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SEED STORAGE PROTEIN PROFILE OF FEW LEGUMINOUS GRAINS GROWN IN INDIA USING SDS-PAGE

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ABSTRACT:

A major part of the human diet all over the world consists of cereals and legumes. Legumes are however, considered as the major source of protein and dietary amino acid for man and farm animals. The detailed evolutionary relations and cluster analysis in this group of plants may help in further manipulations and modifications of plants leading to enhanced nutritional supplementation for consumption.
Seven varieties of leguminous seeds namely *Pisum sativum*, *Cajanus cajan*, *Glycine max*, *Phaseolus vulgaris*, *Vigna radiata*, *Lens culinaris* and *Cicer arientum*; belonging to three different major tribes (Phaseoleae, Viciae & Cicereae) were studied for their evolutionary relationships using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The cluster analysis was then done using the unweighed pair group method with arithematic means (UPGMA).
Interestingly, the seven plants clustered in only two major clustures differentiating clearly Phaseoleae and Viciae. Another revelation was found that *Cicer arietinum* clustered with accessions of Viceae tribe and did not form a separate cluster as expected. This suggests the possible adaptations acquired by the plant during the process of domestication.

Keywords: SDS PAGE, Protein profile, Seed protein, Isolation method, Legumes, Plants, UPGMA.

[I] INTRODUCTION

The classification of Leguminosae, containing more than 21,000 species is not well defined. The grain legumes, belonging to a "subfamily" of *Papilionidae* or *Faboidae*, were recently grouped into the five following tribes[1]: Phaseoleae, Vicieae, Cicereae, Aeschynomenae, and Gemistae. Most of the grain legumes belong to the first three tribes.

The productive features, isozymes and protein polymorphisms of most grain legume crops are well documented [2,3,4,5,6,7]. However, the comparative study of protein variation in these species is not well demonstrated. Hence, it is

desirable to increase our knowledge of the genetic resemblance among the most important grain legumes by employing variations in seed storage proteins, which are their main common characteristics.

The protein profiling of germplasm and use of genetic markers have been widely and effectively used to determine the taxonomic and evolutionary aspects of several crops [8,9,10,11].

The systematic methodology especially based on morphology chiefly has been improved by the incorporation of physiology, ecology or biochemical traits[12,13]. It was reported that biochemical and molecular analysis, particularly of electrophoretic analysis of seed proteins as revealed by SDS-PAGE (Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis) have provided valid evidence for detecting intraspecific variation and assessing interspecific relationships [14,15,16,17,18].

Table1: List and characteristics of grain legumes studied (after Sammerfield and Roberts, 1985).

Sl. No	Species	Tribe	Common name	Origin or site of domestication
1	*Phaseolus vulgaris*	Phaseoleae	Common/kidney bean	Mexico, Peru
2	*Cajanus cajan*	”	Pigeon pea	India
3	*Vigna radiate*	”	Mung bean	Ethiopia, Africa
4	*Glycine max*	”	Soybean	China
5	*Lens culinaris*	Vicieae	Lentil	Middle East
6	*Pisum sativum*	”	Pea	Near East
7	*Cicer arietinum*	Cicereae	Chickpea	Middle East, Iran, South-east Turkey

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 by identifying and/or eliminating duplicates in the gene stock and helps in the establishment of core collection [19]. Cultivar identification is useful for describing a new cultivar, testing genotype purity and speeding up DUS (distinctness uniformity stability) test for candidate cultivar [20]. One practical application of knowledge of genetic diversity is in the design of populations for genome mapping experiments [21].

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is most economical simple and extensively used biochemical technique for analysis of genetic structure of germplasm. Seed storage proteins have been used as genetic markers in four major areas: 1) Analysis of genetic diversity within and between species, 2) Plant domestication in relation to genetic resources conservation and breeding, 3) Genome relationship and 4) A tool in crop improvement[11]. The present study was taken to understand the genetic variability among seven leguminous plant seeds cultivated in India using 1-D SDS PAGE profile.

