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Effect of Mutagens on Germination Percentage, Seeding Height and Seedling Injury in M_1 Generation of *Vicia faba*L

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Abstract: ***Vicia faba L. is a flowering plant belonging to family Fabaceae which reflects all properties of a legume crop such as soil improvement and benefit of human health. It has an outstanding production scale in approximately 58 countries and rating third in engenderment. Faba beans magnification is facilated in any climatic and soil condition. It functions as a precursor of dopamine a neurotransmitter which can be used to cure Parkinson's disease and also participates in biological nitrogen fixation thereby incrementing soil fertility. Vicia faba seeds contains high nutritional value and opulent in protein, carbohydrates, in volute vitamins, folic acid, niacin and vitamin C, dietary fibres and macro and micro nutrients. The present research work was conducted to study morphological changes and chlorophyll mutation in leaves of Vicia faba by single and combined treatment of Gamma rays and Ethyl Methanesulphonate. Screening of mutation was carried out in M1 generation of Vicia faba variety Wal Kokan Bhushan. The seeds were given individual doses/concentrations of Gamma rays (240 Gy, 300 Gy, 360 Gy, 420 Gy) and EMS (0.25%, 0.50%, 0.75%, 1%). Seeds were sown to raise M1 generation. Germination percentage and seedling height was increases with decreased in doses/concentrations of different mutagens. Highest Seedling injury was observed in higher doses of Gamma rays combined with lower doses of EMS. Different chlorophyll mutation observed were xantha, viridis, aurea, maculate. The frequency of chlorophyll mutation increased with decreases doses/concentrations of both single and combined treatments.***

Keywords: Mutagens, Mutation, Vicia faba, Ethyl Methanesulphonate, Xantha, Viridis, Maculata, Chlorophyll mutation

1. Introduction

The use of medicinal plants has been documented in various scriptures around the world (Dhami and Mishra, 2015). Worldwide, Medicinal Plants and Plant Extracts were widely used by many populations across the world. It pays a particular attention in the scientific community owing to its characteristics like simplistic preparation, less in cost and treatment where no side effects involved. In the medicinal plants, leaves stem, root, fruit, and seed can be studied discretely or whole plant can be taken as sample and extractions can be made in different solvents utilizing various techniques (Gajalakshmi *et al*., 2013; Van, 2015). Medicinal plant products like glue, resins, metabolites and latex have been utilized as elements in medicine and is commercially available in markets as powders, tablets, oils, emollients, etc (Charaka and Sofovora, 2000).Medicinal plants were possessed with a substantial amount of phytochemicals. It is believed these phytochemicals are responsible for its biological activities.

Categorically the compounds present in medicinal plants can be studied further and investigated for its property (Gajalakshmi *et al*., 2011). Like medicinal plants, legumes are the nutritional products with high consequentiality, which got many health benefits to mankind. Legumes are the major source of alimentation for humans. Legumes are opulent in nutrition quantity, concretely high in proteins and carbohydrates. Medicinally, legumes are believed to decrement the incidence rate of the major diseases like cancer, heart and neurodegenerative diseases. The reason for its medicinal property could be due to the presence of the poly phenols, with a high antioxidant activity (Ramos, 2007).

Broad bean is a significant vegetable consumed by the majority of people in the world. The legume is botanically called as *Vicia faba*, is shown in Photoplate 1. Faba bean belongs to genes *Vicia* and family Fabaceae (Leguminosae) (Akpinar *et al*., 2001). The fruit has many prevalent names such as Broad bean, Horse bean, Windsor bean, Tick bean, Fava bean, etc. In Hindi, the national language of India, *V. faba* is called as 'kalamatar and bakala' (Singh *et al*., 2013). It has four subspecies, namely minor, equaine, Major and paucijuga (Hossain and Mortuza, 2006). Faba bean is an annual legume botanically known as *Vicia faba* L. (Hanelt and Mettin, 1989; Harlan, 1969).

