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ASSESSMENT OF MUTAGENIC EFFECTIVENESS AND EFFICIENCY OF EMS AND GAMMA RAYS IN *VICIA FABA* L. VAR. PRT-12

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Abstract

The success of any mutation breeding programme for genetic improvement of crop plant depends on the effectiveness and efficiency of the mutagen used. In the present investigation five different concentrations of ethyl methane sulphonate (EMS) i.e., 0.05, 0.75, 0.10 and 0.25 and 0.50mol/mL and 50Gy, 100Gy, 150Gy, 200Gy and 250Gy of gamma rays were used to induce genetic variations *Vicia faba* L. var. PRT-12 which can be used as viable and economically important character in future breeding programme of this crop. The standard formulae were used to quantify the mutagenic efficiency and effectiveness of the EMS and gamma rays. Overall, the findings showed that as mutagenic treatments increased, so did their efficacy and efficiency. Furthermore, it was discovered that EMS and lower-or intermediate-level gamma-ray treatments were more successful in producing the greatest number of mutations while causing the least amount of biological damage.

Keywords: EMS, Gamma Rays, Mutagenesis, Genetic Improvement, V*icia faba.*

INTRODUCTION

For any plant breeding program, the availability of genetic variability in the gene pool of a particular crop is of foremost importance because it provides a spectrum of variations for an effective and better selection process. This variability could be increased through recombination hybridization, and mutations [7, 8]. Induced mutation provides the possibility of creating desired attributes that either is not present in nature or have been lost during the selection process [9, 10]. Induced mutagenesis has been established as an important method in many plant improvement programme by treating seeds and other vegetative plant parts with mutagens [11] and it has immensely contributed to global agriculture by producing over 3248 mutants with desirable qualitative and quantitative traits in different plant species (FAO/IAEA 2017) [12]. The creation of high frequency of desirable mutations depends upon the selection of an effective and efficient mutagen [13]. This study ensures the assessment of the effectiveness and efficiency of gamma ray and EMS. The broad bean, Vicia faba L. (2n = 12), belongs to the Fabaceae family. Uses for this significant pulse crop include vegetable, silage, fodder, and feed the stock. It is a popular breakfast dish in China, Ethiopia, the Middle East, and the Mediterranean region. Because Vicia faba self-pollinates, its genotype is homozygous; consequently, it needs to be further improved. This can be achieved by mutagenesis, which can introduce new genetic variabilities into the genotype.

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MATERIALS AND METHODS

Certified seeds of faba bean (*Vicia faba* L.) variety PRT12 were procured from the Sale Counter of National Seed Centre, Indian Agriculture Research Institute (IARI), New Delhi, India. Dry and healthy seeds (10-12% moisture content) of variety were treated with different doses/ concentrations of gamma ray (50, 100, 150, 200 and 250 Gy) and EMS (0.05, 0.075, 0.10, 0.25 and 0.50%). 150 seeds was used for each dose/concentration including the control. Thereafter, the treated as well as untreated seeds were sown in 30 cm diameter earthen pots (50 seeds in each pot) for raising the seedlings. Seeds from M1 plants were harvested separately. For raising the M2 generation, 35 M1 plants and 35 seeds from each plant were selected for each treatment, including the control. Recommended agronomical practices were employed for the preparation of field, sowing and subsequent management of M1 and M2 generation [14- 20].

Frequency of Morphological Mutation

The morphological mutations were scored on the basis of characters in control plants and their deviations in the treated populations at older stage (75 days old). Following formula was adopted to calculate the frequency of mutation in M2 generation.

Mutagenic Effectiveness and Efficiency

$$Mutation\ frequency\left(M_p\right)=\frac{No.of\ mutated\ plants\ at\ older\ stage}{Total\ no.of\ germinated\ seedlings}X100$$

The methods of calculating mutagenic effectiveness and efficiency were suggested [21]. Mutagenic effectiveness is a measure of the frequency of mutation induced by unit dose of a mutagen (kR in case of gamma ray or time × concentration in case of chemical mutagen), while mutagenic efficiency represents the proportion of mutation in relation to the biological damage.

The formulae suggested by Konzak, were used to evaluate mutagenic effectiveness and efficiency of the mutagens [22].

$$Mutagenic\ effectiveness\ \left(in\ case\ of\ physical\ mutagen\right)=\frac{Mutation\ rate(M_2\ family\ or\ population\ basis)M_p}{Dose\ in\ Kilo\ Rontgen(kR)}$$

Where: M_p = Percentage of mutated plant progenies (mutation rate in M_2)

$$Mutagenic\ effectiveness:\ \left(in\ case\ of\ chemical\ mutagen\right)=\frac{Mutation\ rate(M_2\ family\ or\ population\ basis)M_p}{Concentration\ of\ Mutagen(C).X\ Duration\ of\ treatment\ in\ hours(T)}$$

$$Mutagenic\ efficiency=\frac{Percentage\ of\ Mutated\ plant\ progenies\ (mutation\ rate\ in\ M_2)M_p}{*Biological\ damage\ in(M_2)\ generation}$$

For measuring the biological damage two different criteria were used.

(i) Injury (I): percentage inhibition in seed germination.

(ii) Sterility (S): percentage reduction in pollen fertility or percentage pollen sterility.

