



EFFECT OF EMS AND SA ON THE FREQUENCY OF PLANTS CARRYING CHLOROPHYLL DEFICIENT SECTORS IN M₁ GENERATION OF WINGED BEAN

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ABSTRACT

Objective of the present investigation has been to induce genetic variability in winged bean especially in its quantitative and qualitative characters. This would help in developing improved varieties of winged bean through of mutation breeding. In the present work, the seeds of winged bean of variety (cultivars) II-EC-178313 and 2I-38825 were treated with two chemical mutagens like Ethyl Methane Sulfonate (EMS) and Sodium Azide (SA) to induce mutations. From present studies, it is quite clear that the varied concentrations of both the EMS and SA have proved effective in inducing the chlorophyll deficient sectorial types in both the varieties of winged bean. The different kinds of sectors such as yellow (xantha), light green (viridis), yellow green (chlorina) and white (albina) types were observed on leaves.

KEY WORDS

Psophocarpus tetragonolobus (L.) DC, induce genetic variability

INTRODUCTION

Winged bean botanically described as *Psophocarpus tetragonolobus* (L.) DC. belongs to family fabaceae. It is also known as Goa bean, four angled bean, four cornered bean, Manila bean, is atropical legume plant native to Papua New Guinea. Winged bean is extensively cultivated in the countries like Myanmar, India, Indonesia, Bangladesh, Srilanka, Cambodia, and Philippines. The systematic and extensive work on taxonomical position of winged bean was carried out by Verdcourt and Halliday (1978). It is a perennial /annual, twining, glabrous, self-pollinating herbaceous plant. The roots are numerous and tuberous, stem is firm and herbaceous, leaves are pinnately trifoliate, petiolate, flower shows the variation in colour like blue, bluish white or purple.

Winged bean has large potential to fulfill the need of staple food that is rich in protein and oil for man and as a fodder for animals. Masfield (1973) was the first person who could highlight the potential utility of this plant. It has been described as wonder legume in the

sense that virtually all parts of this plant are edible and immensely nutritious. The winged bean seed rivals the soybean in quantity and quality of its protein. The seeds contain high amount of proteins (29 – 42%) and good quality edible oil (15- 20%), NAS (1975).

Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement, Acharya et. al. (2007). Both the chemical mutagens such as EMS and SA proved to be most effective and powerful mutagens. In plants EMS usually causes point mutations, Okagaki (1991), while SA is marginally mutagenic in different organisms, Arenaz et. al. (1989). Genetic variability is one of the prerequisites for crop improvement. As a consequence, lack of required amount of variability, limits the scope for the selection of better genotypes. Artificially induced mutations are the best way to enlarge genetic variability, considerably within a short time, Patil et. al. (2003). Frequency of natural mutation is very low and hence artificial mutations are induced and genetic variability is best enhanced with the application of mutagens. It is

essential to restore the lost genetic variability of different varieties from different part of the world through different techniques like induced mutagenesis. Thus, mutation breeding or induced mutation is having great potential for the improvement of traditional agricultural crops like winged bean.

Despite possessing multipurpose utility and immense potential as detailed above the winged bean has remained unfamiliar among society because of the high amount of antinutritional factors, absence of market demands, labour intensive nature of crop and long duration of its life cycle. To overcome these, negative properties, it's an urgent need to develop improved varieties of winged by using the novel approach of mutation breeding.

MATERIALS AND METHODS

The experimental plant material selected for the present investigation comprised two varieties (cultivars) of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) namely II-EC-178313 and 2I-EC-38825. Germplasm of these two cultivars was procured from National Bureau of Plant Genetic Resources, Regional Station PKV, Akola, (M.S.).

The chemical mutagens viz., Ethyl Methane Sulfonate (EMS) and Sodium Azide (SA), manufactured by Sigma chemical company Ltd., U.S.A. was used in the present investigation. The pilot experiments were conducted for determining the suitable concentrations for further studies.

Prior to mutagenic treatments seeds were immersed in distilled water for 6 hours. The presoaking enhances the rate of uptake of the mutagen through an increase in cell permeability and also initiates metabolism in the seeds for treatment. Such presoaked seeds were later on immersed in the mutagenic solutions for 6 hours with an intermittent shaking. Seeds soaked in distilled water for 12 hours served as control. All the chemical mutagenic treatments were given at room temperature of $25 \pm 2^{\circ}$ C.

The different concentrations used for the chemical treatments were 0.05%, 0.10% and 0.15% for EMS and 0.01%, 0.02% and 0.03% for SA respectively. Immediately after the completion of treatment the seeds were washed thoroughly under tap water. Later

on, they were kept for post soaking in distilled water for 2 hours.

