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ORIGINAL ARTICLE

Induction of Quantitative Variability Through EMS and SA Treatment in Winged Bean [P*sophocarpus tetragonolobus* (L.) DC.]

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ABSTRACT
Winged bean botanically described as Psophocarpus tetragonolobus (L.) DC. belongs to fabaceae family. Winged bean is a multipurpose underutilized legume. The present study was conducted to find out the effect of Ethyl Methane Sulphonate (EMS) and Sodium Azide (SA) in M_2 and M_3 generations on different quantitative parameters of winged bean. The parameters studied days of flowering, days to first pod bearing, days to maturity and yield per plant. Both positive and negative shifts in mean values of quantitative parameters were recorded as a result of EMS and SA treatments. Winged bean being a self-pollinated protein rich crop has very limited genetic variability therefore induced mutation can provide an additional source of mutation in recent plant breeding programme. Hence an experiment was conducted to evaluate the extent genetic variability in quantitative characters in M_2 and M_3 generation following mutagenesis with EMS and SA. By inducing mutation in winged bean, it may be possible to identify new beneficial traits for higher yield. Thus aim of the present investigation is to identify and select mutants with useful morphological attributes.
Key words: *EMS, SA, Winged bean, Quantitative parameters, M_2 and M_3 generations, Mutations.*

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INTRODUCTION

Legumes constitute an economically important group of plants which stand in next in importance to cereals. Since the legumes produce more proteins in their seeds than the cereals, they occupy prime importance in any strategy meant for direct use of plant proteins. The proteins play an important role in human diet. The deficiency of dietary proteins along with other growth factors usually leads to symptoms like growth retardation, bone malformation, degeneration of reproductive organs and loss of immune response to infection. Nutritionists have specified the recommended daily intake of proteins for better health. But unfortunately many people suffer from either undernutrition, malnutrition or both in poor/developing countries mainly due to the shortage of proteins. To overcome these severe problems, winged bean (*Psophocarpus tetragonolobus* (L.) DC.) offers exceptional promise as a high protein food source for developing world. Winged bean is a tropical, climbing, dicotyledonous and perennial legume plant native to New Guinea. It grows abundantly in hot, humid, equatorial countries of Southern Asia, Melanesia and the pacific area [1]. It can be consumed as young pods, flowers, leaves, green seeds, dried seeds and also tuberous roots, are all edible and highly nutritious [2, 3]. It has been described as wonder legume in the sense that virtually all parts of this plant are edible and immensely nutritious. Winged bean could be regarded as an ideal for the people of poor and developing countries as it contains high quality protein and a reasonable quantity of oil as a source of energy. The winged bean seeds 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 %) [4]. Though winged bean is considered as a miracle bean for its high yield potential and nutritional value when compared to soybean [5]. It has been neglected all over the world

due to some undesirable features possessed by the plant. The main negative attributes observed in winged bean are the presence of antinutritional factors, long duration, climbing nature of the plant and absence of market demands. To overcome these negative attributes, induced mutations are the best techniques to induce variability in quantitative traits.
Recently induced mutagenesis has been widely employed to create desired genetic variability in crop improvement [6]. The mutagens may cause genetic changes in an organism break the linkage and produce many new promising traits for improvement of crop plants [7]. Chemomutagens include a broad variation of morphological and yield structure parameters in comparison to normal plants [8]. Mutation breeding technique has been also successfully employed in creating genetic variability for desirable traits and used these variants for breeding several crops including cereals, pulses and oil seeds [9, 10, 11]. Thus the present study was conducted to investigate the mutagenic effect of EMS and SA as means of increasing the variability in quantitative parameters within the cultivars and hence improve its nutritional value and productivity through selection.

MATERIAL AND METHODS

For the present mutation breeding research, the seeds of two cultivars (varieties) such as II-EC-178313 and 2I-EC-38825 of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) were used for induction of mutations. The seeds were obtained from the National Bureau of Plant Genetic Resources (NBPGR), Regional Station, PKV, Akola (M.S.) India.

