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SEED STORAGE PROTEIN QUANTIFICATION BY BRADFORD'S METHOD IN WILD CHICKPEA AND ITS INDUCED MUTANTS

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ABSTRACT

The chickpea is known as one of important worldwide crops. it is one of the chief sources of protein and protein content extending from 20% in pea to 40% lupine. The protocol of Mutagenesis is extensively used to mute the quantitative and qualitative seed protein profile. A legume seed protein is considered to be associated with enhancement of the nutritional quality as structure, texture, flavour and colour. The present study has been executed to assess and evaluate the mutagenic effect of the physical and chemical mutagens on seed storage protein content in M_2 generation.

KEYWORDS: *Wild chickpea, Bradford Assay, Sodium Azide, Induced Mutants.*

INTRODUCTION

Legume seed proteins constitute water-soluble albumin and salt soluble globulins and their proportion can be altered under the impact of mutated genes which result into the improvement of nutritional value (Amirshahi and Tavakoli, 1970). Legume seed proteins improve the qualitative nutritional aspect such as the structure, texture, flavour 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 various doses and concentration in physical and chemical mutagens has been reported to cause the morphological variation and seed storage protein in *Phaseolus vulgaries* (Belele *et al.,* 2001), 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 many chemical and physical mutagenic agents are applied in the mutation breeding process.

MATERIAL AND METHOD

The wild germplasm of chickpea *Cicer reticulatum* for present study was procured from the ICRISAT, Patancheru, India. The physical and chemical mutagenic treatment was given to the seeds independently and in combination. Sodium azide was used for the chemical mutagenic treatment while x ray for physical mutagenic treatment in the present study. The seeds treated separately with different concentration of Sodium Azide as 0.1%, 0.2%, and 0.3% were encoded as T_2, T_3, T_4, treatments respectively. The another set of seeds first treated with various concentration of SA, followed by different doses of x rays viz. 0.1% SA +5KR, 0.2% SA +10KR, 0.3% SA +15KR as combined treatment encoded as T_5, T_6, T_7 respectively. The seeds of 3rd set were treated with various doses of x radiation viz. 5KR, 10KR, 15KR and encoded as T_8, T_9, T_{10} treatments respectively whereas the untreated normal seed formed as control treatment T_1. The treated

seeds were sown to raise M_1 and M_1 seeds yield were sown to raise M_2 generation to derive M_2 seed yield for the present study of protein estimation. The test seeds of T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10} of M_2 generation were used for the protein quantification in M_2 generation.

The seed flour of test seeds of M_2 generation was formed thereafter 25 mg of seed powder was mixed with 1ml of Protein Extraction Buffer (0.05 M Tris -H CL , 0.2 % SDS , 5 M Urea an d 1% ß - Mercaptethanol with pH-6.8-7.00) followed by centrifugation a15000×g rpm for 7 Minutes at 4^0C for seed storage protein extraction . The Supernatant thus collected was stored in the refrigerator for protein estimation by dye-binding method (Bradford, 1976). The estimation of soluble protein was performed with Red dye Coomassie Brilliant Blue G-250, which turned blue when added to the protein. The Bovine Serum Albumin (BSA) was used as standard protein in the present study. The fresh reagent was prepared at the every time of use during experimentation. The Protein reagent (0.01%) was used in the present study to assess the seed protein content of mutant and control by using Bradford assay (1976) against BSA as standard. The 10µl (0.1ml) protein extract in PEB aliquot was taken and adjusted volume 1ml with phosphate saline buffer (PSB) followed by each test seed sample assayed with 5ml of CBB G-250. The protein quantification in unknown sample was assessed for all the treatments using standard protein curve of BSA Bovine Serum albumen following Bradford (1976) dye-binding method (Prasad *et al.*, 1986). Each sample was taken in triplicate and absorbance was measured as an optical density. The protein estimation by Bradford assay for all the treatments of M_2 generation has been represented in Table 1.

RESULT AND DISCUSSION

The seed storage protein content for all the treatment as compared to the control was found to be increased by Bradford assay and represented in the **Table 1**. The higher seed protein content was observed in T_2 treatment as 30 µg . The enhancement of protein content has been reported in *Phaseolus* treated with the mutagens (Prasad *et al.,* 1986); in *Cicer arietinum* treated with different concentration of sodium azide (SA), ethyl methane sulphonate (EMS) and gamma radiation (GR) in M_3 generation (Barshile and Apparao, 2009); in *Cicer reticulatum* treated with EMS and gamma radiation independently and in combination in M_1 and M_2 generation (Kamble *et al.,* 2015 a, b) . The highest increase in protein content have been reported in 5KR and 10KR in two different *Phaseolus* variety and this induction of high protein mutant may be assigned to the micromutation with positive effects (Prasad *et al,* 1986). Tallberg (1981) confirmed that the change of protein composition is due to mutated genes. The proteins are the direct gene products therefore mutation in gene(s) might be reflected in the polypeptides (Prasad et al., 1986). Gamma ray induced protein mutants reported in *Cicer* (Sheikh *et al.,* 1978). The treatment with EMS, gamma rays and Sodium Azide in *Vigna* has been reported high protein mutant (Tahir Nadeem *et al*., 1978). The 21-34.95% high protein has been reported in M_5 generation of *Vicia* treated with gamma rays (Abo-hegazi, 1979). The similar result relative to enhancement of seed protein in the induced mutant treated with chemical and physical mutagen independently and in combination has been observed in the present study.

CONCLUSION

The seed storage protein profile of the induced mutants in M_2 generation represented variation as compared to the untreated control in the present quantitative study. The chemical and physical mutagens have potential to bring about mutation in the chickpea. The quantitative variation was observed between control and its induced mutants. The induced mutants may be harnessed in the improvement breeding programme.

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Table 1: Protein Quantification in M_2 Generation of *Cicer reticulatum* L. and its 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	15 µg	6.00
2	T_2	10µl	90µl	5ml	0.2934	30 µg	11.9
3	T_3	10µl	90µl	5ml	0.2348	26 µg	10.2
4	T_4	10µl	90µl	5ml	0.2045	21 µg	8.6
5	T_5	10µl	90µl	5ml	0.1907	19 µg	7.9
6	T_6	10µl	90µl	5ml	0.2045	21 µg	8.6
7	T_7	10µl	90µl	5ml	0.1854	18 µg	7.5
8	T_8	10µl	90µl	5ml	0.2833	27 µg	11.4
9	T_9	10µl	90µl	5ml	0.1436	16 µg	6.4
10	T_{10}	10µl	90µl	5ml	0.1436	16 µg	6.4