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Comparative Study in Relation to Protein and Protein Profiling of *Rhizobium* Inoculated Desi and Kabuli Chickpea (*Cicer arietinum* L.) Genotypes

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ABSTRACT

Fifteen genotypes of chickpea control and *Rhizobium* inoculated were investigated for protein content and genetic divergence based on seed protein profile using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Protein content of desi type was relatively higher than kabuli type. Protein content fifteen genotypes ranged from 18.76-23.58% in control, *Rhizobium* inoculated 19.83-24.54%. Desi and Kabuli genotype of chickpea *Rhizobium* inoculated gave some unique band in comparison to control genotype of chickpea. Desi genotypes gave some unique band in comparison to Kabuli genotypes of chickpea. Highest number of protein band found in PUSA 362, KWR 108 and followed by H82-2 and Phule G 5 whereas minimum number of protein bands were found in L550. It is clear from the result obtained that the chickpea genotype having highest number of protein band having highest quantity of protein. Maximum bands are of medium molecular weight followed by highest molecular weight and low molecular weight. Electrophoresis (SDS-PAGE) of seed storage protein can economically be used to assess, genetic variation and relation in genotypes. Thus, specific bands of seed storage protein profile may be used as markers for identification of the mutants/genotypes. The research results about the biochemical characteristics of desi chickpea varieties are expected to provide guidelines for the researchers confronted with the need to use such typical food seeds in India as well as in the rest of the world.

Key words: SDS-PAGE, genotypes, protein, protein profile, electrophoresis

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most widely grown grain legume in the world after bean and soyabean.The Mediterranean origin of the crop imparts special significance to chickpea in the agriculture of this area, where it has multiple functions in the traditional farming systems. Besides being an important source of human and animal food, the crop also plays an important role in the maintenance of soil fertility, particularly in the dry and rainfed area. In addition, it is also widely used as green manure. Chickpea seeds contain about 20.6% protein, 61.2% carbohydrates and 2.2% fat (Saini *et al*., 2004; Werner and Newton, 2005; Togay *et al*., 2008). Chickpea is an important source of vegetable protein (19-30%) in major part of the world. It is consumed as green vegetables (whole pods or immature seed) in Asian countries and dry seed in Europe, Australia, America and Mediterranean regions. Legume crops, "the meat of poor", are cultivated on a large

area of Pakistan. Legumes, being rich sources of protein, calories, certain minerals and vitamins play an important role in human nutrition (Shad *et al*., 2009). Food legumes are crops of the family Leguminosae also called Fabacae. World widely, these are mainly grown on large area for their edible seeds and thus are also named grain legumes. Iqbal *et al*. (2006) reported that legumes are helpful in enhancing the protein content and improving the nutritional status of the cereal-based diets. Iqbal *et al*. (2003) investigated that cereal proteins are deficient in certain essential amino acids, particularly lysine. Diversity in the genotypes is the foundation on which improvements are built. If the genotypes do not have information on characterization, evaluation and biochemical analyses, their utilization is limited. Genotypes without utilization for crop improvement mean the wastage of resources. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is used due to its validity and simplicity for describing genetic structure of crop genotypes, but its implication has been limited mainly to cereals due to less polymorphism in most of the legumes (Ghafoor *et al*., 2002). Seed storage proteins have been used as genetic markers obtained by electrophoresis to resolve the taxonomic and evolutionary problems of several crop plants (Gupta and Namdeo, 1997; Das and Mukherjee, 1995). Researchers can use genetic similarity information to make decisions regarding the choice for selecting superior genotypes for improvement or to be used as parents for the development of future cultivars through hybridization. The present study was initiated to study genetic diversity on the basis of seed protein profile and its relationship with protein content in chickpea.

