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AS Sonavane
Department of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar, Baramati, Dist. Pune, Maharashtra, India

Correspondence
AS Sonavane
Department of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar, Baramati, Dist. Pune, Maharashtra, India

Effect of EMS and SA on meiotic aberrations induced in m_1 generation of winged bean

AS Sonavane

Abstract
In the present work the seeds of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) of variety II-EC-178313 and 2I-EC-38825 were treated with two chemical mutagens namely Ethyl methanesulfonate (EMS) and Sodium azide (SA) to induce mutations. These treated seeds were sown in field to raise M_1 generation for study of different parameters along with meiotic aberrations/anomalies. The meiotic metaphase I, anaphase I and telophase II were scored for determining the frequency of meiotic chromosomal aberrations. Various types of aberrations/anomalies such as precocious movement, stickiness, non disjunction, bridges, laggards and multiple groupings could be scored in the pollen mother cells.

Keywords: Mutagens, aberrations, EMS, SA, non disjunction, stickiness

1. Introduction
Psophocarpus tetragonolobus (L.) DC. belongs to family Fabaceae, popularly known as winged bean, Goa bean and Asparagus pea. It is a tropical, climbing and perennial legume plant native to New Guinea. It grows abundantly in hot, humid equatorial countries, from the Philippines and Indonesia to India, Burma, Thailand and Sri Lanka. It is now being grown in more than 70 countries. Is has been described as wonder legume in the sense that virtually all parts of this plant are edible and immensely nutritious. The various parts like leaves, flowers, tuberous roots and seeds were fit for human consumption. These parts are rich sources of the protein, vitamin, minerals and calories so often in short supply in tropical countries. It is an especially good source of vitamin A, deficiencies of which cause blindness in many children in tropical countries. 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) [6]. Studies have shown that like many other legumes, when combined with corn it has the protein value of milk and can adequately nourish a protein starved infant.
Despite outstanding nutritional potential, the winged bean has failed to gain popularity and acceptance among farmers. This has happened due to some undesirable features possessed by the plant. These features are- labour intensive nature of crop production, the lack of sufficient field trials for varietal selection, absence of market demands, photosensitivity and relatively long duration of the crop and the presence of some antinutritional factors in various plant organs.
Keeping this in view, a mutation breeding programme was initiated at our end for the improvement of such a highly versatile and multipurpose crop for achieving its genetic improvement and creating of new variability through the established method of induced mutation in case of winged bean.

Materials and Methods
The seed material of winged bean (*Psophocarpus tetragonolobus* (L.) DC. variety 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.

Mutagens Used
The chemical mutagens like 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 investigation.

Details of Mutagenic Treatments

The pilot experiments were conducted for determining the suitable concentration 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 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.

Meiotic aberrations were studied in squash preparations of pollen mother cells. Buds were collected in morning from plants growing in experimental field (M_1 generation). Buds were fixed in 1:1:1 proportion of acetic acid, chloroform and absolute alcohol. Anthers were dissected and squashed in drop of acetocarmine and the different meiotic chromosomal abnormalities were scored.

Results and Discussion

The meiotic metaphase I, anaphase I, and telophase II were scored for determining the frequency of meiotic chromosomal aberrations/anomalies. Various types of anomalies such as precocious movement, stickiness, non-disjunction, bridges, laggards and multiple grouping could be scored in the pollen mother cells. The frequency of meiotic aberration carrying cells enhanced linearly with an increase in concentration of EMS and SA in both II-EC-178313 and 2I-EC-38825 varieties of winged bean. The highest percentage of abnormal cells (5.76% and 5.99%) could be noted at 0.15% concentration of EMS in variety II-EC-178313 and 2I-EC-38825 of winged bean respectively.

