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Evaluation of Genotypic variation using SDS-PAGE

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Abstract
The objective of this study was to assess the genetic divergence available in four different genotypes of chickpea based on their analysis of seed storage proteins, for the identification of genetically diverse and agronomically superior genotypes of chickpea seeds. In this study, total 4 cultivars of chickpea obtained from Sehore M.P. have been studied for their analysis of seed storage protein profiles to examine their relationship by SDS-PAGE technique. The Proteomic assay comprised a total of 64 reliably score able protein alleles identified in the 4 accessions of legumes. There is no polymorphic band found in this result so these accessions cannot be further differentiated different genotypes of chickpea and not used further in breeding programmes.

Keywords: SDS-PAGE, Polymorphic band, Seed storage proteins, Proteomic assay.

1. Introduction
The mature seed provides a stable and convenient system for biochemical analysis to establish relationship between parents and hybrids (Singh *et al.,* 2012) [1]. Characterization of germplasm using biochemical fingerprinting has got special attention due to its increased use in crop improvement and the selection of desirable genotypes for breeding crops (Nisar *et al.,* 2007) [2]. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is economical, simple and extensively used biochemical technique for analysis of genetic structure of germplasm. The use of genetic markers and protein profiling has also been successfully used to resolve the taxonomy and evolutionary problems of several crop plants (Ghafoor *et al.,* 2002) [3].
Seed storage protein analysis helps in protein identification and characterization of diversity in crop varieties and their wild relatives. Variation in protein band provides information on the relationship among the seeds collected from various geographic regions. Storage proteins play main role that includes nitrogen and energy source and defense against insect and pathogens such as bacteria and fungi (Ehsanpour *et al.,* 2010) [4]. The present study was initiated to study genetic diversity on the basis of seed protein profile and its relationship with agronomic traits in chickpea.

2. Materials & Methods
Four chickpea genotypes were selected to study the genetic variation of genotypes using SDS-PAGE. Chickpea seed were collected from College of Agriculture, Sehore, Madhya Pradesh India.

2.1. Protein extraction
Seed coats were removed and seed storage proteins were extracted as described by (Jha and Ohri, 2002) [5]. The seed material was homogenized using 0.1 M Tris-HCl buffer (pH: 7.5) in the ratio 1:10 (W/V). Total protein was extracted after centrifugation at 17,600 g for 20 min at 4 °C and clear supernatants were used for analysis.

2.2. Protein profiling using SDS-PAGE
Protein profiling of samples was performed using SDS-PAGE as described by (Laemmli, 1970) [6]. Equal quantities of proteins (150 µg) from each sample along with protein molecular weight marker (SM0671, Fermentas) were loaded into 10% gels and electrophoresis was performed at constant voltage (100 volts). Staining of gels was done in 0.025% Coomassie Brilliant Blue 250 containing 40% methanol and 7% acetic acid, while destaining was done in the same solution without dye.

2.3. Statistical analysis
All work was done in triplicates and the data presented are means ± S.D. of three independent determinations. Significance was accepted at $p \leq 0.05$.

3. Results and Discussion
In the present study, seeds of *Cicer arietinum* L. are rich in seed storage proteins with a number of stable bands in the gel (Fig.1). Considerable variation was observed with low (1.8±0.02 mg/gm) and high (3.18±0.03 mg/gm) levels in the protein content which was though not reflected in the protein banding patterns. No additional bands were observed in the protein rich lines and no missing bands could be detected in the protein deficient lines. Further, the intensity of the various bands in the profile was not appreciably greater in the protein rich line than the lines with low protein content. Such observations have been reported by (Bhagyawant *et al.*, 2009) [7] in chickpea. The seed protein profiles of four chickpea accessions from different geographically cultivated regions of Madhya Pradesh showed diversity in the banding pattern. The types of band were depicted on the basis of their colour intensity like dense, medium and light. The protein banding pattern of all the *Cicer* species studied are represented in the schematic drawing (Fig. 1). The SDS-PAGE fractionated proteins showed distinction in the number and molecular weight of these polypeptides. The major components of all the species were in the molecular weight range of approx 110 to 10 kDa, with the variation in relative mobility values. The maximum numbers of bands (16) were present in all the cultivars. There is no polymorphic bands are observed in this four cultivars so they cannot be differentiated into different species using SDS-PAGE. It is either due to extensive breeding or mixing in transportation. Our results are in agreement with the results of [4] characterized the seed storage protein patterns of four Iranian Pistachios using SDS- PAGE depicting no variation in different cultivars of pistachios seeds with regard to their total seed protein profile. The total protein content of pistachios seeds in all cultivars did not show any significant results however in SDS- PAGE, patterns of a few protein bands were up- regulated whereas some other bands showed down regulation. On the basis of their studies they concluded that the identified protein patterns may be used as protein markers.

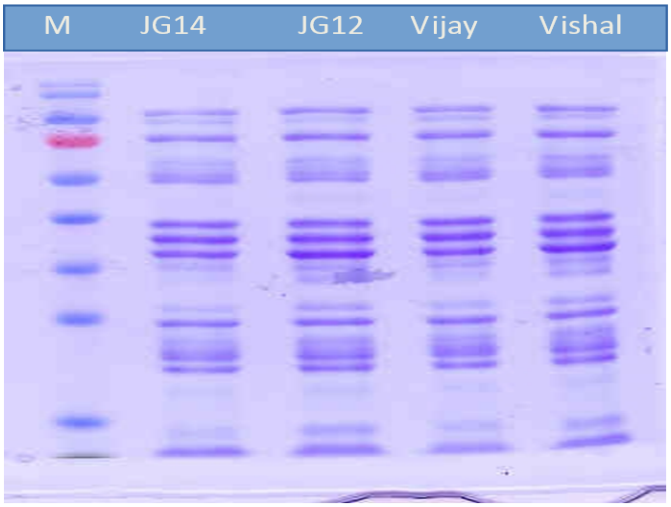

Fig 1: SDS-PAGE pattern of four chickpea cultivars.

Table1: Agronomic details of chickpea genotypes used in SDS-Page.

S.No.	Cultivars	Protein (mg/gm)	Agronomic details
1	JG12	2.58±0.04	*Wilt resistant*
2	JG14	1.8±0.02	*Wilt resistant*
3	Vijay	2.78±0.05	*Wilt resistant*
4	Vishal	3.18±0.03	*Wilt resistant*

4. Conclusion
The overall pattern of seed storage proteins did not show any diversity in chickpea cultivars as substantiated by the SDS-PAGE. It is either due to low sample numbers. In our opinion such association needs to be further explored using more sophisticated techniques of proteomics like 2D-gel electrophoresis coupled with MALDI - TOFF and second generation sequencing approaches.

5. Acknowledgement
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