Induction of Mutations in *Vicia faba* L. through Physical and Chemical Mutagens.

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Abstract

Vicia faba, L. also known as the Broad bean, It belongs to family Fabaceae. Mutation breeding is the potential tool for creating variability. It is modern and useful tool employed by mutation breeders with help of physical and chemical mutagens like different doses/ concentrations of Gamma rays, EMS and NMU were used by several workers. Induced mutation breeding is one of the most efficient and modern scientific techniques. Inducing desirable mutations and exploiting them for crop improvement is known as Mutation breeding. The induced mutation through physical and chemical mutagen is very effective tool to induce variations for significant characters. Broad bean is rich in Carbohydrates, Proteins, Vitamins (Riboflavin, niacin), Fats, Minerals and Fibres. 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 treatments were given to individual. Doses/concentrations of gamma rays (240 Gy, 300 Gy, 360 Gy, 420 Gy) and EMS (0.25%, 0.50%, 0.75%, 1%), and combination of Gamma rays and EMS (240Gy+0.25%, 300Gy+0.50%, 360 Gy+0.75%, 420 Gy+1% EMS). Different types of chlorophyll mutation were observed like *xantha, viridis, aurea, maculate*. The frequency of chlorophyll mutation was increased with decrease in doses/concentrations of both single and combined treatments. Also two types of flower colour mutations were observed and recorded like light yellow and creamish white colour.

Keywords: Ethyl Methanesulphonate, Gamma rays, Xantha, Viridis, Maculate, Chlorophyll mutation, NMU.

Introduction

The use of medicinal plants since 5000 BC 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. Medicinal plant products like glue, resins, metabolites and latex have been utilized as elements in medicine and which commercially available in markets in the form of powders, tablets, oils, emollients, etc (Charaka and Sofovora, 2000). Medicinal plants were possessed with a substantial amount of phytochemicals. Like medicinal plants, legumes are the nutritional products with high consequentiality, which received 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 Figure 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). The plant *V. faba* was originated from the East and it is widely consumed in South America and interestingly

it has capability to grow in all climatic conditions. It is popularly cultivated in India (Marcello and Elena, 2017).

Particularly, the cold climatic conditions are very auspicious for them to grow in countries like Jujuy and Argentina, where the temperature is usually low. The crop was cultivated ecumenical and its output was majorly optically discerned in many countries. Among those, china upper side in the engenderment (60%) of *V. faba* crop than any other country in the world. The countries like Ethiopia and England were the major importers. Egypt was the major importer of *V. faba* in the form of dry beans from Australia (Azaza *et al*., 2009; Chillo *et al.*, 2010; Kaur *et al*., 2014). Despite of its ability in growth, additionally, a study conducted between 2015 and 2016, shows intercropping of vegetables like carrot and cabbage can be done in *V.faba* Plantation in order to ensure beneficial horticulture production (Liga *et al*., 2017).

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 plant got faculty to fine-tune atmospheric nitrogen, which ultimately increases the soil fertility, as well, it is prominently known to be covered crop as it averts the soil erosion. Apart from these, the special feature of *V. faba* is, frugal in cost and a good source of protein. The protein content was equipollent to the protein content of meat and fish. *V. faba* fruit exposed that, 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%) as shown in Figure 3. The fractions of proteins were isolated from the components the *V. faba*, and it was composed of globulins (79%), albumins (7%) and glutelins (7%). Smaller amount of anti-nutrition contents like vicine and covicine, ABTS radical-scavenging activity, high lipid contents were also found. In addition high amounts of poly phenols were abundant in hexane extraction (Vioque *et al*., 2012). Poly phenols were high in the seeds were abundant (Pastor *et al*., 2011). Apart from major nutrition, micro nutrients were also present in the fruit. 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 listed in Table 1.

Chemical Constituents	Type and amount present
Carbohydrates	51 to 68%
Protein	20 to 41 % (globulin (79%), albumin (7%), glutelins (7%)
Lipids	2.30 to 3.91%
Unsaturated fatty acids	Myristic, oleic, linoleic acid
Vitamins	Folic, niacin
Saturated fatty acids	Pamitic, stearic acid
Minerals	Ca, P,K,B,Cu, Fe, Ga, Cr, Li, Mn, Ni, Pb, Ba, Al, S

Table 1. Chemical constituents of *Vicia faba*

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 persistent 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.

Country	No. of mutant cultivars	% of total
China	605	26.8
India	259	11.5
USSR	210	9.3
Netherland	176	7.8
Germany	138	6.1
USA	125	5.5
Japan	120	5.3

Table No. 1.2: Mutant cultivar released in top seven countries of the world

Source: Source: Kharakwal and Shu (2009). Chemical mutagens are of three types, these are alkylating agents, base analogues and acridine dyes. Alkylating agents are commonly used for the induction of mutation in plants; they transfer the alkyl group to nitrogen base or phosphate groups of DNA. Ethyl Methanesulphonate (EMS), Methyl Methanesulphonate (MMS), Ethyl imines (EI) are some commonly used alkylating agents; they resemble the actions of radiations so known as radiomimetic chemicals. These radiomimetic agents have bio functional alkyl reactive groups that react with DNA, causes extensive cross linkage of DNA, chromosome breakage, chromosome mutations and gene mutations (Vasu and Hasan, 2011). The new method for the induction of mutation is the use of combination treatments of physical and chemical mutagens. The interaction effect of combination treatments on mutation frequency was calculated by the formula: K = (a + b) / (a) + (b), where (a) and (b) are the mutation frequencies induced by the two mutagens when applied singly, whereas (a + b) is the mutation frequency produced by the two mutagens in

combination treatments and 'K' is the hypothetical interaction coefficient (Sharma, 1970). This interaction effect is known as synergistic effect of combination treatment. A synergistic effect may cause if the sites of action protected during treatment with first mutagens are exposed to the action of the second (Arnason *et al*. 1963). An additive effect or even lowering of the effect may result; in case their actions are independent or the two mutagens compete for the same site (Aastveit, 1968). The combination of mutagenic agent is another useful method for showing the mechanism of their action (Khan, 1981).

