STUDY OF M1 BIOLOGICAL PARAMETERS IN LIMA BEAN (*Phaseolus lunatus* L.) INDUCED BY CHEMICAL AND PHYSICAL MUTAGENS

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ABSTRACT:

Phaseolus lunatus (L.) belongs to family Fabaceae. Lima beans, like many other legumes, which are a good source of dietary fibre, and a virtually fat free source of high – quality protein. The most abundant mineral in the raw lima bean is potassium, followed by calcium, phosphorous, magnesium, sodium, and iron. When lima beans germinated, there was increased calcium and phosphorus. Additionally, it is a good source of vitamin B-6. Lima bean is commonly grown for its edible seeds. It is one of the more important vegetable legume in the United States. Lima bean is a perennial herb grown as an annual. Leaves have three leaflets. Seeds and leaves are said to have astringent properties, the vines are used as cattle feed, the young leaves are used to prepare hay, and immature dried seeds can be made into flour. The green beans contain 1.3g protein, green seeds;8.4g and green leaves;0.6g per 100g edible portion. Seeds contain cyanogenic glucoside linamarin which may cause poisoning when eaten raw.

The cultivar of lima bean, 'King of Garden'(Pole) variety seeds were 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%.

Mutagens play an important role to assess the effect of mutagenic treatments. In present investigation all the mutagenic treatments were successful in inducing mutagens in lima beans. In M1 biological parameters like seed germination percentage, seedling height and seedling injury were studied. These biological parameters decreased with increases in different mutagenic treatments which were recorded. Seedling height and seedling injury was decreased with increases in different mutagenic concentration and doses of EMS and Gamma rays.

KEYWORDS: EMS, Gamma rays, lima bean, seedling height and seedling injury.

INTRODUCTION:

Phaseolus lunatus L. variety *King of Garden* (Pole) belongs to family Fabaceae. It is also called as lima bean, butter bean, double bean etc. which predominantly a self-fertilizing plant. Lima bean seeds and pods are rich in proteins and vitamins. It is an ancient crop which is widely distributed in the tropical countries like India. It is most drought resistant crop that can grow in diverse environmental condition. Most of varieties are twining habit and few are bushy, prostrate or semi-erect in habit. Leaves are trifoliate and flowers are white to yellow and borne on loose racemes. Pods are flat or inflated, linear or broad, up to 9 cm long. Seeds are long and oval to kidney shaped, but in most cultivars the seeds are quite flat and in some spherical.

Lima beans came originally from Central and South America. In India, lima bean crop is mostly cultivated in Karnataka, Tamil Nadu and Maharashtra. It is widely used as a seed legume and vegetable. The crop is also used as an animal fodder and green manure. As a green manure, it provides the organic matter and minerals. The legume crops can fix free atmospheric nitrogen into the soil and improve crop yield. The lima beans have a great potentiality to be used as a resource for tropical agricultural systems and improving human food and animal feed stuff. The green pods which are used as vegetable and for animal feed is cut into hays or mixed as silage. Lima bean can be used as cover crop where it protects the soil from the sun's harmful radiations during the dry season and which decreases the erosion of the soil due to the wind or rainfall. The lima bean crop is cultivated either as a pure crop or intercropped with other non-legume crops.

Photo plate No. 1- Seeds of *Phaseolus lunatus* L. (Lima bean)

MATERIAL AND 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, India and developed by Frank S. Platt (New Haven, U.S.A) from an old variety called 'Large White' in 1883.

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±2ºC. 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%.