MATERIALS AND METHODS

The present investigation on "Biochemical and molecular studies on *Rhizobium* inoculated chickpea (*Cicer arietinum* L.) genotypes grown in eastern U.P." was carried out at Student's Instructional Farm and in the laboratory of Department of Biochemistry during *Rabi* season of 2010-11 and 2011-12. After harvesting the seeds were collected and stored in desiccators for the analysis of various biochemical parameters. The protein content in chickpea seed was determined by the Lowry *et al*. (1951). One gram sample was taken and homogenized in presence of 10% TCA. The whole content was finally centrifuged at 4000 rpm for 15 min. The residue was discarded. Thereafter, 1 ml supernatant was taken and mixed with 1 mL 10% TCA. It was kept for 30 min and the residue obtained was dissolved in 5 mL 0.1 N NaOH. 0.5 and1.0 mL sample extract was taken in another test tube and volume was made upto 1 mL with distilled water. Then, 5 mL alkaline copper reagent was added and it was mixed properly. After 10 min 0.5 mL folin's reagent was added and it was kept at room temperature for 30 min. Finally, colour intensity was recorded at 660 nm on spectronic-20 against the blank solution. Calculation was done by standard curve using BSA as standard protein and results were expressed as amount of protein in percent. Protein profiling was done by method given by Laemmli (1970). The 0.25 g of chickpea seed was ground in 1 mL sample extraction buffer with help of mortar and pestle. Then, transferred the crushed material in to centrifuge tube of 15 mL capacity and centrifuged at 10,000 rpm (C-24 remi, 12×15 mL Angle head) for 10 min, In cooling centrifuge (4°C). Finally supernatant was transferred in to clean microfuge tube. Sample were kept in the water bath for at 100°C for 5-7 min. Then it was centrifuged at 5000 rpm (C-24 remi, 12×15 mL Angle head) for 5 min. Thoroughly cleaned glass plates and spacers were assembled properly and were clamped in on upright position on a gel casting unit with 1% agar on bottom to seal chamber leak proof between glass plates. The gel solution was poured in the chamber between glass plates carefully leaving space from the top and layer of distilled water was added on top of the gel and allowed to polymerize for 30-60 min. After

removing water from the gel, stacking gel 4% was poured; comb was placed and kept for polymerizing (30-45 min). The 60 µL protein sample were mixed with 40 µL of loading dye. From this 70-80 µL was loaded into well with the help of micropipette after proper loading the electrophase unit was connected with power supply and run at 30 mA at 220 V. When the tracking dye reached of the end of running gel after complete separation of molecules, power supply turned off. The gel was gently removed from the space between the plates, immerged in the staining solution contained in a tray. The gel was destained by putting it into the destaining solution. The process was continued until the back ground was colorless. Dye not bound to protein was removed. The relative mobility of the different protein band were recorded by comparing the bands with their parents loaded with gel.

RESULTS AND DISCUTTION

The protein content of chickpea seeds have been presented in Table 1 recorded protein content ranged from 18.76-23.58% in control, *Rhizobium* inoculated 19.83-24.54%. The protein content of chickpea seeds was estimated and the maximum content of protein noticed in PUSA 362 (23.52%) followed by KWR108 (22.90%), Phule G 5 (22.50%) in control, *Rhizobium* inoculated PUSA 362 (24.52%) followed by C-235 (24.23%) KWR108 (24.20%) and lowest value was in KKG 306 (19.54%) followed by L550 (19.25%), KAK 2 (18.75%), in control whereas, *Rhizobium* inoculated KKG 306 (20.86%) followed by L550 (20.40%), KAK 2 (19.80%) during 2010-11. In the second year maximum protein content was reported in PUSA 362 (23.64%) followed by KWR108 (22.85%), Phule G 5 (22.60%) in control, *Rhizobium* inoculated PUSA 362 (23.58%) followed by KWR108 (22.88%), Phule G 5 (22.56%) and lowest value was in KKG 306 (20.90%) followed by L550 (20.45%), KAK 2 (19.85%), in control whereas, *Rhizobium* inoculated KKG 306 (20.90%) followed by L550 (20.45%), KAK 2 (19.85%) during 2011-12. The results regarding protein content

Table 1: Percentage of protein content in chickpea seeds

	Protein content (%)					
	Mean				Pooled mean	
Genotypes	2010-11 (C)	2010-11 (R)	2011-12 (C)	2011-12 (R)	(C)	(R)
Pusa-362	23.52	24.52	23.64	24.55	23.58	24.54
KWR 108	22.90	24.20	22.85	24.25	22.88	24.13
H82-2	21.52	22.65	21.50	22.67	21.51	22.66
NDG-54	22.32	23.89	22.30	23.89	22.31	23.89
Phule G5	22.50	23.55	22.60	23.64	22.56	23.60
RSG 888	21.68	22.50	21.65	22.54	21.67	22.53
C-235	21.45	24.23	21.44	24.26	21.45	24.25
DCP 92-3	21.75	22.60	21.80	22.65	21.78	22.63
Uday	20.86	21.82	20.84	21.85	20.85	21.84
Pant G 186	21.72	22.77	21.70	22.80	21.71	22.79
BG 2083	20.65	21.71	20.60	21.75	20.63	21.73
KKG 306	19.54	20.86	19.70	20.90	19.62	20.88
ICCV 10	21.54	22.60	21.53	22.64	21.54	22.62
L 550	19.25	20.40	19.26	20.45	19.26	20.43
KAK 2	18.75	19.80	18.76	19.85	18.76	19.83
CD at 5%	2.21	2.34	2.22	2.37		

