

## Changes on Protein and Methionine Content of Black Gram (*Vigna mungo* (L.) Hepper) Induced by Gamma Rays and EMS

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**Abstract:** Legumes are considered as the major source of protein, however, the protein and their amino acid constituents are less when compared to animals. Especially, legumes are deficient in essential amino acid, methionine which is important for synthesizing other some of the amino acids. The attempts were made to improve the protein quantity and quality of legumes with various strategies and achieved a little improvement. However, it is not sufficient to fulfill the portion requirement. In this connection, the present study was investigated to improve the quantity and quality (methionine) of protein of black gram (*Vigna mungo* L.) through the mutation using physical gamma rays and chemical EMS in M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> generation. The protein and methionine content were estimated through the induced genetic effects by the mutagens. The results showed some level of improvement in protein content in 0.1% EMS and 60 kR of gamma rays.

**Key words:** Mutation • Protein • Methionine • Gamma rays and EMS

### INTRODUCTION

A major part of the human diet all over the world consists of cereals and legumes [1]. Legumes are considered as the major source of protein and dietary amino acid for man and farm animals [2]. According to the estimation of FAO, 70% of human food comprises cereals and legumes and the remaining 30% comes from animals [3]. Animal proteins being more expensive, people in developing countries virtually depend on seed protein alone for their entire protein requirement. But unlike, animal proteins like casein and egg albumins which are nutritionally more balanced in terms of all essential amino acids, plant proteins are generally deficient in lysine and tryptophan where as legumes are deficient in sulphur containing amino acid-methionine and cysteine [1]. Methionine is one of the several essential amino acids needed in the diet; the human body cannot synthesize it from simpler metabolites. Methionine supplies sulfur and other compounds required by the body for normal metabolism and growth. It is an important source of dietary sulfur. It will be essential to confirm that the increased total methionine (or other amino acid) is digestible to the animal to at least the same degree as conventional cultivars [4].

Black gram (*Vigna mungo*) is an important pulse crop occupying unique position in Indian agriculture. Among the pulses, it stands fourth in production and acreage [5]. However, genetic base of the present day collection remains poor [6] due to lack of genetic variability owing to their autogamous nature. So, that creation of variability is difficult through hybridization due to its high self-pollination and more flower drop [5]. Research on this species is lagging behind than that of cereals and other legumes. In order to improve yield and other polygenic characters, mutation breeding can be effectively utilized [5]. Mutation induction has become an establishment tool in plant breeding to supplement existing germplasm and to improve cultivars in certain specific traits [7]. Induction of mutation forms an important part of breeding programme as it widens the gene pool through creation of genetic variability. Therefore, the genetic variability is the basic requirement for making progress in crop breeding [8].

Genetic variants, showing differences in composition of seed protein have been reported in many species and genera of higher plants [9-11]. Mutation breeding, involving refined selection and detection method is of great use in crop improvement [12]. It has hoped that induced changes in the protein complement could be used

in varietal description and assessment of the distinctness of the cultigens [13]. The increasing demand for protein rich new material for animal feed or intermediary products for human nutrition have let to a greatest interest in the pulse crop as a protein source [14].

The methionine concentration averages about 1% within amino acid spectrum, a value similar throughout the whole *Phaseolus* genus. However, genetic alterations of specific protein fractions provide a means for increasing total protein content, raising limiting essential amino acid concentration [2].

## MATERIAL AND METHODS

In the present study, black gram variety Vamban-1 was subjected with physical and chemical mutagen to induce mutation.

### Mutagenic Treatment

**Physical Mutagen:** Five sets of hundred well-matured seeds of black gram were subjected for gamma irradiation. These sets of seeds treated with 20, 40, 60, 80, 100 and 120 kR of gamma rays. Irradiation was accomplished in Sugarcane Breeding Institute (ICAR) at Coimbatore, India. The labeled Cobalt ( $^{60}\text{Co}$ ) was used for source of gamma rays.