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RESULTS

The effectiveness decreased in all doses of gamma ray from 1.30 to 0.90 in 5 - 25 kR gamma ray. In MMS the effectiveness increased from 9.4 in 0.05% MMS to 13.89 and 13.50 at 0.075% and 0.1% concentrations, respectively, but in still higher conc. it decreased to 3.10 at 0.50% conc. (Table 1). The most effective dose/concentrations in *Vicia faba* L. var. PRT-12 were: 5 kR gamma ray, 0.075% MMS. The mutagenic efficiency with regard to inhibition (Mp/I) was higher at low dose of gamma ray (5 kR) and low concentration of MMS (0.05%) and, thereafter, it decreased at higher doses/ concentrations (Table 1). The most efficient dose/concentrations in *Vicia faba* L. var. PRT-12 were: 5 kR gamma ray and 0.05% MMS. The mutagenic efficiency based on pollen sterility (Mp/S) was the highest in lower dose/concentrations of gamma ray (5 kR) whereas in MMS it increased in lower conc. and decreased in still higher concentrations (Table 1). The most efficient dose/concentrations with regard to pollen sterility in *Vicia faba* L. var. PRT-12 were: 5 kR gamma ray, 0.075% MMS.

Table 1: Effectiveness and efficiency of gamma ray and ems in *Vicia faba* L. variety PRT12

Doses/ Concentrations	Percentage of Mutant Plant Progenies in M2 (Mp)	% Inhibition in Germination (I)	% Pollen Sterility (S)	Mutagenic Effectiveness Mp/ CT or kR	Mutagenic Efficiency	
					Mp/I	Mp/S
Control	-	-	-	-	-	-
Gamma Rays						
50Gy	6.61	3.96	1.14	1.30	1.67	5.80
100 Gy	10.80	11.11	5.32	1.08	0.97	2.03
150 Gy	14.71	19.05	7.50	0.98	0.77	1.96
200 Gy	18.56	23.01	13.94	0.93	0.81	1.33
250 Gy	22.89	34.13	22.43	0.90	0.67	1.02
EMS						
0.05%	11.76	5.56	11.74	9.40	2.12	1.00
0.075%	25.00	17.46	17.18	13.80	1.43	1.46
0.10%	32.97	25.39	27.87	13.72	1.30	1.18
0.25%	35.29	32.53	31.01	5.88	1.08	1.14
0.50%	37.80	36.51	34.40	3.10	1.04	1.10

DISCUSSION

The mutagen's utility in plant breeding is contingent upon its capacity to recover a high frequency of desired mutations, in addition to its efficacy [28]. In this experiment, the mutagenic efficacy generally declined as gamma ray and MMS concentrations increased, although it increased at lower and intermediate MMS concentrations. Based on altered plant progenies in the M2 generation, the order of mutagenesis efficacy was MMS>gamma ray. Furthermore, it was discovered that alkylating chemicals were more efficient than gamma rays. The early removal of badly injured plants or seedlings was the reason for the decrease in the mutagenesis effectiveness at higher doses. The swift removal of seedlings and the subsequent poor mutagenic efficiency may have been caused by the harmful effect of the higher

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doses/conc. of the mutagens on plants. Thus, it was discovered to be inversely related to the increasing concentrations of mutagens. Reddi and Suneetha reported similar outcomes with MMS in rice, which demonstrated the efficacy did not always raise linearly with increasing concentrations; rather, each concentration had its own effectiveness, irrespective of the other higher and lower concentrations [29]. In *Vigna unguiculata* L. Walp [30], *Trigonella foenum-graecum* L. [31], *Vigna umbellata* Thunb, Ohwi and Ohashi [32], mutagenic effectiveness was found to be higher at lower doses/concentrations of physical and chemical mutagens [30], *Vigna unguiculata* L. Walp [30], *Trigonella foenum-graecum* L. [31], *Vigna umbellata* Thunb, Ohwi and Ohashi [32] and colleagues concluded that in all of the aforementioned plants, the chemical mutagens exhibited greater efficacy. The amount of genetic damage observed in the M2 generation in relation to the biological harm produced in the same generation is indicated by the mutagenesis efficiency. MMS>gamma ray was the order of mutagenic effectiveness with respect to inhibition in germination (Mp/I), and MMS>Gamma ray was the order of efficiency with respect to pollen sterility (Mp/S). According to Wani (2017), a mutagen's increased effectiveness suggests that, in comparison to the mutation it causes, there is comparatively less biological damage (such as sterility or injury to seedlings). The suppression of growth regulators and metabolic disruptions during germination may be the cause of the inhibition of seed germination that occurs after mutagenic treatments. According to Kumar and Rai, mutagens have an adverse effect on the meristematic tissues of seed embryos and can cause chromosomal damage, which results in a decrease in seed germination. Because chiasmata are in charge of maintaining the bivalents that allow for proper chromosome segregation, which in turn maintains pollen fertility, low chiasma frequency may be one of the reasons for reduced pollen fertility [33–36].

As a result of mutagenesis treatments, altered gene loci, intra- or intergenic disruptions, or both, the chiasma frequency decreases, indicating the caused heterology that may ultimately result in pollen sterility. The alterations at the chromosomal and DNA levels, such as deletion, inversion, duplication, and translocation, may also be responsible for the decline in chiasma frequency. It was also discovered that the lower or intermediate treatments were the most effective at causing mutations with comparatively less harm to the biological system. The reduced toxicity of lower or intermediate treatments on pollen fertility and seed germination may account for the increased efficiency attained at these levels.

Alkylating compounds were also discovered to be more effective than gamma rays in the current investigation. These findings concur with those of past researchers studying *Glycin max* L. Merrill. Wani studying *Cicer arietinum* L., Tripathy studying *Lathyrus sativus* L., Mangaiyarkarasi studying *Catharanthus roseus*, and Ambli studying *Pennisetum typhoides*. In this case, it is determined that alkylating chemicals were found to be more successful and efficient than gamma rays in producing the greatest number of viable mutations in the Pusa Uttam aubergine variety. Several of those mutations were eliminated because they were thought to be more advantageous and significant from an agronomic and economic standpoint in a later generation. In the future, these mutagens might also be suggested in programmes aimed at breeding mutations to produce desired mutants in various crops.

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