For each treatment, a batch of 450 presoaked seeds was used. Out of 450 seeds, a lot of 300 seeds from each treatment was sown in field following randomized block design (RBD) with three replications along with control as the M_1 generation. The seeds were sown at a distance of 30 cm between the plants and 90 cm between the rows. The field experiments were carried out at the experimental field of Botany Department, Dr. Babasaheb Ambedkar Marathwada University Aurangabad (M.S.), during kharif season. Further the M_1 generation was observed carefully to record plants with different types of chlorophyll deficient sectors in leaves and frequency of such plants was calculated.

RESULTS AND DISCUSSION

The critical screening of the M_1 generation of winged bean revealed the induction of chlorophyll deficient sectors of different types in the leaves at all the concentrations of both the mutagens such sectors were of yellow (*xantha*), light green (*viridis*), yellow green (*chlorina*) and white (*albina*) types. They were found at the margins of leaflets, at the apex or distributed throughout the leaf lamina. From the results, it is quite clear that the varied concentrations of both the EMS and SA proved effective in inducing the chlorophyll deficient sectorial types in both the varieties of winged bean. The frequency of chlorophyll deficient sectors increased with the increasing concentration of mutagens in both the varieties of winged bean.

The frequency of chlorophyll deficient sectorial plants ranged from 3.53% to 5.00% and 3.33% to 6.03% after EMS treatments in variety II-EC-178313 and 2I-EC-38825 of winged bean respectively. While in case of SA treatments the frequency values ranged from 4.31% to 5.88% and 2.58% to 4.76% in the pertinent varieties of winged bean (**Table – 1 and 2**).

The mutagen induced chlorophyll deficient sectors embody one of the prime biological effects. This effect however, is restricted to the M_1 generation only. In recent years, it has been very much appreciated that the in-depth study of such sectors (also described as leaf chlorophyll chimeras) would prove immensely helpful in having a rapid estimation of effectiveness of mutagenic

agent and new treatment conditions right in M₁ generation itself.

Stadler (1930) was first to observe that mutations induced by seed irradiation appeared in the form of sectors in M₁ plants. Anderson et. al. (1949) demonstrated that the analysis of mutated sectors can greatly help in tracing the ontogeny of organs in M₁ plants. Motto et.al. (1975) reported chlorophyll chimeras in french bean after EMS treatments. Recently Mahamune and Kothekar (2012) observed chlorophyll chimeras in French bean after EMS and SA treatments. Goud (1967) opined that differential response of the embryonic cells to mutagen causes chimerism. Developments of chlorophyll chimeras in plants become possible when a sector of the multicellular embryo become mutated. The embryo contains different sets of meristematic initials which are potentially capable of producing certain portion of the mature plant. A

differential response of such embryonic cells to the mutagen causes chimerism.

Chary (1983) recorded higher frequency of chlorophyll deficient sectors after chemical mutagenic treatments than gamma rays and magnetic field treatments. He further stated that EMS was the most effective mutagen among all chemical mutagens in producing chlorophyll deficient sectors.

CONCLUSION

From above study, it is concluded that the different types of chlorophyll deficient sectors such as *xantha*, *viridis*, *chlorina* and *albino* could be detected in the M₁ generation of winged bean. The frequency of sectorial plants showed an increasing trend with the increasing concentration of EMS and SA in both the varieties of winged bean.

Table – 1: Effect of EMS on the frequency of plants carrying chlorophyll deficient sectors in M₁ generation of *Psophocarpus tetragonolobus* (L.) DC.

Variety	Concentration	Frequency of plants carrying chlorophyll deficient sectors (%)	± S.E.
II-EC-178313	Control	-	-
	0.05%	3.53	0.33
	0.10%	4.62	0.66
	0.15%	5.00	0.33
2I-EC-38825	Control	-	-
	0.05%	3.33	0.33
	0.10%	5.04	0.57
	0.15%	6.03	0.88

± S.E.= Standard Error

Table – 2: Effect of SA on the frequency of plants carrying chlorophyll deficient sectors in M₁ generation of *Psophocarpus tetragonolobus* (L.) DC.

Variety	Concentration	Frequency of plants carrying chlorophyll deficient sectors (%)	± S.E.
II-EC-178313	Control	-	-
	0.01%	4.31	0.66
	0.02%	4.54	0.66
	0.03%	5.88	0.57
2I-EC-38825	Control	-	-
	0.01%	2.58	0.33
	0.02%	4.42	0.66
	0.03%	4.76	0.33

± S.E.= Standard Error

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