Quantitative Assessment of the Seed Storage Protein in Induced Mutants.

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ABSTRACT

The chickpea is one of important leguminous crops in world and Indian subcontinent. The grain legumes serve as important sources of proteins and protein content ranging between 20% in pea to 40% lupine. Mutagenesis is harnessed to change the quantitative and qualitative properties of the seed protein. Legume seed proteins primarily enhance the nutritional quality including structure, texture, flavor and colour. The present study has been focused to study the mutagenic effect of the physical and chemical mutagens on protein content.

Key words: - Wild chickpea, *Cicer*, Bradford Assay, Sodium Azide, Mutants.

Introduction

Legume seed proteins contains water-soluble albumin and salt soluble globulins and their proportion can be changed under the influence of mutated genes which result into the improvement of nutritional value (Amirshahi and Tavakoli, 1970). Legume seed proteins enhance the nutritional quality including structure, texture, flavor and colour to food products. 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 create the morphological variation and seed storage protein in *Phaseolus vulgaries* (Belele *et al.,* 2001). The various concentration of EMS has been reported to induce variation in cowpea (Odeigah *et al*., 1998). *Cicer reticulatum* is annual wild species of the cultigens and the wild progenitor of cultivated chickpea (Ahmad and Slinkard, 1992). The number of chemical and physical mutagenic agents is used in the mutation breeding.

Material and Method

The seeds of *Cicer reticulatum* were obtained from the ICRISAT, Patancheru, India. The seeds treated with different concentration of Sodium Azide viz. 0.1%, 0.2%, and 0.3% and encoded as T_2, T_3, T_4, treatments respectively. The seeds of another set first treated with chemical mutagen and thereafter subjected to physical mutagenic treatment with various concentration of SA and doses of x rays such as 0.1% SA +5KR, 0.2% SA +10KR, 0.3% SA +15KR forming treatment T_5, T_6, T_7 respectively. The seeds of 3rd set subjected to various doses of x radiation viz. 5KR, 10KR, 15KR, formed treatment T_8, T_9, T_{10} respectively while the untreated normal as control formed treatment T_1. The treated seeds were sown to raise M_1 generation to derive M_1 seeds yield for the present study. The test seeds of T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10} treatments were used for the protein quantitation in M_1 generation.

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 eppendorff 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 further experimentation. The soluble protein was estimated by dye-binding method (Bradford, 1976). Red dye Coomassie Brilliant Blue G-250 turned blue on addition to the protein and the absorbance of working dye was maintained 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. Every time, the fresh reagent was prepared at the time of use. The seed protein content of mutant and control was estimated using Bradford assay (1976) against BSA as standard. The 10 µl (0.1ml) protein extract in PEB aliquot was taken and volume adjusted 1ml with phosphate saline buffer (PSB) in each test tubes thereafter, each test seed sample assayed with 5ml of CBB G-250. The amount of protein in unknown sample was calculated for all the treatments using standard protein.

Absorbency measurement of each sample was taken in triplicate and the mean of three readings were 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 storage protein content 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 31 µg was observed in T_2 treatment. The protein content has been reported enhanced in *Phaseolus* treated with the mutagens (Prasad *et al.,* 1986). The seed protein content of mutants has been reported increased in *Cicer arietinum* 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). The similar observation has been reported in chickpea treated with EMS and gamma radiation independently and in combination in M_1 and M_2 generation (Kamble *et al.,* 2015 a, b). Relative increase in protein content and the highest increase have been reported in 5KR and 10KR in two different *Phaseolus* variety (Prasad *et al,* 1986). The induction of high protein mutant may be attributed to the micromutation with positive (+ve) effects (Prasad *et al.,* 1986).

Tallbery (1981) confirmed that the alteration of protein composition is due to mutated genes. The proteins are the direct gene products and hence mutation in gene(s) responsible for its synthesis might be appeared in the polypeptides (Prasad *et al.,* 1986). The +ve alteration in seed protein contents indicate that induced changes are as a result of mutated genes (Prasad *et al.,* 1986). 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 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 seed storage protein profile of all the mutants in M_1 generation showed variation with respect to the untreated control in the present study. The chemical and physical mutagen showed the potential to cause the mutation in the chickpea. The variation was observed between control and its induced mutants. The induced mutants may be utilized in to the improvement breeding programme.

Acknowledgement

The authors are very much thankful to ICRISAT, Patancheru, (AP) India for supplying the wild germplasm and UGC, New Delhi for providing financial assistance to the present study. The author expressed thanks to Dr. H. J. Petkar, Head and Assistant Professor, MCA department, Dr. BNCPE, Yavatmal for providing the computer facility.

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Table 1: Protein Estimation in M_1 Generation of *Cicer reticulatum* L. and its induced mutants (Bradford's Assay).

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	0.2943	31µg	12.00
3	T_3	10µl	90µl	5ml	0.2348	26µg	10.2
4	T_4	10µl	90µl	5ml	0.2015	21µg	8.6
5	T_5	10µl	90µl	5ml	0.1928	20µg	8.1
6	T_6	10µl	90µl	5ml	0.2089	22µg	8.9
7	T_7	10µl	90µl	5ml	0.1865	19µg	7.8
8	T_8	10µl	90µl	5ml	0.2894	29µg	11.7
9	T_9	10µl	90µl	5ml	0.1436	16µg	6.4
10	T_{10}	10µl	90µl	5ml	0.1437	16µg	6.4