Spectrum of Chlorophyll Mutations in *Lablab purpureus* (L.)Sweet through EMS and Gamma rays mutagens

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Abstract

Lablab purpureus (L.) belongs to family Fabaceae. Fabaceae is second largest agriculturally important family after the Family Poaceae in terms of the food and vegetable protein source and of fodder. *Lablab purpureus* is the single species of the genus *Lablab*. It is known as a Dolichos bean and economically important as grain legume, vegetable, animal fodder and green manure. In the present study seeds of *Lablab purpureus* (L.) variety *Phule Suruchi* were treated with Ethyl Methane Sulphonate (EMS) at concentration of 10mM, 20mM, 30mM, 40mM, Gamma rays doses with 100Gy, 200Gy, 300Gy, 400Gy, and combination of EMS and Gamma rays Concentration/doses at 100Gy+40mM, 200Gy+30mM, 300Gy+20mM, 400Gy+10mM.

In the present investigation the frequency of the chlorophyll mutants in the EMS treatment was in the range of the 1.23-3.33% in M_2, 3.48-5.68% in M_3 and 3.47-5.53% in M_4 Generation. In Gamma rays was 1.25-3.48% in M_2, 3.10-4.86 in M_3, 3.85-5.16% in M_4 Generation and in Combination treatment the frequency of the chlorophyll mutant was 3.10-6.06% in M_2, 4.15-6.03% in M_3 and 5.24-6.92% in M_4 Generation. The highest frequency of the chlorophyll mutants 5.68% in EMS was observed at the 40mM concentration in M_3 generation *and* lowest at 10mM EMS treatment with frequency percentage was 1.23% in M_2 Generation. In Gamma rays the highest frequency percentage of chlorophyll mutants 5.16 % were recorded in the 400Gy Gamma radiation in M_3 Generation and lowest 1.25% at the 100Gy radiation. In Combination treatment the highest frequency of the chlorophyll mutants was observed to be 6.92% at the 400Gy+10mM treatment in M_4 Generation and lowest 3.10 at 100Gy+40mM treatment in M_2 Generation. Amongst the all the three mutagenic treatments the highest frequency of chlorophyll mutant was 6.92 % observed in 400Gy+10mM treatment in M_4 Generation and lowest was 1.23% at 10mM EMS treatment in M_2 Generation.

Keywords: Gamma rays, EMS, *Chlorophyll Mutants, Albino, Xantha, Chlorina,* and *Viridis* etc.

Introduction:

Pulses are the main source of the supplementary protein to the daily diet of the vegetarian population. Pulses are grown in different parts of the world and well adapted to the diverse climatic conditions. Their low input requirement, fast growth, nitrogen fixing ability makes them suitable to the diverse environment. They have short growing period and photosensitive. Pulses have the wide range of adaptability to the latitude, longitude and climatic variables. Pulses include all those legume species whose kernels either whole or split are used as a food for the human consumption. It includes majority of the grain legumes. Pulses are the important source of the proteins, vitamins and minerals. The family Fabaceae comprises more than 600 genera and about 18,000 species of cultivated plants.

Fabaceae is second largest agriculturally important family after the Family Poaceae in terms of the food and vegetable protein source and of fodder. *Lablab purpureus* is the single species of the genus *Lablab*. There are three subspecies of the Genus *Lablab purpureus. Lablab purpureus* subsp *.bengalensis*, found in Tropical areas like Africa, Asia and America has tender fruits. *Lablab purpureus* subsp. *purpureus*

grown in Asia as a field crop for seeds and fodder. It is semi-erect bushy perennial usually grown as an annual crop. The fruits are relatively short. The plants flushed with purple in all the parts. The plant has strong and unpleasant smell. *Lablab purpurus* subsp. *uncinatus* is of East African origin has relatively small fruits.

The *Lablab* as a wild distributed in Grassland and bush land forest. As a cultivated crop lablab has many favorable traits grow in adverse environmental conditions. Lablab remains green during the dry season when other fodder crops dry. The wild Lablab found from the sea level 2000-2400 meter altitude but for cultivation prefers low Altitude.

