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Physical and chemical mutagenic effect on pollen fertility in M_1 generation of Garden Bean [*Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb) 14]

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ABSTRACT

Mutation breeding has been widely used to develop a large number of desirable varieties in field and horticultural crops. The present study was performed by exposing the seeds with Gamma rays and Ethyl Methane Sulphonate to assess the pollen fertility in M_1 generation. It was observed, that the pollen fertility decreased with increasing dose/ concentrations of mutagens. The results showed that the treatment of EMS was more effective in reducing pollen fertility as compared to gamma rays and control. Pollen fertility percentage was better in control when compared to Gamma rays and EMS. Lower dose/ concentrations of these two mutagens produced less biological damage and would be suitable for inducing desirable attributes in Garden bean.

Key words: Pollen fertility, EMS, Gamma rays and *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb)14.

INTRODUCTION

Lablab purpureus (L.) Sweet belongs to the family Fabaceae. The genus *Lablab* was originated from India [1]. *Lablab purpureus* has two distinct botanical forms namely *Lablab purpureus* (L.) Sweet var. *typicus (*Garden Bean) and *Lablab purpureus* (L.) Sweet var. *lignosus* (Field Bean). Garden bean was grown for its tender pods and Field bean was mainly cultivated for its seeds.

Lablab purpureus (L.) Sweet was mostly grown for its tender pods and it is also grown as a green manure crop or as a forage [2]. The beans are naturally rich in minerals and vitamins [3] with high concentration of protein (20- 25%). In fact, it is considered as a multipurpose crop since it is used for food, forage, soil improvement,weed control and soil protection [4].

Mutation breeding in crop plants is an effective tool in the hands of plant breeders especially in crops having narrow genetic base. Many mutants have been identified as donors of desirable traits in breeding program. Mutation breeding of plants is useful to improve the character if the character you want is not located in a plant germplasm of a species, and also for generating variability in the existing varieties [5].

Induced mutation using physical and chemical mutagens is one method to create genetic variation resulting to new varieties with better characteristic features [6]. Various mutagenic agents were used to induce favorable mutations at

high frequency; the use of ionizing radiation, such as X-rays, gamma-rays and neutrons as well as chemical mutagens for inducing variation is well established [7, 8].

Ionization radiations still remain most suitable agents for inducing genetic variability [9, 10, 11]. Application of radiation has been most frequently used for induction of mutation resulting in direct development of 89% mutant varieties [12]. Gamma irradiation is one of the main physical mutagens for mutation studies in plants. It had adverse effect on traits of plants and this depended on plant species or varieties and the dose of irradiation [13]. These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds [14, 15]. Gamma irradiation has provided number of useful mutants and still shows an elevated potential for improving vegetative plants [16]. A great majority of mutant varieties (64%) were developed by the use of gamma rays [17].

Chemical mutagens are the one cause of mutations in living organisms. Many of these chemicals have clastogenic (chromosome damaging) effects on plants via reactive oxidative radicals [18]. These effects can occur both spontaneously and artifically following induction of mutagens. Among the chemical mutagens, EMS, MMS and MES have been reported to be the most effective and powerful mutagens. These sulphonate compounds have bifunctional alkyl reactive groups that react with DNA, causes extensive cross linkage of DNA, chromosome breakage, chromosome mutations and gene mutation. Chemical mutagen generally produce induced mutations which leads to base pair substitution, especially GC→AT resulting in amino acid changes, which change the function of proteins but do not abolish their function as deletion or frame shift mutation mostly do [19]. These chemomutagens induce a broad variation of morphological and yield structure parameters in comparison to normal plants.EMS is the most commonly used chemical mutagen in plants. EMS alkylates guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C- to-A/T transitions [20]. It produces a range of novel traits and broadening of genetic diversity of plants [21].

Mutation breeding has contributed significantly to plant improvement. So far, 3218 number of crop varieties has been developed through induced mutagenesis [22]. In India, at least 300 cultivars have been developed in at least 55 plant species [23].

Based on above, the present experiment was conducted to study the effect of two mutagens *viz*., gamma rays and EMS on pollen fertility in Garden bean.

MATERIALS AND METHODS

The seeds of Garden bean (*Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb) 14) were procured from Tamil Nadu Agricultural University, Coimbatore. In the present study, the two mutagens (Gamma rays and Ethyl Methane Sulphonate) were employed for the treatments of seeds. Healthy, dry and uniform seeds of Garden bean were irradiated with 5KR, 10KR, 15KR, 20KR, 25KR, 30KR, 35KR, 40KR, 45KR and 50KR of gamma rays from ^{60}CO source at Sugarcane Breeding Institute, Coimbatore. For the treatment of EMS, the selected seeds were presoaked in distilled water for 6 hours and the wet seeds were treated with different concentrations of EMS (such as 5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM and 50mM) for 4 hours. After the treatment, the seeds were rinsed in running tap water to remove the excess of EMS solution from seed surfaces. In the laboratory experiment, the treated seeds were transferred onto the Petri dishes containing absorbent cotton for the seed germination test. The seeds were continously assessed following the next day of treatments. The LD_{50} for germination was 25KR of gamma rays and 30mM of EMS. Based on the LD_{50} value, the three treatments of gamma rays and EMS around LD_{50} value were fixed for further studies.

