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# STUDY OF MITOTIC INDEX, POLLEN STERILITY, FLOWER COLOR MUTATION AND SEED COAT COLOR MUTATION IN LIMA BEAN (*PHASEOLUS LUNATUS* L.) INDUCED BY MUTAGENS

Botany

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## ABSTRACT

Lima bean Phaseolus lunatus (L.) belongs to family Fabaceae. The cultivar of lima bean, 'King of Garden'(Pole) variety seeds was treated with chemical mutagen Ethyl Methenesulphate (EMS) at the concentration of 0.25%,0.50%,0.75%,1% and physical mutagen Gamma rays at the dosage of 240Gy,300Gy,360Gy,420Gy and also with combination of both (Gamma rays and EMS) like 240Gy+1%,300Gy+0.75%,360Gy+0.50%,420Gy+0.25%.

Mutation plant breeding now days is an effective tool in improvement of economically important crops. The study was performed by exposing the seeds with Gamma rays and Ethyl Methanesulphonate to assess the mitotic index and pollen sterility in M1 generation in lima beans.

## KEYWORDS

Gamma rays, Ethyl Methenesulphate (EMS), mutation breeding, mitotic index, pollen sterility

## INTRODUCTION:

Induced mutation plays a very important and significant role in crop improvement of agricultural crops. It is an important and essential tool for induction of variations in quantitative and qualitative characters of the plants. **Legume,** is called as pod fruit of plants in the family Fabaceae. The fruits come in a variety of size and shape; may be long and narrow and produce their seeds in a single line. Many pulses are grown for their edible seeds, which are high in protein and contain the essential amino acids. The term "pulses" is only used for crops harvested for dry grains, after crops harvested green for food, which are known as vegetable crops. Pulses serve as an important source of protein for a large portion of the global population, pulses contribute to healthy soils and climate change through nitrogen-fixing properties.

*Phaseolus lunatus* L. variety *King of Garden* (Pole) belongs to family Fabaceae, which is native to tropical America and now widely cultivated throughout the tropics of the world, including India and other countries, mainly found under cultivation for its pods and seeds. There are wild and cultivated types of *Phaseolus lunatus* L., generally referred to as *Phaseolus lunatus* var. *Silvester Baudet* and *Phaseolus lunatus* var. *Lunatus* respectively. In lima beans, there are two main groups like large -seeded lima beans and small-seeded Sieva Beans. Lima bean is a minor grain legume. Lima bean can produce up to 2000 to 8000 kg of fresh seeds which depend on cultivar type and cultivation conditions. In the tropics, in experimental conditions, climbing types grown in pure stands may yield 750kg/hector dry seeds whereas bushy types may yield 2000-2500 kg/hector. *Phaseolus lunatus* L. variety *King of Garden* (Pole) are used as fodder, hay or silage. Lima bean is also used for green manure and as a cover crop. Lima beans also used in intercropping systems, for specific and suitable for cultivars. The lima beans are rich in protein and contain a good amount of minerals. The seeds are astringent and used as diet in fever.

### Morphology of *Phaseolus lunatus* L.:

The Lima bean is an annual and perennial crop which is propagated through its seeds. They are herbaceous, twining climber crops. The vegetative period of lima bean is in the range of 80 days -90 days or 120 days. It has two types of growth habits; one is tall growing pole types and other dwarf bush forms. The bush forms have been originated as mutants of tall pole types. On the basis of leaf and pod characters, there are five forms which have been recognized. They are like forma *macro carpus* (Flat Lima), forma *salicis* (Willow leaved Lima), forma *lunonanus* (Bush sieve), forma *limenanus* (Bush Lima), and forma *solanoides* (potato Lima). It has thin tap root system. The stem may be up to 4.5-8m long. The leaves are alternate and tri foliate, ovate to lanceolate leaflets. Inflorescences are 2cm long and produce white to yellow or violet bisexual flowers which are less than 1 cm in length. The fruits are 3.5-12 cm long, dehiscent pods with 2 to 4 seeds. Seeds vary in shape, size and color. The seeds are mostly kidney shaped, which are 1.50 cm, long, flat and thin, white, creamy, red or speckled.