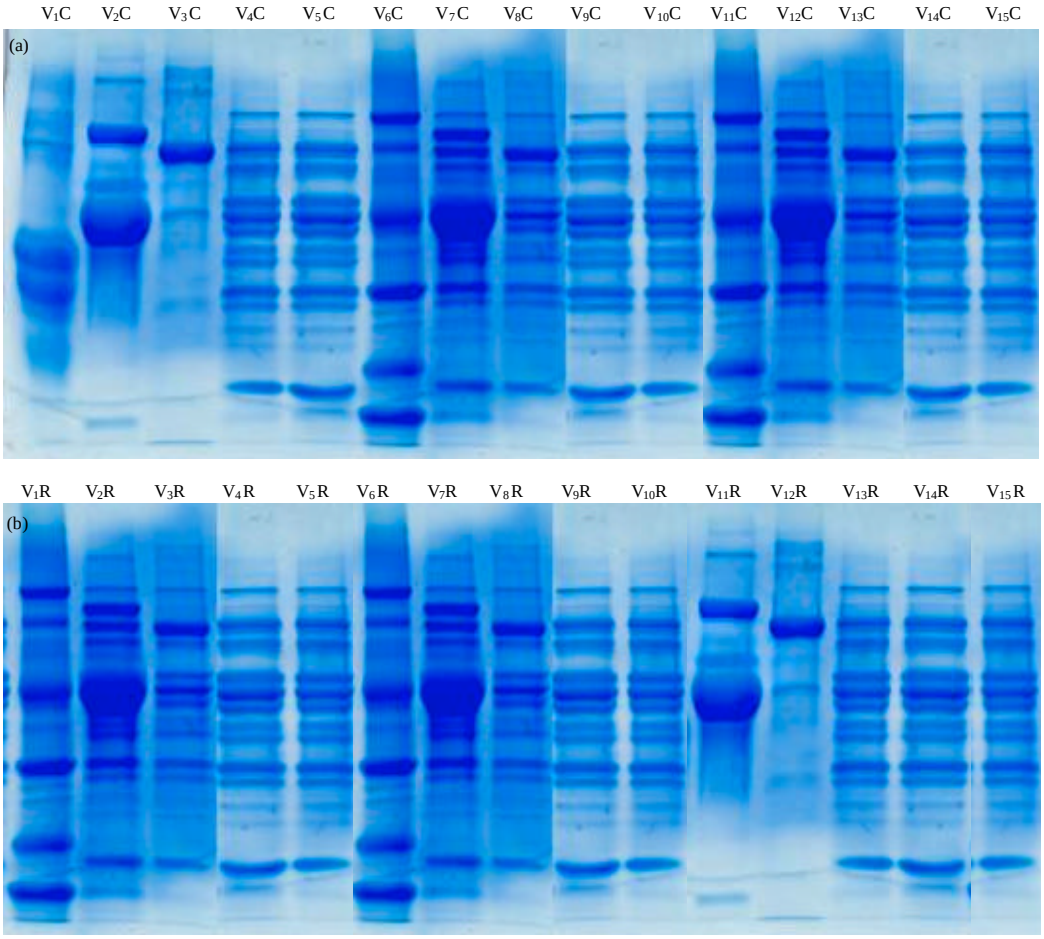

Fig. 1(a-b): Protein profile by SDS-PAGE of chickpea seeds of fifteen genotypes of chickpea, (a) Without *Rhizobium* inoculated and (b) With *Rhizobium* inoculated, V1: Pusa-362, V2: KWR 108, V3: H82-2, V4: NDG-54, V5: Phule G5, V6: RSG 888, V7: C-235, V8: DCP 92-3, V9: Uday, V10: Pant G 186, V11: BG 2083, V12: KKG 306, V13: ICCV 10, V14: L 550, V15: KAK 2

of chickpea seeds varied significantly. Out of 15 genotypes, genotype PUSA 362 was found superior genotype for both of the years. The results are incoherence with Gupta and Namdeo (1997).

Protein profiling have been presented in Fig. 1, the banding patterns produced by the chickpea genotypes were less diverse. Desi genotypes gave some unique band in comparison to Kabuli genotypes of chickpea. Highest number of protein band found in PUSA 362, KWR 108 and followed by H82-2 and Phule G 5 whereas minimum number of protein bands were found in L550. It is clear from the result obtained that the chickpea genotype having highest number of protein band having highest quantity of protein. Maximum bands are of medium molecular weight followed by highest molecular weight and low molecular weight.

Maximum similarity among the desi-chickpea genotypes Pusa-362, KWR 108, H82-2, NDG-54, Phule G5 in control, *Rhizobium* inoculated Pusa-362, KWR 108, H82-2, NDG-54 was recorded 96% similarity. Maximum similarity among the kabuli chickpea genotypes ICCV 10, KAK 2 in control, *Rhizobium* inoculated L 550, KAK 2 was recorded with 94% similarity.

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