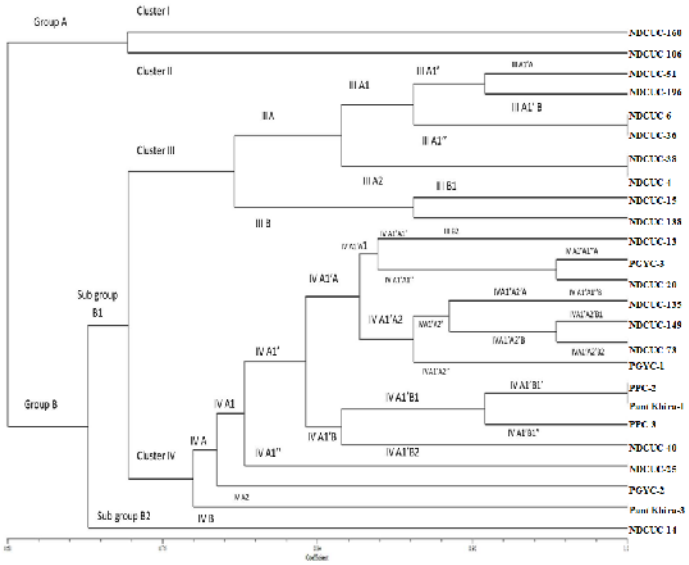

Fig. 1. Dendrogram of 25 different genotypes of cucumber.

CONCLUSION

Protein profiling is an effective method for identification of genetic diversity, combining biochemical approaches with molecular approaches would help plant breeder to understand the diversity/ variance among plants so it is concluded from the study that NDCUC-16 and NDCUC-16 and NDCUC-14 are most diverse among all genotypes and may be further utilized as potential source in cucumber breeding programme for the successful varietal development.

FUTURE SCOPE

Seed Storage proteins are highly independent of environmental fluctuations. The high stability of seed protein profile and its additive nature makes it a promising tool for distinguishing genotypes of particular plant species. Diversity and variation is pre-requisite tool for plant breeding. Without diversity, hybridity cannot exploit in heterosis. SSD-Page profiling is a cheap and effective tool to determine the difference in genotypes. NDCUC-16 and NDCUC-16 and NDCUC-14 are more diverse and have potential to use as a parent in breeding program.

Acknowledgements. Author thanks to the department of biotechnology and department of vegetable science, ANDUA&T, Kumarganj, Ayodhya for providing facility for my laboratory experiment.

Conflict of Interest. None.

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How to cite this article: Shashank Shekhar Singh and Khushboo Kathayat (2022). Study of Seed Protein Profiling of Cucumber (*Cucumis sativus* L.) Genotype using SDS-PAGE for Determination of Genetic Relationship. *Biological Forum – An International Journal*, *14*(1): 627-631.