**Photo plate No.1- *Phaseolus lunatus* L. plant with pods**

### Nutrient contents

The lima beans are rich in protein and contain a good amount of minerals. A sample of dried ripped lima beans of a white-seeded type shows: moisture-13.3, albuminoids-19.7 carbohydrates-58.0, fibre-3.7, ash-3.4%, folic acid -0.034 mg/100 g., in green beans. The lima beans also contain a proteinase of the papain type, carotene oxidase, lecithin (0.62%), cephalin (0.09%), gum, and tannin. With maturity of fruits there is increase in starch and protein and a decrease in sugar and ascorbic acid contents of the beans.

## MATERIALAND METHODS:

### Collection of Genotype:

The Experimental genotype selected for the present investigation was *Phaseolus lunatus* L. variety *King of Garden* (Pole). It is commonly known as Double bean in Marathi. The experimental seed material was collected from Sheti Udyog Bhandar, Swargate, Pune, Maharashtra.

### Mutagens Used:

Physical mutagen gamma rays, chemical mutagen EMS and combination of both mutagens were used for the treatment.

### Gamma Rays Treatment:

Healthy, uniform size and dry seeds of *Phaseolus lunatus* L. variety *King of Garden* (Pole) were packed in the polythene bags and sealed for Gamma radiation. Electromagnetic ionizing radiations were applied from $CO^{60}$ source of irradiation. The seed samples were exposed to different doses of Gamma rays like 240Gy, 300Gy, 360Gy and 420Gy. Gamma radiation was carried out at Nuclear Chemistry Division, Department of Chemistry, Savitri bai Phule University of Pune, Ganeshkhind, Pune -411007.

### 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/$cm^3$. In present experimental research work to determine the lethal doses of $LD_{50}$ at suitable concentration of mutagens. EMS treatments were administered at room temperature at 25±2°C. About

500 healthy, uniform size and dry seeds of the *Phaseolus lunatus* L. variety *King of Garden* (Pole) were selected for the present treatment.

**Combination:**

For the combination treatment, Gamma rays irradiated seeds of different doses 240Gy, 300Gy, 360Gy and 420Gy were used. After the physical mutagenic treatment, chemical mutagenic treatment of EMS was conducted on the seed samples. In combination treatment Gamma rays and EMS mutagens used like 240Gy+1%, 300Gy+0.75%, 360Gy+0.50%, and 420Gy+1%.

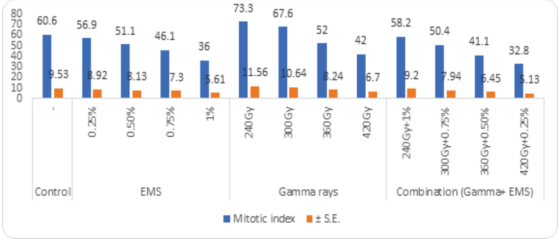
**EXPERIMENTAL OBSERVATION:**

**Mitotic index:**

**Table No.1-Effect of Mutagens on Mitotic Index in $M_1$ Generation of *Phaseolus lunatus* L.**

| Mutagens | Concentration / Dose | Mitotic index | ± S.E. |
|---|---|---|---|
| Control | - | 60.6 | 9.53 |
| EMS | 0.25% | 56.9 | 8.92 |
| | 0.50% | 51.1 | 8.13 |
| | 0.75% | 46.1 | 7.3 |
| | 1% | 36 | 5.61 |
| Gamma rays | 240Gy | 73.3 | 11.56 |
| | 300Gy | 67.6 | 10.64 |
| | 360Gy | 52 | 8.24 |
| | 420Gy | 42 | 6.7 |
| Combination (Gamma+ EMS) | 240Gy+1% | 58.2 | 9.2 |
| | 300Gy+0.75% | 50.4 | 7.94 |
| | 360Gy+0.50% | 41.1 | 6.45 |
| | 420Gy+0.25% | 32.8 | 5.13 |

