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Mutagenic effectiveness and efficiency of Gamma rays and EMS in *Lablab purpureus* (L.) Sweet var. *typicus*

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Keywords

Mutagenic effectiveness, Mutagenic efficiency, *Lablab purpureus* (L.) Sweet var. *typicus*, Gamma rays; EMS

Abstract

Mutation has been successfully employed in breeding of several food crop varieties, ornamentals and export crops. The aim of this study was undertaken to assess the effectiveness and efficiency of EMS and gamma rays on *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO(Gb)14. Genetically pure, uniform and dry seeds were treated with different doses/ concentrations of gamma rays (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 KR) and EMS (5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM and 50mM). Mutagenic effectiveness and efficiency of gamma rays and EMS was calculated on the biological damage (lethality, injury and pollen sterility) in the M_1 generation and both chlorophyll and morphological mutations observed in the M_2 generation. The results indicated that, mutagenic effectiveness and efficiency decreased with increase in mutagenic treatments.

Introduction

Agricultural production has witnessed a sharp rise at the global level due to application of various tools of improvement including induced mutagenesis. Sweden has greatly advanced in mutation breeding since 1929 due to efforts of scientists like Gustaffsson at Svaloff Research Station. Mutagenesis provides a powerful technique to improve plant breeding and assist functional and genomic analyses of crop plants. This technique was first introduced with the use of x-ray and radium radiations followed by fast neutron and gamma radiation (Serrat *et al.*, 2014). Because such application of physical mutagens required specialized equipment, chemical mutagens were introduced later. Chemical mutagens are used widely because they are easier to handle and increase mutation frequency (Sikora *et al.*, 2011). It is a coherent tool used in mutation breeding program for creating new alleles (Laskar and Khan, 2014). Various chemical mutagens have been prepared, such as sodium azide, ethyl methanesulphonate (EMS) and N-ethyl-N-nitrosourea, which produce different side effects on the genetic structure of treated populations. These chemicals can cause point mutations, insertions, and/or deletions in the genomic strands, leading to phenotypic changes, which could be desirable traits for important crops (Greene *et al.*, 2003; Flibotte *et al.*, 2010).

EMS, an alkylating agent, commonly is used as a chemical mutagen for DNA lesions. Unlike N-ethyl-N-nitrosourea, EMS induces a biased spectrum of G/C-to-A/T transitions. These transitions most likely occur due to the alkylation at the O^6 or N^7 position of guanine, which leads to the replacement of cytosine with thymine base pairing (Lawley and Martin, 1975) known as EMS

canonical base substitutions, the high frequency of G/C-to-A/T changes has been observed upon EMS exposure in different organisms, including *Arabidopsis thaliana* (Till *et al.,* 2011), *L. japonicus* (Perry *et al.,* 2009), *Caenorhabditis elegans* (Thompson *et al.,* 2013), *Solanum lycopersicum* (Minoia *et al.,* 2010), and *Saccharomyces cerevisiae* (Shiwa *et al.,* 2012) at different rates. EMS also tends to produce random point mutations and induces a low level of chromosomal breaks and lethal effects. These effects provide a competent survival rate and allow subsequent analyses to be performed for both forward and reverse genetics. EMS can be used as a supplementary approach to improve desired identifiable characters such as yield related characters (Botticella, 2011). Mutation frequency, detected using various techniques, displays a wide range of variation according the EMS treatment conditions.

Gamma rays are the most energetic form of electromagnetic radiation; their energy level is from ten to several hundred kilo electron volts and they are considered as the most penetrating compared to other radiations (Kovacs and Keresztes, 2002). These radicals can damage or change important components of plant cells. They have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf, 2003). It is known to be the most popular mutagens for their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Gosal, 2002).

Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen while mutagenic efficiency is an estimate of biological effects induced such as lethality, injury and sterility. The observation of non-random pattern of variation in mutagenic effectiveness and efficiency demonstrates that the genotypic response to different mutagens is of genetic origin and depends upon the physical and chemical properties of the mutagen (Khan, 1981). Therefore, this study was undertaken to gather information on effectiveness and efficiency of different doses/ concentrations of gamma rays and EMS in *Lablab purpureus* (L.) Sweet var. *typicus*.