M1 Generation parameters:

1.Seed Germination percentage

2.Seedling height and Seedling injury

3.Chlorophyll mutations

4.Leaf morphological changes

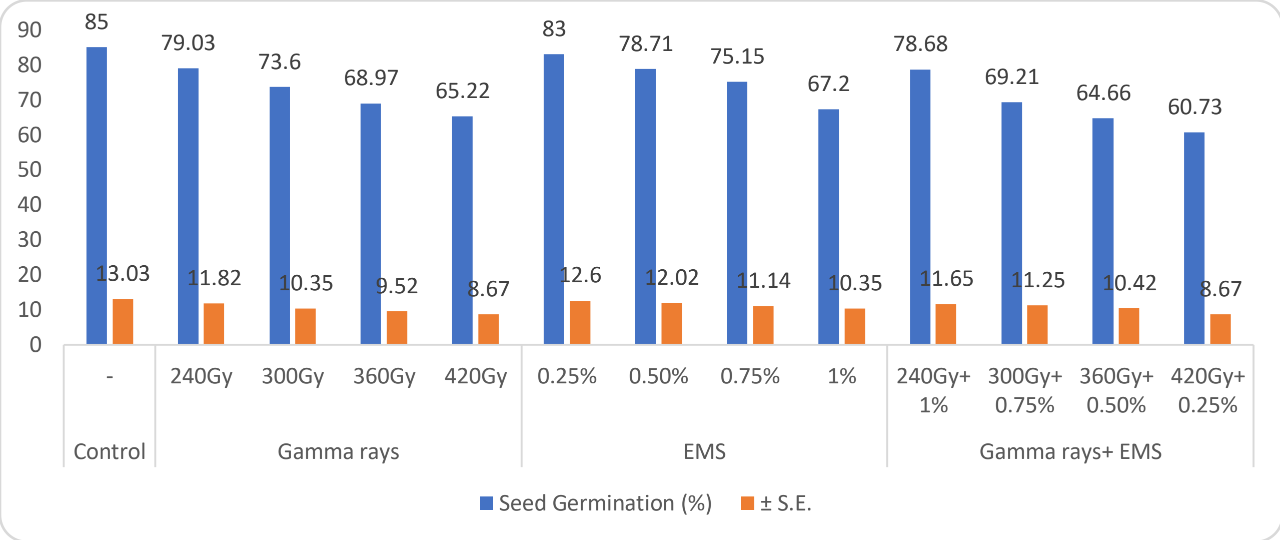
EXPEREMENTAL OBSERVATIONS:

1.Seed germination percentage:

Table No-1.1-Effect of Mutagens on seed germination percentage in M1 generation of *Phaseolus lunatus* L.

Mutagens	Concentration / Dose	Seed Germination (%)	± S.E.
Control	-	85	13.03
Gamma rays	240Gy	79.03	11.82
	300Gy	73.60	10.35
	360Gy	68.97	9.52
	420Gy	65.22	8.67
EMS	0.25%	83.00	12.6
	0.50%	78.71	12.02
	0.75%	75.15	11.14
	1%	67.20	10.35
Gamma rays+ EMS	240Gy+ 1%	78.68	11.65
	300Gy+ 0.75%	69.21	11.25
	360Gy+ 0.50%	64.66	10.42
	420Gy+ 0.25%	60.73	8.67

Fig. No. 1.1-Effect of Mutagens on percentage of germination of seeds in M_1 Generation of *Phaseolus lunatus* L.



