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INDUCTION STUDIES FOR CHLOROPHYLL PIGMENTS AND PROTEINS CONTENT IN *LABLAB PURPUREUS* (L.) SWEET THROUGH ETHYL METHANESULPHONATE AND GAMMA RAYS

S.S. Jagtap[1] and A.D. More[2]
1. Department of Botany, Waghire College, Saswad, Pune-412301, India.
2. Department of Botany, Fergusson College, Pune-411004 Savitribai Phule Pune University, Pune- 411 007, India
Email: swap2004@yahoo.co.in

ABSTRACT:
In the present investigation the estimation of total chlorophyll content, and protein content were estimated for the improvement of the nutritive value of the *Lablab purpureus* (L.) Sweet. The chlorophyll 'a' and 'b' content was estimated of the leaves, and protein content of the pods and seeds were estimated in the M_4 generations. The highest chlorophyll content was estimated in late flowering mutant and lowest in early flowering mutants. The highest protein content was estimated in dark green leaf mutant and lowest at the dwarf mutants. Promising results were observed in tall, spreading, dark green, luxuriant and early flowering mutants as far as bio chemical content is concerned.

Keywords: *Mutation; EMS, Gamma rays, Mutant, Chlorophyll content; protein content*

INTRODUCTION:

Pulses are the important source of the proteins, vitamins and minerals. By products of the Pulses are fed to the animals as a dry and fresh fodder. *Lablab purpureus* is grown as a pulse crop in Asia, Africa and Caribbean. The immature seeds, pods and young leaves are edible and cooked as vegetables. *Lablab purpureus* is used as a forage, hay and silage crop. As forage it is grown with sorghum and millet.[12]The nitrogen fixing legume is valuable as a green manure.[5,2] It is also used as a stimulant to reduce fever flatulence to stimulate digestion and as an antispasmodic[18], in Namibia the root has been used to treat heart condition[15] Mutation breeding is a novel technique to impart biochemical changes in plants. This method may bring out many positive results leading to crop improvement[4, 8, 9].

MATERIAL AND METHODS:

The Experimental genotype selected for the present investigation was Dolichos bean *Lablab purpureus* .L (Sweet). It is commonly known as a Wal in Marathi. The experimental seed material was collected from College of Agriculture, MPKV, Shivajinagar, Pune, Maharashtra, India. Physical Mutagen Gamma Rays and Chemical Mutagens Ethyl Methanesulphonate was employed for induction of mutation.

Mode of the Mutagenic Treatment:

1. Gamma rays- Healthy and uniform size of dry seeds of the Dolichos bean variety *Phule suruchi* were treated with CO^{60} irradiation. The seed samples were exposed to doses of 100Gy, 200Gy, 300Gy, and 400Gy of Gamma rays.

2. Ethyl Methanesulphonate (EMS molecular weight 124.16 g/mol and its density 1.20g/cm^3) was used to determine the lethal dose (LD $_{50}$) at suitable concentration of mutagen for the further study. The different concentrations used for the chemical mutagenic treatment were 10mM, 20mM, 30mM, and 40mM.

3. Combination treatment- For the combination treatment Gamma rays irradiated seeds were treated by EMS. The concentration/ dose for combination treatment were 100Gy+40mM, 200Gy+30mM, 300Gy+20mM, and 400Gy+10mM. For each treatment 500 seeds were used.

Seeds of each treatment along with the control were sown in field as per Complete Randomized Block Design (CRBD) with three replications to raise the M_1 generation plants. Screened mutants of M_4 generation were tested for the Biochemical studies.

Chlorophyll estimation:

The extraction of the chlorophyll was carried out in 80% Acetone. The amount of chlorophyll a and chlorophyll b was estimated by using absorption coefficients as per Sadashivam and Manikam, 2008.

Protein content:

The freshly harvested green leaves were used for the protein extraction. This fine powder was used for extraction of protein by Lowery *et. al;* 1951 method.

