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Improvement in Protein and Chlorophyll Content through Physical and Chemical Mutagens in *Phaseolus vulgaris* L.

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A B S T R A C T

In the present studies, selected different seven morphological mutants in M_4 generation induced through Gamma rays, EMS and Combination of both mutagens for their better performance. They were estimated for chlorophyll content, leaf protein and seed protein. The estimated values of all recorded mutants ranged from 1.34 to 2.44 mg/gm. The maximum amount of total chlorophyll content 2.44 mg/gm estimated in late flowering mutant, while the minimum amount of total chlorophyll content 1.47 mg/gm. was estimated in sterile mutant. The estimated values of leaf protein content for all observed mutants were ranged from 3.32% to 4.81 %. The maximum estimated amount of total leaf protein content was 4.81% in robust mutant, while the minimum estimated amount of total leaf protein content 3.32 % was estimated in dwarf mutant. The estimated values of seed protein content for all studied mutants were ranged from 25.60 %to 20.8 %. The maximum estimated amount of total leaf protein content was 25.60% in robust mutant, while the minimum estimated amount of total leaf protein content 20.8 % was estimated in late flowering mutants.

Introduction

In modern plant mutation breeding is one of the major trends has been supporting the traditional methods by biochemical investigations to obtain a better estimate of the mutation breeding values of a varieties. The economic importance of different plants, simply not restricted to the number and weight of seeds produced. Many substances are stored in the seeds such as proteins, oils, carbohydrates and minerals and secondary metabolites like alkaloids, tannins etc. In particular, the leaf and seed proteins and seed oils are of considerable significance for both human and animal nutrition. Legumes are not only the major source of proteins, but they are also important as a source of dietary fibres which belong to the carbohydrate. Minerals are also necessary compounds in human nutrition.

They are most important factors in maintaining physiological processes and act as catalysts for many biological reactions.

In leaf and seed material of plants and their nutritional improvement of legumes through mutation breeding have attracted substantial attention of the world (Milner, 1972). It has been increasingly realized that the genetic variability available in legumes can be exploited for increasing the proteins properties to generate nutritionally improved varieties (Jain, 1969; Swaminathan and Jain, 1972). The nutritional characteristics of different legumes have been thoroughly understood and the need for improvement in certain factors in plant system can be highlighted (Baldev *et al.*, 1988).

In last few decades, the aspects of genetically influence on proteins composition have been widely worked out. A new aspect in the agriculture has been the improvement of some such crucial materials, which are vital from nutritional point of view, through the mutation breeding approach.

Induced mutations are source of producing variations in plants. Naturally occurring mutation rate is too low for practical applications. Therefore, physical and chemical mutagens have proven useful to increase the frequency of mutations and variations. In this regard, mutation breeding is considered complementary to the conventional method.

Materials and Methods

Selected mutants in M_4 generation were used for biochemical studies. Biochemical investigation was carried out as follows:

Chlorophyll estimation

The chlorophylls are the essential components for photosynthesis and occurred in chloroplast as green pigments in all green plants. They are made up of proteins and which are extracted in organic solvent like acetone. Chemically, each chlorophyll molecule consists of porphyrin (tetrapyrol) and magnesium at the centre and a long chain of hydrocarbon (phytyl) which is attached through a carboxylic acid group. Chlorophyll a and b occur in higher plants (Witham *et al.*, 1971)

Chlorophyll was extracted in 80% acetone. The 663nm and 645nm wavelength of spectrophotometer was used for different readings of chlorophyll a and chlorophyll b and by using the absorption coefficients; the amount of chlorophyll a and chlorophyll b was estimated.

Weight 1g of finely cut leaves and mixed well into a clean mortar. Grind the sample to a fine pulp by adding of 20 ml of 80% acetone. The extract was centrifuge at 5000 rpm for 5 minutes and transferred the supernatant to a 100 ml volumetric flask. Grind the residue with 20 ml of 80% acetone and again centrifuge and transfer the supernatant to the same volumetric flask.

This procedure was repeated for changed the residue green colour to colourless. Washed the mortar and pestle thoroughly with 80% acetone and collected in the volumetric flask. To make final volume of extract up to 100 ml with 80% acetone. The absorbance of the samples was recorded at the 645, 663 and 652 nm against the solvent 80% acetone as a blank. Calculate the amount of chlorophyll a and b present in the extract.