**Fig. No.1- Effect of Mutagens on Mitotic Index in $M_1$ Generation of *Phaseolus lunatus* L.**



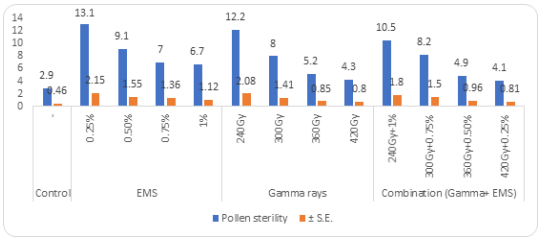

The mitotic index was 60.60% in the control of lima bean. The mitotic index was decreased with the increases in the dose/concentration of all the three mutagenic treatments like EMS, Gamma rays and Combination treatment (EMS+ Gamma rays). The mitotic index in the EMS was ranged from 36.0% to 56.9%. In Gamma rays the mitotic index was ranged from 42.0% to 73.3%, while in the combination treatment the mitotic index range was from 32.8% to 58.2%. The highest mitotic index was 73.3% observed at 240Gy Gamma rays, and lowest 32.8% at combination of 420Gy+0.25%.

**Pollen Sterility:**

**Table No.2- Effect of Mutagens on Pollen sterility in $M_1$ generation of *Phaseolus lunatus* L.**

| Mutagens | Concentration / Dose | Pollen sterility | ± S.E. |
|---|---|---|---|
| Control | - | 2.9 | 0.46 |
| EMS | 0.25% | 13.1 | 2.15 |
| | 0.50% | 9.1 | 1.55 |
| | 0.75% | 7 | 1.36 |
| | 1% | 6.7 | 1.12 |
| Gamma rays | 240Gy | 12.2 | 2.08 |
| | 300Gy | 8 | 1.41 |
| | 360Gy | 5.2 | 0.85 |
| | 420Gy | 4.3 | 0.8 |
| Combination (Gamma+ EMS) | 240Gy+1% | 10.5 | 1.8 |
| | 300Gy+0.75% | 8.2 | 1.5 |
| | 360Gy+0.50% | 4.9 | 0.96 |
| | 420Gy+0.25% | 4.1 | 0.81 |

**Fig No.2-Effect of Mutagens on Pollen sterility in $M_1$ generation of *Phaseolus lunatus* L.**




The pollen sterility was increased with the increases in the concentration/dose of the different mutagenic treatments like EMS and Gamma rays. The pollen sterility in control was 2.9%. In EMS treatment the pollen sterility was in ranged from 6.7% to 13.1.%. Pollen sterility was minimum at 1% and maximum at 0.25% of EMS. In Gamma rays the pollen sterility was in the range of the 4.2% to 12.2%, the minimum pollen sterility was observed at the 420Gy and highest pollen sterility was observed at 240Gy. In Combination treatment the pollen sterility was observed at 4.1% to 10.5%, it was minimum in 420Gy+0.25% and maximum at 240Gy+1%. All the three mutagenic treatment the highest pollen sterility was 13.1% observed at 0.25% EMS and minimum at 4.1% at 420Gy+0.25% of combination.

**Flower color mutations:**

In $M_1$ generation, different types of flower color mutations were observed and studied. For control flower was white in color. The flowers color mutants like white and yellow were observed.

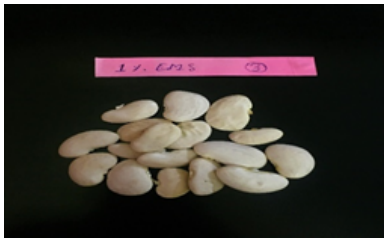
**Yellow color**

**Mix of Yellow and white color**

**Photo plate No. 2- Flower color mutation in *Phaseolus lunatus* L.**

**Seed coat color mutations:**

The seed coat color variation was recorded in $M_1$ generation. In control, presence of light brown color seed coat. The mutant seed coat color shows Creamy white seed coat color. The observation showed significant trend of seed coat color.