Materials and Methods

Uniform and dry seeds of *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO(Gb)14 was taken for the induction of mutation using gamma rays and EMS. The gamma radiation was given to the dry seeds with different doses of gamma rays (5KR, 10KR, 15KR, 20KR, 25KR, 30KR, 35KR, 40KR, 45KR and 50KR) at the Sugarcane Breeding Institute, Coimbatore, Tamil nadu, India. For EMS treatment, the seeds were presoaked in distilled water for 6 hours, were subjected to chemical mutagens, Ethyl Methane sulphonate (EMS) for 4hours. The concentrations used for EMS were 5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM, 45mM and 50mM. After the EMS treatment, the treated seeds were washed throughly in running tap water to terminate the residual effect of the mutagenic chemicals.

For raising M_1 generation, the treated seeds were sown along with control at the Botanical garden, Department of Botany, Annamalai University, Tamil nadu, India in a randomized block design with three replication. The spacing was maintained at 15 cm (Plant to plant in a row) and 30 cm (between the rows) in the field. In the M_1 generation seedling height, plant survival and pollen fertility was studied. The data on biological damage (Injury, Lethality and sterility) was computed as the reduction in plant height, reduction in plant survival and reduction in pollen fertility. All the surviving M_1 plants were harvested separately and the seeds of different treatments were sown to raise M_2 generation with three replications in a randomized block design. Screening was done for chlorophyll and viable mutation. Chlorophyll mutations were classified in accordance with the system of Gustaffson (1940) and Blixt and Gottschalk (1975). Frequency of viable mutations was calculated on M_1 and M_2 plant basis. Data on biological abnormalities such as injury, lethality and sterility in M_1 generation and chlorophyll and morphological mutation frequency in M_2 generation were used to determine the mutagenic efficiency and effectiveness of mutagen.

Mutagenic effectiveness and efficiency

Mutagenic effectiveness means mutations induced by a unit dose of mutagen (KR (or) Concentration x Time) while mutagenic efficiency gives an idea of the damage such as lethality, injury and pollen sterility. The formulae proposed by Konzak *et al.,* 1965 were followed for the calculations of mutagenic effectiveness and efficiency by incorporating the mutation frequency values recorded for each mutagenic treatment.

$$\text{Mutagenic effectiveness (Physical mutagen)} = \frac{\text{Mutation Frequency (MF)}}{\text{Dose of Physical mutagen (KR)}}$$

$$\text{Mutagenic effectiveness (Chemical mutagen)} = \frac{\text{Mutation Frequency (MF)}}{\text{Conc. of chemical mutagen x duration of treatment}}$$

$$\text{Mutation efficiency} = \frac{\text{Mutation Frequency (MF)}}{\text{Biological damage in } M_1 \text{ generation}}$$

$$= \text{MF/L, MF/I, MF/S}$$

Where,

MF - Chlorophyll or Viable or total mutation per 100 M_2 plants
KR - Dose of mutagenic radiation in Kilo rad
I - Percentage of injury or reduction in seedling height.
L - Percentage of lethality or reduction in plant survival
S - Percentage of sterility or reduction in pollen fertility

Results and Discussion

The effect of different doses or concentrations of gamma rays and EMS on the biological damage caused by the mutagens in M_1 generation [plant survival reduction (lethality), seedling height reduction (Injury) and reduction in pollen fertility (sterility)], mutation frequency, mutagenic effectiveness and efficiency are depicted in Table-1.

