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SEED STORAGE PROTEINS AND INTRASPECIFIC RELATIONSHIPS IN *CICER ARIETINUM* L.

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ABSTRACT

Phylogenetic aspects of storage seed protein analysis by polyacrylamide gel electrophoresis indicated that both the *desi* and *kabuli* types are the varieties of the same species *Cicer arietinum* L. and corroborated the earlier findings that there is no evidence to classify kabuli types as *Cicer kabulicum*. The results indicated that all the four varieties have very great homology, the number of bands resolved was 9 in each case and the pattern was also the same except minor differences in position and distance of bands.

Key words : Chickpea, seed storage proteins, seed globulin, PAGE electrophoresis, homology

The storage or reserve proteins constitute the major portion of the proteins in the seed. For most seeds, except cereals, storage proteins are predominantly globulins [1]. The mature seed provides a stable and convenient system for biochemical analysis to establish phylogenetic relationships in plants [2, 3]. This is because of the fact that, proteins, owing to their unique molecular status are a very special chemical constituent of an organism. Their amino acid sequences are derived directly from genotypic information and permit the deduction of nucleotide sequences in the DNA and their physical properties arising from variation in size, shape and change of the molecule lend them admirably to useful forms of analysis like electrophoresis.

In the present study, electrophoretic analysis of seed protein extracts of varieties representing two *desi* (G 130 & H 214) one green seeded (L 345) and one *kabuli* (C 104) variety of chickpea (*Cicer arietinum* L.) are reported here.

MATERIALS AND METHODS

Four commercially released and established varieties of chickpea viz., two *desi* (G 130 & H 214), one green seeded (L 345) and one *kabuli* (C 104) with 100 seed weight ranging from 12 to 22 g and crude protein content ranging from 20 to 25% were selected for this study.

Seed proteins soluble in potassium sulphate buffer were electrophoressed on polyacrylamide gel under cationic system following Reisfield *et al.* [4].

Preperation of sample: Seed from each variety were powdered and passed through a 60-mesh/m sieve in a Willey Mill. Lipids and phenols were removed from the powdered sample by washing three times using 25 ml. of acetone, chloroform and methanol (2:1:1:) mixture. During the second washing the mixture was allowed to stand overnight. The filtered sample was air-dried. One gram of sample was homogenised with 5ml cold potassium sulphate solution (5 percent w/v) containing 0.7% 2-mercaptoethanol and 100mg of polyvinyl pyrolidone (PVP, 2 percent w/v). The homogenate was allowed to stand for one hour at 4°C, then centrifuged at 15,000 r.p.m. for 20 minutes. One ml of the clear supernatant was pipetted out immediately and made upto 40 percent sucrose by dissolving 0.4 g sucrose. This dense extract corresponding to approximately 200 mg protein was used for electrophoresis under the cationic system.

Electrophoresis was performed in cationic system according to Reisfeld et al. [4]. The electrophoretic unit was placed in a cold chamber at 4°C. The current was initially adjusted to 2 ma per gel tube for the first ten minutes, then raised to 4 ma per tube. Electrophoresis was carried out for one and half hours at 4°C when the green ring of the tracking dye on the moving front reached the lower end of the gel.

Fixation, staining and grouping of bands in gel: After completion of the electrophoretic run, the gels were removed from the tubes, fixed and stained for one hr in 0.5% Amido Black-B dissolved in 7% acetic acid. After an hour, the excess unbound stain was eluted out of the gels by frequent changes of fresh 7% acetic acid. Destained gels with well resolved protein bands were labelled and stored in 7% acetic acid in a refrigerator.

Destained gels were compared for protein bands by visual observations and the bands were grouped into several categories :

A) dense and broad, B) dense and narrow, C) light and broad

D) light and narrow and E) very faint.

The gels were photographed immediately.

Rf computation: The variation in the position of bands in gels were expressed in Resolution factor (Rf) values. The distance migrated by each band from the origin (the junction between separating and stacking gels) was measured. The relative migration rate of each protein band was calculated according to the formula:

$$Rf = \frac{\text{distance of protein band from origin}}{\text{distance of tracking dye from origin}} \times 100$$

The Rf values were expressed with a '+' prefix denoting the mobility of protein towards the cathode under cationic syatem.

