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Effect of EMS and SA on mitotic anomalies induced in M_1 generation of *Psophocarpus tetragonolobus* (L.) DC.

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Abstract

In the present study,the mutagens Ethyl methanesulfonate (EMS) and Sodium azide (SA) succeeded in inducing the chromosomal aberrations/anomalies in variety II-EC-178313 and 2I-EC-38825 of winged bean (*Psophocarpus tetragonolobus* (L.) DC.). The mitotic metaphases and anaphases were scored for determining the frequency of chromosomal aberrations/anomalies. Different types of aberrations such as stickiness, mis-orientation, precocious movement, laggards and bridges could be scored in case of root meristem cell of both the varieties of winged bean.

Keywords: Winged bean, Aberrations, EMS, SA, Laggards

Introduction

The winged bean (*Psophocarpus tetragonolobus* (L.) DC.) being a humid tropical crop has been grown as backyard crop in Soth-East Asia, South Asia, the Indian ocean and the pacific Islands for many centuries. It has been grown in these areas mainly as a green vegetable but it is also grown widely on a larger scale as a tuber crop in Papua New Guinea and Burma. Burkhill (1935) reported that tubers of winged bean plant are the potential source of both protein and carbohydrate, this rare combination makes the winged bean tuber unusual among the tropical root crops. It has been called as "one species supermarket" because all of the plant is edible. The tender pods, which are the most widely eaten part of the plant can be harvested within two to three months of planting.

The flowers of winged bean are often used to colour rice and pastries. The young leaves can be picked and prepared as a leaf vegetable, similar to spinach. Each part of the winged bean provides a source of vitamin A, vitamin C, calcium iron and other vitamins. The seeds contain 29-42% protein and 20% oil NAS (1981).

As winged bean possess several positive attributes, but due to some peculiar shortcomings its wide scale popularization and ready usage has been neglected throughout the world. The shortcomings like labour intensive nature, absence of market demands, long duration of the crop and presence of some antinutritional factors in various plant organs.

Thus keeping in mind, the excellent nutritional potential of a rather unfamiliar grain legume crop like winged bean, efforts

were initiated to achieve the genetic improvement of that system through mutation breeding under our local conditions. The pertinent efforts have culminated in development of a range of true breeding productive mutants.

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 viz., ethyl methanesulfonate (EMS) a monofunctional alkylating agent and sodium azide (SA) manufactured by sigma chemical company Ltd., U.S.A. was used in the present work.

Details of mutagenic treatments

To begin with the pilot experiments were conducted for determining the suitable concentration for further studies. The chemical mutagenic treatments were administered at room temperature of 25 ± 2^0 C. The fresh aqueous solutions of the mutagens were prepared prior to treatment.

Treatment

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 the 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.

Further one hundred seeds from each treatment were allowed to germinate in petridishes lined with moist paper at room temperature (25 ± 2^o C). Root tips of 1 to 15 cm. length were fixed in fixative containing 1:3 acetic alcohol for 24 hours. They were subsequently traneferred to 70% alcohol and stored in refrigerator. The root tips were stained in 2% hematoxylin by using ferric alum as a mordant. They were later on squashed in a drop of 45% acetic acid. At least 30-35 slides were prepared for each concentration and the chromosomal aberrations were scored.

Results and discussion

The mitotic metaphases and anaphases were scored for determining the frequency of chromosomal aberrations/anomalies. Different types of aberrations such as stickiness, mis-orientation, precocious movement, laggards and bridges could be scored in case of root meristem cell of the treated material. The frequency of cells having chromosomal abnormalities increased linearly with an increase in the concentration of EMS and SA in both the varieties of winged bean. The highest frequency of mitotic anomalies carrying cells could be noted at 0.15% concentration of EMS in both the varieties (II-EC-178313 and 2I-EC-38825), where the frequency values for mitotic anomalies were 10.52% and 12.19% respectively. (Table 1 & 2).

The frequency of mitotic anomalies carrying cells ranged from 8.06% to 10.52% and 3.21% to 5.26% after EMS and SA treatments in variety II-EC-178313, while in variety 2I-EC-38825 the values were 9.11% to 12.19% for EMS and 3.23% to 4.70% SA treatments respectively. It could be observed that the frequency of metaphase aberrations was more than that of anaphase at majority of the mutagenic treatments.