The following formula was used for estimation:

$$\text{mg chlorophyll } a\text{/ g tissue} = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg chlorophyll } b\text{/ g tissue} = 22.9\ (A_{645}) - 4.68\ (A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{mg total chlorophyll / g tissue} = 20.2\ (A_{645}) - 8.02\ (A_{663}) \times \frac{V}{1000 \times W}$$

Where A = absorbance at specific wavelengths
V = final volume of chlorophyll extract in 80% acetone
W = fresh weight of tissue extracted.
(Source: Sadasivam and Manikam, 2008)

Estimation of leaf protein and seed protein by Lowery's method

Material used: Healthy leaves and dry seeds of French bean.

Procedure for Extraction

For leaf protein extraction, the freshly harvested green leaves were used. The sample was washed with tap water and removed any dust or dirt. For extraction of seed protein, dry seeds were washed with tap water and grinds to them in a fine powder with grinder. The fine powder was used for the extraction of protein by (Lowery *et al.*, 1951) method.

Weight exactly 0.5g of the material and homogenized them in an 80% hot ethanol. The collected homogenate sample was transferred in a test tube and extract was boiled in water bath for about 15-20 minutes. The centrifuged sample at 5000 rpm for 10 minutes then discard the supernatant that contains the low molecular weight compounds like sugars, free amino acids, chlorophyll etc. After centrifuged, sample was collected with 5% PCA. Centrifuged the sample again at 5000 rpm for 10 minutes than discard the supernatant that contains high molecular weight compounds like DNA and RNA. Residue was extracted for 30 minutes at 30°C in 5-6ml of 2% Na_2CO_3 prepared in 0.1N NaOH. Centrifuged the sample at 5000 rpm for 10 minutes then discard the residue and saved the supernatant. Final volume became to 10 ml with distilled water. This prepared crude protein extract was used for present estimation of leaf protein and seed protein.

Estimation of protein

This colorimetric method is used to determine the protein contents from the plant materials. This method is very simple and sensitive. It is based on the principle that different proteins and amount of aromatic amino acids like tryptophan and tyrosine. These amino acids reduce the phosphomolybdic-phosphotungstic components in the folin-phenol reagent giving a blue colored product. A bluish

purple colour is developed by the biuret reaction of the proteins with the alkaline cupric tartarate. The intensity of these colours products is measured calorimetrically or spectophotometric at 660 or 750nm.

Prepared 1 to 8 numbers of test tube as a series of standard protein by pipette solution of BSA from 0.1 to 0.5 ml. adjusted the volume in all the tubes to 0.5 ml by addition of appropriated volume of distilled water. Extract was pipette out 0.1ml and 0.2 ml of protein in test tube no. 7 and 8. Add 5ml of freshly prepared regent C in all the tubes. Mix the content thoroughly and incubated the tubes at room temperature for 20 minutes and add 0.5 ml of reagent D in all the tubes and again incubated them at room temperature for 20 minutes. The absorbance of the samples was recorded at the750nm on a spectrophotometer. Use test tube no.1 (blank) to standardize the instrument. Plot the graph of amount of proteins v/s absorbance at 750nm and from the graph calculated the amount of proteins present in the sample.

The following formula was used for estimation

0.1ml of plant extract contains = X µg protein (from the graph)

∴ 10ml of extract (= total volume of extract) contains = 10X / 0.1

= Y µg protein

This 10ml extract was prepared from 0.5g of plant material.
∴ 0.5gplant material contains = Y µg protein

∴ 1g plant material contains = Y/ 0.5 = Z µg protein

(Source: Sadasivam and Manikam, 2008)

Results and Discussion

Experimental Observations

Chlorophyll Content: (Table No.1)

Total chlorophyll content were estimated in morphological mutant like 1) Dwarf 2) Tall 3) Luxuriant 4) Robust, 7) Large leaves 8) Dark leaves 5) Early flowering 6) Late flowering and 9) Sterile mutants in French bean.

In control plant, estimated value was 1.97 mg/gm of total chlorophyll. The estimated values of all recorded mutants ranged from 1.34 to 2.44 mg/gm. The maximum amount of total chlorophyll content 2.44 mg/gm estimated in late flowering mutant, while the minimum amount of total chlorophyll content 1.47 mg/gm. was estimated in sterile mutant. Besides the total chlorophyll content were specific in Chl-a and Chl-b estimated values was in fluctuations. This value of fluctuation can be directly effect on photosynthesis process in leaves of the plants. The most of morphological viable mutants showed positive shift in mean estimated values against the control and the dwarf mutant observed negative shift in mean estimated value in compared to the control.