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.

Material and Methods Mutagens Used

Physical Mutagen – Gamma Rays

Chemical Mutagen – Ethyl Methane Sulphonate (EMS)

Combination of Gamma Rays and Ethyl Methane Sulphonate (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

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.

Morphological Changes Observed in Leaves of *Vicia faba*

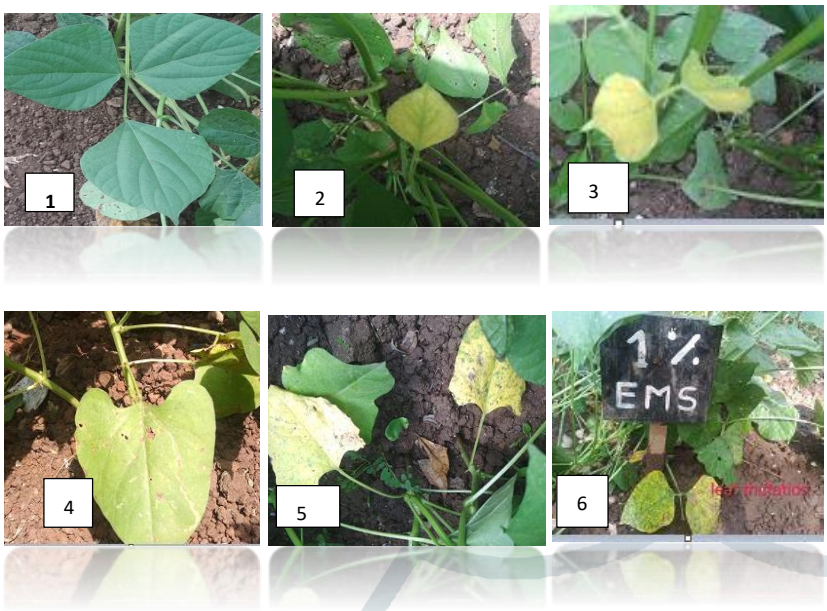
In M1 generation, the plants of *Vicia faba* besides carrying chlorophyll deficient sectors, also exhibited considerable variation in morphology with respect to the shape and size of leaflets. The variations comprised enhancement in the size of the leaflets, reduction in the size of the leaflets, broadening 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)

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 be originated 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 was 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)

Flower Colour Mutation in *Vicia faba*

Two types of flower colour mutations were observed in *Vicia faba*. Light yellow and creamish colour flowers in combination treatments 240Gy+1%EMS, 300Gy+0.75%.

(Photo plate-3 1) Control, White colour flowers 2)1%EMS Creamish white flowers, 3)240Gy+1%EMS Light yellow flowers, 4) 300Gy+0.75 % EMS Light yellow flower)

Results and Discussions

Chemical and physical mutagens can induced the treated plant in morphological, genetically and biochemical changes. In the present investigation, the chlorophyll chimeric plants produced due to the physiological changes may also be contributing to the formation of leaf chimeras. In M1 generation, the plants of *Vicia* besides carrying chlorophyll deficient sectors, also exhibited considerable variation in respect of the shape and size of leaflets. Various morphological changes in leaves were found in different mutagens like 1% EMS, 300 Gy, 0.25% EMS. Chlorophyll mutations were recorded in 240Gy, 300Gy, 1%EMS, 0.75%EMS, 240Gy+1%EMS. Chlorophyll mutation frequency was increased with decrease in dose or concentration in all the mutagenic treatments. Combination treatments were showed the less frequency of chlorophyll mutants than that of individual treatments. Two types of flower colour mutations were observed that is light yellow and white colour flowers in 1%EMS,

240Gy+1%EMS, 300Gy+0.75%EMS,

Conclusions

The different chlorophyll deficient sectors like ***xantha, albina, chlorina, maculate and viridis*** were recorded in the leaflets like totally, partially and at the margins. All treatments were effectively induced the chlorophyll deficient sectors in *Vicia*. The maximum frequency of such chlorophyll chimeras carrying plants was in low doses of gamma rays and high concentration of EMS treatment. Many researchers found that the chlorophyll mutants are not heritable. It has been observed that all mutagenic treatments induced morphological changes in leaves like bifoliate, tetrafoliate, pentafoliate, large sized leaves and small sized leaves. Four types of chlorophyll mutations were observed in *Vicia faba* like, ***xantha, chlorina, maculata, tigrina and viridis***. The variety of *Vicia* responds to EMS, Gamma rays mutagens and combination of both gamma and EMS. There was no specific trend according to increase or decrease in concentration /dose of mutagens, but EMS treatment shows the highest frequency of chlorophyll mutation than gamma rays and combination. The lower concentration /doses of combination treatment show high frequency of chlorophyll mutations than individual 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 disturbing 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).

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