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Effect of mutagens on Mitotic Index and mitotic aberrations in M_1 generations of *Lablab purpureus* (L.) Sweet

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Abstract:

In the present investigation the effect of the different Chemical and Physical mutagens like EMS, Gamma rays and combination of both EMS and Gamma rays concentration and doses were studied for the mitotic index and mitotic abnormalities in the M_1generation . The Mitotic index is decreased with the increased in the concentration and doses of the mutagens. All the three mutagenic treatments EMS, Gamma rays and combination treatment induced the reduction in mitotic index. The maximum induction of mitotic index is observed in EMS followed by Combination and Gamma rays radiation. The different mitotic abnormalities like micronuclei, mis-orientation, stickiness, precocious movements, multiple bridges. The percentage of the mitotic abnormalities was increased with the increases in the dose or concentration of the mutagenic treatments. The highest percentage of the mitotic abnormalities was observed at EMS treatments followed by the Gamma rays and Combination treatments.

Keywords: Gamma rays, EMS, Mitotic index. micronuclei, mis-orientation, stickiness, precocious movements, multiple bridges etc.

Introduction:

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. Mass *et. al*; (2010) were observed that *Lablab purpureus* suffer from low yield when grown as a cash crop it is mostly popular as a home garden and mixed cropping. *Lablab purpureus* is used as a forage, hay and silage crop. As forage it is grown with sorghum and millet. The nitrogen fixing legume is valuable as a green manure.

Lablab purpureus (L.) Sweet is summer growing annual or short lived perennial forage legume. It growing habit is twining, climbing, or upright herbaceous plant that grows up to 3-6 mt in length. The plant has deep tap root system and glabrous or pubescent stem. The leaves are alternate and trifoliate. The leaflet is rhomboidal in shape and 7.5-15 cm long and 8-14cm broad. The leaflet is acute at the apex. The upper surface of leaflet is smooth and the lower surface rough. The inflorescence is raceme with many flowers on the elongated peduncle. The flowers are white, blue or purple in colour.

Seed Production:Grain maturation on forage cultivars is not uniform but crops have more synchronous maturity. High grain yields of 1-2.5 t/ha depending on the cultivar. In mixed cropping the grain yields of 0.5 t/ha. Late seedling varieties affected by early frosts. India is one of the major producer and consumer of the pulses in the world. The total 29% of the area is under cultivation of the pulses with the 19% of the total production of the world. India is also the largest importer and processor of the pulses in the world.

Economic importance

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As Commercial crop:*Lablab purpureus* is grown as a pulse crop in Africa, Asia and Caribbean. It is also consumed as a green vegetable as its tender pods and leaves are high in nutrients. Compared to other crops it is low in yield therefore it is cultivated as garden crop or mixed crop. Protein from the Dolichos bean can be used as a food additive (Maass *et. al*; 2010).

As Forage crop: *Lablab purpureus* is used as forage and hay. The leaves are edible as fodder and seeds moderately edible. It is one of the most palatable legumes for animals (Valenzuela and Smith; 2002).

Nutrient contents:There is considerable variation in the composition of the pods and the seeds of the Dolichos bean according to cultivar, climatic conditions and the standard of crop management. The proportion of seed to pod-husk is approximately 1.1:1. The approximate composition of the immature pods has been given as: moisture-82.4%; protein-4.5%; fat-0.1%; fibre-2%; carbohydrate-10%; ash-1%, calcium-0.05%; phosphorus-0.06%; iron-10 mg / 100g and nicotinic acid-0.8 mg / 100g: Vitamin C: uncooked samples-0.77-1.12 mg / 100 g and cooked samples-7.33-10.26 mg / 100 g., 4%; carbohydrate- 60.1 % ; ash-3.2%; calcium-0.06%; phosphorus-0.45%; iron-2 mg /100g and nicotinic acid-1.8 mg / 100 g. The chief protein is a globulin and ***Dolichosin*.** The amino acid content (mg / g N) has been reported as: isolencine-256, leucine-436, lycine-36, methionine-36, cystine-57, phenyl alanine-299, tyrosine-197, threonine-207, valine-294, arginine-393, histidine-186, alanine-266, aspartic acid-727, glutemic acid-978, proline-288 and serine.

Materials and Method

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: Ethyl Methanesulphonate 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^oC. 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.

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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.

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 were 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.