Lablab is a summer growing annual and short-lived perennial fodder legume. Which sown for grazing and conservation in tropical environments with a summer rainfall. It is a vigorously trailing, twining herbaceous plant, resistant to disease and insect attack (Milford and Minson; 1968, Cameron; 1988). Stems are trailing to upright, reach to 3 m in length and are robust. Leaves are large and trifoliate, with the leaflets having a broad ovate-rhomboid shape measuring 7 to 15 cm long. The dorsal side of the leaf is smooth with the underside being hairy (Cameron, 1988).

Material and Methods:

Experimental Genotype

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.

Mode of the Mutagenic Treatment:

1. Gamma rays:

Healthy and uniform size of dry seeds of the Dolichos bean variety *Phule suruchi* were packed in the polythene bags and sealed for the Gamma radiation. Electromagnetic ionizing radiations were applied from CO^{60} source of irradiation. Gamma radiation was carried out at Nuclear Chemistry Division, Department of Chemistry, SPPU, Ganeshkhind, Pune -411007.The seed samples were exposed to doses of 100Gy, 200Gy, 300Gy, and 400Gy of Gamma rays.

2. Ethyl Methanesulphonate (EMS):

Ethyl Methanesulphonate (EMS) was obtained from Spectrochem. Pvt. Ltd. Mumbai (India) with a molecular weight 124.16 g/mol and its density 1.20g/cm^3 to determine the lethal dose (LD_{50}) at suitable concentration of mutagen for the further study. Chemical mutagenic treatments were administered at room temperature at 25±2°C. Healthy and dry seeds of the Dolichos bean variety *Phule suruchi* having uniform size were selected for the treatment. Seeds were surface sterilized with 0.1% mercuric chloride solution for about one to two minutes then washed thoroughly and soaked in distilled water for 6 hours for pre –soaking of the seeds, which were made the seed coat permeable for the mutagenic treatment.

The aqueous solution of the mutagen was prepared prior to the treatments. The different concentrations used for the chemical mutagenic treatment were 10mM, 20mM, 30mM, and 40mM.After the pre soaking seeds were immersed in the mutagenic solution for the four hours with the continuous shaking. The volume of the chemical solution used was five times more than of the seeds to facilitate uniform absorption. Seeds soaked in distilled water for 6 hours served as a control. Immediately after the completion of the treatment, the seeds were washed thoroughly under running tap water for 3 to 4 times. The seeds later on kept for post –soaking in distilled water for 4 Hours.

500 seeds were used for each treatment. Out of 500 seeds, 100 seeds from each treatment were plotted between the folds of the filter paper and kept in the dark room at room temperature. It is used to record the germination percentage and seedling injury. Another slot of 100 seeds were kept in the filter

paper and germinated in the petriplates after three days to raise the root tips required for the study of the cytological preparation like the mitotic index and screening of the chromosomal abnormalities. The remaining 300 seeds of each treatment along with the control were sown in field by Complete Randomized Block Design (CRBD) with three replications to raise the M_1 generation plants.

3. Combination treatment:

For the combination treatment Gamma rays irradiated seed samples were used. After the Physical mutagenic treatment, chemical mutagenic treatment of EMS was conducted on the seed samples. In the combination treatment Gamma rays and EMS mutagens used like 100Gy+40mM, 200Gy+30mM, 300Gy+20mM, and 400Gy+10mM. For each treatment 500 seeds was used. From each treatment 100 seeds were plotted between the folds of filter paper and kept in dark at room temperature, which was used to record the germination percentage and seedling injury. Another 100 seeds were kept in filter paper and germinated in petri plates after three days to raise the root tips required to study cytological preparations for the mitotic index and screening of chromosomal abnormalities. The remaining slots of 300 seeds of each treatment along with the control (untreated seeds) were sown in field by Complete Randomized Block Design (CRBD) with three replications in order to raise the M_1 generations.