RESULTS AND DISCUSSION

Most of the protein applied on the gel migrated into the gel where it resolved into different bands of varying width and intensity at various positions in the gel. The maximum number of bands obtained were 9 in all the varieties. The results on the relative mobility groups, band number and Rf values under cationic system are given in Table 1 and are presented as gel photographs in Fig. 1 and diagramatic representation of the densitograms on the basis of band patterns in Fig. 2.

Table 1. Relative mobility values for various bands electrophoresed in seed protein extracts of *desi*, green seeded and *kabuli* varieties of chickpea

	Chickpea varieties							
	desi				green seeded		kabuli	
	G 130		H 214		L 345		C 104	
Mobility group	Band No.	Rf value (+)	Band No.	Rf value (+)	Band No.	Rf value (+)	Band No.	Rf value (+)
A	1	10.8	1	12.5	1	17.5	1	12.5
B	2	21.7	2	27.5			2	27.5
	3	22.5	3	29.2			3	29.2
	4	30.0						
C	5	36.7	4	36.7	2	32.5	4	36.7
					3	34.2		
					4	40.0		
D	6	44.2	5	41.7	5	45.0	5	41.7
	7	47.5	6	45.8	6	46.7	6	45.8
			7	49.2			7	50.0
E	8	52.5	8	55.0	7	50.8	8	55.8
					8	57.5		
F	9	67.7	9	67.5	9	70.8	9	70.8

The electrophororetic spectrum was divided on the basis of mobility into 6 groups (A, B, C, D, E and F). Group A comprised the slow mobility group, B, C and D the medium mobility, E the fast and F the fastest mobility group (Table 1).

(1) *Group A*: This least mobile group extending from 10.8. to 17.5 had a total of four faint bands, one from each variety. The slowest band was observed in *desi* var. G 130 and the fastest in green seeded var. L 345.