Photoplate 1. The structure of the fruit (*Vicia faba)*

The crop is known by many names, most of which refer to a particular subgroup rather than the whole species (Hawtin and Hebblethpiait, 1983; Zohary and Hopf, 1973). It is among the oldest crops in the world, worldwide it is third most important feed grain legume after soybean (*Glycine max* L.) and pea (*Pisum sativum* L.) area and production

(Mihailovic et al., 2005). The area under faba bean crop in India is very less and that is why it is still categorized as minor, unutilized, underutilized, less utilized, and still not fully exploited crops. Though, its green pod is mainly used as vegetables, dry cotyledons are one of the excellent and cheap sources of lysine rich protein for poor's (Bond, 1976; Hawtin and Hebblethpiait, 1983; Abdel, 2008). Faba bean is also a good source of *levadopa (L-dopa*), a precursor of dopamine, can be potentially used as medicine for the treatment of Parkinson's disease (Oplinger, 1982; Vered et al., 1997). It is one of the best crop that can be used as green manure and one of the best bio factory of nitrogen by fixing 130 to 160 kg N/h

Nutritional properties

In many countries, only eighteen species of this plant have been cultivated widely. But, globally, it has been found that there are more than 80 different species of *V. faba*. the fruit contains a high nutritional value and opulent in proteins, carbohydrates, in volute vitamins, folic acid, niacin, and vitamin C, dietary fiber and macro and micro nutrients. Majorly, the seedy part of the *V. faba* was opulent in carbohydrates (51-68%), followed by proteins (2041%). The fractions of proteins were isolated from the components the *V. faba*, and it was composed of globulins (79%), albumins (7%) and glutelins (7%). The proteins were extracted from the seeds of *V. faba* using various solvents. Among, maximum of 92% of protein was extracted from the seed flour through alkaline extraction. Where, smaller amount of antinutrition contents like vicine and covicine, ABTS radical-scavenging activity, high lipid contents were also found. The consequential minerals were such as Ca, P, K, Mg, Na, S, Al, B, Ba, Co, Cr, Cu, Fe, Ga, Li, Mn, Ni, Pb, Sr, Zn.

Biological properties and Clinical applications

Biologically, the legume *V. faba* has faculty to decrement the complication and the progress of certain diseases. It plays a consequential role as adjuvant in remedying of consequential diseases like AIDS, hypertension, heart, renal, liver and Parkinson's disease (Fernandes and Banerji, 1995; Jordinson *et al.*, 1999; Ye *et al*., 2001; Hornykiewicz, 2002; Ye and Ng, 2002; Ellwood *et al*., 2008).

Anti-fungal activity

The anti-microbial activity with the potential antioxidant activity of victuals can be acclimated to discover a novel medical formula (Saha and Rajeswari 2015). The 15KDa trypsin inhibitor from *V. faba* was isolated through liquid chromatography and termed it as Egypt trypsin inhibitor (VFTI-E1), which showed a vigorous antifungal activity against fungus valsamali (Fei *et al*., 2011).

Resistance against human cytomegalovirus (HCMV)

The *V. faba* plant was resistant to human cytomegalovirus (HCMV), the HCMV is a pathogen that causes infections in people and its astringency in those who are immune deficient and withal causes birth defects. By utilizing PCR and dot-blot hybridization it is identified that three out of five *V. faba* plant shown resistant against HCMV and the protein responsible for resistance is pp-150.

Broad bean in world trade

World exports of dry faba bean seeds in 1998 to 2002 amounted to 475, 000 tones. The main exporting countries were Australia (201, 000 tonnes), the United Kingdom (114, 000 tonnes), China (63, 000 tonnes) and France (53, 000 tonnes). The main importers in this period were Egypt (197, 000 tonnes), Italy (169, 000 tonnes) and Spain (52, 000 tonnes). The exports from African and Asian countries are negligible (FAO, 2009).

