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SEED STORAGE PROTEIN PROFILES IN CULTIVARS OF *CAPSICUM ANNUUM* L.

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Abstract
SDS-PAGE protein profile patterns were studied from seed material of 10 cultivars of *C. annuum* L. A total of 15 protein polypeptide bands with molecular weights ranging from 22.4 to 80.8kD were recorded. On the basis of seed protein banding nature three varieties could be identified clearly viz., (CA2, CA4 and CA5). Among the varieties CA9 manifested maximum (9) number of protein bands. The greatest similarity (100%) was observed between CA6 and CA7, while the lowest (40%) was noted between (CA3-CA6), (CA3-CA7) and (CA3-CA10). The UPGMA dendrogram represented low genetic diversity. The study revealed that large intra-specific differences were not found in the cultivars, but presence of considerable variations in protein profiles of the cultivars suggested that these selected varieties can be a good source for crop improvement through hybridization programs.

Key Words: *Capsicum*, SDS-PAGE, similarity index, UPGMA

Introduction
Information about genetic diversity of germplasm is a useful tool in gene bank management and in planning experiments, as it facilitates efficient sampling and utilization of germplasm. *Capsicum annuum* L. is an economically important crop plant belonging to the family Solanaceae, two main consumption types of pepper spice and vegetable are prevalent throughout the world. Varieties have been a land mark in the genetic improvement of chili pepper (*Capsicum annuum* L.), as it resulted in increase in its potential for fruit yield. Most of the varieties or cultivars within a *Capsicum annuum* L. complex show resemblance with their morphometrics. Conventionally, morphological descriptors are routinely used for establishing the identity of varieties. But these morphological descriptors suffer from many draw backs such as influence of environment on trait expression, epistatic interactions, pleotropic effects etc. Furthermore, the evaluation of plant material is often laborious and time consuming especially, when a large number of accessions are to be analyzed. Considering these difficulties the introduction of biochemical techniques has made possible and a more accurate evaluation of genetic variation, bringing greater precision to measures of genetic diversity. Among the biochemical techniques, DNA molecular markers currently in use are too expensive as compared to protein molecular markers. SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) is a most economical, simple and extremely used biochemical technique for describing the seed protein diversity of crop germplasm [1-3]. Genetic and taxonomic relationships in the genus *Capsicum* have been investigated with electrophoresis of seed storage protein banding patterns [4-8]. Genotypic variations of seed protein profiles in chili peppers have been reviewed [9-12]. However, polymorphism of seed storage protein profiles in *Capsicum annuum* L. and *Capsicum frutescens* L. germplasm has been associated with geographical origin [13-14]. Keeping in view the importance of protein profiling, the present study was conducted to characterize and estimate genetic diversity in 10 cultivars of *C. annuum* L., and this data may provides a scientific basis for future selection and germplasm management.

Materials and Methods

Seed material
Ten varieties of *Capsicum annuum* L. were denoted by these numbers i.e., (CA1, CA2, CA3, CA4, CA5, CA6, CA7, CA8, CA9 and CA10) were obtained from Sutton and Seeds, Calcutta, India. They were grown in randomized design with three replicates at the experimental farm of Andhra University, Visakhapatnam, which allowed production under the same cultivation conditions to obtain seeds with the same physiological quality. They were harvested manually and dried in the sun light.

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Seed protein extraction

About 200mg seeds from each genotype homogenized with the help of mortar and pestle using 0.01M Tris-Hcl buffer (pH 7.5). The resulting homogenates were centrifuged at 15000rpm for 10 minutes then the supernatants were filtered with 541 Whatmann filter paper and the obtained residues were boiled at 90^0C for five minutes with 1:1 ratio of 1.0M Tris (pH 6.8), 10% SDS, 2% β-mercaptoethanol, 10% glycerol and 0.002% bromophenol blue.

SDS-PAGE

Extracted soluble proteins were fractionated by one dimensional SDS-PAGE [15]. Ten and five percent running and stacking gels were used, gels measured (18cmX16cmX0.1cm). Electrophoresis was conducted at a constant current 35mA until the tracking dye reached the bottom of the gel. After electrophoresis, the gels were stained overnight in 0.25% Coomassie brilliant blue-R250, followed by de-staining in methanol and acetic acid for 45 minutes. The gels were further de-stained until the back ground was clear enough for bands scoring. Marker proteins (RPMW: Medium range protein molecular weight marker, obtained from Genei, Bangalore, India) were used as references. Molecular weights of protein bands were estimated by their relative mobilities. In order to eliminate differences in electrophoretic conditions as a cause of variation in the protein profiles, each variety protein sample was separated from three independent electrophoretic runs and two separate extractions.