M_1 Generation

The field experiment was conducted in the Botanical Garden, Department of Botany, Annamalai University. The seeds treated with two mutagenic agents along with control were sown in the field in a randomized block design with three replications. All the necessary cultural operations such as timely irrigation, weeding and plant protection measures were ensured.

Pollen fertility

Pollen fertility was analysed in 10 randomly selected plants belonging to each mutagenic treatments and control. For this purpose the pollen grains were collected on clean glass slides by dusting the freshly dehisced anthers from

young flower buds. Aceto- carmine test was used to determine the pollen fertility. It was determined by staining the pollen grains with 1% acetocarmine. Pollen grains that stained fully and had a regular outline was considered as fertile, while partially stained, shrunken and empty were considered as sterile. The values are expressed in percentage.

RESULTS AND DISCUSSION

In the present investigation, effect of gamma rays and EMS on pollen fertility was observed. Pollen character is one the important stable and genetically controlled characters. The percentage of pollen fertility was high in control plants (98.19). The pollen fertility decreased with increasing dose/ concentration of mutagens. The pollen fertility range in two mutagenic treatments was 93.43% to 87.70%. In Gamma rays, the pollen fertility ranged from 93.43% to 89.67% and in EMS, it ranged from 92.25% to 87.70%. The highest percentage of reduction was observed in 35mM of EMS (87.70).

The pollen fertility percentage was decreased with increasing dose/ concentrations of mutagens (Table-1). The negetive effect of mutagens on pollen fertility percentage in mutagenic treatment plants may be due to meiotic aberrations that were induced by mutagens leading to the formation of aberrant pollen grains [24, 25, 26, 27].

The sterility of the pollen may be due to physiological and genetic changes or may be due to meiotic aberrations [28, 29]. A gradual decrease in pollen fertility percentage was reported earlier in *Vigna radiata* [30, 31, 32,] and in *Lens culinaris* [33] in horsegram [34] and in pigeonpea [35].

Table-1: Effect of Gamma rays and EMS on Pollen fertility in M_1 generation of *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO (Gb)14

Mutagens	Treatments (dose/conc)	Pollen fertility (%)	Percentage of reduction over control
Control		98.19	-
Gamma rays	20KR	93.43	4.84
	25KR	91.72	6.58
	30KR	89.67	8.67
EMS	25mM	92.25	6.04
	30mM	89.37	8.98
	35mM	87.70	10.68

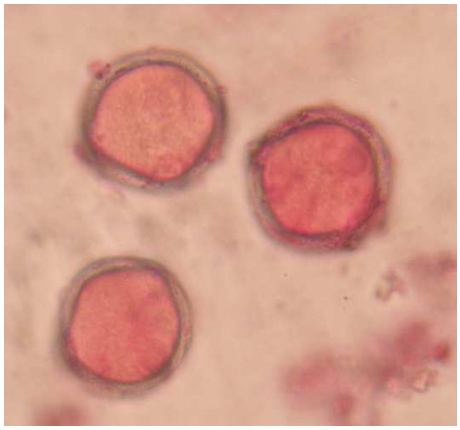

Figure1: Fertile pollen grains

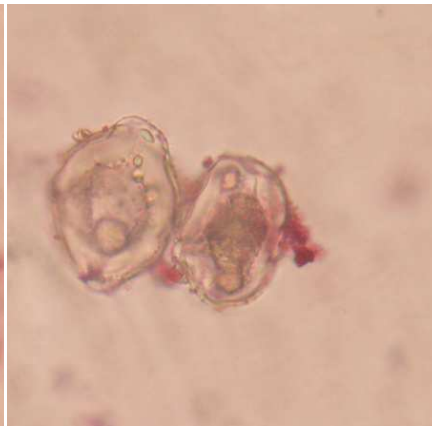

Figure2: Sterile pollen grains

CONCLUSION

From the experiment, it is concluded that the pollen fertility decreased with increasing dose/concentrations of gamma rays and EMS. The percentage of pollen fertility was less in gamma rays treatment plants than EMS. Based on the results, it is advocated that the lower dose/ concentrations of gamma rays and EMS may be suitable for developing the cultivar with desirable alleles.

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