Mutation Breeding

Broad bean (*Vicia faba*) is diploid species with 2n=12. Mutation breeding research on Cowpea in India was initiated in late sixties. With release of more than 305 mutant cultivars belonging to 56 plant species. Varieties of *Vicia* with persistant brown grains have been developed by breeding programme in USA that is versatile product for frozen vegetable applications. Developing cultivars with sustainable resistance to insects is a key objective of breeding programme throughout the world because insect damage is the number one problem for broad bean grain production. Most broad bean breeders employ back cross, bulk breeding methods to handle segregating population because it is self-pollinating crop. The main objectives of breeding programme have to develop disease resistant varieties, desirable quality and high yielding of grains. India is one of releasing mutant varieties of broad in the world in the field of mutation breeding. India has also become major recognized centre for work on induced mutations and second largest contributor for the mutant varieties in the world. Several countries like China, Japan, India, Netherland USA, took up the task of crop improvement through mutation breeding. Most of the mutant varieties have been developed using physical mutagens (X-rays, gamma rays, thermal and fast neutron. Through Gamma rays about 60% varieties have been developed. Chemical mutagens like EMS were the first to be used for inducing mutations in broad bean. Induction of mutations is achieved with some agents known as mutagens. These mutagens may be physical or chemical. Physical mutagens are the radiations having mutagenic properties form different radioactive substances. These radiations are of two types that are ionizing and non-ionizing radiations. Non-ionizing radiations are the low energy radiations, which are capable of causing excitations at the level of nitrogen bases of genetic material. UV rays are the commonly used non-ionizing radiation. Ionizing radiations are high-energy radiations, which are capable of causing excitation and ionization of nitrogen bases. X-rays, gamma rays, alpha particles, beta particles, fast and thermal neutrons are some ionizing radiations.

Experimental Genotype

Experimental genotype selected for the present investigation was [*Vicia faba (*L.).] commonly known as broad bean and in Marathi it is known as Aabai. The experimental seed material of broad bean variety – *Wal Kokan Bhushan*

collected from Sheti Udyog Bhandar, Swargate, Pune, Released by Agrilabh Bheej Ltd. Indore, Madhya Pradesh.

2. Material and Methods

Mutagens Used

Physical Mutagen – Gamma Rays
Chemical Mutagen – Ethyl Methanesulphonate (EMS)
Combination of Gamma Rays and Ethyl Methanesulphonate (EMS)

Modes of Treatments Gamma radiation

Healthy, uniform size and dry seeds of the *Vicia faba* variety (**Wal Kokan Bhushan)** were packed in polythene bags and sealed them 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, University of Pune, Ganeshkhind, and Pune - 411007. The seed samples were exposed to doses of 240 Gy, 300Gy, 360Gy and 420Gy. of Gamma rays.

Modes of Treatment for Ethyl methanesulphonate (EMS)

Ethyl Methanesulphonate (EMS) was obtained from Spectrochem Pvt. Ltd. Mumbai (India) with a molecular weight 124.16 g/mol and density 1.20 g/cm^3. To determine the lethal dose (LD_{50}) and suitable concentrations of mutagens for the further studies. Chemical mutagenic treatments were administered at room temperature of 25 ± 2°C. Healthy and dry seeds of the *Vicia faba* variety– (*Wal Kokan Bhushan***)** having uniform size were selected for the treatment. Seeds were surface sterilized with 0.1% mercuric chloride solution for about one to two minutes than washed thoroughly and soaked in distilled water for 6 hours for pre soaking of seeds, which were made the seed coat permeable for the mutagenic treatment. The fresh, aqueous solutions of the mutagen were prepared prior to treatments. The different concentrations used for the chemical mutagenic treatments were 0.25%, 0.50%, 0.75% and 1%. After the pre - soaking seeds were immersed in the mutagenic solution for 4 hours with continuous shaking. The volume of the chemical solution used was five times more than that of the seeds to facilitate uniform absorption. Seeds soaked in distilled water for 6 hours served as control. Immediately after the completion of treatment, the seeds were washed thoroughly under running tap water for 3 to 4 times. Later on they were subjected to post - soaking in distilled water for 4 hours.