Leaf protein content: (Table No.2)

Total leaf protein content were estimated in morphological mutant like 1) Dwarf 2) Tall 3) Luxuriant 4) Robust, 7) Large leaves, 8) Dark leaves, 5) Early flowering 6) Late flowering and 9) Sterile mutants in French bean.

In control plant, estimated value was 3.25 % of total proteins. The estimated values of leaf protein content for all observed mutants were ranged from 3.32% to 4.81 %. The maximum estimated amount of total leaf

protein content was 4.81% in robust mutant, while the minimum estimated amount of total leaf protein content 3.32 % was estimated in dwarf mutant. The estimated values of viable mutants for total leaf protein content was to be found highest value in robust followed by large leaves and late flowering mutants. All morphological viable mutants showed positive shift in mean estimated values against the control.

Seed protein content: (Table No.2)

Total seed protein content were estimated in morphological mutant like 1) Dwarf 2) Tall 3) Luxuriant 4) Robust 7) Large leaves 8) Dark leaves 5) Early flowering 6) Late flowering and 9) Sterile mutants in *Phaseolus vulgaris* L.

In control plant, estimated value was 22.30 % of total proteins. The estimated values of seed protein content for all studied mutants were ranged from 25.60 %to 20.8 %. The maximum estimated amount of total leaf protein content was 25.60% in robust mutant, while the minimum estimated amount of total leaf protein content 20.8 % was estimated in late flowering mutant. The estimated values of morphological viable mutants for total seed protein content was to be found maximum value in robust followed by luxuriant and dark green leaves mutants. The most of viable mutants showed positive shift in mean value against the control and late flowering mutant observed negative shift in mean estimated value in compared to the control.

The total chlorophyll content was estimated in different morphological viable mutants observed in the M_4 generation. These morphological viable mutant like 1) Dwarf 2) Tall 3) Luxuriant 4) Robust, 5) Large leaves 6) Dark leaves 7) Early flowering 8) Late flowering and 9) Sterile induced through the EMS, Gamma rays and combination of both mutagens treatments in *Phaseolus vulgaris* L. in the majority of viable mutants the estimated values of the chlorophyll pigment increased after mutagenic treatments and few viable mutant shows the pigment decrease in the leaves. The chlorophyll a and chlorophyll b content was significantly increased as a result of mutagenic treatments and in some mutants the content of chlorophyll was linearly decreased (Mejri *et al*., 2011) reported that chlorophyll a and chlorophyll b content was significantly decreased after Gamma radiations. Increased in the frequency of chlorophyll mutants depends on the dose or concentration of the mutagen. Chlorophyll a and b content increased significantly after Gamma radiations. Gamma radiation causes the mutation of leaflets and chlorophyll deficiency in Faba beans by (Mejri, *et al*., 2011) Similar result was reported by many researchers in viable mutants of different crop plants like *Solanum* by (Kothekar, 1983), in Winged bean by (Hakande, 1992), in Alfafa by (More, 1992), in Pea by (Satpute and Dhulgande, 2010), in Cluster bean by (Shinde, 2013) and in Cowpea by (Gaikwad, 2013).

The leaf and seed protein content in the present investigation have revealed an enhancement in majority of morphological viable mutants observed in the M_4 generation. These morphological viable mutant like 1) Dwarf 2) Tall 3) Luxuriant 4) Robust 5) Large leaves 6) Dark leaves 7) Early flowering 8) Late flowering and 9) Sterile induced through the EMS, Gamma rays and combination of both mutagens treatments in *Phaseolus vulgaris* L. A positive shift of mean value in leaf and seed protein content was observed in majority of viable mutants. Total estimated protein content in leaf and seed was slight higher than control. The estimated leaf and seed proteins observed in highest values in high yielding mutants. After mutagenic

treatments on seed material the quantitative and qualitative changes was observed due to which estimated soluble protein has been increased in majority of mutants. The estimated leaf and seed protein content in high yielding mutant showed positive shift in mean value over the control after mutagenic treated with Gamma rays EMS and combination. In some mutants estimated negative values in total seed protein content induced by mutagens.