Table - 1. Mutagenic Effectiveness and Efficiency of *Lablab purpureus* (L.) Sweet var. *typicus* in M_2 generation

Treatment dose (or) concentrations		Survival reduction 30th day (L)	Height reduction 30th day (I)	Pollen Sterility (S)	Mutation frequency	Effectiveness M / KR (or) ct	Efficiency		
Mutagens							L= MF/L	I= MF/I	S= MF/S
Gamma rays	20KR	30.03	11.52	4.84	5.15	0.257	0.171	0.447	1.064
	25KR	39.92	20.64	6.58	8.68	0.347	0.217	0.420	1.319
	30KR	47.02	34.19	8.67	4.19	0.139	0.089	0.122	0.483
EMS	25mM	35.96	16.68	6.04	7.00	0.280	0.194	0.419	1.158
	30mM	40.31	29.21	8.98	12.52	0.417	0.310	0.428	1.394
	35mM	50.59	42.53	10.68	5.06	0.144	0.100	0.118	0.473

Mutagenic effectiveness and efficiency

In mutation breeding it is necessary to determine the effectiveness and efficiency of mutagen. Frequency of mutations induced by mutagenic treatment is an index of the effectiveness of mutagen. By observation of the values, the major trends pertaining to this parameter influenced by different doses/concentrations of mutagen can be understood. In general, the effectiveness decreased with increasing doses/concentrations of mutagens. The maximum mutagenic effectiveness was observed in 30mM of EMS (0.417) and the minimum mutagenic effectiveness was recorded in 30KR of gamma rays (0.319). This was in confirmation with the findings of Thilagavathi and Mullainathan (2009) in Black gram, Khan and Tyagi (2010) in Soya bean, Satpute *et al*.(2012) in Soya bean, Bhosale and Kothekar (2010) in Cluster bean, Sikder *et al* (2013) in Tomato, Burghate *et al* (2013) in Ground nut, Mangaiyarkarasi *et al* (2014) in *Catharanthus roseus*, Kulthe and Mongle (2014) in Winged bean, Ambli and Mullainathan (2016) in Pearl Millet.

Mutagenic efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as injury, lethality and sterility induced by the mutagen. Efficient mutagens and their treatments are indispensable for the cost effective use of the mutagen as a tool for the induction of mutations and their direct and indirect utilization in successful breeding programme. On the basis of lethality the maximum mutagenic efficiency was recorded at 30mM of EMS (0.310) followed by 25KR of gamma rays (0.217) and the minimum mutagenic efficiency was observed in 30KR of gamma rays (0.089). On the basis of injury, the mutagenic efficiency was high in 30mM of EMS (0.428) followed by 25KR of gamma rays (0.420) (Table-1). On the basis of sterility, highest mutagenic efficiency was observed at 30mM of EMS (1.394) followed by 25KR of gamma rays (1.319). Mutagenic efficiency decreased with an

increase in the dose/ concentration of mutagens. Similar results were reported earlier in Sunflower (Raja Ramesh Kumar and Venkat Ratnam, 2010), Pigeon pea (Sangle and Kothekar, 2013) Black gram (Bhosale and Hallale, 2013) and Green gram (Mishra and Singh, 2014).

When the mutation rates based on efficiency were compared, EMS was found to be more efficient than gamma rays in *Lablab purpureus* (L.) Sweet var. *typicus*. Similar observation has been recorded by Girija and Dhanvel (2009), Sharma *et al.,* (2005), Ramya *et al.* (2013) and Bashir *et al.,* (2013). Such difference in effects of mutagen on different materials might be due to seed metabolism and onset of DNA synthesis. Kundi *et al.* (1997) reported differential sensitivity within crop and even genotype. It was opined that the sensitivity depends upon the genetic architecture and mutagens employed besides the amount of DNA, its replication time in initial stages and degree of heterochromatin (Blixt, 1970).

Conclusion

The present investigation was carried out to study the mutagenic effectiveness and efficiency of Gamma rays and EMS in *Lablab purpureus* (L.) Sweet var. *typicus*. The mutagenic effectiveness and efficiency decreased with increased in doses/ concentrations of mutagens. The results indicate that lower doses/ concentrations of mutagens are effective in induction of mutations for crop improvement in *Lablab purpureus* (L.) Sweet var. *typicus*

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