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# Comparative Study on Protein of $M_2$ Generation in Wild Chickpea Treated with EMS and Gamma Radiation Independently and in Combination

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**Abstract:** ***The chickpea is one of important leguminous crop and India is single largest producer. The grain legumes serve as an important source of proteins as the protein content of seed is more. Therefore, the grain legume is a good substitute to animal proteins in the diet. Mutation breeding is employed to mute the quantitative and qualitative aspects of the seed protein in many crops. Legume seed proteins play an important role to enhance the nutritional quality including structure, texture, flavor and colour to food products. The present work has been undertaken to assess the mutagenic effect of gamma radiation and ethyl methane sulphonate on protein content in wild chickpea and its induced mutants in $M_2$ generation.***

**Keywords:** EMS, Gamma radiation, Wild chickpea, Protein, Bradford Assay

## 1. Introduction

The proteins present in the legume seed are composed of water-soluble albumin and salt soluble globulins and their proportion may be modified with respect to either of the two under the influence of mutated genes and such alteration improves nutritional value [4]. Legume seed proteins primarily improve the nutritional quality and impart a variety of functional properties as 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 [11].The gamma radiation has been reported to generate variation and seed storage protein in *Phaseolus vulgaries* [7]. The various concentration of EMS has been reported to induce variation in cowpea [14]. The chromosomal rearrangements or even doubling of the chromosome numbers have been reported with no or very small effects on the seed protein profile [13]. *Cicer reticulatum* is the wild progenitor of cultigen [3]. The more or less similar pattern of albumin and globulin proteins has been reported in all the *Pisum* species [16].

## 2. 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. The M1 seeds sown to raise M2 generation in order to obtain M2 seed yield. The test seeds of M2 generation of all the $T_1$, $T_2$, $T_3$, $T_4$, $T_5$, $T_6$, $T_7$, $T_8$, $T_9$, $T_{10}$, $T_{11}$, $T_{12}$, $T_{13}$, $T_{14}$, and $T_{15}$ treatments were used for the extraction and estimation of protein in $M_2$ generation.

The seed storage Protein was extracted using Protein Extraction Buffer (PEB).The M2 test seeds of all the treatments were powdered and the 25 mg of seed flour of each treatments 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 [5]. The extracted crude proteins are recovered as a clear supernatant and store in refrigeration for estimation. The soluble protein was estimated by dye-binding method [8]. Coomassie Brillant Blue G-250 (CBB 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 was maintained 1.18 [17]. 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 double distilled water. Every time, the fresh reagent was prepared at the time of use.The seed protein content of all the treatments was evaluated using Bradford assay (1976) against BSA as standard at 595 nm on UV spectrophotometer. The standard graph was plotted between absorbance and quantity of BSA.


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10 µl protein extract in PEB aliquot was assayed with 5ml of CBB G-250. The amount of protein in unknown sample was calculated for all the treatments using standard. 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 following Bradford assay [8] dye-binding method [15]. The protein estimation by Bradford assay for all the treatments of $M_2$ generation is represented in **Table 1**.

## 3. Result and Discussion

The seed storage protein in all the treatments estimated by Bradford assay was found to be enhanced in all the treatment with respect to the untreated control and tabulated in the **Table 1**. The higher amount of protein as 35 µg was observed in $T_2$, $T_3$ and $T_{12}$ treatment of $M_2$ generation. The protein content has been reported as increased in *Phaseolus* followed by the mutagenic treatment [15]. The seed protein content of mutants has been reported as an increased in *Cicer arietinum* over the 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 [6]. Relative increase in protein content and the highest increase have been reported in 5KR and 10KR in 2 different *Phaseolus* variety [15]. The alteration of protein composition is due to mutated genes as has been reported by Singh and Shashtry [19] and Tallbery [21].The induction of high protein mutant may be attributed to the micromutation with positive (+ve) effects while low seed yield to micro-mutations with negative (–ve) effects [15].

The proteins are the direct result of gene therefore, mutation in gene(s) responsible for its synthesis may be reflected in the polypeptides [15]. The ratio of two subfractions water soluble albumin and salt soluble globulin can be altered in favour of either of two under the influence of mutated genes [12]. The +ve alteration in seed protein contents indicate that the induced changes are as a result of mutated genes [15, 10].

High protein and high amino acids were reported in mutant treated with different mutagen high protein and high amino acid content in *Vicia* treated with EMS and gamma rays [9], 21-34.95% high protein in gamma treated mutant in $M_5$ generation [1]. Gamma ray induced protein mutants reported in different crop *Cicer* [18] 13.1% high protein [2] in *Vigna* high protein reported following treatment with EMS, gamma rays and Sodium Azide by Tahir Nadeem *et al.* [20].

## 4. Conclusion

The protein content of all the induced mutants in $M_2$ generation showed variation with respect to the untreated control parents in the present study. The chemical and physical mutagen showed the potential to change the protein content through the mutation in the wild chickpea. The variation was observed between control and its induced mutants in present study.

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**Table 1:** Protein Quantitation by Bradfords Assay in $M_2$ Generation of wild *Cicer reticulatum* L. and its mutants

| Sr No | Treatment | ProteinSample Extract (in PEB) | Phosphate Saline Buffer (PSB pH=7.00) | Protein Reagent | Optical Density at 595 nm | Quantity of Protein µg/250µg Seedflour | Mg/100 mg w/w |
|---|---|---|---|---|---|---|---|
| 1 | $T_1$ | 10µl | 90µl | 5ml | 0.1429 | 15µg | 6.00 |
| 2 | $T_2$ | 10µl | 90µl | 5ml | 0.3299 | 35µg | 14.00 |
| 3 | $T_3$ | 10µl | 90µl | 5ml | 0.3212 | 35µg | 14.00 |
| 4 | $T_4$ | 10µl | 90µl | 5ml | 0.2829 | 31µg | 12.4 |
| 5 | $T_5$ | 10µl | 90µl | 5ml | 0.2460 | 26µg | 10.4 |
| 6 | $T_6$ | 10µl | 90µl | 5ml | 0.2448 | 26µg | 10.4 |
| 7 | $T_7$ | 10µl | 90µl | 5ml | 0.1975 | 21µg | 8.4 |
| 8 | $T_8$ | 10µl | 90µl | 5ml | 0.1701 | 18µg | 7.2 |
| 9 | $T_9$ | 10µl | 90µl | 5ml | 0.1576 | 16µg | 6.4 |
| 10 | $T_{10}$ | 10µl | 90µl | 5ml | 0.1782 | 18µg | 7.2 |
| 11 | $T_{11}$ | 10µl | 90µl | 5ml | 0.2135 | 23µg | 9.2 |
| 12 | $T_{12}$ | 10µl | 90µl | 5ml | 0.3272 | 35µg | 14.00 |
| 13 | $T_{13}$ | 10µl | 90µl | 5ml | 0.2699 | 28µg | 11,2 |
| 14 | $T_{14}$ | 10µl | 90µl | 5ml | 0.1892 | 19µg | 7.6 |
| 15 | $T_{15}$ | 10µl | 90µl | 5ml | 0.2571 | 27µg | 10.8 |


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