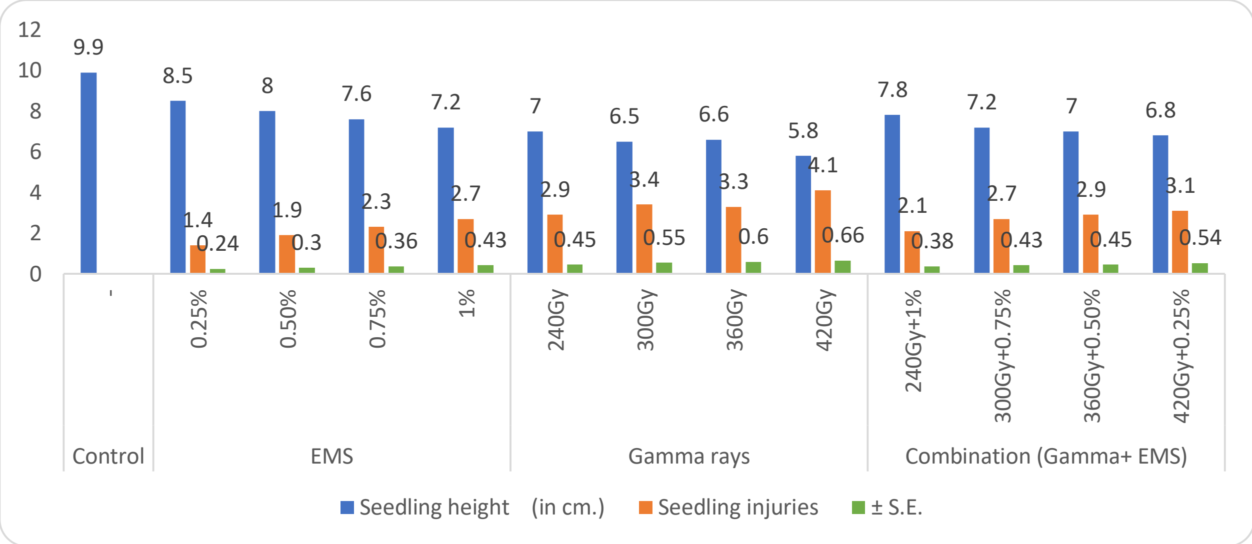
The seeds of *Phaseolus lunatus* L. variety *King of Garden* (Pole) were germinated after 7 days of sowing. The Average germination percentage in the control was 85%. The germination percentage was decreased at lower concentration/doses of mutagens and increased at the higher concentration/doses of the mutagens. In EMS treatment the germination percentage was decreased with the increases in the concentration of the different mutagens. The germination percentage was 83% at 0.25% of EMS concentration and 67.2% at 1% concentration of EMS. In Gamma rays treatment the highest germination percentage was 79.03% reported in the 240Gy while lowest germination percentage 65.2% was reported in the 420Gy treatment of Gamma rays. In combination of (Gamma rays +EMS) treatment the highest germination percentage 78.6% was observed at the 240Gy+1% treatment and the lowest germination percentage 60.73% was observed at the 420Gy+0.25% treatment. All the three mutagenic treatments, EMS treatment with 0.25% concentration was the highest germination percentage of the seed i.e. 83, while the combination treatment with the 420Gy +0.25% treatment observed in lowest germination percentage of the seeds i.e. 60.73%.

2.Seedling height and Seedling injury:

Table No. 1.2. Effect of Mutagens on Seedling height and Seedling injury in M_1 Generation of *Phaseolus lunatus* L.

Mutagens	Concentration / Dose	Seedling height (in cm.)	Seedling injuries	± S.E.
Control	-	9.9	-	-
EMS	0.25%	8.5	1.4	0.24
	0.50%	8	1.9	0.3
	0.75%	7.6	2.3	0.36
	1%	7.2	2.7	0.43
Gamma rays	240Gy	7	2.9	0.45
	300Gy	6.5	3.4	0.55
	360Gy	6.6	3.3	0.6
	420Gy	5.8	4.1	0.66
Combination (Gamma+ EMS)	240Gy+1%	7.8	2.1	0.38
	300Gy+0.75%	7.2	2.7	0.43
	360Gy+0.50%	7	2.9	0.45
	420Gy+0.25%	6.8	3.1	0.54

Fig.No.1.2 Effect of Mutagens on Seedling height and Seedling injury in M_1 Generation of *Phaseolus lunatus* L.



