Comparative Protein Quantitation in Wild Chickpea and Its Induced Mutants

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ABSTRACT

The chickpea is one of important leguminous cereal crop and India is largest producer of the crop. The grain legumes play important and primary role as a sources of proteins due to the protein content of seed ranging from 20% in pea to 40% lupine, in the third world. Thus, the grain legume is considered as a good substitute to animal proteins in human diet. Mutagenesis is utilized to alter the quantitative and qualitative aspects of the seed protein in many cereal crops. Legume seed proteins primarily increase the nutritional quality and impart a variety of functional properties, including structure, texture, flavor and colour to food products. The present study has been undertaken to assess the mutagenic effect of physical and chemical mutagens on protein content in wild chickpea and its induced mutants.

Key words: - Protein, Wild chickpea, Bradford Assay, Mutagens. Mutant.

Introduction

Legume seed proteins are composed of water-soluble albumin and salt soluble globulins and their ratio can be altered with respect to either of the two under the influence of mutated genes and such alteration improve nutritional value (Amirshahi and Tavakoli, 1970). The enhanced use of soyabean protein (Kinsella, 1979) paved way to concentrate increased research for the utilization of legume seed protein in foods (Kim *et al.,* 1990). Legume seed proteins primarily increase the nutritional quality and impart a variety of functional properties, including structure, texture, flavor and colour to food products.

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alteration improve nutritional value (Myers and Gritton, 1988). Inter and intra specific variation in seed protein have been reported in wheat, barley and their wild relatives (Masood *et al.,*1994).. The gamma radiation has been reported to generate the morphological variation and seed storage protein profile in *Phaseolus vulgaries* (Belele *et al.,* 2001). The various concentration of EMS has been reported to induce variation in cowpea (Odeigah *et al*., 1998). The chromosomal rearrangements or even doubling of the chromosome numbers have been reported albeit, no or very small effects on the seed protein profile (Nakai, 1977). *Cicer reticulatum* is the wild progenitor of cultivated chickpea (Ahmad and Slinkard, 1992). The more or less similar pattern of albumin and globulin proteins has been reported in all the *Pisum* species (Przybylska *et al.,* 1977).

Material and Method

The seeds of *Cicer reticulatum* of Accession Number ICC 17121 were procured from the ICRISAT, Patancheru, India. The seeds of 1st set treated with various concentration of EMS viz. 0.1%, 0.2%, 0.3% and 0.4% formed treatment T_2, T_3, T_4, T_5 respectively. The seeds of 2nd set first treated with chemical mutagen and thereafter subjected to physical mutagenic treatment with various concentration of EMS and doses of gamma rays in 0.1% EMS +5KR, 0.2% EMS +10KR, 0.3% EMS +15KR and 0.4% EMS +20KR forming treatment T_6, T_7, T_8, T_9 respectively. The seeds of 3rd set subjected to various doses of gamma radiation viz. 5KR, 10KR, 15KR, 20KR, 25KR, 30KR formed treatment T_{10}, T_{11}, T_{12}, T_{13}, T_{14} and T_{15} respectively while the untreated normal 4th set scored as control formed treatment T_1.The treated seeds were sown to raise M1 generation to derive M1 seeds yield for the present study. The test seeds in T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, and T_{15} treatments were used for the estimation of protein in M_1 generation.

Protein Extraction

The M1 seeds of all the treatments were powdered separately and the 1000µl of 0.1M NaOH was added to the 30 mg of seed flour of each treatments and mixed by vortex. Thereafter, centrifuged at 15,000 × g. rpm for 7 minute at 4°C. The extracted crude proteins are recovered as a clear supernatant and store in refrigeration for further use. The soluble protein was estimated by dye-binding method (Bradford's method) as it showed highest sensitivity for protein. Coomassie Brillant Blue G-250 is one of the dyes that combines with protein to give an absorption maximum in the region of 595nm wave length. Red dye CBB G-250 turned blue on addition to the protein sample and the absorbance of working dye is 1.18 (Sadasivum and Manickam, 1996). The Bovine Serum Albumin was used as standard protein .The Protein reagent (0.01%) was used in the present study was made by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 ml of 95% alcohol and 100 ml of 85% (wt. / vol.) Ortho-phosphoric acid followed by diluted to 1 Liter with the distilled water. The seeds of M_1 generation were powdered and the 25 mg of seed flour was mixed with 1ml of Protein Extraction Buffer (0.05 M Tris -HCL, 0.2% SDS, 5 M Urea and 1% β-Mercaptethanol with pH-6.8-7.00) in the eppendorf tube to extract the seed storage protein thereafter, centrifuged at 15000×g rpm for 7 Minutes at 4°C in cooling centrifuge. Supernatant was collected and stored in the refrigerator for protein estimation and (Asgar *et al.,* 2003). Every time, the fresh reagent was prepared at the time of use.