**Chemical Mutagen:** Five hundred well-matured healthy seeds were subjected to the mutagenic treatment. The seeds were pre-soaked in distilled water for five hours at room temperature ( $28\pm 2^\circ\text{C}$ ) prior to treatment. After pre-soaking the excess of moisture in the seeds was removed by filter paper. Then, the seeds soaked in the freshly prepared aqueous solution which about three times than that of volume of seeds with corresponding concentrations of EMS viz, 0.02, 0.04, 0.06, 0.08, 0.1 and 0.12 % for six hours at room temperature ( $28\pm 2^\circ\text{C}$ ) with an hour intermittent shaking. The pH of aqueous solution was adjusted to 8.5 by using 0.2 M solution of sodium tetra borate (Borax). After the treatment, seeds were washed thoroughly with distilled water. The untreated seeds pre-soaked in water were used as control. Both treated and control seeds were sown in the field in randomized block design (RBD) with three replication to raise the  $M_1$  generation.  $M_1$  plants were harvested and grown in successive seasons to develop  $M_2$ ,  $M_3$  and  $M_4$  generations. The biometrical and biochemical analysis were studied and the data was significantly analyzed with ANNOVA for RBD using the software NPRC STAT developed from National Pulse Research Centre, Vamban, Tamilnadu, India.

**Biochemical Analysis:** Estimation of seed protein (15) content (%) ( $M_2$ ,  $M_3$  and  $M_4$  generation) 0.5 g seeds from the same plant of various dose/concentration of  $M_2$ ,  $M_3$  and  $M_4$  generation were separately dehulled and ground in a pestle and mortar and the extracts were defatted by washing with three changes of cold acetone for 4 to 6 hr. The acetone was removed by filtration and the extracts were air-dried at room temperature. The protein from the defatted meal were precipitated with 10% trichloroacetic acid and recovered by centrifugation at 5000 rpm for 30 min at  $4^\circ\text{C}$ . The protein content was then determined calorimetrically according to the method of Lowry *et al.* [15] using bovine serum albumin (BSA) as standard.

**Estimation of Methionine Content (16) in Seed Meal ( $M_2$ ,  $M_3$  and  $M_4$  Generation):** For the estimation of methionine content 0.5g of defatted sample (seed meal) was weighed and transferred into a 50 ml conical flask and 6 ml of 2N HCl was added to the conical flask and autoclaved for 1 hr. A pinch of activated charcoal was added to the hydrolysate (autoclaved sample) and heat to boil. It was filtered when hot and washes the charcoal with hot water. The filtrate was neutralized with 10N NaOH to pH 6.5. Sample was made up to the volume to 50 ml with water after cooling to ambient temperature. The solution was made up to 25 ml and transferred into the 100 ml conical flask. Then 3 ml of 10% NaOH was added in the conical flask followed by 0.15 ml sodium nitro prusside. After 10 min 1 ml of glycine, solution was added and again after another 10 min 2 ml of Ortho-phosphoric acid was added and shaken vigorously. The intensity of red colour was read at 520 nm against a blank prepared in the same way but without nitroprusside. Standard curve: 0, 1, 2, 3, 4 and 5 ml of standard methionine solution was pipette out and made up to 25 ml with water. The 0 level serves as the blank. A standard curve was drawn and the methionine content calculated from the graph. Methionine content in the sample = (Methionine content from the graph X 4) mg/g

## RESULTS AND DISCUSSION

**Seed Protein Content (%):** As an estimation of the extent of variability induced in  $M_2$  generation will be great value in providing useful information for carrying out further selection. Seed protein content was gradually increased with increasing dose/concentration of both gamma rays and EMS compared to respective control. Seed protein content was high at 0.1% EMS followed by 60 kR of gamma rays than control in  $M_2$  and  $M_3$  generation in the

Table 1: Effect of mutagens on seed protein (%) in M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> generation