Chlorophyll mutations:

The chlorophyll mutations were screened and recorded in the field when the seedlings were 7-10 days old. The types of chlorophyll mutations scored like *albino*, *Xantha*, *Chlorina*, and *Viridis*. These are classified according to the terminology of (Gustafsson; 1940). The frequency of chlorophyll mutants was calculated according to (Gaul; 1960) i.e. number of mutants/100 M_2plants. The M_2 generation was raised from the harvested and collected seeds of the M_1 generation plants. The chlorophyll mutants were scored 10-15 days old seedling of plants. The chlorophyll mutants like, *Albina, Xantha, Chlorina and Viridis* were recorded in Dolichos bean of M_2 plants.

The following characteristics of chlorophyll mutations.

Albina*: albina* mutant was completely white in colour such seedling cannot survive more than 10-15 days after seed germination.

Xantha*: xantha* mutant was yellowish in colour, this mutant survives for 20-25 days and growth of the seedling was stunted further.

Chlorina*: chlorina* mutant was yellowish green in colour few of them again reverted to normal green colour and survive up to 40-45 days.

Viridis*: viridis* mutant was light green in colour the seedling can survive up to 50-55 days.

Experimental observations:

The Experimental results recorded in the present investigation on "Induction of genetic variation *Lablab purpureus* (L) Sweet (Dolichos bean) by physical and chemical mutagens." in variety *Phule suruchi*.

Albina **Chlorina** **xantha** **viridis**

Table No. 1. Effect of mutagens on the spectrum of Chlorophyll mutants in M_2 generation of *Lablab purpureus* (L.) Sweet.

Mutagens	Dose /Conc.	Frequency of Chlorophyll mutants				
		Albina	*Xantha*	*Chlorina*	*Viridis*	Total
Control	-	-	-	-	-	-
Ethyl Methane sulphonate	10mM	-	0.411	0.411	0.411	1.23
	20mM	0.427	0.427	0.854	-	1.70
	30mM	-	0.888	0.444	0.444	1.77
	40mM	0.476	0.952	0.952	0.952	3.33
Gamma rays	100Gy	0.416	0.416	0.416	-	1.25
	200Gy	-	0.877	0.877	0.438	2.19
	300Gy	0.462	0.925	0.925	0.462	2.77
	400Gy	0.497	0.995	0.995	0.995	3.48
Combination Treatments	100Gy+40mM	0.444	1.33	0.888	0.444	3.10
	200Gy+30mM	0.462	0.925	1.388	0.925	3.70
	300Gy+20mM	0.490	1.470	1.470	0.980	4.41
	400Gy+10mM	1.01	2.02	1.515	1.515	6.06

Table No. 2. Effect of mutagens on the spectrum of Chlorophyll mutants in M_3 generation of *Lablab purpureus* (L.) Sweet.

Mutagens	Dose /Conc.	Frequency of Chlorophyll mutants				
		Albina	*Xantha*	*Chlorina*	*Viridis*	Total
Control		-	-	-	-	-
EMS	10mM	0.696	1.393	1.045	0.348	3.48
	20mM	0.729	1.094	1.459	0.729	4.01
	30mM	1.115	1.115	1.858	1.115	5.20
	40mM	1.136	1.893	1.515	1.136	5.68
Gamma Rays	100Gy	0.344	0.689	1.379	0.689	3.10
	200Gy	0.709	1.063	1.418	1.063	4.25
	300Gy	0.719	0.438	1.438	0.719	4.31
	400Gy	0.749	1.123	1.872	1.123	4.86
Gamma Rays+EMS	100Gy+40Mm	0.346	1.038	1.384	1.384	4.15
	200Gy+30mM	0.716	1.075	1.433	1.433	4.65
	300Gy+20mM	0.735	1.470	1.838	1.470	5.51
	400Gy+10mM	1.132	1.886	1.886	1.132	6.03

Table No. 3. Effect of mutagens on the spectrum of Chlorophyll mutants in M_4 generation of *Lablab purpureus* (L.) Sweet.