For the improvement of proteins content in leaf and seed material through genetic approach can be a reliable source. Efforts are required for the manipulation of various protein components in the leaf and seed of crops. (Mertz *et al*., 1964) reported that the genetic alteration of protein quality can be considered as useful technique for the rapid removal of protein malnutrition. In recent years the protein synthesis in seeds is regulated by a series of specific genes in the proteins due to genetic alterations in most of the crops. The total seed protein content and protein pattern with relative proportions of the protein groups are influenced by the different mutant genes (Gottschalk and Muller, 1970).

In present estimation of leaf and seed proteins in different viable mutants the leaf protein content was increased after different mutagenic treatments. The similar result was reported by many researchers.

According to them leaf protein content were estimated in two varieties of chickpea after Gamma radiation then leaf protein content were slightly decreased in some doses and leaf protein content was increased at higher doses. (Arulbalchandran and Mullainathan, 2009) in Black gram. Seed protein content was gradually increased with increase in dose or concentration of EMS and Gamma ray treatments against control.

Total protein content in leaf and seed was slight higher than control recorded Mutagenic treatments produced recessive mutations and these mutations have affected a number of associated quantitative characters which improve yield and proteins. The similar result was reported by many researchers like (Jijiya, 1986 and Sudhakaran, 1971) and (Desai and Rao, 2014).

Tah, 2006 reported that seed protein content was increased in two varieties of Moonbeam against the control. (Auti and Apparao, 2008) reported that seed protein content increased in viable mutants after mutagenic treatments in Mungbean. (Wani and Anis, 2008) reported that reduction in protein content in high yielding mutant of Chickpea. (Pavadai *et al.,* 2010) reported that increased seed protein content in Soybean. (Sri Devi and Mullainathan, 2012) recorded that increased seed protein content of mutant in Black gram.

Similar result was reported by many researchers in viable mutants of different crop plants like *Solanum* by (Kothekar, 1983), in Winged bean by (Hakande, 1992), in Alfafa by (More, 1992), in Pea by (Satpute and Dhulgande, 2010), in Cluster bean by (Shinde, 2013) and in Cowpea by (Gaikwad, 2013).

The nutritional improvement of legumes through mutation breeding program has immense important in world of food crisis.

Table.1 Effect of mutagen on Chlorophyll content in the morphological mutant of *Phaseolus vulgaris* L.

Morphological mutants	Chlorophyll-a Mg/gm	Chlorophyll-b Mg/gm	Total Chlorophyll Mg/gm
Control	1.02	0.94	1.97
Tall Mutant	1.28	1.02	2.30
Dwarf Mutant	0.77	0.57	1.34
Luxuriant Mutant	1.19	1.04	2.23
Robust	1.11	0.91	2.02
Early flowerings	1.01	0.96	1.98
Late flowering	1.30	1.13	2.44
Dark green	1.19	0.80	1.99
Large leaves	1.09	0.91	2.00
Sterile	1.13	0.34	1.47

Table.2 Effect of mutagen on Leaf protein and Seed protein content in the morphological mutant of *Phaseolus vulgaris* L.

Morphological Mutants	Seed protein %	Leaf protein %
Control	**22.30**	**3.25**
Tall Mutant	**23.00**	**3.32**
Dwarf Mutant	**23.40**	**3.41**
Luxuriant Mutant	**24.60**	**3.34**
Robust	**25.60**	**4.81**
Early flowerings	**23.50**	**3.36**
Late flowering	**20.8**	**3.65**
Dark green	**24.00**	**3.54**
Large leaves	**24.1**	**4.21**
Sterile	**-**	**-**

Induction of mutation is an important part of breeding programmed as it creates the gene pool through creation of genetic variability. Therefore, the genetic variability is the basic requirement for making improvement in crop breeding programmed.

Many researcher have worked in the field of mutation breeding and estimation of biochemical studies like (Deshpande, 1980), (Kothekar, 1983), Hakande, 1992), (More, 1992), (Satpute, 1994), (Elangovan and Selvraj, 1994), (Shen *et al*., 2008), (Sagade, 2008), (Mundhe, 2008), (Yathaputanon *et al*., 2009), (Giri, 2010) and (Satpute and Dhulgande, 2010) and (Shinde, 2013).

In conclusion, from the above estimation, there is a lot of scope for improvement through induced mutation breeding and biochemical studies like chlorophyll content, protein content in French bean. The estimation of biochemical components indicated the application of mutation breeding in the development of superior genotypes carrying improved nutritional values in French bean.

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