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Cytological studies:

From each treatment/dose treated with mutagen about one hundred seeds were kept in moist filter paper at room temperature (25 ± 2^0C) About 1-1.5cm root tips length were fixed in 1:3 Carnoy's fluids fixative (1 part of Glacial Acetic Acid and 3 parts of Alcohol) for 12 hours. They were later on transferred to 70% Ethanol and stored in Refrigerator. Before preparation of slide, the root tips were hydrolyzed in 1N HCL at 60^0C in water bath for 7-10 minutes and then wash with water and stained in 1% Hematoxylin by using 4% ferric alum as a mordent. They were later on squashed in a drop of 45% acetic acid. At least 20-25 slides were prepared for each concentration. The mitotic aberrations were screened and recorded. Cytological study includes the stickiness of the chromosome, mis-oriented metaphase, precocious movement of chromosome, sticky bridge, multiple Anaphase Bridge and micronuclei.

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.* The chemical mutagen Ethyl Methanesulphonate, Physical mutagen like Gamma rays and combination treatment like EMS and Gamma rays in Dolichos bean is discussed below.

Mitotic Index: (Table No.1)

The mitotic index in the control of Dolichos bean was 19.00%. The mitotic index decreased with the increases in the dose/concentration of all the three mutagenic treatments like EMS, Gamma rays and Combination treatment (EMS+ Gamma rays). The mitotic index in the EMS range was from 14.67-18.65%. In Gamma rays the mitotic index range was from 14.35-17.80%, while in the

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combination treatment the mitotic index range was from 15.40-18.50%.The highest mitotic index 18.65% was observed at 10mM concentration and lowest 14.35% at 400Gy Gamma rays treatment

Table No.1- Effect of Mutagens on Mitotic Index in M_1 Generation of *Lablab purpureus*

Mutagens	**Dose /Concentration**	**Mitotic Index**	**S.E**
Control	-	19.00	-
EMS	10mM	18.65	0.6
	20mM	17.70	0.5
	30mM	15.50	0.3
	40mM	14.67	0.5
Gamma Rays	100Gy	17.80	0.3
	200Gy	16.20	0.4
	300Gy	15.40	0.7
	400Gy	14.35	0.9
Gamma Rays+ EMS	100Gy +40mM	18.50	0.6
	200Gy +30mM	17.20	0.5
	300Gy +20mM	16.10	0.8
	400Gy+10mM	15.40	0.4

Mitotic abnormalities :(Table No-2)

The mitotic metaphase and anaphase stages of the mitotic cell division were observed and calculated the total percentage of the abnormalities. The different types of the abnormalities such as the micronuclei, mis-orientation, precocious movements, stickiness and multiple bridges were observed in the different treatments of the mutagens. The frequency of the mitotic abnormalities increased with the increases in the concentration/dose of the mutagens. The frequency of the mitotic abnormalities in EMS treatment in the range was 5.9-14.73%. In Gamma rays radiation the frequency of the mitotic abnormalities was in the range of the 5.34-10.08%, while in the combination treatment the frequency of the mitotic abnormalities were in the range of the 5.48-10.07%. The maximum frequency of mitotic abnormalities was observed in 40mM of EMS treatment followed by the 400Gy Gamma rays treatment and 400Gy +10mM Combination treatment. The lowest frequencies of the mitotic abnormalities were observed at the 100Gy Gamma rays radiation. The frequencies of the mitotic abnormalities were more than anaphase abnormalities in all the treatments.

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Table No. 2.Effect of mutagens on mitotic aberrations in M_1 generations of Lablab *purpureus* (L.)Sweet

Mutagens	Dose / Conc.	Pro Phase	% of Abnormalities in Metaphase			% of Abnormalities in Anaphase		Total % of Abnormalities
	-	Micro Nuclei	Mis Orient Tation	Stickiness	Precocieous movement	Stickiness	Multiple Bridge	
Control	-	-	-	-	-	-	-	-
EMS	10mM	0.78	2.57	0.80	0.45	0.77	0.53	5.9
	20mM	0.9	2.98	0.90	0.90	1.12	0.87	7.67
	30mM	2.16	3.56	1.15	1.38	1.18	0.7	10.13
	40mM	3.12	4.44	2.54	2.13	0.58	1.92	14.73
Gamma Rays	100Gy	0.85	0.80	1.54	0.87	0.74	0.54	5.34
	200Gy	0.87	1.92	0.65	1.43	1.33	0.85	6.4
	300Gy	1.45	1.73	1.83	1.56	1.76	0.70	9.03
	400Gy	2.80	2.89	0.95	0.98	1.34	1.12	10.08
Gamma Rays+ EMS	100Gy +40mM	0.90	1.92	0.67	0.89	0.45	0.65	5.48
	200Gy +30mM	1.27	2.77	0.59	0.76	0.83	0.67	6.89
	300Gy +20mM	0.84	2.90	0.93	1.54	0.93	1.67	8.81
	400Gy+ 10mM	2.67	3.67	1.23	0.73	1.23	0.54	10.07

The effect of the Physical and chemical mutagens evidenced in various growth phenomenon such as and cytological abnormalities in the M_1 generation of the *Lablab purpureus* (L.) Sweet. The physiological damage is due to toxicity of the mutagens. There is disintegration of the cell physiology, showing the M_1 generation imbalance in growth. The factor mutation affects genetic material like DNA and RNA which are heritable from one to another generation. The chromosomal aberrations affects the cell division process and results into the decreased in seed germination percentage and increases the lethality of the plants.