Mutagens	Dose /Conc.	Frequency of Chlorophyll mutants				
		Albina	*Xantha*	*Chlorina*	*Viridis*	Total
Control	-	-	-	-	-	-
Ethyl Methane sulphonate	10mM	0.347	1.041	1.388	0.694	3.47
	20mM	0.741	1.423	1.779	1.067	5.01
	30mM	1.086	1.086	1.449	1.449	5.07
	40mM	0.738	1.476	2.214	1.167	5.53
Gamma rays	100Gy	0.350	1.052	1.403	1.052	3.85
	200Gy	0.357	1.428	1.071	1.428	4.28
	300Gy	0.722	1.805	1.444	1.083	5.05

	400Gy	0.738	1.476	1.845	1.107	5.16
Combination Treatments	100Gy+40mM	0.699	1.748	1.748	1.048	5.24
	200Gy+30mM	1.086	1.449	1.811	1.449	5.79
	300Gy+20mM	1.115	2.230	1.486	1.486	6.31
	400Gy+10mM	0.769	1.923	2.307	1.923	6.92

Chlorophyll mutations (Table No. 1 to 3)

In the present investigation the frequency of the chlorophyll mutants in the EMS treatment was in the range of the 1.23-3.33% in M_2, 3.48-5.68% in M_3 and 3.47-5.53% in M_4 Generation. In Gamma rays was 1.25-3.48% in M_2, 3.10-4.86 in M_3, 3.85-5.16% in M_4 Generation and in Combination treatment the frequency of the chlorophyll mutant was 3.10-6.06% inM_2, 4.15-6.03%in M_3 and 5.24-6.92% in M_4 Generation. The highest frequency of the chlorophyll mutants 5.68% in EMS was observed at the 40mM concentration in M_3generation *and* lowest at 10mM EMS treatment with frequency percentage was 1.23% in M_2 Generation. In Gamma rays the highest frequency percentage of chlorophyll mutants 5.16 % were recorded in the 400Gy Gamma radiation in M_3 Generation and lowest 1.25% at the 100Gy radiation. In Combination treatment the highest frequency of the chlorophyll mutants was observed to be 6.92% at the 400Gy+10mM treatment in M_4 Generation and lowest 3.10 at 100Gy+40mM treatment in M_2 Generation. Amongst the all the three mutagenic treatments the highest frequency of chlorophyll mutant was 6.92 % observed in 400Gy+10mM treatment in M_4 Generation and lowest was 1.23% at 10mM EMS treatment in M_2 Generation.

The relative percentage of the *albina* mutant was ranged from 0.41-1.01% and in *xantha* range from 0.41-2.02%, in *chlorina* range from 0.41-1.51%, and *viridis* range from 0.41-1.51% in M_2 Generation. The relative percentage of the *albina* mutant was ranged from 0.34-1.13% and in *xantha* range from 0.43-1.88%, in *chlorina* range from 1.04-1.88%, and *viridis* range from 0.34-1.47% in M_3 Generation.The relative percentage of the *albina* mutant was ranged from 0.34-1.11% and in *xantha* range from 1.04-2.23%, in *chlorina* range from 1.38-2.30%, and *viridis* range from 0.69-1.51% in M_4 Generation.

Discussion:

In the present investigation the chlorophyll mutants were scored in the M_2 generation in the research field after the 10-12 days of old seedlings of the *Lablab purpureus* (L.) Sweet. The observed chlorophyll mutants were classified into *albina*, *xantha*, *chlorina* and *viridis* according to the classification was given by the (Gustafason, 1940). The frequency of the chlorophyll mutants was increased with the increases in the concentration or dose of the mutagens in all treatments. The highest frequency of the chlorophyll mutants was observed at the 400Gy+10mM of Combination treatment followed by the 40mM EMS treatment and 400Gy Gamma rays treatment. Frequency of *chlorina* mutants was observed maximum in most of the treatment followed by the *xantha*, *viridis* and *albina*.