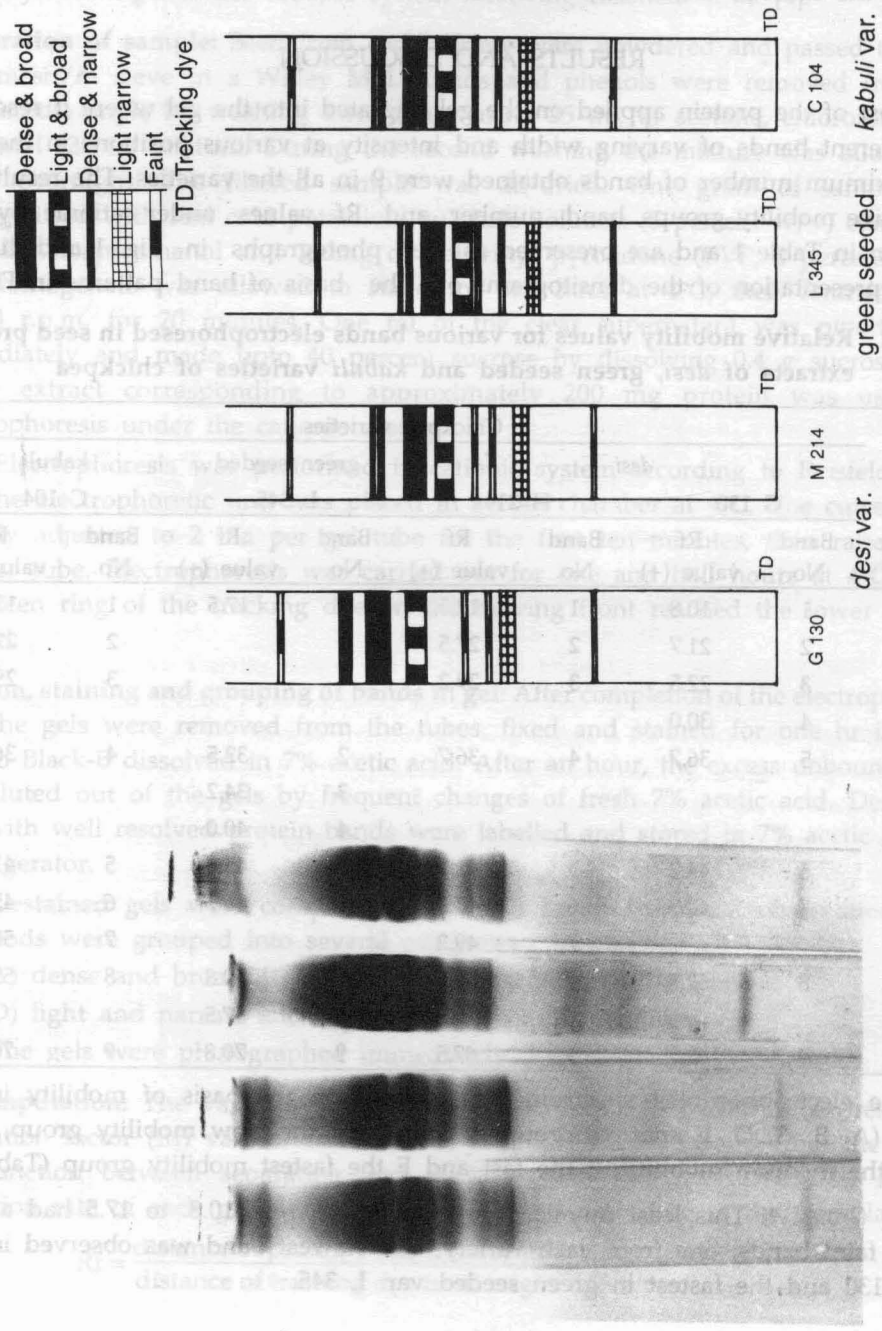

Fig. 1. Protein band pattern in polyacrylamide gels of var. G 130, H 214, L 345 and C 104

Fig. 2. Densitograms for protein band pattern under cationic system

(2) *Group B*: This group was characterised by a dense narrow band in *desi* varieties G 130, H 214 and *kabuli* var. C 104 and also one dense and broad band in *desi* var. G 130. The green seeded var. L 345 had no band in this mobility group. The mobility ranged from 21.7 to 30.0.

(3) *Group C*: This medium mobility group was characterised by the presence of most prominent protein band found in all the four varieties. A light and broad band at the Rf position (36.7) in *desi* var. G 130, a dense narrow at the same position in *desi* var. H 214 and two bands, dense narrow, dense broad and light broad in green var. L 345 and *kabuli* var. C 104 were found in this group. The Rf range was 32.5 to 40.0 and covered 6 bands in this group.

(4) *Group D*: The second group under medium mobility had maximum number of bands (10), all the four varieties having at least two bands, although there were three in *desi* var. H 214 and *kabuli* var. C 104. All bands except one dense narrow in green seeded var. L 345, were dense broad and light broad. The Rf ranged from 41.7 to 50.0.

(5) *Group E*: This group is the first of the two groups with fast mobility and consists of light narrow bands in all the four varieties and one faint band in case of green seeded var. L 345. The range of Rf in this group was from 50.8 to 57.5.

(6) *Group F*: This group constituted the fastest of all the groups and range in Rf was observed from 67.5 to 70.8. All the four varieties had one faint band in this group. The bands in case of the two *desi* and the other two culinary varieties were at the same position in each group, the Rf in *desi* varieties was 67.7 and in *kabuli* it was 70.8.

Under the cationic system, *desi* var. G 130 and H 214 showed identical proteins and green seeded var. L 345 and *kabuli* var. C 104 also showed similarity in this regard. Overall protein band pattern did not differ largely and indicated close relationship between the *desi* and *kabuli* varieties.