Combination treatment of Gamma rays and EMS

For the combination treatments Gamma rays irradiated seed samples were used. After the physical mutagenic treatment, the chemical mutagenic treatment of EMS was conducted on the same seed samples. In the combination Gamma rays and EMS used like 240Gy+1%, 300Gy+0.75%, 360Gy+0.50% and 420Gy+0.25%. For each treatment, a batch of 500 seeds was used. 100 seed from each were plotted between the folds of filter paper, 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 lots of 300 seeds of each treatment along with control (untreated seeds) were sown in research field by Complete Randomized Block Design (CRBD) with three replications in order to raise the M_1 generation.

Experimental Observations

Experimental results recorded in the present investigation in the variety (*Wal Kokan Bhushan*) EMS, Gamma rays and combination of both EMS and gamma radiation are discussed below.

A) Effect of Mutagens on Seed Germination percentage in M1 generation of *Vicia faba*

Seed germination percentage was decreases with increase in dose/concentration of mutagens. Gamma rays as a physical mutagen shows seed germination percentage as 240 Gy with 81.66%, 300 Gy with 81.00%, 360 Gy with 79.00% and 420 Gy with 77.00% respectively. EMS as a chemical mutagen at different concentrations 0.25%, 0.50%, 0.75% and 1% shows 83.00%, 80.335, 78.66% and 76.00% seed germination respectively. Combination treatment of gamma rays and EMS, 240Gy+1% with 80.00%, 300Gy+0.75% with 78.66%, 360Gy+0.50% with 76.00%, 420Gy+0.25%with 74.00% seed germination respectively. At each treatment the seed germination percentage declines with increased in dose/concentration of mutagen.

Table 1: Effect of Mutagens on Seed Germination Percentage

Mutagens	Dose/Conc	Seed Germination %	±S.E
Control		85.00	0.69
Gamma rays	240Gy	81.66	0.05
	300Gy	81.00	0.02
	360Gy	79.00	0.01
	420Gy	77.00	0.50
EMS	0.25%	83.00	0.50
	0.50%	80.33	0.01
	0.75%	78.66	0.02
	1%	76.00	0.50
Gamma rays +EMS	240Gy+1%EMS	80.00	0.50
	300Gy+0.75%	78.66	0.50
	360Gy+0.50%	76.00	0.01
	420Gy+0.25%	74.00	0.50

