Indian J. Hort. 67(3), September 2010: 343-347

Biochemical characterisation of parthenocarpic gynoecious cucumber lines, hybrids, monoecious varieties and wild relatives

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ABSTRACT

To accentuate the field study, biochemical characterization of total fifteen genotypes including four parthenocarpic gynoecoius cucumber lines and their three hybrids indigenously developed at pantnagar, four monoecious varieties (*Cucumis sativus* L.), three wild relatives (*C. sativus var. harwickii*) and a backcross [(PCUCP 3 × Poinsette) PCUCP 3] were subjected to seed protein analysis through SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis). Similar protein banding pattern was observed for *C. sativus* L. and *C. sativus* var. *hardwickii* genotypes. However, specific protein bands B_2 (0.31) and B_4 (0.35) present in *C. sativus* var. *hardwickii* 2316, precisely distinguished this genotype from other genotypes of *C. sativus* as well as *C. sativus* var. *hardwickii.* The presence of three distinct protein bands (A_1, B_9 and B_{10}) in parthenocarpic gynoecious genotype PCUCP 4 notably differentiated this genotype from all other genotypes used in the study. All four field bred monoecious varieties of cucumber used in the study can be differentiated on the basis of presence or absence of one or other band. On the basis of UPGMA analysis, fifteen genotypes were categorized into two major groups. Cluster one comprised of 11 genotypes while cluster two consisted of 4 genotypes.

Key words: Parthenocarpy, *C. sativus* L., *C. sativus* var. *hardwickii*, SDS-PAGE, cluster analysis.

INTRODUCTION

Seed protein and isozyme variants that migrate at different rates under electrophoresis have been extensively used as molecular genetic markers for characterization of species and cultivars. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) provides the best resolution among all the available electrophoretic methods to identify cultivars by protein banding pattern (Smith and Simpson, 7). Seed protein profile has been increasingly utilized for the varietal identification and characterization of genotypes. The high stability of seed protein profile, its additive nature and negligible effect by environmental conditions or seasonal fluctuations makes it a unique and powerful tool (Ladizinsky and Hymowitz, 4). Moreover, the biochemical techniques are quicker, less labor intensive and more reliable than the traditional methods, since the expression of isozyme loci are co-dominant and not altered by environmental factors (Smith and Smith, 8). Thus, a total of fifteen genotypes including the parthenocarpic gynoecious line and hybrids, monoecious varieties, wild relatives and a backcross were subjected to seed protein analysis through SDS-PAGE.

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MATERIALS AND METHODS

The seed protein electrophoresis using SDS-PAGE as per Leammli (3) was carried out in Biotechnology and Seed Tech. Lab, Department of Vegetable Science, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar. Four parthenocarpic gynoecious cucumber lines (PCUCP 1, PCUCP 2, PCUCP 3 and PCUCP 4) and three parthenocarpic gynoecious hybrids (PCUCP 1 × Poinsette, PCUCP 3 × Poinsette and PCUCP 4 × Poinsette) indigenously developed at pantnagar, four monoecious lines (Poinsette, PCUCP 8, PCUCP 15 and PCUCP 28), three wild relatives (*C. sativus* var. *hardwickii* 2314, *C. sativus* var. *hardwickii* 2315, *C. sativus* var. *hardwickii* 2316) and a backcross (PCUCP 3 × Poinsette) × PCUCP 3 were used for the study.

Seed coat was removed with the help of a scraper. A sample of 0.1 g of seed cotyledon was ground in a mortar pestle, until well mixed. One ml of extraction buffer (1M Tris-HCl- pH 8.0, 2% SDS, 10% glycerol, 1mM PMSF- phenyl methyl sulphonyl fluoride and 2% mercaptoethanol) was added and further crushed. The ingredients were then transferred to an eppendorf tube. The sample was homogenized and incubated in boiling waterbath at 100°C for five min. The contents were centrifuged at 10,000 rpm for thirty minutes. The supernatant was then transferred to a fresh tube and stored at 4°C. Loading sample was prepared by appropriately diluting the extracted protein with sample

buffer (Tris-pH 7.4, 2% SDS, 2% mercaptoethanol and bromophenol blue) and further heating in boiling water bath for 5 min. at 65°C just before loading the gel.