Mutagens	M <sub>2</sub> generation		M <sub>3</sub> generation		M <sub>4</sub> generation	
	Mean (mg)	% over control	Mean (mg)	% over control	Mean (mg)	% over control
Gamma rays						
Control	22.51±1.47	-	22.64±1.22	-	23.59±1.55	-
40 kR	22.62±1.28	0.49	23.66±2.31	0.10	22.71±1.37	-4.96
60 kR	23.98±2.41	6.53	24.21±2.23	6.93	22.16±2.11	-6.95
80 kR	22.37±1.27	-0.62	23.56±1.34	4.06	21.44±1.56	-3.76
EMS						
0.08%	23.62±2.49	4.93	23.71±1.42	4.73	23.45±1.22	-3.81
0.1%	24.21±2.36	7.55	24.45±1.33	7.99	23.48±1.41	-0.46
0.12%	22.69±1.89	0.79	22.98±2.22	1.50	22.21±1.18	-5.84
	SE 0.985	SED 1.236	SE 1.101	SED 1.965	SE 0.934	SED 1.368

Table 2: Effect of mutagens on methionine content (mg met/g seed meal) in M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> generation

Mutagens	M <sub>2</sub> generation		M <sub>3</sub> generation		M <sub>4</sub> generation	
	Mean (mg)	% over control	Range	% over control	Mean (mg)	% over control
Gamma rays						
Control	8.68±0.31	-	5.97-6.21	-	7.49±0.14	-
40 kR	7.59±0.22	12.55	8.55-8.92	-10.85	8.88±0.22	18.56
60 kR	8.56±0.31	-1.38	9.16-9.43	7.82	9.40±0.34	25.50
80 kR	7.00±0.22	-19.35	6.44-6.66	-23.92	6.44±0.41	-17.01
EMS						
0.08%	7.88±0.41	-9.21	7.92-8.24	-5.25	8.40±0.24	12.15
0.1%	8.64±0.11	-0.46	9.56-9.88	12.01	9.68±0.36	29.24
0.12%	6.84±0.24	-21.19	7.59-7.91	-10.85	7.68±0.14	2.54
	SE 0.3658	SED 0.5482	SE 0.3942	SED 0.5692	SE 0.3699	SED 0.5221

present study (Table 1, Fig. 1 & 2). Among the dose/concentration of mutagens, 0.1% EMS and 60 kR of gamma rays which enhanced protein content than control and other dose/concentration in M<sub>2</sub> generation. Similarly, in M<sub>3</sub> generation, significant increase was observed in 0.1% EMS and 60 kR gamma rays than control and other dose/concentration of mutagens. Whereas, seed protein content was decreased in M<sub>4</sub> generation at all dose/concentration of EMS and gamma rays compared to control (Table 1, Fig. 1 & 2). A positive shift of mean values in seed protein content in M<sub>3</sub> generation was observed in Black gram by Rehman *et al.* [17]. Yield and productive traits of M<sub>4</sub> generation with effect of EMS was recorded in black gram [18]. Total protein content was slightly higher than control recorded in black gram mutant [19]. This is clearly a case of mutagenic recessive mutation. However, the mutation has affected a number of associated quantitative traits which improves the yield and protein.

**Methionine Content (mg met/g Seed Meal):** Methionine (Met) is the primary limiting essential amino acid in grain legumes [20]. The mutagens influenced the methionine content with different level of variation. A little reduction of methionine content was found at all dose/concentration of gamma rays and EMS treated population than control in M<sub>2</sub> and M<sub>3</sub> generation. These values were significantly lower than control (Table 2, Fig. 3 & 4). The most significant effect was noticed on methionine content M<sub>4</sub> generation with various dose/concentrations of gamma rays and EMS. Among them, 0.1% EMS enhanced the methionine followed by 60 kR gamma rays compared control (Table 2, Fig. 3 & 4). Data indicated that the introduction of a chimeric gene encoding a methionine-rich seed protein into crop plants, particularly legumes whose seeds are deficient in the essential sulfur-containing amino acids, represents a feasible method for improving the nutritional quality of seed proteins [21]. Genetic alteration of specific protein

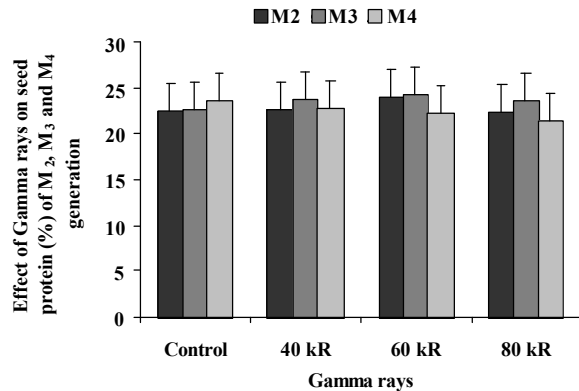


Fig. 1: Effect of Gamma rays on seed protein (%) of M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> generation

