

Studies on Induced Mutations in Winged Bean : Frequency and Spectrum of Chlorophyll Mutations



Botany

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* A. S. SONAVANE

Assistant Professor, Department of Botany, Shardabai Pawar Mahila Mahavidyalaya Shardanagar, Baramati, (M.S.) India, * Corresponding author

ABSTRACT

The seeds of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) variety II- EC- 178313 and 2I- EC-38825 were treated with different concentrations of chemical mutagenic agents like Ethyl methane sulfonate (EMS) and Sodium azide (SA) separately. Mutagenic treatments resulted in induction of four types of chlorophyll mutants such as xantha, chlorina chloroxantha and viridis. In M₂ generation. Among the two chemical mutagens, EMS proved to be very much successful in inducing the highest frequency of chlorophyll mutants in both the varieties of winged bean. The mutagen EMS failed to produce the xantha mutant in both varieties (II- EC-178313 and 2I- EC-38825) of winged bean.

INTRODUCTION

Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) popularly known as a "Supermarket on a stalk" because it combines the desirable characteristics of the green bean, garden pea, spinach, mushroom, soybean, bean sprout and potato, save for the stalk, virtually the entire plant is fit for human consumption—from flowers and leaves to tuberous roots and seeds. As it is a potential legume crop of the tropics with high protein and oil content in the seeds, used as a staple food, but it could not get popularity and acceptance among the farmers because of the labour intensive nature of the crop, long duration of its life cycle, antinutritional factors in its seeds/ tubers. To overcome these negative properties the conventional breeding programme is not enough. We have to make many changes in genetic constitution of the species. Although it possess several positive attributes, it is amazing that still the crop has remained obscure and neglected in India as well as all over the globe. Thus keeping this view in mind, the approach of induced mutations can be used quite beneficially for achieving the desirable varieties in winged bean.

Since the pioneering work of Stadler (1928), chlorophyll mutations have been used to estimate the frequencies of induced mutations. Chlorophyll mutations are important parameter, which are used to estimate the effectiveness of the mutagens. It is also the induction of factor mutations.

Thus the present investigation deals with the studies on induced chlorophyll mutations by Ethyl methane sulfonate (EMS) and Sodium azide (SA)

MATERIALS AND METHODS.

The seed material of two varieties of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) namely II-EC-178313 and 2I-EC-38825 obtained from the National Bureau of Plant Genetic Resources, Regional station PKV, Akola, was used in the present study. The chemical mutagens such as Ethyl methane sulfonate (EMS) and Sodium azide (SA) were used in the present investigation.

DETAILS OF MUTAGENIC TREATMENTS:

To begin with the pilot experiments were conducted for determining the suitable concentrations for further studies. Prior to mutagenic treatment seeds were immersed in distilled water for 6 hours. The presoaking enhances the rate of uptake of the mutagen through increase in cell permeability and also initiates metabolism in the seeds for treatment such presoaked seeds were later on immersed in mutagenic solution for 6 hours with an intermittent shaking. Seeds soaked in distilled water for 12 hours served as control.

The different concentrations used for the chemical mutagenic 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 running tap water. Later on they were kept for post soaking in distilled water for 2 hours. After the completion of treatment , the treated seeds were sown in field following randomized block design (RBD) three replication along with control as the M₁ generations. From M₁ generations, the plants were selected depending upon their morphological variations as compared with the control plants. Thus seeds were harvested from such plant separately and stored in polythene bags and used for sowing M₂ generation.

Scoring of Chlorophyll Mutations:

The M₂ population was grown from the seed progenies of M₁ plants. The M₂ population was screened keenly for different types of chlorophyll mutations. The frequency and the spectrum of mutations were calculated. The identification and classification procedure for chlorophyll mutations was followed Gustafsson (1940) and Blixt (1961). The frequency of chlorophyll mutants was calculated according to Gaul (1960) i.e. number of mutants/100 M₂ seedlings.

RESULTS AND DISCUSSION

Chlorophyll mutations in M₂ generation had been proved to be most dependable indices for evaluating the genetic effects of mutagenic treatments (Gustafsson and Von-Wettstein, 1956).

In present studies, the four different types viz.. xantha, chlorina, chloroxantha and viridis of chlorophyll mutations were observed. It was found that both the mutagens succeeded in inducing the chlorophyll mutants. The results on chlorophyll mutations induced by various chemical and physical mutagens, alone and in combination were recorded by several workers (Mahamune and Kothekar 2012, and Girija and Dhanavel, Ramezani and More 2014).

The mutants such as chlorina, chloroxantha and viridis survived till maturity and formed a distinct group of viable chlorophyll mutants. But when compared with the normal plants, they showed less seed yield and delayed flowering pattern. Most of them breed true in the subsequent M₃ generation. It is observed that both the mutagens succeeded in inducing various types of chlorophyll mutants. The spectrum of induced chlorophyll mutants was quite broad in both the varieties of winged bean and the response of the varieties towards the different mutagens was differential.

In present investigation, EMS proved to be very much successful in inducing the highest frequency of chlorophyll

mutants in variety II-EC-178313 and 2I-EC-38825 of winged bean as compared with that of SA.