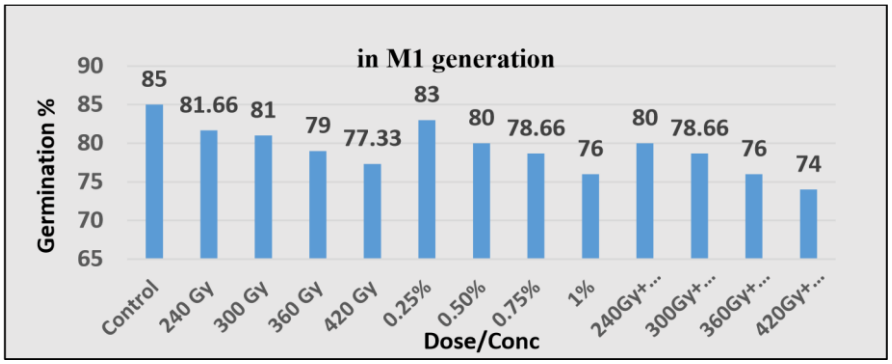


Figure 1: Effect of Mutagens on Seed germination %

B) Effect of Mutagens on Seedling Height and Seedling Injury

Seedling height is highest in gamma rays treated seeds; 240Gy shows 9.06cms, 300Gy with 7.55 cms, 360Gy with 5.46 cms and 420 Gy with 4.87 cms respectively. EMS treatment at different concentration shows like 0.25% shows 8.48%, 0.50% shows 8.18 cms, 0.75% shows 5.32 cms and 1% with 4.5 cms seedling height respectively. Combination treatment 240Gy+1% with 6.38 cms, 300Gy+0.75% with 6.19 cms, 360Gy+0.50% with 4.15 cms, 420Gy+0.25% with 3.99 cms seedling height respectively. Seedling height shows increase with low dose/concentration of mutagens as the dose/concentration increases the seedling height decreases. Highest seedling injury is recorded in 420 Gy+0.25% EMS that is 63.49%. Lowest seedling in jury is recorded in gamma rays treated seeds that is 17.10 %

Table 2: Effect of Mutagens on Seedling Height and Seedling Injury

Mutagens	Dose/ Conc	Seedling height (cms)	Seedling injury	% Seedling injury	± S.E
Control		10.93			0.699
Gamma rays	240Gy	9.06	1.87	17.10	0.503
	300Gy	7.55	3.38	30.92	0.351
	360Gy	5.46	6.06	55.44	
	420Gy	4.87	5.47	50.04	0.458
EMS	0.25%	8.48	2.45	22.41	0.465
	0.50%	8.18	2.75	25.16	0.672
	0.75%	5.32	5.61	51.32	0.910
	1%	4.5	6.43	58.82	0.465
Gamma rays +EMS	240Gy+1%EMS	6.38	4.55	41.62	0.357
	300Gy+0.75%EMS	6.19	4.74	43.36	0.358
	360Gy+0.50%EMS	4.15	6.78	62.03	0.413
	420Gy+0.25%EMS	3.99	6.94	63.49	0.387

