A Case Study

ADVANCE RESEARCH JOURNAL OF
C R P
IMPROVEMENT
Volume 5 | Issue 2 | Dec., 2014 | 229-231
e ISSN-2231-640X

DOI :
10.15740/HAS/ARJCI/5.2/229-231
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Electrophoretic characterization of genotypes of cucumber (*Cucumissativus* L.) based on seed protein profiles

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ABSTRACT : Twenty germplasm lines of cucumber (*Cucumber sativus*) were characterized by sodium dodecyl sulphate polyacrylamide vertical slab gel electrophoresis (SDS-PAGE). The seed protein could be resolved into total 11 bands distributed in 3 zones *i.e.* A, B and C. Zone A has 5 bands, zone B has 3 bands and zone C included 3 bands. Based on electrophoregrams genotypes were classified into 6 dissimilar groups. It was possible through seed protein profiles to distinguished morphologically similar genotypes. Hence, seed protein profiles proved useful in identifying cucumber genotypes.

Key **W**ords : Electrophoretic characterization, Genotypes, Cucumber, Seed protein

How to cite this paper : Kandasamy, R. (2014). Electrophoretic characterization of genotypes of cucumber (*Cucumissativus* L.) based on seed protein profiles. *Adv. Res. J. Crop Improv.*, **5** (2) : 229-231.

Paper **H**istory : **Received :** 12.11.2014; **Accepted :** 25.11.2014

Cucumber (*Cucumissativus* L.) is an important summer vegetable crop of tropical India. A wide range of genetic variability is available in cucumber. Releasing large number of varieties and increasing morphological similarities between them, it would make bit of confusion among plant breeders and producers. So it is necessary to differentiate one cultivar form the other cultivars. Seed protein and isozyme variants that migrate different rates have been extensively used as a marker of characterization of cucurbits (Dane, 1983; Knerr *et al.*, 1995). Seed protein has the advantage of being scorable, from inviable organ or tissues and the electrophoretic protocols for bulk protein assay are generally simpler than for isozymes (Gepts, 1990). The following experiment was carried out to characterize the twenty genotypes of cucumber through seed protein profiles.

RESEARCH PROCEDURE

Twenty culinary melongenotypes were collected from different locations in Tamilnadu and used for electrophoresis. Approximately 5-6 seeds was ground in 1 ml extraction buffer [0.0625 N TrisHCl, 2% Sodium Dodecyl Sulphate (SDS), 10% glycerol, 1mM EDTA and 2% 2-mercaptoethanol]. The ground material was heated in water bath at 100^0C for 5 minutes followed by centrifugation at 1000 rpm for 15 minutes at 4^0C temperature. Supernatant were stored at -85^0C for further use.

The separating gel of 15 per cent and stacking gel of 4 per cent was prepared (Laemmli, 1970) with slight modification. Separating gel was prepared by mixing 10 ml of 30 per cent (w/v) acrylamide and 0.8 per cent (w/v) N, N, tetra methylene trisacrylamide, 4.7 ml distilled water, 5 ml of 1.5 MTris, 0.01 ml of 10 per cent APS and 0.1 ml of N. N-Tetra methylene diamine (TEMED). Gel was left for one hour at room temperature to polymerize. Equal volume of protein sample (10 µl) and sample buffer (10µl) (Tris buffer 1.5 g), Glycerol (20 ml), SDS (2 g), mercaptoethanol (2 mg), bromophenol blue (2 mg) and final volume was made (100 ml) was mixed in an eppendorf tube and it was heated at 70^0C for 5 minutes. Based upon the Bradford method of protein value above samples were loaded in individual well. 1000 ml of electrode buffer (3 g Tris, 14.4 g glycine, 1 g SDS and volume made to 1000 ml) was poured in upper and lower buffer tank. Electrophoresis was performed at room temperature for 4 hours at constant voltage 100 v/gel. After completion of electrophoresis the gel was transferred to staining solution containing 0.1 g co-omassie Brilliant Blue, 40 ml methanol, 10 ml of glacial acetic acid and 50 ml of distilled water, staining continued overnight and it was transferred to

destaining solution (same composition without staining agent). The electrophoregrams of protein profile were prepared and gel was photographed. The presence and absence of data bands was used for similarity index and unweighted pair group method using arithmetic averages (UPGMA) analysis.

RESEARCH ANALYSIS AND REASONING

The examination of gels clearly showed that they were 3 major separation zones *i.e.* A, B and C in seed protein of 20 germplasm collection (Table 1). Zone A represented the heaviest molecular weight proteins was sub divisible into 5 thin and distinct bands *i.e.* A_1, A_2, A_3, A_4 and A_5. Zone B was sub divisible into 3 bands B_1, B_2 and B_3 represented thin and thick bands. Zone C represented dark to light bands and was sub divisible into 3 bands *i.e.* C_1, C_2 and C_3. Thus, 20 cucumber genotypes resolve a total of 10 bands as summarized in Table 2. Grouping of genotypes mainly based on A zone banding pattern. Among the zone the light and sharp band A_1 was invariably present in genotype 19 and absent in 1 genotype. So the dissimilar groups were mainly based on presence/ absence of A_2, A_3, A_4 and A_5 bands.