The seed protein content of mutant and control was evaluated using Bradford assay (1976) against BSA as standard at 590 nm on UV spectrophotometer. The standard graph was plotted between absorbance and quantity of BSA. Protein solution containing 10 to 100 µg protein in a volume up to 0.1 ml was pipetted into test tubes. The volume was adjusted to 0.1 ml with phosphate saline buffer in each test tubes. 5(ml) milliliters of protein reagent was added and mixed either by inversion or vortexing. The absorbance was measured at 595 nm after 2 min and before 1

hour against a reagent blank prepared from 0.1 ml of the phosphate saline buffer and 5 ml of protein reagent.10 µl protein extract in PEB aliquot was assayed with 5ml of CBB G-250. A standard graph was plotted and the amount of protein in unknown sample was calculated for all the treatments.

Absorbency measurement of each sample was taken in triplicate and the mean of three reading taken as the optical density of the sample. The quantity of each fraction was evaluated in relation to standard curve for BSA Bovine Serum albumen following Bradford (1976) dye-binding method (Prasad *et al.,* 1986). The protein estimation by Bradford assay for all the treatments of M_1 generation is depicted in **Table 1**.

Result and Discussion

The seed protein content in present study, estimated by Bradford assay was found to be increased in all the treatment as compared to the control and represented in the **Table 1**. The higher amount of protein 36 µg was observed in T_{12} treatment of M_1 generation. The protein content has been reported increased in *Phaseolus* followed by the mutagenic treatment (Prasad *et al.,* 1986). The seed protein content of mutants has been reported in *Cicer arietinum* over the parent control followed by the mutagenic treatment with different concentration of sodium azide (SA), ethyl methane sulphonate (EMS) and gamma radiation (GR) in M_3 generation (Barshile and Apparao, 2009). Relative increase in protein content and the highest increase have been reported in 5KR and 10KR in 2 different *Phaseolus* variety of bountiful and giant respectively (Prasad *et al,* 1986). The induction of high protein mutant may be attributed to the micromutation with positive (+ve) effects and low seed yield to micro-mutations with negative (–ve) effects (Prasad *et al.,* 1986).

Singh and Shashtry (1977) and Tallbery (1981) confirmed that the alteration of protein composition is due to mutated genes. Proteins are the direct gene products and hence mutation in gene(s) responsible for its synthesis may be reflected in the polypeptides (Prasad *et al.,* 1986). The +ve alteration in seed protein contents and profile indicate that induced changes are as a result of mutated genes (Prasad *et al.,* 1986). Legume seed protein is mainly composed of water soluble albumin and salt soluble globulin. The ratio of two subfractions can be altered in favour of either of two under the influence of mutated genes (Muller, 1977). And such alteration in mutants improves their nutritive value (Nelson, 1969). Gamma ray induced protein mutants reported in different crop *Cicer* (Sheikh *et al.,* 1978) 13.1% high protein (Abo-hegazi, 1980) in *Vigna* high protein reported following treatment with EMS, gamma rays and Sodium Azide by Tahir Nadeem *et al.* (1978).High protein and high amino acids were reported in mutant treated with different mutagen high protein and high amino acid content: 17-28% arginine, 14-22% glycine 5-14% aspartic acid, 7-9% leucine, 0-5% other in *Vicia* treated with EMS and gamma rays (Hussein and Abdalla, 1979), 21-34.95% high protein in gamma treated mutant in M_5 generation (Abo-hegazi, 1979).

Conclusion

The protein profile of all the mutants in M_1 generation showed variation with respect to the untreated control parents on comparison. in the present study. The chemical and physical mutagen showed the potential to cause the mutation in the wild chickpea. The variation was observed between control and its induced mutants. The induced mutants with suitable qualitative and quantitative traits which may be utilized in to the improvement breeding programme.

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Table 1: Protein Estimation by Braddfords Assay in M_1 Generation of wild *Cicer reticulatum* L. and its mutants .

Sr No	Treatment	Protein Sample Extract (in PEB)	Phosphate Saline Buffer (PSB pH=7.00)	Protein Reagent	Optical Density at 595 nm	Quantity of Protein µg/250µg Seed flour	Mg/100 mg w/w
1	T_1	10µl	90µl	5ml	0.1430	15µg	6.00
2	T_2	10µl	90µl	5ml	0.3218	35µg	14.00
3	T_3	10µl	90µl	5ml	0.3003	33µg	13.2
4	T_4	10µl	90µl	5ml	0.2502	27µg	10.8
5	T_5	10µl	90µl	5ml	0.2353	25µg	10.00
6	T_6	10µl	90µl	5ml	0.2188	23µg	9.2
7	T_7	10µl	90µl	5ml	0.1928	21µg	8.4
8	T_8	10µl	90µl	5ml	0.1665	17µg	6.8
9	T_9	10µl	90µl	5ml	0.1424	15µg	6.00
10	T_{10}	10µl	90µl	5ml	0.1577	16µg	6.4
11	T_{11}	10µl	90µl	5ml	0.2065	22µg	8.8
12	T_{12}	10µl	90µl	5ml	0.3336	36µg	14.4
13	T_{13}	10µl	90µl	5ml	0.2388	25µg	10.00
14	T_{14}	10µl	90µl	5ml	0.1673	17µg	6.8
15	T_{15}	10µl	90µl	5ml	0.2450	26µg	10.4

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