Data analysis

The gels were scored as presence (+) or absence (-) of protein polypeptide bands and their staining intensities i.e., faint, medium and intensed. Depending upon the presence or absence of polypeptide bands, Similarity index (SI) [16] between the genotypes was calculated by the following formula:

$$SI = \frac{2Z}{X+Y} \times 100$$

Where, Z = Number of similar bands between the genotypes and X+Y = Total number of bands in the two genotypes compared. Cluster analysis was performed on the similarity index by UPGMA (Unweighted pair group method with arithmetic averages) by using statistical software SPSS for windows package (Version 10).

Results and Discussion

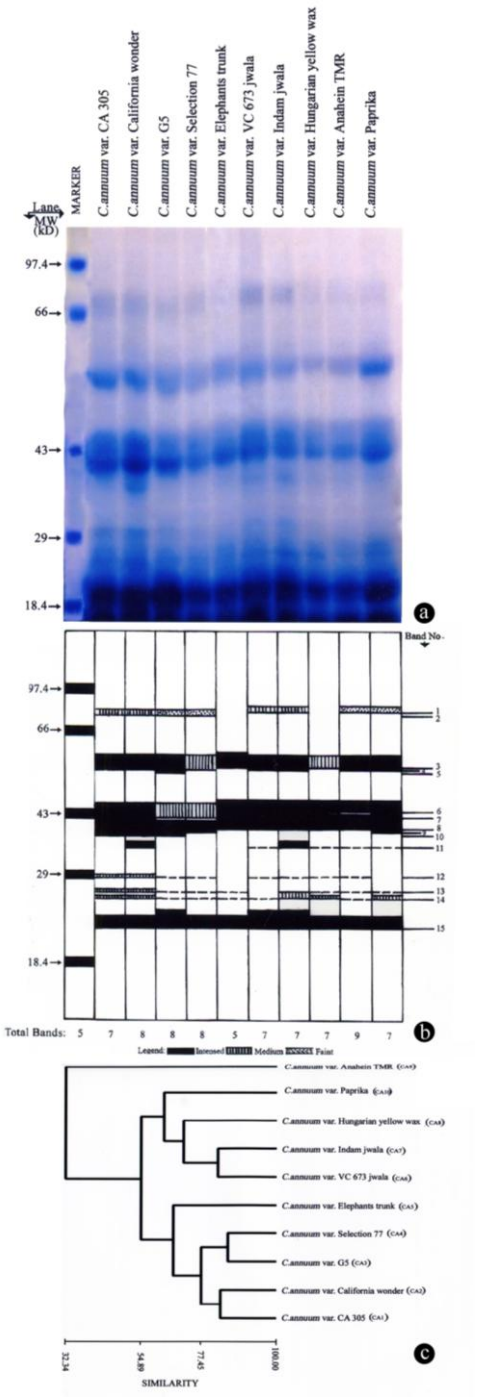
Results of SDS-PAGE seed storage protein profiles of 10 cultivars of *Capsicum annuum* L. were summarized in (Table 1 and Fig. 1a&b). A total of 15 protein polypeptide bands of diverse molecular weights ranging from 22.4 to 80.8kD were identified. The genotypes showed considerable variation in protein band number ranged from 5-9 in agreement with previous observations [5,11,7]. Among the cultivars studied CA9 represented maximum (12) protein bands while, the minimum (5) were recorded in CA5. Band no. 14(25.6kD) and 15(22.4kD) was found in all cultivars. However, band no. 5(60.0kD) is recorded in CA3 and 6(44.0kD) is present in cultivar CA9 and these bands can be considered as cultivar specific (Table 1, Fig. 1a&b). Based on molecular weights (kD) the protein polymorphism was observed in three regions i.e., low (0-25.0kD), medium (25.1-50.0kD) and high (50.1-75.0kD) consisted of 1, 9 and 5 bands respectively. Most of the protein bands were recorded faint and intensed in staining. The results are in accordance to Panda et al [4] Zubaida et al [8] Anu and Peter [14]. Similarity index for ten cultivars ranged from 40 to 100% (Table 2). Highest percent similarity was recorded between CA6 and CA7 while, the minimum (40.0%) was recorded between (CA3 – CA6), (CA3 – CA7) and (CA3 – CA10). Similar results were also reported in cultivars of *C. annuum* L. by Anu and Peter [14]. Cluster analysis of banding pattern of examined cultivars of *C. annuum* L. based on similarity index and UPGMA resulted two distinct clusters each comprising four cultivars and the remaining two cultivars i.e., CA5 and CA9 showed distinct affinities to cluster I and II respectively (Fig. 1c). The dendrogram as a whole revealed low genetic diversity at protein levels because most of the varieties are in the same cluster. Fufa et al [3] reported that the genetic diversity estimates based on seed storage protein were lowest because they were the major determinants of end-use quality, which is a highly selected trait. According to the results of the SDS-PAGE, the overall blueprint of seed storage proteins show low degree of heterogeneity may be attributed to cultivar homogeneity or purity [13]. Our results suggest that 3 varieties i.e., CA2, CA4 and CA5 showed better demarked protein profiles with reference to band presence or absence, staining and molecular weights can be recommended in future breeding programs to develop chili pepper varieties. It is suggested that cultivars with similar banding patterns should be further characterized by 2-D electrophoresis.