RESULTS AND DISCUSSION:

Total chlorophyll estimation and protein content was recorded in the morphological mutants like 1) Dwarf 2) Tall 3) Luxuriant 4) Dark green leaf mutant 5) Spreading 6) Early flowering and 8) Late flowering mutant in Lablab purpureus (L.) Sweet Variety Phule suruchi. (table no.1 and.2)

Mutation breeding now days is an effective tool in improvement of economically important crops[1]. Gamma rays inhibit the plant growth and development by inducing cytological, biochemical, genetical, morphological and physiological changes in the cells and tissues[6]. The total chlorophyll content estimated in different morphological viable mutants observed in the M $_4$ generation. are 1) Dwarf 2) Tall 3) Luxuriant 4) Spreading 5) Early flowering 6) Late flowering and 7) Dark green leaf mutants. These viable mutants induced through the treatment with the EMS, Gamma rays and combination treatments in *Lablab purpureus* (L.) variety *Phule suruchi.* Mutagenic treatment increased the chlorophyll content in the viable morphological mutants. The chlorophyll a and b content was significantly increased as a result of the mutagenic treatment. Increase in the chlorophyll depends on the dose or the concentration of the mutagen. Chlorophyll a and b content increased significantly after the gamma irradiations. Similar results were reported by the researchers in the viable mutants of the other economically important crops like *Solanum* [10], Winged bean [7]and Pea [16].

The leaf, Pod and dry seed protein content estimation revealed an increase in the total protein content in majority of the viable morphological mutants. The five mutants showed an increased in the total protein content while two mutants showed a reduction in the total protein content. Increase in the soluble protein in the majority of the mutants could be due to qualitative and quantitative change by the mutagenic treatment of the EMS, Gamma rays and combination treatment. The genetic approach can be the reliable source for the improvement of the protein content in the leaf, pod and seed

material[13]. The mutagenic treatment may produce recessive mutations and these mutations affect the number of the quantitative characters which altered the yield and protein content. Similar results were reported in Mungbean[3,19], Reduction in the protein content reported in chickpea[20], increased in protein content were reported in Soyabean[14], and increased in seed protein content were observed in the Black gram[17].

CONCLUSION:

Physical and chemical mutagens are capable in inducing mutations the by altering genetic material of the plant. The mutagens like EMS and Gamma rays have successfully induced genetic variability in different mutants. The mutants like Dark green leaves, Spreading and Luxuriant was observed highest yield in contributing parameters and even more protein and carbohydrate content as compared to control. These mutants could be promoted for cultivation after successful completion of seed certification process.

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Table No: 1: Effect of mutagen on Chlorophyll content in the morphological mutant of *Lablab purpureus* (L.) Sweet variety *Phule suruchi*.

Morphological Mutants	**Chlorophyll a mg/gm**	**Chlorophyll b mg/gm**	**Total Chlorophyll mg/gm**
Control	1.5	0.77	2.27
Dwarf Mutant	1.52	0.838	2.35
Tall Mutant	1.6	0.92	2.52
Luxuriant Mutant	1.82	1.21	3.03
Dark Green leaf Mutant	1.82	1.32	3.14
Spreading Mutant	1.51	0.843	2.35
Early flowering mutant	1.59	1.30	2.89
Late flowering mutant	1.50	0.82	2.32

Table No.2: Effect of mutagen on Leaf protein and Seed protein content in the morphological mutant of *Lablab purpureus* (L). Sweet variety *Phule suruchi*.

Morphological mutants	**Immature pod proteins (%)**	**Dry seed proteins (%) in 100 gms.**	**Leaf proteins (%)**
Control	2.47	22.30	2.25
Dwarf	2.2	21.00	2.32
Tall	3.1	23.40	3.01
Luxuriant	4.2	23.60	3.34
Spreading	4.12	24.00	3.31
Early flowering	3.4	22.50	2.36
Late flowering	2.1	20.8	2.45
Dark green leaves	4.5	24.30	3.54