Figure 2: Effect of Mutagens on Seedling height and Seedling Injury

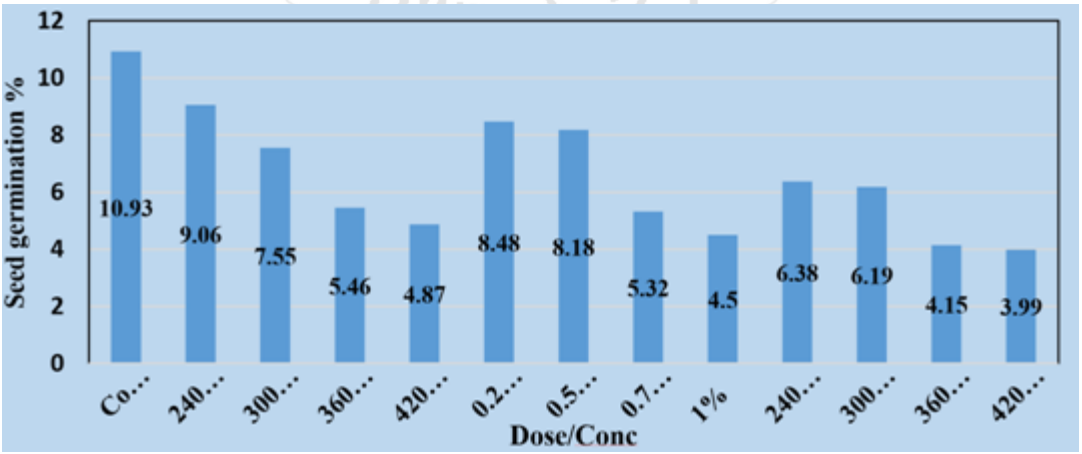


Figure 2: Effect of Mutagens on Seedling height and Seedling Injury

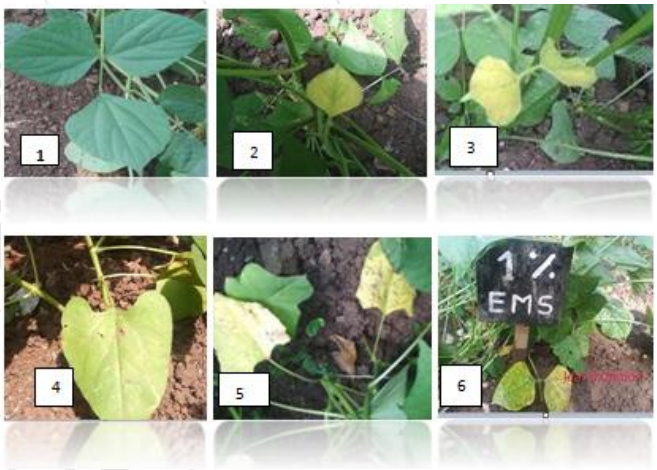
C) Morphological Changes Observed in Leaves of *Vicia faba*

In M1 generation, the plants of *Viciafaba* besides carrying chlorophyll deficient sectors, also exhibited considerable variation in morphology with respect to the shape and size of leaflets. The variations comprised enhancement in the size of the leaflets, reduction in the size of the leaflets, broadening and expansion of the leaflets. The changes in morphology of leaflets have been produced due to the alterations in physiological and metabolic activities of the developing primordial and the irregularities produced in the meristem. (Prasad, 1967). Joshua *et al*. (1972) have attributed the leaf abnormalities to the pleiotropic action of mutated gene.The variations comprised enhancement in the size of the leaflets, increase in the size of the leaflet lamina, dark green color, Bifoliate, Unifoliate leaves etc. All the mutagens succeeded in inducing all above mentioned leaf variations.

Photo plate 1, 1) Control 2)Unifoliate leaves 0.25% 3) Unifoliate leaves 360 Gy+0.75%, 4)1% EMS Bifoliate leaves)

D) Chlorophyll deficient sectors in leaves

In the M generation of Vicia faba, the chlorophyll deficient sectors were recorded in all the mutagenic treatments. Different chlorophyll deficient sectors like xantha, chlorina, and viridis, maculate, tigrina were detected in the leaflets totally, partially and at the margins. All mutagenic treatments were effectively induced chlorophyll deficient sectors in *Vicia faba*. The frequency of such chlorophyll chimeras carrying plants was maximum in lower dose of gamma rays and in EMS treatments. A chlorophyll chimeric plant can originate when a sector of the embryo becomes mutated. The embryo contains different sets of meristematic tissues, which are capable for giving rise to certain parts of the mature plant. A differentials responses of such embryonic cells to the mutagen caused chimerism. In the present investigation, the chlorophyll chimeric plants produced due to the physiological changes may also be contributing to the formation of leaf chimeras. It is quite possible that such chimeras would be raised due to developmental disturbances caused by generative changes.



(Photo plate 2, 1) Control 2) Aurea mutant 300Gy 3) Xantha mutant 0.50% 4) Viridis mutant 300Gy 5) Xantha mutant 240Gy+1%EMS 6) Tigrina mutant 1%EMS)

E) Flower Colour Mutation in *Vicia faba*

Two types of flower colour mutations were observed in *Vicia faba*. Light yellow and white colour flowers in combination treatments240Gy+1%EMS, 300Gy+0.75%. The flower colour mutation can be exploited as genetic markers in the mutation breeding experiment. (Kumar *et .al*; 2009) was reported that induced flower colour mutations in Mungbean varieties “PS16” and “Sona” by using Gamma rays and EMS treatment. White flower colour mutations induced by chemical and physical mutagens were reported by (Datta and Sengupta, 2002. Atta *et. al*; 2003., Barshile, 2006.,) in *Cicer arietinum*.L. (Borkar and More, 2010) was reported that white, red, purple, blue, yellow colour of flowers in *Phaseolus vulgaris* .Linn induced through the EMS, Gamma rays and Combination of EMS and Gamma rays treatment. (Girija and Dhanavel, 2013) observed on the

basis of seedling in M_2 generation in increased the frequency of chlorophyll mutation with increases in all mutagenic dose or concentrations of EMS and Gamma rays in *Vicia Faba.*