MUTAGENS USED

The following two potent chemical mutagens were used for the present research work-

ETHYL METAHNE SULPHONATE (EMS)

Ethyl Methane Sulphonate ($CH_3SO_2OC2H_5$) a monofunctional alkylating agent with molecular weight of 124 manufactured by Sigma Chemical Company Ltd., USA.

SODIUM AZIDE (SA)

Sodium Azide (NaN_3) having molecular weight of 65.01 obtained from Sigma Chemical Company Ltd., USA.

DETAILS OF MUTAGENIC TREATMENTS

Healthy, dry, mature and uniform seeds of two winged bean cultivars (varieties) such as II-EC-178313 and 2I-EC-38825 were selected for the mutagenic treatments. These selected seeds were presoaked in distilled water for 6 hours. Such presoaked seeds were treated with different concentrations of freshly prepared EMS solution (such as 0.05 %, 0.10 % and 0.15 %) and SA solution (such as 0.01 %, 0.02 % and 0.03 %) for 6 hours. Seeds soaked in distilled water for 12 hours served as control (untreated seeds). After the completion of 6 hours treatment with EMS and SA mutagens, the seeds were washed thoroughly under running tap water. Later on they were kept for post soaking in distilled water for 2 hours. 300 seeds of each treatment were sown in the field following randomized block design (RBD) with three replications along with control for raising the M_1 generation. Various morphological parameters were studied from M_1 generation. After maturity, M_1 plants (seeds) were harvested separately on the plant basis. The separately collected seeds were sown as to raise M_2 generation. The seeds of M_2 were collected and sown to raise M_3 generation. The M_2 and M_3 populations were screened for quantitative traits to study the induced variability. From each treatment 25 plants were randomly selected for recording data on different quantitative parameters in both the M_2 and M_3 generations. Similarly 25 plants were picked up from the control for comparative assessment.

RESULTS AND DISCUSION

In the present investigation, an attempt was made to estimate the induced variability with reference to quantitative parameters in M_2 and M_3 generations. Induced variability was thoroughly studied in winged bean cultivars II-EC-178313 and 2I-EC-38825.for both the M_2 and M_3 generations in regard to days to flowering, days to first pod bearing, days to maturity and yield per plant. A thorough statistical analysis of the data on individual quantitative characters was done to analyze the effect of mutations in shifting the mean and variance in either direction.

DAYS TO FLOWERING: (Table 1, 2, 3 & 4)

Data on this quantitative parameter was recorded by considering the number of days taken by the plant from the date of sowing to the opening of first flower bud on the plant. From the present investigation, it is observed that as compared with control majority of the treated plants showed a general tendency towards early flowering. This feature was quite evident at all the EMS/SA treatments in both, II-EC-178313 and 2I-EC-38825 varieties of winged bean. The values for days to flowering in control ranged from 105.80 to 105.00 in II-EC-178313 and 102.10 to 102.30 in 2I-EC-38825. A negative shift in mean values was observable at majority of EMS/SA treatments in both the varieties of winged bean. In case of variety II-EC-178313, EMS (0.15 %) and SA (0.02 % and 0.03 %) treatments revealed significant negative shift in mean values in M_2 generation while in case of variety 2I-EC-38825, EMS (0.10 % and SA (0.02 % and 0.03 %) treatments revealed significant negative shift in mean values in M_2 generation. In M_3 generation, this feature was indicated by the 0.15 % EMS and 0.01 % and 0.02 % SA in variety II-EC-178313, while by all the treatments of EMS/SA IN variety 2I-EC-38825. Earlier similar results were reported by [12] in wheat, [13] in mungbean, [14] in soybean and [15] in field pea.