For the evaluation of the M_1 biological effect some important parameters were undertaken for the study of Mitotic index and mitotic abnormalities.

Result and Discussion:

Mitotic index:

In *Lablab purpureus* (L.) Sweet the mitotic index was decreased with the increases in the dose or concentration of the all three mutagenic treatment. The decreased in the mitotic index because of

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the formation of cytochemical substances, physiological changes in protoplasm and increased in the chromosomal aberrations after the mutagenic treatments**.**

All the three mutagenic treatments like EMS, Gamma rays and combination of EMS and Gamma rays showed the reduction in the mitotic indices .The maximum induction of mitotic index was observed in the EMS followed by the combination and Gamma rays radiation. The similar results were reported by the (Bhatta and Sakya, 2008) in *Allium cepa* L. where mitotic index decreased with the increases in concentration of the Magnesium sulphate treatment.

Mitotic abnormalities:

In the present investigation of *Lablab purpureus* (L.) Sweet various types of the mitotic abnormalities were studied such as micronuclei, mis-orientation, stickiness, precocious movements, multiple bridges. The percentage of the mitotic abnormalities was increased with the increases in the dose or concentration of the mutagenic treatments. The highest percentage of the mitotic abnormalities was observed at EMS treatments followed by the Gamma rays and Combination treatments. In *Sesamum indicum* (L.) was investigated the chromosomal aberrations induced by EMS. The chromosomal aberrations like stickiness, laggard and multivalent abnormalities was maximum in higher concentration of the EMS reported by (Kumar and Yadhav, 2010).The chromosomal aberrations developed due to physiological and chromosomal damage was reported by (Singh *et.al;* 1997, Nilan *et.al;* 1964). Sometime the cell division like mitosis delayed also caused the chromosomal aberrations observed by (Yadhav, 1987).

The chromosomal aberrations induced by biochemical activities of certain enzymes like catalase and Lipase was observed in reduction of germination percentage and survival at the maturity of the plants was reported by (Ananthaswamy *et.al;* 1971).The mitotic index was decreased in different concentrations of Magnesium sulphate. The drop in Mitotic index was steep in the higher concentration of Magnesium sulphate was reported by (Bhatta and Sakya, 2008) in *Allium cepa*.The reduction in mitotic activities due to blockage of G_1 cell cycle which suppressed DNA synthesis was reported by (Mohandas and Grant,1972) in higher plants.

The chromosomal abnormalities induced by the chemical, physical mutagens and the combination of both were reported by the (Sree Ramalu, 1973; Mehra and Mann, 1974). (Goswami and Dave, 1975) was observed that the breakage of the spindle fibre unilaterally or through the developments of fibres across the spindle leads to the mis-orientaion type of the abnormality during the mitosis. The two hit exchanges resulting in the inversion forms the single double or multiple bridge formation during the Anaphase was reported by (Patil and Bora, 1961). The chances of the bridges are due to stickiness of the chromosome observed by (Sudhakaran, 1971).

The similar results were reported by the (Shinde and More, 2013) in *Cymopsis tetragonoloba* (Linn)Taub, (Salve and More, 2014) in *Coriandrum sativum* Linn, (Bhosale and More, 2014) in *Withania somnifera* Dunal , (Gaikwad and More,2015) in *Vigna unguiculata* (L.)Walp, (Borkar and More, 2015) in *Phaseolus vulgaris* , (Ramezani and More , 2015) in *Lathyrus sativus* Linn.

Summary and Conclusion:

The mitotic index was decreased with the increases in the dose or the concentration of the Gamma rays, EMS and Combination treatment. The pollen sterility percentage was increased with the increases in the dose or concentration of the physical and chemical mutagens. The different chromosomal abnormalities like the micronuclei, mis-orientation, stickiness, precocious movements,

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multiple bridges were observed in the prophase, metaphase and anaphase stages of the mitosis. It was observed that the percentage of the chromosomal abnormalities increased with the increases in the dose or the concentration of the Gamma rays and EMS mutagens.

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