Frequency of chlorophyll mutation could be enhanced linearly with dose or concentration was observed by (Konzak, *et .al*; 1965). An increased in the frequency of chlorophyll mutant with different doses of Gamma rays was recorded by Reddy, (1974). EMS acts on genes responsible for the chlorophyll development was suggested by Reddy and Gupta, (1989). Natarajan and Upadhya, (1964) was correlated the high incidence of the chlorophyll mutations after EMS treatment due to the specificity of the mutagens to the certain regions of the chromosomes. Chlorophyll mutation was related to the gene or chromosome alterations in the system of the plants were observed by Asencion *et. al*; (1994). (Shavarnikov *et.al;* 1976) were observed the difference in the chlorophyll mutation frequency and spectrum of chlorophyll mutation was depends on mutagen, plant genotype and physiological state of the organisms during the process of mutagenic treatments. Ryan and Heslot, (1963) was stated that induction of chlorophyll mutation was dependent on randomized action of physical mutagens while EMS has some specificity with the loci of chromosome in Barley.

Frequency of chlorophyll mutation was increased with increases of dose or concentrations in Gamma rays, Sodium Azide and Combination treatments were reported by Srinivas and Veerabadhiran, (2010) in *Lablab purpureus* (L.) Sweet. They suggested that strong mutagens canreached their saturation point even at low dose in the genotype having highly mutable allelic sites and strong mutagens becomes more toxic than higher doses relatively weak mutagens. (Gnanamurthy*et.al;*2011) were observed frequency of chlorophyll mutants in M_2 generation, the maximum chlorophyll mutation frequency were recorded at 50mM concentration of EMS and minimum chlorophyll mutation frequency was observed at 30mM concentration of Sodium Azide. The mutation frequency shows decreased with increases in the concentration of all the mutagenic treatments in *Zea mays* (L.). Girija and Dhanavel,(2013) were reported that on the seedling basis of M_2 generations progressive increased in the frequency of chlorophyll mutation was increases in all mutagenic dose or concentration of Gamma rays and EMS in *Vigna unguiculata* (L.)Walp.They observed chlorophyll mutation of *albino*, *xantha* and *viridis* in all mutagenic treatments but predominant occurred of *viridis* mutant in all mutagenic treatments.In M_2 generation the frequency of chlorophyll mutation was progressive increased with increases in all mutagenic dose of mutagenic treatments or concentrations in *Catharanthus roseus*(L.) was reported by (Mangaiyarkarasi *et.al*; 2014)

Similar observations were reported by many researchers in different plants like (Kothekar in 1978) in *Solanum*, (Deshpande,1980) in *Mormodica*, (Hakande,1992) in Wingbean, (More,1992) in *Medicago sativa*, (Shinde , 2013) in *Cymopsis tetragonoloba* (Linn)Taub, (Salve, 2014) in *Coriandrum sativum* Linn, (Bhosale, 2014) in *Withania somnifera* Dunal , (Gaikwad,2015) in *Vigna unguiculata* (L.)Walp, (Borkar, 2015) in *Phaseolus vulgaris*, (Ramezani , 2015) in *Lathyrus sativus* Linn.

CONCLUSION:

The chlorophyll deficient sectors of the leaflets in the M_1 generation produced the different types of the chlorophyll mutants like *albina* (White), *viridis* (Light green), *xantha* (Yellow) and *chlorina* (Yellow green). These was found on the margin of the leaflet or on the entire leaflet. The combination treatment was succeeded in production of the highest number of the chlorophyll deficient sectors followed by the Gamma rays and EMS. The seeds were harvested and collected from the M_1 generation were raised to grow the M_2 generation plants. The spectrum of the frequency of the chlorophyll mutations like *albina*, *viridis*, *xantha* and *chlorina* were recorded. The maximum mutant frequency was reported from the Combination followed by the Gamma rays and EMS.

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