Table 1. Rm values, molecular weights and band presence or absence in ten cultivars of chili pepper (*Capsicum annuum* L.).

S.No.	Rm value	MW(kD)	Band presence(+)/absence(-)									
			Capsicum annuum L. cultivars									
			CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8	CA9	CA10
1	0.208	80.8	-	-	-	-	-	+	+	-	+	+
2	0.215	80.0	+	+	+	+	-	-	-	-	-	-
3	0.346	61.6	-	-	-	-	+	-	-	+	-	-
4	0.354	60.8	+	+	-	+	-	+	+	-	+	+
5	0.361	60.0	-	-	+	-	-	-	-	-	-	-
6	0.461	44.0	-	-	-	-	-	-	-	-	+	-
7	0.477	43.2	-	-	+	+	-	-	-	-	-	-
8	0.507	39.2	-	-	-	-	+	+	+	+	+	-
9	0.515	38.4	-	-	-	+	-	-	-	-	-	+
10	0.523	36.8	+	+	+	-	-	-	-	-	-	-
11	0.554	33.6	-	+	-	-	-	+	+	+	+	+
12	0.631	29.6	+	+	+	+	-	+	+	+	+	-
13	0.669	26.4	+	+	+	+	+	-	-	+	+	+
14	0.685	25.6	+	+	+	+	+	+	+	+	+	+
15	0.761	22.4	+	+	+	+	+	+	+	+	+	+
Total number of bands			7	8	8	8	5	7	7	7	9	7

Table 2. Percentage similarities between the cultivars of chili pepper (*Capsicum annuum* L.).

Cultivars		CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8	CA9	CA10
C.annuum var. CA 305	**CA1**	100.00	93.33	80.00	80.00	50.00	57.14	57.14	57.14	62.50	57.14
C.annuum var. C.Wonder	**CA2**		100.00	75.00	75.00	46.15	66.66	66.66	66.66	70.59	66.66
C.annuum var. G5	**CA3**			100.00	75.00	46.15	40.00	40.00	53.33	47.06	40.00
C.annuum var. Selection 77	**CA4**				100.00	46.15	53.33	53.33	53.33	58.82	66.66
C.annuum var. Elephants trunk	**CA5**					100.00	50.00	50.00	83.33	57.14	50.00
C.annuum var. VC 673 Jwala	**CA6**						100.00	100.00	71.43	87.50	71.43
C.annuum var. Indam Jwala	**CA7**							100.00	71.43	87.50	71.43
C.annuum var. Hung. Y.Wax	**CA8**								100.00	75.00	57.14
C.annuum var. Anahein TMR	**CA9**									100.0	75.00
C.annuum var. Paparika	**CA10**										100.00



Figs.1a-c: SDS-PAGE seed protein profiles in ten cultivars of chili pepper (*Capsicum annuum* L.). Fig. 1a: 10% SDS polyacrylamide gel with resolved protein bands. Figs. 1b: Explanatory electrophorogram. Fig. 1c: UPGMA dendrogram.

Conclusions

It is therefore concluded that seed storage protein profiles could be useful markers in cultivar identification. The present study revealed that large intra-specific variations were not found in the varieties of *C. annuum* L. However band no. 5 (CA3) and 6 (CA9) identified as cultivar specific. Based on the resolved protein profiles CA2, CA4 and CA5 cultivars represented better demarked protein banding patterns could be suggested to future chili breeding programs for cultivar development.

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