The frequency of meiotic aberration carrying cells ranged from 4.02% to 5.76% 3.91% to 5.99% after treatments in variety II-EC-178313 and 2I-EC-38825 of winged bean, while after SA treatments the values varied from 3.90% to 5.55% and 3.57% to 4.87% respectively.(Table-1& 2)

The frequency of metaphase I aberrations was found to be more than anaphase I and telophase II at majority of the mutagenic treatments in both the varieties of winged bean.

In the present investigation both the mutagen (EMS and SA) have succeeded in inducing the various types of meiotic abnormalities in both the varieties of winged bean. As the concentration of mutagen is increased, there is a enhancement in percentage of aberration carrying cells. Earlier similar results were reported by Sree Ramlu (1971) [9], Nerkar (1977) [7], Grower and Tyagi (1980) [3], Panda (1983) [8] and Sharma and Singh (1990) [11] after mutagen/pesticide treatments.

Stickiness could be observed in both the varieties of winged bean at all the treatments. The stickiness could have resulted due to the depolymerisation of nucleic acids Kawai (1969) [4] and Amer and Ali (1961) [1]. After irradiation treatment the better sensitivity was found in pollen mother cells than the somatic cells Swanson (1957) [10].

In the present study the formation of groups due to chromosomal stickiness has been observed. At metaphase the association of chromosomes constituting multiple structures may have been to translocations of haphazard origin. Shaikh and Godward (1972) [12] in *Lathyrus sativus* and *Vicia ervilia* and Sinha and Godward (1972) [12] in *Lens culinaris* obtained similar results.

In the present investigation the single and double bridges at anaphase I could be observed. This may be due to the inversion of various types Levis and John (1966) [5]. According to Gaul (1964) [2] a bridge could appear due to fusion of two centric fragments.

Bridges were reported in number of plants after treatment of several mutagens like EMS, gamma rays and NEU by Sax (1960) [14], Saylor and Smith (1966) [15] and Sudhakaran (1971) [16].

Conclusion

Different types of meiotic aberrations could be observed after the mutagenic treatments in the present study. They comprised mis-orientation, precocious movement, stickiness, laggards, bridges and multiple groupings. Such aberrations were noticeable at metaphase, anaphase and telophase stages of the cell cycle. It was observed that EMS induced slightly more meiotic chromosomal aberrations than SA, in both the varieties of the winged bean.

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

Variety	Concentration	% Anomalies at Metaphase I		% Anomalies at Anaphase I			% Anomalies at Telophase II	Total percentage of anomalies
		Stickiness	Precocious movement	Non disjunction	Laggards	Bridges	Multiple grouping	
II-EC-178313	Control	-	-	-	-	-	-	-
	0.05 %	0.84	1.22	-	0.37	0.62	0.97	4.02
	0.10%	0.74	1.65	0.52	0.13	0.40	1.39	4.83
	0.15%	1.27	1.67	0.43	0.27	0.58	1.54	5.76
2I-EC-38825	Control	-	-	-	-	-	-	-
	0.05 %	1.17	1.40	0.27	-	0.28	0.79	3.91
	0.10%	0.87	1.22	0.26	0.48	-	1.39	4.21
	0.15%	0.94	1.87	0.40	0.68	0.39	1.71	5.99

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

Variety	Concentration	% Anomalies at Metaphase I		% Anomalies at Anaphase I			% Anomalies at Telophase II	Total percentage of anomalies
		Stickiness	Precocious movement	Non disjunction	Laggards	Bridges	Multiple grouping	
II-EC-178313	Control	-	-	-	-	-	-	-
	0.01 %	0.86	1.07	0.36	0.26	-	1.35	3.90
	0.02%	1.03	1.56	-	0.68	0.14	1.17	4.60
	0.03%	1.28	1.42	0.27	0.52	0.38	1.68	5.55
2I-EC-38825	Control	-	-	-	-	-	-	-
	0.01 %	1.37	1.11	-	-	0.24	0.85	3.57
	0.02%	1.28	1.02	-	0.25	0.40	1.41	4.36
	0.03%	1.08	1.53	0.45	0.60	0.25	0.96	4.87

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