B zone consisted of B_2 and B_3 band were present among all the genotypes. In B zone, grouping of genotypes was based on the presence/absence of B_1 band. In C region the dark and thick band C_1, C_2 and C_3 were commonly present in all the genotypes. Group I had 8 bands *i.e.* A_1, A_3, B_1, B_2, B_3, C_1, C_2 and C_3. This group had 5 genotypes. Group II was characterized by missing of A_4 and A_5 bands. Only one band of A_5 was found lacking in group III. This group consisted of 3 genotypes like CS_4, CS_{11} and CS_{20}. Group IV was obtained on the basis of missing totally three band *i.e.* A_2 and A_3 bands in A zone and B_1 band in B zone. In group V did not had A_3, A_4 and A_5 bands in A zone and all other bands in B and C zones are present.

Table 1 : SDS-PAGE banding pattern for seed protein in different genotypes of cucumber

Sr. No.	Genotype	A_1	A_2	A_3	A_4	A_5	B_1	B_2	B_3	C_1	C_2	C_3
1.	CS_1	+	-	+	-	-	+	+	+	+	+	+
2.	CS_2	+	-	+	-	-	+	+	+	+	+	+
3.	CS_3	+	+	+	-	-	+	+	+	+	+	+
4.	CS_4	+	+	+	+	-	+	+	+	+	+	+
5.	CS_5	-	-	-	-	-	+	+	+	+	+	+
6.	CS_6	+	-	+	-	-	+	+	+	+	+	+
7.	CS_7	+	+	+	-	-	+	+	+	+	+	+
8.	CS_8	+	+	+	-	-	+	+	+	+	+	+
9.	CS_9	+	-	+	-	-	+	+	+	+	+	+
10.	CS_{10}	+	+	+	-	-	+	+	+	+	+	+
11.	CS_{11}	+	+	+	+	-	+	+	+	+	+	+
12.	CS_{12}	+	+	+	-	-	+	+	+	+	+	+
13.	CS_{13}	+	+	+	-	-	+	+	+	+	+	+
14.	CS_{14}	+	-	+	-	-	+	+	+	+	+	+
15.	CS_{15}	+	+	-	-	-	+	+	+	+	+	+
16.	CS_{16}	+	-	-	+	+	-	+	+	+	+	+
17.	CS_{17}	+	+	+	-	-	+	+	+	+	+	+
18.	CS_{18}	+	-	-	+	-	-	+	+	+	+	+
19.	CS_{19}	+	+	+	-	-	+	+	+	+	+	+
20.	CS_{20}	+	+	+	+	-	+	+	+	+	+	+

Table 2 : Genotype showing dissimilar protein profiles

Group	Genotypes	Number	Bands											No. of protein bands
			A_1	A_2	A_3	A_4	A_5	B_1	B_2	B_3	C_1	C_2	C_3	
I	CS_1, CS_2, CS_6, CS_9, CS_{14}	5	+	-	+	-	-	+	+	+	+	+	+	8
II	CS_3, CS_7, CS_8, CS_{10}, CS_{12}, CS_{13},CS_{17}, CS_{19}	8	+	+	+	-	-	+	+	+	+	+	+	9
III	CS_4, CS_{11}, CS_{20}	3	+	+	+	+	-	+	+	+	+	+	+	10
IV	CS_{16}, CS_{18}	2	+	-	-	+	+	-	+	+	+	+	+	8
V	CS_{15}	1	+	+	-	-	-	+	+	+	+	+	+	8
VI	CS_5	1	-	-	-	-	-	+	+	+	+	+	+	6

However, all the bands of A zone was absent in group VI. Thus, this technique is very useful to distinguish valuable cultivars. The utility of this technique has been demonstrated by many workers in cultivar identification (Cooke, 1984; Henn *et al.*, 1992). When this technique was used in cucumber lines to identification, four to six isozyme phenotypes were found to be common to the cultivated types and the ancestral species (*Cucumissativus* var. *hardwickii*) by (Isshiki *et al.*, 1972). Thus, minor genetic differences may not give rise to variation for all protein profiles. The cultivars which were based on morphological features could be distinguished through electrophoresis of protein/enzymes have been reported in bottle gourd (Upadhyaya *et al.*, 1998) and in muskmelon (Choudhary and HariHar Ram, 2000). Therefore, it may be concluded that the efficiency of protein profiles in distinguishing several genotypes of cucumber was successful in most of the case. Similar results in indigenous genotypes have also been reported (Singh and Ram, 2000; Anita Singh and Harihar Ram, 2005).

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