With the advent of refind electrophoretic techniques [4-9] large amounts of phylogenetic information have been extracted from seed proteins and isoenzymes. Boulter *et al.* [7] examined the storage globulins of the tribe *Viceae* and have established that a tribal protein band pattern exists. In a subsequent study Boulter *et al.* [8, 9] showed that 36 species of *Crotolaria* could be derived into at least two groups on the basis of their protein band patterns and there was reasonable correlation with the generic subdivisions and with the distribution. The conclusions on phylogenetic aspects of seed protein analysis are based on the fact that of the many compounds found in living systems, sementides are potentially the most informative taxonomic characters of an organism. Proteins are classified as tertiary sementides and the

similar amino acid sequence in the *homologue* proteins of different organisms corresponds to the similar sequence of nucleotides in the DNA.

The conclusions of the present investigation are largely based on the fact that the storage proteins of legumes usually have two major components with sedimentation coefficients of 7S and 11S, respectively [10]. The 7S and 11S components of *Pisum sativum, Vicia faba, Cicer arietinum* and *Arachis hypogaea* consist of subunits since they can be broken down by treatment with urea or sodium dodecyl sulphate into a variable number of components which can be separated on polyacrylamide gels as distinct bands [10, 11]. The results of the present study on electrophoretic analysis of seed storage protein extracts of chickpea varieties representing two *desi*, one green seeded and one *kabuli* variety indicated similarity in protein band patterns of these varieties. These results also corroborate the earlier evidence gathered from data on pollen size, cytology, karyotype and radiosensitivity studies and clearly support the conclusion that *desi* and *kabuli* types of chickpea are the varieties of the same species *Cicer arietinum* L. and that there is no evidence wheatsoever, to classify *kabuli* types as *'Cicer kabulicum'* [12, 13].

REFERENCES

1. T. G. Krishna and C. R. Bhatia. 1985. Vicilin from *Cajanus cajan* seeds. Phytochemistry, **24**: 2201-2203.
2. G. Ladizinsky and T. Hymowitz. 1979. Seed Protein electrophoresis in taxonomic and evolutionary studies. TAG, **54**: 145-151.
3. T. G. Krishna and R. Mitra. 1988. The probable genome donors to *Arachis hypogaea* L. based on arachin seed storage protein. Eyphytica, **37**: 47-52.
4. R. A. Reisfield, U. J. Lewis and D. E. Williams. 1963. Disc Electrophoresis of basic proteins and peptides on polyacrylamide gels. Nature **195**: 281-83.
5. Yogesh R. Mawal, Madhumalti R. Mawal and P. K. Ranjekar. 1990. Unusual denaturation properties of vicilin from *Cajanus cajan*. Biochemical and Biophysical Research Communications., **166**: 1446-1452.
6. A.K. Singh, S. Sivaramakrishnan, H. Melak Mangesha and C.D. Ramaiah. 1991. Phylogenetic relations in section *Arachis* based on seed protein profile. TAG, **82**: 593-597.
7. D. Boulter, D. A. Thurman and E. Derbishire. 1967. A disc electrophoretic study of globulin proteins of legume seeds with reference to their systematics, New Phytol., **66**: 27-36.
8. D. Boulter, E. Derbyshire, J. A. Frahm Leliveld, R. M. Polhill. 1970. Observations on the cytology and seed proteins of various African species of *Crotolaria* (Leguminosae), New Phytol., **69**: 117-131.
9. D. Boulter and E. Derbyshire. 1971. Taxonomic aspects of the structure of legume proteins, *In*: "Chemotexonomy of the Leguminosae" (Ed. J.B. Harborne, D. Boulter, B.L. Turner) 285-308.
10. P. Jackson, D. Boulter and D. A. Thurman. 1969. A comparison of some properties of vicilin and legumin isolated from seeds of *Pisum sativum, Vicia faba* and *Cicer arietinum*, New Phytol., **68**: 25-33.
11. D. R. Grant and J. M. Lawrence. 1964. Effects of sodium dodecyl sulphate and other dissociating reagents on the globulins of Peas. Arch. Biochem. Biophys., **108**: 552-61.
12. L. S. Cobly. 1965. An introduction to the botany of tropical crops, Longmans, London, 150-151.
13. P. D. Dixit. 1932. A note on the cytology of *kabuli* and *desi* gram. Indian J. Agric. Sci., **2**: 385-90.