In case of EMS treatments the increase in the frequency of chlorophyll mutants was rather gradual from 5.69% at 0.05% to 7.01% at 0.15% concentration in variety II-EC-178313 while in variety 2I-EC-38825 it was 4.61% at 0.05% to 6.77% at 0.15% concentration. (Table-1).

After the SA treatment the values of chlorophyll mutants varied from 4.34% at 0.01% to 6.77% at 0.03% concentration in variety II-EC-178313, while in variety 2I-EC-38825, the values were varied from 3.89% at 0.01% to 5.76% at 0.03% concentration. (Table-2)

Table-1: Effect of EMS on the frequency of chlorophyll mutants in M₂ generation of (*Psophocarpus tetragonolobus* (L.) DC.

Variety	Concentration	Frequency of plants Chlorophyll mutants (%)
II-EC-178313	Control	-
	0.05%	5.69
	0.10%	6.75
	0.15%	7.01
2I-EC- 38825	Control	-
	0.05%	4.61
	0.10%	4.67
	0.15%	6.77

Table-2 : Effect of SA on the frequency of chlorophyll mutants in M₂ generation of (*Psophocarpus tetragonolobus* (L.) DC.

Variety	Concentration	Frequency of plants Chlorophyll mutants (%)
II-EC-178313	Control	-
	0.01%	4.34
	0.02%	4.54
	0.03%	6.77
2I-EC- 38825	Control	-
	0.01%	3.89
	0.02%	5.19
	0.03%	5.76

As regards the xantha mutant the 0.03% concentration of SA proved to be the most effective in inducing the highest relative percentage (16.66%) in variety 2I-EC-38825. The same mutants however was not observable in Variety II-EC-178313 of winged bean. The mutagen EMS failed to produce the xantha mutant in both the varieties of the winged bean. The results on the induction of chlorophyll mutations due to sodium azide was reported by (Makeen et al. 2013 and Lal et al. 2009) in black gram.

It was found that the chlorina type of mutant could get induced maximally at majority of the mutagen treatments in both the varieties of winged bean. In case of EMS treatment , the highest frequency of chlorina mutant (50.00%) could be recorded at 0.15% concentration in both the varieties of winged bean. However in case of SA treatments the maximum relative percentage (50.00%) could be noted at 0.01% and 0.03% concentration in variety II-EC-178313, while in variety 2I-EC-38825 it could be recorded at 0.02% and 0.03% concentration of SA.

As regards the chloro-xantha mutant the 0.05% concentration of EMS proved to be the most effective one in inducing the highest relative percentage (66.66%) in variety 2I-EC 38825 while in variety II-EC-178313, the highest relative percentage (50.00%) could be noticed at 0.01% concentration of SA.

The *viridis* type of chlorophyll mutant revealed its highest percentage (80.00%) in 2I-EC-38825 at 0.10% concentration of EMS. The lowest relative percentage (25.00%) was noticeable at 0.15% , 0.02% and 0.03% concentration of both EMS and SA mutagens in both the varieties of winged bean respectively. (Table-3 and 4).

Table-3: Effect of EMS on the spectrum of chlorophyll mutants in M₂ generation of (*Psophocarpus tetragonolobus* (L.) DC.

Variety	Concentration	Relative percentage				
		Al-bina	Xan-tha	Chlorox-antha	Chlo-rina	Vir-idis
II-EC-178313	Control	-	-	-	-	-
	0.05%	-	-	42.85	28.57	28.57
	0.10%	-	-	20.00	40.00	40.00
	0.15%	-	-	-	50.00	50.00
2I-EC-38825	Control	-	-	-	-	-
	0.05%	-	-	66.66	33.33	-
	0.10%	-	-	-	20.00	80.00
	0.15%	-	-	25.00	50.00	25.00

Table-4: Effect of SA on the spectrum of chlorophyll mutants in M₂ generation of (*Psophocarpus tetragonolobus* (L.) DC.

Variety	Concentration	Relative percentage				
		Al-bina	Xan-tha	Chlorox-antha	Chlo-rina	Vir-idis
II-EC-178313	Control	-	-	-	-	-
	0.01%	-	-	50.50	50.50	-
	0.02%	-	-	33.33	33.33	33.33
	0.03%	-	-	25.00	50.00	25.00
2I-EC-38825	Control	-	-	-	-	-
	0.01%	-	-	-	33.33	66.66
	0.02%	-	-	25.00	50.00	25.00
	0.03%	-	16.66	33.33	50.00	-

Thus in the present investigation, a wide spectrum of chlorophyll mutants has been recorded. The chlorina was found to be the most abundant type in most of the mutagenic treatments. Ehrenberg (1960) recorded viridis as the most common type after chemical mutagenic treatments.

The present study has shown that the *chlorina* and *viridis* types of mutants were the predominant types in both the varieties of winged bean. Ashri Goldin (1965) observed similar type of results in peanut. In the present study, the *xantha* mutant could not be detected in the variety II-EC-178313, however, it could be detected in variety 2I-EC-38825 of winged bean. Hakande (1992) noted a minor varietal difference in frequency and spectrum of chlorophyll mutations in winged bean.

It is also clear that, considerable varietal difference towards mutagens was found in the present investigation on winged bean. This may be due to differences in genetic make up concerned with the development and expression of chlorophyll in case of the two varieties of winged bean.

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