Table 1: Effect of EMS on Mitotic anomalies induced in M_1 generation of *Psophocarpus tetragonolobus* (L.) DC.

Variety	Concentration	% Anomalies at Metaphase				Anomalies at Anaphase %		Total percentage of anomalies
		Stickiness	Mis orientation	Precocious movement	Bridges	Laggards	Precocious movement	
II-EC-178313	Control	-	-	-	-	-	-	-
	0.05 %	2.26	0.92	2.21	2.01	-	0.66	8.06
	0.10%	2.78	1.47	2.24	1.62	0.47	0.56	9.14
	0.15%	2.86	1.30	2.73	2.24	0.57	0.82	10.52
2I-EC-38825	Control	-	-	-	-	-	-	-
	0.05 %	2.44	1.64	2.22	2.39	0.42	-	9.11
	0.10%	2.52	2.25	3.00	2.41	0.28	0.86	11.32
	0.15%	3.00	2.56	3.30	1.90	0.54	0.99	12.19

Table 2: Effect of SA on Mitotic anomalies induced in M_1 generation of *Psophocarpus tetragonolobus* (L.) DC.

Variety	Concentration	% Anomalies at Metaphase				Anomalies at Anaphase %		Total percentage of anomalies
		Stickiness	Mis orientation	Precocious movement	Bridges	Laggards	Precocious movement	
II-EC-178313	Control	-	-	-	-	-	-	-
	0.01 %	0.66	0.60	0.49	0.39	-	1.07	3.21
	0.02%	0.62	1.27	0.86	0.55	0.75	0.54	4.59
	0.03%	0.64	1.11	1.02	0.39	0.92	1.18	5.26
2I-EC-38825	Control	-	-	-	-	-	-	-
	0.01 %	0.32	0.40	0.53	0.62	0.94	0.42	3.23
	0.02%	0.47	0.44	1.14	0.64	0.92	0.49	4.10
	0.03%	0.46	0.48	0.96	0.74	0.90	1.16	4.70

The chromosomal aberrations have been believed to be intimately associated with mutagenicity of radiations and chemicals Different researchers like Sparrow (1951), Bacq and Alexander (1955), Bhaskaran and Swaminathan (1962) and Kothekar (1987) have described the cytological effects of radiations. The induction of chromosomal aberrations through the use of physical/chemical mutagens either singly or in combination has been reported by many workers such as Conger (1965), Mehra and Mann (1974), Deshpande (1980) and Datta (1992).

In the present work, the highest frequency of chromosomal aberrations carrying cells was noticeable at 0.15% of EMS and 0.03% of SA in both II-EC-178313 and 2I-EC-38825 varieties of winged bean. The most common anomaly was stickiness of chromosomes which could be noticed in all the treatments of mutagens. It may have resulted due to changes in the surface and contour of chromosomes. Goswami and Dave (1975) observed similar abnormalities in *Pisum*.

Precocious movement and mis-orientation at metaphase comprised another more frequently observed aberration in the present studies. They can be interpreted as arising from disturbance in the formation of spindle. Similar types of results were observed by Prasad (1974).

Single, double and cris-cross bridges at anaphase were observed in most of treatments in the present investigation. Such aberrations can arise due to breakages induced in chromosomes by mutagen. Researchers like Kaul (1994), Kothekar (1987) and Harsulkar (1994) reported breakages of chromosomes after treatment with radiations/ chemicals/ pesticides.

The lagging of chromosomes was also observed in the present studies. Kallo (1973) reported that the lagging of chromosomes get developed mainly on account of mutagen affected spindle fibres.

In the present investigation, the persistent nucleolus was observed after the chemical mutagenic treatments in both the varieties of winged bean. Shashibala and Prasad (1986) also reported persistent nucleolus in *Vigna* species.

Conclusion

Different types of mitotic aberrations could be observed after the mutagenic treatments in the present study. Such aberrations were noticeable at metaphase and anaphase stages of the cell cycle. The frequency of aberrations carrying cells increased linearly with an increase in concentration of both the mutagens, namely EMS and SA. It was observed that the EMS induced slightly more mitotic chromosomal aberrations than the SA, in both the varieties of the winged bean.

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