The SDS solubilized protein samples were then subjected to vertical SDS-PAGE with 12.5 % separating and 4% stacking gels using Tris-glycine electrode buffer (Tris-glycine and SDS, pH 8.6). The samples were then electrophoresed at a constant voltage of 100 V. The run was stopped when dye front was approximately 0.5 cm from the bottom of the gel. The gels were then immersed in overnight 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 solutions (3% NaCl). The gels were intermittently and carefully shaken and destaining solution was changed till the blue colour of the background of the bands disappeared. The gels were then visualized on a Syngene Gel Documentation system and photographed. The clustering was performed with Jaccard's coefficient of similarity and UPGMA clustering using NTSYS PC (Version 2.0i).

RESULTS AND DISCUSSION

The seed protein fragments (Fig. 1) exhibited appreciable polymorphism amongst the fifteen genotypes used for the study and the diagrammatic representation has been depicted in zymogram, (Fig. 2). A total of sixteen protein bands were obtained which were further categorized under three distinct zones A, B and C depending on their decreasing molecular weights and increasing Rf values. The maximum numbers of protein bands were resolved in parthenocarpic gynoecious genotype PCUCP 4 with eleven out of total sixteen protein fragments, while the minimum number were reported in monoecious genotype PCUCP 15 with seven fragments.

The zone A represented heaviest molecular weight proteins ranging from above 97 to 66 KDa. The band A_1 was resolved in eleven genotypes except PCUC 15, PCUC 28, *C. sativus* var*. hardwickii* 2314 and the backcross (PCUC 3 × Poinsette) × PCUC 3, while unique band A_2 (0.21) was present only in PCUCP 4. The zone B comprised of ten bands and the major differences in the protein banding pattern are mainly confined to this zone. The protein band B_1 (Rf 0.27) was discernibly present in all the fifteen genotypes with variable band intensity. B_2 was present in thirteen genotypes except genotypes PCUCP 1 × Poinsette and *C. sativus* var*. hardwickii* 2316, while B_6 was present in twelve genotypes except PCUCP1 × Poinsette, PCUC 8 and PCUCP 15. Similarly, protein band B_8 (Rf-0.43) was found to be present in all the fourteen genotypes except PCUCP 4. The wild relative *C. sativus* var. *hardwickii* 2316 exhibited the presence of unique bands B_2 (Rf-0.31) and B_4 (Rf-0.35). Parthenocarpic

Table 1. Presence (+) and Absence (-) pattern of protein bands in fifteen genotypes as inferred from protein profile.

Genotype	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
PCUCP 1	+	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+
PCUCP 2	+	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+
PCUCP 3	+	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+
PCUCP 4	+	+	+	0	+	0	0	+	0	0	+	+	+	+	+	+
PCUCP 1 × Poinsette	+	0	+	0	0	0	+	0	+	+	0	0	+	+	+	+
PCUCP 3 × Poinsette	+	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+
PCUCP 4 × Poinsette	+	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+
Poinsette	+	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+
PCUC 8	+	0	+	0	+	0	0	0	+	+	0	0	+	+	+	+
PCUC 15	0	0	+	0	+	0	0	0	0	+	0	0	+	+	+	+
PCUC 28	0	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+
C. hardwickii 2314	0	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+
C. hardwickii 2315	+	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+
C. hardwickii 2316	+	0	+	+	0	+	0	+	0	+	0	0	+	+	+	+
(PCUCP 3 × Poinsette) PCUCP 3	0	0	+	0	+	0	0	+	0	+	0	0	+	+	+	+

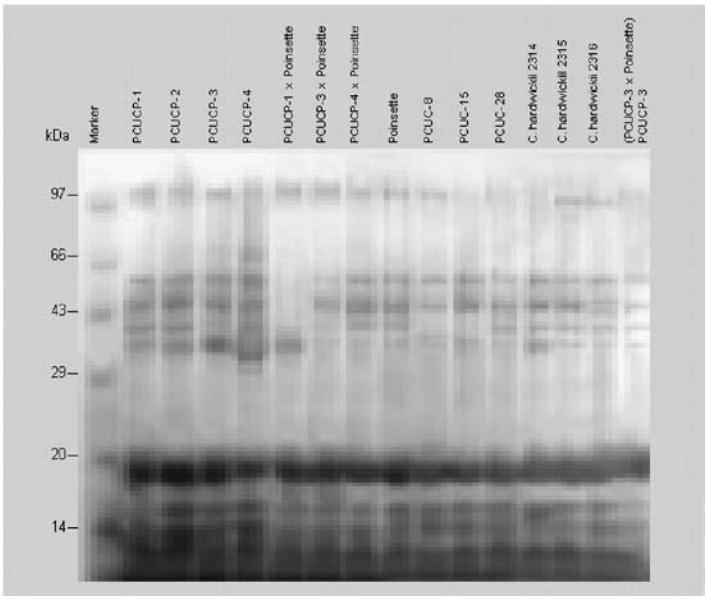

Fig. 1. Seed protein profile for parthenocarpic lines (PCUCP-1, PCUCP-2, PCUCP-3, PCUCP-4), parthenocarpic hybrids (PCUCP-1 × Poinsette, PCUCP-3 × Poinsette, PCUCP-4 × Poinsette), field bred varieties (Poinsette, PCUC-8, PCUC-15, PCUC-28), wild relatives (*C. hardwickii* 2314, *C. hardwickii* 2315, *C. hardwickii* 2316 and back cross (PCUCP-3 × Poinsette) PCUCP-3.