**Creamy white seed coat color**

**Photo plate no.3- Seed coat color mutation in *Phaseolus lunatus* L.**

**RESULT AND DISCUSSION-**

**1.Mitotic index: (Table No- 1)**

In *Phaseolus lunatus* L. variety *King of Garden* (Pole) the mitotic index was decreased with the increases in the dose/concentration of the all three mutagenic treatments. The mitotic index was decreased due to the formation of cytochemical substances, physiological changes in

protoplasm and in 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 index. The maximum induction of mitotic index was observed in the Combination of EMS and Gamma rays followed by EMS. The similar results on mitotic index were observed and reported by Shinde and More, 2010 in *Cymopsis tetragonoloba* (Linn) Taub, by Borkar and More, 2013 in *Phaseolus vulgaris*.

*Lima et al*., 2015 were reported that the mitotic index was 4.1%, the interphase nucleus was non-reticulated, and 19% of dividing somatic cells showed abnormal behaviour. Meiosis showed irregularities resulting in a meiotic index of 44.6%. Viability of pollen grains was 94.3%. These results indicated that the common bean cultivar *BRSMG Talisma* consists of repair mechanisms that compensate for changes by producing a large number of pollen grains. Interphase nuclei showed a consisted pattern and were classified as non-reticulated i.e., without condensed euchromatin and with distinct chromocenters, while the rest of the nucleus was almost non-stainable was reported by (Guerra, 1985).

**2.Pollen sterility: (Table No-2)**

In the present investigation in *Phaseolus lunatus* L. variety *King of Garden* (Pole) the pollen sterility was increased with increases in the dose/concentration of the mutagenic treatments. In all the mutagenic treatments of EMS, Gamma rays and combination treatment the pollen sterility percentage was increased in $M_1$ generations in *Phaseolus lunatus* L. variety *King of Garden* (Pole). The reduction in percentage of pollen sterility in Gamma rays and EMS was reported by Panigrahi *et al*., 2015 in *Vigna mungo*. The similar work on pollen sterility in different plants by many researchers was reported like Hakande, 1992 in Wing bean, More, 1992 in *Alfalfa,* Shinde and More, 2010 in *Cymopsis tetragonoloba* (Linn)Taub. The pollen sterility in Soya bean (*Glycine max*) was reported by Satpute and Fultambekar, 2012 and Monica and Seetharaman, 2015 worked on effect of EMS and Gamma rays on pollen fertility in *Lablab purpureus* (L.) Sweet. The pollen sterility percentage was increased due to the meiotic aberrations which was induced by mutagens as a result to formation of the aberrant pollen grains was reported by Khan and Wani, 2005. The pollen sterility can be increased due to physiological and cytogenetical changes in pollen mother cells. The pollen sterility was increased with increases in concentration/dose of EMS and Gamma rays in *Lablab purpureus* (L.) Sweet was reported by Jagtap and More, 2014.