The seedling height in lima bean was recorded in all the three mutagenic treatments. The seedling height in control was 9.9 cm. The seedling height in all the mutagenic treatment was decreased with increases in concentrations/doses as compared to the control. In EMS treatment the maximum seedling height was 8.5 cm observed at 0.25% EMS concentration and lowest seedling height was 7.2 cm at 1% EMS concentration. In Gamma rays, the maximum seedling height was 7 cm observed at 240Gy and while the lowest seedling height was 5.8 cm at 420Gy. In combination treatment, maximum seedling height was 7.8 cm observed in 240Gy+1%, while the lowest seedling height was 6.8 cm observed at 420Gy+0.25% of the combination. All the three mutagenic treatments were increased in the seedling injury which recorded in EMS and Gamma rays treatment. The seedling injury in EMS treatment was observed 1.4% -2.7 % and in Gamma rays it was ranged from 7.66% -30.74%. In combination treatment the seedling injury was 2.1% at 240Gy+1% and seedling injury was 3.1% at 420Gy+0.25%.

3. Chlorophyll mutations

The chlorophyll mutants were observed after 10-15 days of old seedling. The chlorophyll mutants like, *Albina, Xantha, Chlorina and Viridis* were recorded in lima bean of M_1 plants.

Characteristics of chlorophyll mutations:

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

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

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

Viridis: Viridis mutant was light green in color, the seedling can survive up to 50-55 days.

Albina (White) Xantha(Yellow) Viridis Chlorina(Yellow green)

Photo plate 2- Chlorophyll mutation in *Phaseolus lunatus* L.

4. Leaf morphological changes:

Leaf morphological changes was observed in the M_1 generation of lima bean. Clift leaflet, torn leaflets, lanceolate leaf, lyrate leaflet, lunate leaflets were observed.

Clift leaflet Lunate leaflet Torn leaflet Lanceolate leaflet

Photo plate 3- Leaf morphological changes in *Phaseolus lunatus* L.

RESULT AND DISCUSSION:

1.Seed germination percentage: (Table No- 1)

The seed germination percentage was decreased with increases in the dose/concentration of the mutagens like EMS, Gamma rays and Combination (EMS and Gamma rays). According to Suresh *et al*., 2017 reported that germination percentage was decreased with the increases in the dose of Gamma rays in Butter bean variety KKL-1. The reduction in germination and survival percentage coupled with delay in germination was observed with increasing dose/concentration of mutagens was reported by Sassi *et al*., 2003 in lima bean variety LBS-1. The seed germination percentage was decreased with the increases in the dose/concentration of the mutagens like EMS and Gamma rays this similar result was reported by Sassi *et al*., 2003. The percentage of seed germination in the var. *Virat* of the chickpea subjected to the treatment of different concentrations of the EMS was considered for seed germination and seedling injury in chickpea (*Cicer arietinum* L.) by Shinde and A.B. Sagade (2016). According to them, different treatments of mutagens have exerted an inhibitory effect on seed germination. Same inhibitory effect on seed germination through mutagens has been reported by Barshile *et al*., (2006) in chickpea. The percentage of seed germination was 100% in control. Seeds were treated with EMS; the percentage of seed germination was decreased from 100% to 69% in 12mM to 53% in 16mM and 45% in 20mM. The seed germination percentage was decreased about 50% reported in 16mM EMS. The reduction in seed germination may be due to physiological and genetical processes was inhibited by mutagen (EMS). The Germination percentage was found to be significantly reduced in all the physical and chemical mutagen treatments was reported by C. Thilagavathi, L. Mullainathan (2011) in *Vigna mungo* (L.) hepper. The 50% germination percentage reduction was recorded at 60Kr of gamma rays (50.47%),15mM of EMS (50.06%), and 25 mM of DES (51.65%). These recorded results indicated that germination percentage reduced under the influence of mutagenic treatment with increases dose/concentration.