(Photo plate-31) Control, White colour flowers 2)1% EMS Creamish white flowers), 240Gy+1% EMS Light yellow flowers, 4) 300Gy+0.75 % EMS Light yellow flower)

3. Results and Discussions

The effect of the Physical and chemical mutagens evidenced in various growth phenomenon such as seed germination, seedling height, seedling injury, Morphological changes in leaves and chlorophyll mutations as well as the change in flower colour in the M_1 generation of the *Vicia faba* L. The seed germination percentage was decreased with the increases in the dose/concentration of the mutagens like EMS, Gamma rays, and combination (EMS and Gamma rays). The similar trend of the decreased in germination percentage was observed in the all the mutagenic treatment. The lowest germination percentage of the seed germination was observed at the 420Gy+ 0.25% EMS combination treatment while the highest germination percentage of the seed was observed at the 0.25% EMS treatment. The same results were studied in seed germination percentage of French bean by (Toker and Cigiranan, 2004; Apparao, 2005). (Ganesan, 1998) in Sesame, (Kumar and Mishra, 2004) in Okra. The mutagenic treatment 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 meristematic region was reported by (Deepika *et. al*; 2016). The chemical mutagens might be disturb the formation of enzymes involved in the germination of the seeds reported by (Kulkarni, 2011). The inhibitory effect of the mutagen on the seed germination was reported by the (Joshi *et .al*; 2011) in onion, (Ramezani and More, 2013) in Grasspea, (Murugan and Dhanvel, 2015) in *Vinca rosea*, (Sarda *et .al*; 2015) in Coriander and (Deepika *et. al*; 2016) in Cluster bean. The reduction in germination percentage was maximum at higher concentration at EMS, NMU and MHz in Alfalfa reported by (More, 1992).The decreased in the seedling emergence, seedling height, seedling survival at maturity with increasing concentration of the mutagen was reported in the mutagenesis studied by (Adamu *et.al*; 2002). The seed germination, seedling height and seedling injury, survival at maturity, plant height, and pollen fertility were reduced with increases in dose or concentration of the mutagens in *Sesanum indicum* L. studied by the (Sheeba *et .al;* 2005).

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

EMS treatment and Gamma rays radiation shows decreased in the seedling height and injury with increases in the 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 the chemical and physical mutation reported by (Ahnstrom, 1974). The inhibition of the cell division and the chromosomal damage are the main causes of the reduction of the seedling height and seedling injury was reported by the (Reddy *et .al*; 1988).

In the present investigation the seedling height was decreased with increases in the dose or concentration of the chemical and physical mutagens. In combination treatment the seedling height was initially increased at the 240Gy+1%EMS treatment and it was decreased again in the same concentration of the mutagen. The seedling injury was increased with the increases in the dose or concentration of the mutagens, except combination treatment where the seedling injury was decreased. The similar results were observed by the (Monica and seetharaman, 2014) in *Lablab purpureus* (L) Sweet by used the EMS and Gamma rays treatment shows the reduction in the root and shoot length at the increasing dose or concentration of the mutagens. The seedling height was decreased with increases in the concentration/ doses of EMS, Gamma rays and combination treatments. All the mutagenic treatments induce in reduction in seedling height in Dolichos bean was reported by (Jagtap and More, 2015). The similar observations were reported by many researchers in different plants like (Shinde and More, 2013) in *Cymopsis tetragonoloba* (Linn)Taub, (Salve and More, 2014) in *Coriandrum sativum* Linn, (Bhosale and More, 2014) in *Withania somnifer*a 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.

Morphological Changes and Chlorophyll deficient sectors in Leaves

The chlorophyll deficient sectors were observed in all the mutagenic treatment in the M_1 generation of the *Vicia faba (*L.). Mutation in the sectors of the leaves results into the chlorophyll chimeric plants. Embryo consists of the

meristematic tissues which develop into the certain parts of the mature plant. The response of embryonic cells to mutagens causes the chimerism was reported by (Gaul, 1958). The leaf chimeras developed due to the certain initial changes in the embryos and the physiological disturbances may also lead to the leaf chimeras.