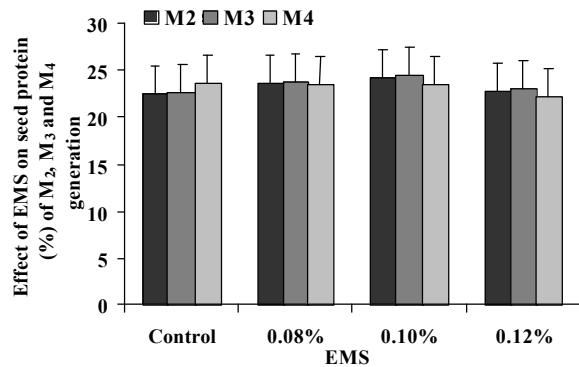


Fig. 2: Effect of EMS on seed protein (%) of M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> generation

fractions provides a means for increasing protein content, raising limiting essential amino acid concentration by differentially regulating fractions with different amino acid composition [2] in the present study. Seed protein variation in electrophoretic profiles of french bean with effect of gamma rays [22]. It revealed appearance and disappearance of band in the protein profile of control seeds and shift in the position of bands in the zygogram of the mutagenized population confirms the alteration in the polypeptide due to gene mutation [21]. In the present investigation, M<sub>4</sub> generation protein content was decreased at all dose/concentration of gamma rays and EMS treated population while, methionine content was increased in M<sub>4</sub> generation at increasing dose/concentration of gamma rays and EMS compared to respective control (Table 1 & 2, Fig. 1-4). It indicated that protein and methionine content were significantly negative correlation with each other. The increase in mean values could due to the occurrence of polygenic mutations with cumulative effects in *Vigna radiata* (L.) [23]. Induced greater

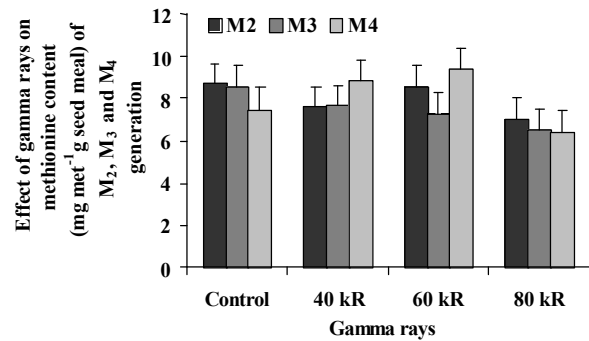


Fig. 3: Effect of Gamma rays on methionine content (mg met<sup>-1</sup> g seed meal) of M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> generation

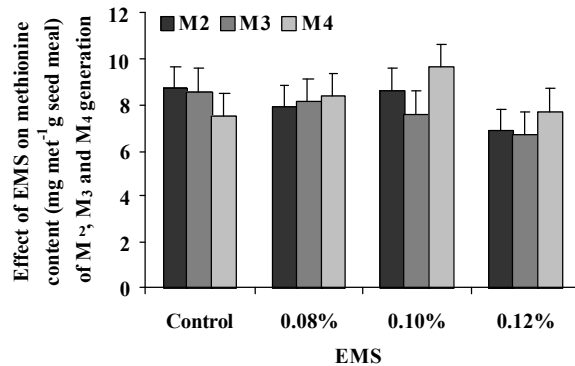


Fig. 3: Effect of Gamma rays on methionine content (mg met<sup>-1</sup> g seed meal) of M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> generation

variability in polygenic traits might due to increased mutations and recombination induced by gamma rays and EMS [24]. Among the dose/concentrations, EMS (0.1%) provided most significant enhancement in mean performance of protein and methionine content. So the study, infers that, protein and their fractions are able to increase through mutagenesis.

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