Gamma irradiation was affected by seed germination of eight cowpea accessions and it was most lethal for life Brown variety at 400 Gy and 500 Gy with seed germination rates varied from 10% to 45%.The another three elite cultivars of cowpea namely (IT86D-719,IT86D-1010 and IT89KD-374-57) reported to resistance to different doses of gamma irradiation with range of 35%-67% seed germination at 400Gy and 500Gy. In eight cowpea accessions ,62% to 85% seed germination was observed at 300Gy treatments. The seed germination percentage at 200Gy was significantly lower than the control treatment in five accessions of cowpea was reported by Festus olakunle *et al*., (2016). The seed germination was decreased due to mutagenic treatments observed in black gram was reported by Deepalakshmi and Thanga Hemavathi (2000). The seed germination was reduced more under chemical mutagens was reported by Gaul (1970) that the damage to the biological material as reflected in seed germination parameter might be considered as an indication of the mutagen effects. The physiological damage in terms of reduction in seed germination and survival percentage of plants revealed that gamma radiation was more deleterious to the *cv. Rajapur-5* as compared to *cv. Dapoli Kulthi -1* was reported by (Datir et al., 2007).

The inhibitory effect on seed germination was directly proportional to the dose/conc of gamma radiation, EMS and their combinations treatments. The inhibitory effects on seed germinations by Gamma radiation were reported earlier by Kulkarni (1978), Rudraswami (1983) and Dalvi (1990) in horse gram. It was decreased in the percent of seed germination with increase concentrations of EMS were reported by Senapati *et al*., (2008), Girija and Dhanvel (2009), Auti (2005) and Barshile (2006) in different legumes. The decreased in seed germination was mainly due to the interference of mutagens with metabolic activities of the seeds was reported by (Sjodin, 1962). Sinha and Godward (1972) showed that the reduction in percentage of seed germination and survival was due to the disturbances caused at the physiological level coupled with chromosomal damage. The disturbance in the formation of enzymes involved in the seed germination process may be one of the physiological effects caused due to mutagenic treatments particularly through chemical mutagens was reported by (Kulkarni, 2011). Gamma radiation, EMS and their combination mutagenic treatments were more effective for their action causing point mutations, enzyme inhibitions and chromosomal aberrations was reported by (Auti, 2005). In seed germination of *Pusakomal* and *V-240 Rambha* variety of cowpea was reduced from 64.00% and 63.20% were recorded in 500Gy+0.25% EMS concentration/dose which may be due to physiological and acute chromosomal damage was reported by (Gaur *et al*., 2003 and Nawale *et al*., 2006).

Reduction in seed germination over control in *Pusakomal* was ranged from 81.30% (500Gy) to 92.00% (100Gy) for the reduction in seed germination percentage in French bean was decreased in germination due to mutagenic action may be attributed to disturbances at cellular level (caused either at physiological level or at physical level) including chromosomal damages or due to the combined effect of both was reported by S.E. Mahamune and V.S. Kothekar (2012).The disturbances in the formation of enzymes involved in the seed germination process may be one of the physiological effects caused by mutagenic treatments particularly chemical mutagens like EMS and SA leading to decrease in seed germination. The decrease in percent of seed germination in French bean was caused by gamma rays, EMS and SA might be due to their effect on cytological, genetical and physiological processes.

In *Phaseolus lunatus* L. variety *King of Garden* (Pole) shows similar trend in seed germination percentage was decreased with increases in concentration of the dose of EMS, Gamma rays and Combination (EMS and Gamma rays). The lowest germination percentage of the seed was observed at the 420Gy+0.25% in combination treatment while the highest germination percentage of the seed was observed at the 0.25% in EMS treatment. The similar results were reported in seed germination percentage of French bean by (Toker and Cigiranan, 2004). The mutagenic treatments showed the inhibitory effect on seed germination percentage. The reduction of the seed germination may be due to the effect of the mutagens on the radicle and plumule occurred at meristematic region. The effect of chemical mutagens can be disturbing by the formation of enzymes involved in the germination of the seeds.

The similar inhibitory effect of mutagen on the seed germination were showed by (Ramezani and More, 2013) in Grass pea. The reduction in germination percentage was maximum at higher concentration at EMS, NMU and MHz in Alfalfa was reported by (More,1992). The decreased in the seedling emergence, seedling height, seedling survival at maturity with increasing concentration of the mutagens like EMS, NMU and MHz was reported in the mutagenesis studies by (More,1992) in *Medicago sativa*. The seed germination, seedling height and seedling injury, survival at maturity, plant height, and pollen fertility were reduced with increases in dose/concentration of the mutagens like EMS, Gamma rays and Combination (EMS and Gamma rays) in *Cymopsis tetragonoloba* (Linn) Taub was reported by (Shinde and More, 2013).