The M_1 population plants with the chlorophyll deficient sectors demonstrated variation in the shape and size of the leaflets. The leaf with rounded apices and leaf broad than length, large sized leaves were also observed. These variations were observed more in the EMS and Gamma followed by the combination treatment.

The bifoliate, unifoliate leaflets in broad bean by EMS and combination treatment were also observed. 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 development of leaf abnormalities to the pleiotropic action of mutated genes. (Chaturvedi and Singh, 1978) was observed bifoliate, tetrafoliate, pentafoliate and wrinkled leaflets in *Phaseolus aureus* after induction of EMS and DES treatment. The similar results were observed 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 somnifer* 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.

Flower Colour Mutation

In the present investigation the creamish white and light yellow colour of flower mutation were observed in the *Vicia faba* L. as compared to white colour flower of control. The flower colour mutation can be exploited as genetic markers in the mutation breeding experiment. (Kumar *et .al*; 2009) was reported that induced flower colour mutations in Mungbean varieties "PS16" and "Sona" by using Gamma rays and EMS treatment. White flower colour mutations induced by chemical and physical mutagens were reported by (Datta and Sengupta, 2002. Atta *et. al*; 2003., Barshile, 2006.,) in *Cicer arietinum*.L. (Borkar and More, 2010) was reported that white, red, purple, blue, yellow colour of flowers in *Phaseolus vulgaris* .Linn induced through the EMS, Gamma rays and Combination of EMS and Gamma rays treatment. (Girija and Dhanavel, 2013) observed on the basis of seedling in M_2 generation in increased the frequency of chlorophyll mutation with increases in all mutagenic dose or concentrations of EMS and Gamma rays in *Vicia faba.*

4. Conclusion

The legumes constitute major portion of the human food as well as source of oil, timber and fiber as a raw material for many products. Grain legume of crops play an important cereal based diet of people from semi-arid tropical countries from Africa, Asia, and Latin America. Legumes play an important role in controlling soil erosion, maintaining soil fertility and solving the nutrient imbalance in the live stocks. Leguminous crops e.g. Pulses and beans play an important role by providing the important protein source. Pulses and beans contain about the 20-30% protein. India grows variety of pulse crops under wide range of agro-climatic conditions. India contributes about the 25% of the total pulses production. *Vicia faba* L. belongs to family Fabaceae. The plant is also called as a broad bean or fava bean and predominantly a self-fertilizing plant. The legume plants can fix atmospheric free nitrogen into the soil and improve crop yield in an economic and environment friendly manner. Broad bean pods and seeds are rich in proteins and vitamins. In the present study M_1 biological parameters was decreased with increases in different mutagenic treatments for the seed germination percentage, seedling height and seedling injury and survival of plants at maturity were reported in Gamma rays and the EMS treatment, While in Combination treatment the results about the seedling height and injury was fluctuating. It was first increased and then decreased at the 420Gy+10.25% EMS. The percentage of the seedling injury was increased with increases in the dose or concentration of the Gamma rays and EMS, while in Combination treatment it showed the damage at the 240Gy+1% EMS and 300Gy+0.75% EMS of Combination treatment.

Morphological changes were also observed in M1 generation. Larged size leaves, rounded leaf apex, unifoliate and bifoliate type of leaves. The chlorophyll deficient sectors of the leaflets in the M_1 generation produced the different types of the chlorophyll mutants like *viridis*, *xantha*, *tigrina*, *aurea*. These was found on the entire leaflet. Gamma rays and EMS succeeded in production of the highest number of the chlorophyll deficient sectors followed by the combination treatment. Two different type of flower colour mutations were also observed. Control with white flowers. The creamish white and light yellow flower colour were observed in M1 generation of *Vicia faba.* The flower colour mutation can be exploited as genetic markers in the mutation breeding experiment.

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