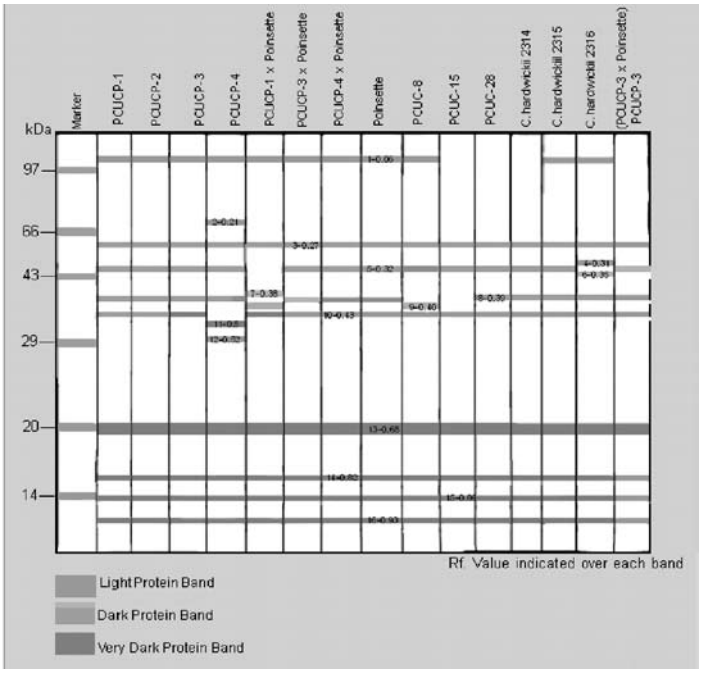

Fig. 2. Zymogram for the protein profile of fifteen parthenscarpic and wild cucumber genotypes.

gynoecious line PCUCP 4 showed distinct protein bands B_9 (0.5) and B_{10} (Rf-0.52) and the parthenocarpic gynoecious hybrid PCUCP 1 × Poinsette showed distinct bands B_5 (Rf-0.38).

Zone C included four protein bands with their corresponding molecular weights ranging from 20 to below 14 KDa and Rf values from 0.68 to 0.93, respectively. The protein band C_1 (Rf-0.68) exhibited

highest intensity and thickest band width among all the sixteen protein bands of the profile. Differences between accessions of the same taxon in darkness and thickness of various bands are the most commonly reported types of variation, suggesting that the formation of many of the bands in the seed protein profile are under control of qualitative gene systems. However darkness and thickness of band may also be due to lack of separation on the gels of several proteins having similar migration rates. All the four fragments of this zone were found to be present homogeneously in all the fifteen genotypes. This similarity probably indicates the common origin of the genotypes which further diversified in the process of evolution.

Based on the presence (+) and absence (-) of protein bands (Table 1) and UPGMA analysis, a dendrogram (Fig. 3) was constructed to group the genotypes on the basis of similarity in their protein banding pattern. Fifteen genotypes were classified into two broad clusters. One of the major clusters was further subdivided into two sub clusters (one major and one minor) and two independent genotypes. The large sub-cluster comprised of seven genotypes with namely three parthenocarpic gynoecious lines PCUCP 1, PCUCP 2, PCUCP 3, two parthenocarpic gynoecious hybrids PCUCP 3 × Poinsette, PCUCP 4 × Poinsette, one monoecious variety Poinsette and one wild relative *Cucumis sativus* var. *hardwickii* 2315. The smaller sub cluster comprised of two genotypes namely one monoecious variety PCUC 28, one wild relative *Cucumis sativus* var. *hardwickii* 2314 which showed genetic similarity between the two morphological distinct genotypes. This study is in similar to Isshiki *et al.* (2), who observed that four out of six isozyme phenotypes were found to be common to the cultivated type (*Cucumis sativus*) and the ancestral species (*Cucumis sativus* var. *hardwickii*). A cultivated plant and its immediate wild progenitor still form a common gene pool and can be considered from the genetic point of view, as members of the same species. Therefore, despite conspicuous morphological differences between them they will share, more or less, the same protein profile. Two other genotypes (PCUCP 3 × Poinsete) × PCUCP 3 and PCUC 15 shared closer affinities with the genotypes of the two sub-clusters, with approx imately 0.82 and 0.74 percent similarity. These eleven genotypes were grouped together in one major cluster which was observably distinct from a second major cluster comprising of a small sub cluster. Approximately 0.66 percent similarities were observed between the two major clusters. The two genotypes PCUCP1 × Poinsette and PCUC 8 were included in the sub-cluster of second major cluster and they shared a similarity percent of just 0.82 percent approx. Two genotypes namely *Cucumis sativus* var. *hardwickii* 2316 and PCUCP 4 emerged outstandingly distinct and