The increased in the lethality in the germination of the seeds with increased at higher radiation of the doses/concentration of the mutagens in *Phaseolus lunatus* L. variety *King of Garden* (Pole) was due to the action of the mutagens on seed metabolic reactions caused by the physiological and cytological changes in the germinating seeds. The similar result was reported by the (Shinde and More, 2010) in *Cymopsis tetragonoloba* (Linn) Taub, (Gaikwad and More, 2013) in *Vigna unguiculata* (L.) Walp, (Borkar and More, 2013) in *Phaseolus vulgaris*, (Ramazani and More, 2015) in *Latharus sativus* Linn, (Jagtap and More, 2015) in *Lablab purpureus* (L.) Sweet.

2. Seedling height and seedling injury: (Table No- 2)

In *Phaseolus lunatus* L. variety *King of Garden* (Pole) effect of mutagens like EMS, Gamma rays and Combination (EMS and Gamma rays) shows decreased in the seedling height and injury with increases in concentration/ dose of the mutagens. Reduction in the seedling height after treatment of the mutagens is common method for the study of the effect of chemical and physical mutation. The inhibition of the cell division and chromosomal damage was the main causes of the reduction of seedling height and seedling injury which was reported by (Reddy *et al*., 1988). The seedling injury was measured as reduction in the height of the different mutagens treated seedling as compared to the height of the 10 days old control seedlings of chickpea was worked by Shinde and A.B. Sagade (2016). They showed the inhibitory effect on the height of seedlings in chickpea. The maximum seedling injury (41.20%) was recorded with higher concentration at 20mM of EMS mutagen. It was increased in seedling injury with increased concentration of mutagens was reported by Toker and Cagirgan (2004) in chickpea. According to Markeen et al., (2007) reduction in seedling height with higher dose of mutagens may be due to the total injury caused by cellular level either due to gene controlled biochemical process or acute chromosomal aberrations or both. It was concluded that EMS was capable of inducing damage and mutations at cellular level in the cultivar *Virat* of chickpea.

The decreases trend of seedling height with increased mutagenic concentration/dose has been reported in French bean by Ellyfa *et al*., (2007). Cheah and Lim (1982) observed the depressed seedling growth due to the effect of gamma irradiation in French bean. Rao and Reddi (1986) observed that reduction in seedling growth by sodium azide due to inhibition of energy supply resulting in inhibition of mitosis which can be associated with seedling growth depression. Sparrow *et al*., (1961) reported that reduction in seedling growth due to gamma radiation leads to inhibition of cell division and extra chromosomal damage. Conger and Stevenson (1969) correlated the increased seedling injury with chromosomal damage. Evans (1965) considered reduction of growth due to cumulative expression of mitotic cell cycle delay and development of chromosomal structural changes.

In the present investigation the seedling height was decreased with increases in the dose/concentration of the chemical and physical mutagens. The seedling injury was increased with the increases in the dose/concentration of the mutagens. The similar results were reported by (Monica and Seetharaman, 2014) in *Lablab purpureus* (L) Sweet. The physical and chemical mutagenic treatments induced in reduction in seedling height in *Lablab purpureus* (L) Sweet was reported by (Jagtap and More, 2015). The similar observations were reported by many researchers in different plants like (Shinde and More, 2010) in *Cymopsis tetragonoloba* (Linn)Taub, by (Gaikwad and More,2013) in *Vigna unguiculata* (L.) Walp, by (Borkar and More, 2013) in *Phaseolus vulgaris*, by (Ramezani and More, 2015) in *Lathyrus sativus* Linn. and by (Jagtap and More, 2015) in *Lablab purpureus* (L.) Sweet.