[II] MATERIALS & METHODS

2.1. Plant material

Seven different varieties of cereals seeds namely *Pisum sativum* (PlantA), *Cajanus cajan* (PlantB) , *Glycine max* (PlantC), *Phaseolus vulgaris* (PlantD), *Vigna radiata* (PlantE), *Lens culinaris* (PlantF) and *Cicer arientum* (PlantG) were obtained from the farmers nearby Visakhapatnam, Andhra Pradesh and Dheng, Bihar. The seeds were collected dried, were rinsed with distill water to remove the dust particles and any other impurities and then dried again for the experiment.

2.2. Reagents

All the chemicals and reagents used were research grade.

2.3. Protein extraction

Method used by [22]. has been shown to be the preferable method for protein extraction from few leguminous plants for SDS PAGE previously[23]. Seeds were grinded to fine powder with the help of mortar and pestle. We added sample buffer (400 µl) to a 0.02 g of fine seed flour as extraction liquid and bromophenol blue (BPB) follow the movement of protein in the gel. The active ingredients used for the extraction of protein buffer contained 0.5 M Tris-HCl (pH 8.0), 0.2% SDS, 5 M urea and 1% 2-mercaptoethanol. When all these chemicals are tightly put together than the solution needs to be purified and homogenate, we mixed the samples thoroughly by vortexing and centrifugation at 15,000 rpm for 5 min at room temperature. After centrifuging samples, the crude proteins were recovered as clear supernatant on the top of the tube. Then the supernatant were transferred into new 1.5 ml eppendorf tubes and were stored at -20°C until gel electrophoresis.

2.4. Protein estimation

The proteins were determined by Bradford's method [24].

2.5. Electrophoresis (SDS PAGE)

One dimensional Sodium dodecyl sulfate polyacrylamide gel were prepared in a concentration of 8% resolving gel and 4.44% stacking gel as suggested by [25]. Electrophoresis was carried out according to [26] after adding sample loading buffer. Gels were stained with staining solution comprising 0.2% (W/V) Comassie Brilliant Blue (CBB) R 250 dissolved in 10% (V/V) acetic acid, 40% (V/V) methanol for about an hour at room temperature. Gels were distained in a solution containing 5 % (V/V) acetic acid and 20% (V/V) methanol.

Drawing the phylogenetic tree:

Electrophoregrams for each variety were scored and the presence (1) or absence (0) of each band noted. Presence and absence of bands were entered in a binary data matrix. Based on electrophoresis band spectra, Jaccard's similarity index (JSI) was calculated by the: S= W/(A+B-W).

Where W is the number of bands of common mobility, A the number of bands in type A and B is the number of bands in type B. The similarity matrix generated was converted to a dissimilarity matrix (Dissimilarity = 1 - similarity) and used to construct dendrogram by the unweighed pair group method with arithematic means (UPGMA). All analysis was carried out using a statistical package NTSYS-pc, version 1.8 (Rohlf, 1993) and STATISTICA.

[III] RESULTS & DISCUSSION

Table2: Amount of protein in seed extracts

Protein isolate	Amount of protein per gram of seed (in mg)
P. sativum	32.525
C. cajan	32.525
G. max	13.700
V. radiate	24.650
P. vulgaris	35.900
L. culinaris	37.475
C. arietinum	84.500

Protein electrophoresis is a powerful tool for population genetics[27]. As storage proteins are not affected by environmental fluctuations, their profiling using SDS-PAGE technology is particularly considered as a reliable tool for economic characterization of germplasm [16,28].

Seed protein patterns can also be used as a promising tool for distinguishing cultivars of particular crop species[29,30].

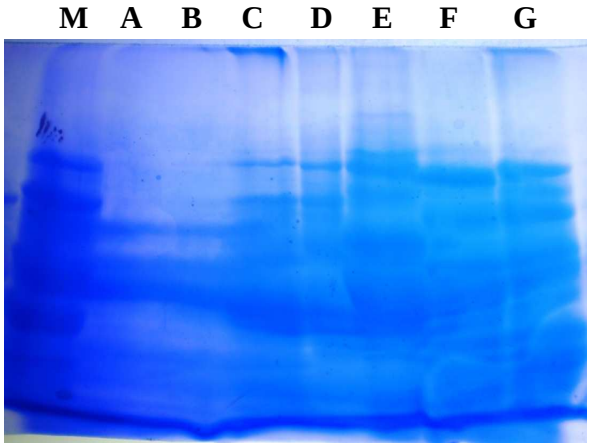

Fig1: Protein bands of Plant A-G with molecular weight marker (M)