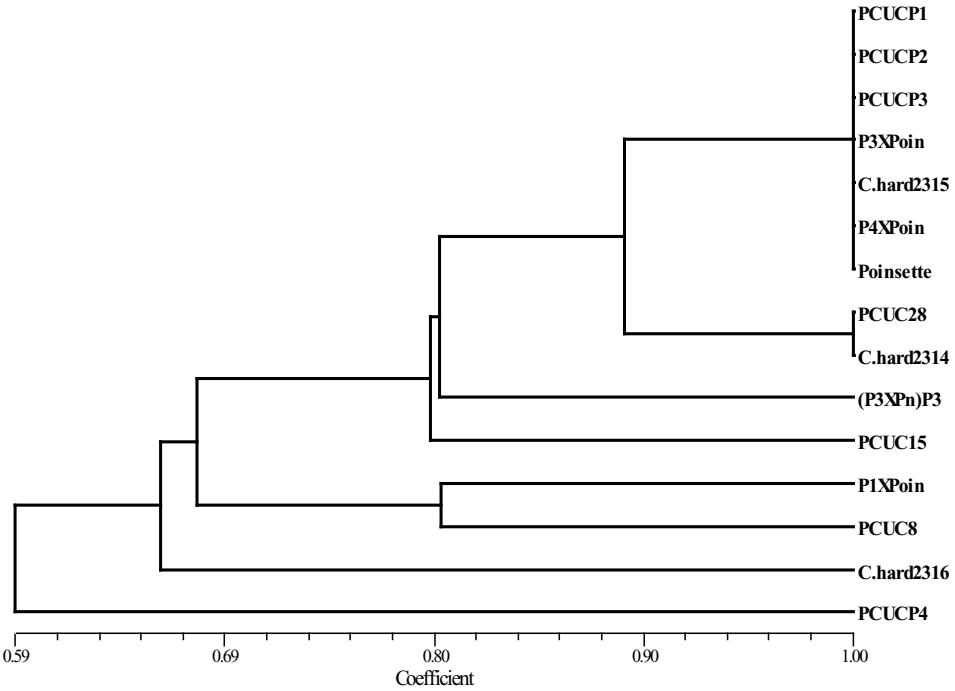

Fig. 3. Dendrogram of fifteen parthenocarpic and some wild cucumber genotypes.

independent of the other genotypes. Both exhibited a relatively low similarity percent of 0.66 and 0.59 with the two major clusters. Thus, these can be concluded as the most diverse genotypes amongst all fifteen. These two genotypes can be further utilized as potent germplasm lines in parthenocarpic cucumber breeding programme for development of parthenocarpic lines and hybrids and incorporation of multiple lateral branches from *Cucumis sativus* var. *hardwickii*.

All four field bred monoecious varieties of cucumber used in the study can be differentiated on the basis of presence or absence of one or other band which showed considerable diversity among them. All four gynoecious and parthenocarpic genotypes can be differentiated from two monoecious genotypes PCUC 15 and PCUC 28 by presence of A_1 band. However, other monoecious genotypes and gynoecious parthenocarpic genotypes had similar protein profile also. Similar protein profile had been reported in cucumber by Singh *et al*. (6) and in *C. melo* by Singh *et al*. (5) and Choudhary and Ram (1) also. Three gynoecious parthenocarpic genotypes had similar protein profile while 4th genotype had three distinct protein bands (A_1, B_9 and B_{10}), which could be differentiated from all other genotypes used in the study. *Cucumis sativus* var. *hardwickii* genotypes shared a common protein profile with *C. sativus* genotypes; however, *Cucumis sativus* var. *hardwickii* 2316 genotype was distinct from all other genotypes with presence of two unique bands.

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Received: October, 2008; Revised: April, 2010;
Accepted : August, 2010