3. Chlorophyll mutations:

In present investigation, chlorophyll mutants were observed after 10-15 days of old seedling. The chlorophyll mutants like *Albina, Xantha, Chlorina and Viridis* were recorded in lima bean of M_1 plants. The chlorophyll mutation frequency was decreased with increases in the concentration/doses of mutagens in *Phaseolus lunatus* L. variety *King of Garden* (Pole). In M_1 generation frequency of the viable mutants was increased with increases in the dose/concentration of all the three mutagenic treatments. The four types of chlorophyll mutants like Albino, Xantha, Chlorina and Viridis were observed in M1 seedlings by V. Ashok Kumar *et al*., (2009) in Cow pea [*Vigna unguiculata* (L.) Walp.]**.** The chimeric mutants were identified and recorded in gamma rays treatment in green gram by Singh and Yadav (2009). Stadler (1930) reported that induced mutations by gamma radiation to seed material which appeared in the form of sectors in M1 plants. Anderson *et al*., (1949) demonstrated that the analysis of mutated sectors can greatly help in tracing the ontogeny of organs in M1 plants. Motto *et al.*, (1975) reported chlorophyll chimeras in French beans after EMS treatments.

4. Leaf morphological changes:

The M_1 population of *Phaseolus lunatus* L. variety *King of Garden* (Pole) plants with the chlorophyll deficient sectors demonstrated variation in the shape and size of the leaflets. The leaf with torn leaflets, clift leaflet, lanceolate leaflet, lunate leaf, elongated leaf lamina was observed. The similar result was observed by (More and Jagtap; 2015) in *Lablab purpureus* (L.) Sweet. These variations were observed maximum in the EMS followed by the Gamma rays and combination treatment. Many researchers reported the same result in plants showing changes in the leaf shape and size of the leaves of the leguminous members were reported by (Hakande, 1992) in *Psophocarous tetragonobolus* (L)**,** by (More, 1992) in Alfalfa and (More and Jagtap, 2016) in *Lablab purpureus* (L.) Sweet. The bifoliate, tetra foliate and Pentafoliate leaflets in *Phaseolus radiata* by EMS treatment was reported by (Apparao and Auti, 2005).

The changes in morphology of leaflets induced by mutagens could be due to changes in physiological and metabolic activities of the developing primordia and alteration in leaf morphology was observed by (Joshua *et al*., 1972). It was correlated with development of leaf abnormalities to the pleiotropic action of mutated genes. The bifoliate, tetra foliate, Pentafoliate and wrinkled leaflets was observed in *Phaseolus aureus* by (Chaturvedi and Singh, 1978) after induction of EMS and DES chemical mutagens treatment. The similar results were observed by (Shinde and More, 2010) in *Cymopsis tetragonoloba* (Linn) Taub, by (Gaikwad and More, 2013) in *Vigna unguiculata* (L.) Walp, by (Borkar and More, 2013) in *Phaseolus vulgaris*, by (Ramezani and More, 2015) in *Lathyrus sativus* Linn and by (More and Jagtap; 2015) in *Lablab purpureus* (L.) Sweet. Ellyfa *et al*., (2007) reported the leaf alterations observed in French bean after gamma radiation. Silva and Barbosa (1996) observed reduced leaf size, small and elongated leaflets and leaflets with folded margins in *Milinionario 1732* variety of French bean after SA treatments. Joshua *et al*., (1972) correlated the development of leaf abnormalities to the pleiotropic action of mutated genes.

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.

ACKNOWLEDGEMENT

I would like to express my gratitude to Dr. R. G. Pardeshi, Principal, Fergusson College and Prof Mrs. S. S. Kate, Head, Department of Botany, Fergusson College, Pune-411004 for their guidance and contribution.

My special thanks to Council of Scientific and Industrial Research Human Resource Development Group (CSIR) for funding my research work and awarding me the Junior Research Fellowship (JRF), New Delhi.

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