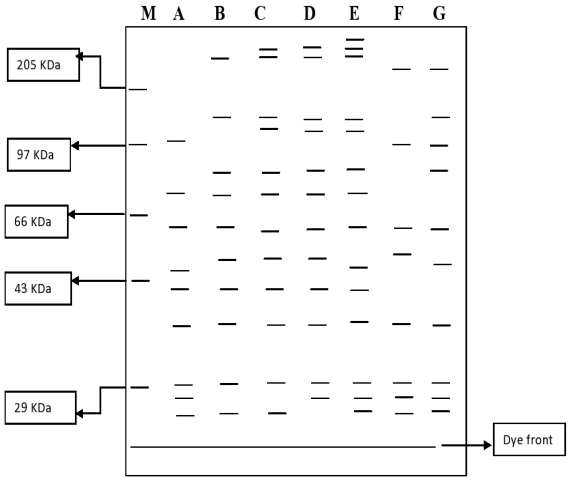

Fig2: Protein banding pattern representation

	A	B	C	D	E	F	G
A	**0**	**0.462**	**0.533**	**0.533**	**0.588**	**0.200**	**0.417**
B		**0**	**0.154**	**0.286**	**0.375**	**0.571**	**0.500**
C			**0**	**0.143**	**0.250**	**0.625**	**0.562**
D				**0**	**0.250**	**0.625**	**0.562**
E					**0**	**0.588**	**0.529**
F						**0**	**0.273**
G							**0**

Table 3: Protein banding pattern

Fig3: Distance matrix using Jaccard method

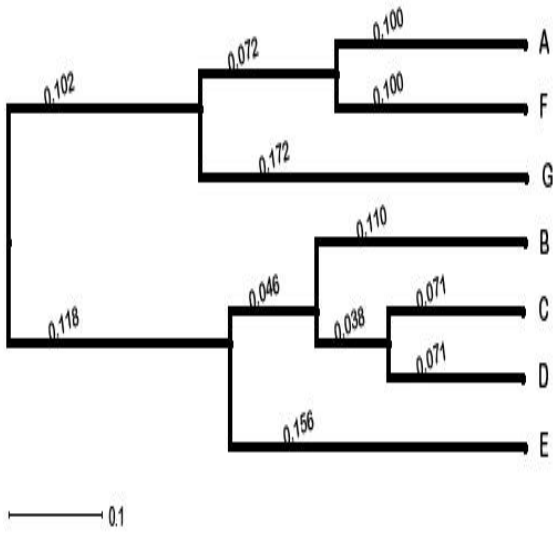

Fig4: Dendrogram showing two major clusters

[IV] CONCLUSION:

In conclusion, electrophoresis (SDS-PAGE) of seed storage proteins can be economically used to assess genetic variation and relation in germplasm and also to differentiate mutants from their parent genotypes. It is suggested that genotypes with similar banding patterns should be further characterized by 2-D electrophoresis. However in present study, no two species showed similar banding pattern.

In the present study, the differences between species are evident. All seven species are clearly identifiable from the protein banding pattern (Fig 1&2). SDS-PAGE of total seed protein profiles is, therefore, an efficient procedure for differentiating grain legume species. Several researchers have confirmed the usefulness of different SDS-PAGE proedures in plant taxonomic, evolutionary and genetic relationship studies[31,32,33]. The cluster analysis using Jackard's coefficient further helped in developing some important conclusions.

Two major clusters were formed upon detailed analysis (Fig 4). In cluster analysis it was found that accessions of the Phaseoleae tribe formed one cluster *(Phaseolus vulgaris, Glycine max, Cajanus cajan* and *Vigna radiata).* Plants from Viciae tribe formed another cluster with *Pisum sativum* & *Lens culinaris* clustered together. *Cicer arietinum* was an exception since it belongs to Cicereae but was clustered with Viceae. This could be attributed to the characters acquired during the domestication process or the adaptive growth in Indian farming conditions.

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