The increased injury, sterility and lethality with increasing doses of mutagens also reported by several investigators, Bhosale and Kothekar (2010), S. Velu *et al*., (2007), Reddy *et al*., (1991) in cluster bean. Similar results were also obtained by Mathur et al., (2001), Sonone *et al.,* (2008) in groundnut and Upadhyaya *et al*., (1984), Khan M.F. and Tyagi S.D. in Soybean. They proved that most of the higher doses of mutagens like EMS and gamma rays showed increased pollen sterility. These results also supported the work on *Phaseolus lunatus* (L) treated with Gamma rays, EMS and Combination in the present study. The probable reason to increased pollen sterility might be meiotic irregularities such as translocations. According to Sudhakaran (1971) and Konzak *et al*., (1961) induction of pollen sterility was due to chromosomal abnormalities caused by mutagens. According to Nilan *et al*., (1964) gross injury due to gene control biochemical process or acute chromosomal aberrations or both may be the reason for pollen sterility. The measure cryptic changes in meiosis due to mutagen treatments can be implicated for pollen sterility. Sato and Gaul (1967) proposed that the radiation induced sterility in M1 might be due to detectable chromosomal aberration and cryptic deficiencies while the sterility induced by EMS can be due to cryptic deficiencies and specific gene mutations. Sudhakaran (1971) has concluded that pollen sterility might be represented as the cumulated result of aberrant meiotic stages as well as physiological and genetical damage induced by breakage chromosomes through the formation of antimetabolic agents in the cells. Khan *et al*., (2009) and Kumar and Rai (2009) stated that the frame shift mutations caused by MMS changed in the protein product as a result of changes in amino acid sequencies which might have affected morphology and fertility of pollen grains. Siddiq and Swaminathan (1969) reported that chromosomal aberration, especially high frequency of translocation was responsible for high sterility in cells.

**3. Flower color mutation:**

In the present research work of lima bean flower color mutations like violet and yellow flower color mutation was observed and recorded in *Phaseolus lunatus* L. variety *King of Garden* (Pole) as compared to white color flower of control. The flower color mutation can be exploited as genetic markers in the mutation breeding experiment. Induced flower color mutations in Mung bean varieties like *PS16* and *Sona* through Gamma rays and EMS treatment was reported by Kumar *et al*., (2009). The study on cyto-morphological, anatomical and biochemical characters of mutant recognized for origin and evolution of the flower color mutation at molecular level like DNA, point mutation and gene mutation in Mung bean was reported by Datta *et al*., (1993). The chromosomal aberrations, changes in chromosome number, gene mutations, changes in the biochemical pathways leading to the pigment formation of flower color mutations.

The similar result like white flower color mutations was induced by chemical and physical mutagens in *Cicer arietinum*. L. were observed and reported by Barshile, (2006). The colors like white, red, purple, blue and yellow flowers was induced through the EMS, Gamma rays and Combination of EMS and Gamma rays treatments in *Phaseolus vulgaris* Linn. was observed and studied by Borkar and More, (2010).

**4.Seed coat color mutation:**

In the present investigation the shiny brown and cream color seed coat was observed in *Phaseolus lunatus* L. variety *King of Garden* (Pole) as compared to the control with white colored seed coat. The similar observations like bold seed, shiny seed coat, dark red seed coat, cream seed coat, wrinkled seed coat was reported by Mahamune and Kothekar, (2011) in *Phaseolus vulgaris* L. The shiny seed coat is one of the rare cases of the dominant mutation in single dominant gene. Recessive mutations in plants was about 99% was reported by Gottschalk and Wolff, (1983). Auti and Apparao, (2009) also reported large number of seed coat color like brown, dark green, yellowish green and black coat color with different doses of Gamma radiation and different concentrations of EMS and SA treatments in Mung bean. The similar observations were reported by many researchers like Auti, (2006) in *Cicer arietinum*, Patil, (2009) in *Phaseolus vulgaris*, Kumar *et al*., (2007) in *Vigna uniguculata*. The chlorophyll mutants were observed in green gram by Singh and Yadav, (1991) which was brownish white seed coat against green. Ashok kumar *et al*., (2009) studied white seed coat color in cowpea (*Vigna uniguculata* (L.) Walp.).

## CONCLUSION

In the present investigation, physical and chemical mutagens like Gamma rays, EMS and Combination were succeeded in inducing the superior genotypes in plant progeny with significant alterations in growth and metabolism of the plant body. In mutation breeding the researcher can use experimental mutagenesis for creation of new varieties of medicinal plants and to obtain higher genetic diversity. This study clearly demonstrates that induced mutation can be successfully utilized to create genetic variability when it is desired to improve specific traits in plants. Such mutants could be promoted for cultivation after successful completion of seed certification.

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