DAYS TO FIRST POD BEARING: **(Table 1, 2, 3 & 4)**

The data on this quantitative parameter was recorded by considering the number of days taken by the plant for the development of first green pod on the plant. As regards the parameter of days to first pod bearing in M_2 and M_3 generations, all the concentrations of EMS/SA showed decreased values. The maximally significant values were found at 0.10 % and 0.15 % of EMS and at 0.02 % and 0.03 % of SA treatments in variety II-EC-178313, while in variety 2I-EC-38825 these values were significant at 0.10 % and 0.15 % concentrations of EMS and 0.02 % and 0.03 % concentrations of SA. Thus a negative shift in values could be recorded at all concentrations of SA and EMS in M_2 and M_3 generation of both the experimental materials. Earlier similar type of results were recorded by [16] in pea, [17] in rice, [18] in *Arabidopsis* and [19] in lentil.

Table – 1: Effect of Ethyl Methane Sulphonate (EMS) on some quantitative parameters of winged bean in M_2 generation.

Variety	EMS Concentration %	Days to flowering		Days to first pod bearing		Days to maturity		Yield per plant	
		Mean	Shift in mean	Mean	Shift in mean	Mean	Shift in mean	Mean	Shift in mean
II-EC-178313	Control	105.80		113.50		142.20		139.20	
	0.05	104.00	-1.8	112.66	0.84	139.85	-2.35	134.22	-4.98
	0.10	103.33	-2.47	111.33	-2.17	144.66	2.46	133.18	-6.02
	0.15	102.33	-3.47	112.29	-1.21	139.00	-3.20	131.35	-7.85
	S.E. (Mean)	0.37		0.46		0.92		0.37	
	F(Replication)	0.67		0.28		1.16		1.98	
	F(Treatment)	1.10		0.11		1.44		10.83	
	C.D. at 1 %	1.71		2.15		4.26		1.73	
	C.D. at 5 %	1.03		1.30		2.57		1.04	
2I-EC-38825	Control	102.10		110.10		138.80		142.20	
	0.05	101.66	-0.44	110.00	-0.10	132.33	-6.47	142.26	0.06
	0.10	100.07	-2.03	108.00	-2.10	131.00	-4.80	144.26	2.24
	0.15	101.33	-0.77	107.66	-2.44	127.21	-11.59	143.42	1.22
	S.E. (Mean)	0.17		0.22		1.40		0.27	
	F(Replication)	2.96		4.30		0.13		2.04	
	F(Treatment)	2.09		12.70		0.47		5.29	
	C.D. at 1 %	0.81		1.02		6.46		1.27	
	C.D. at 5 %	0.49		0.61		3.90		0.77	

DAYS TO MATURITY: **(Table 1, 2, 3 & 4)**

Data on the days to maturity was recorded by considering the number of days taken by the plant for the development of the first fully matured pod on the plant. The mean value in regard to days to maturity demonstrated a shift towards negative direction at majority of the treatments in M_2 of II-EC-178313 and in M_3 generation of both the varieties of winged bean. In case of EMS treatment, variety II-EC-178313 showed a significant negative shift in mean values except for the 0.10 % EMS concentration in M_2 generation. As regards the SA treatments, the M_2 and M_3 generations of both II-EC-178313 and 2I-EC-38825 varieties demonstrated a significant negative shift in mean values. The variety II-EC-178313 and 2I-EC-38825 in M_3 generation revealed a negative shift in all the treatments, indicating earliness in maturity (4-12 days) than the control. Earlier similar results were obtained by [20] and [21] in mungbean.

YIELD PER PLANT: **(Table 1, 2, 3 & 4)**

Regarding to this parameter, the total seed yield per plant was recorded. In the present studies, reduced mean values could be evidently seen at most of the treatments of EMS and SA in M_2 and M_3 generations of variety II-EC-178313 and variety 2I-EC-38825. Both the varieties of winged bean demonstrated a shift in mean values in positive as well as negative directions in M_2 and M_3 generations. In M_2 generation, the variety II-EC-178313 demonstrated a significantly negative shift in mean values at all the concentrations of EMS and SA, while the variety 2I-EC-38825 demonstrated a significantly positive shift in mean values except at 0.01 % and 0.02 % concentration of SA. In the EMS/SA treatments, the shift in mean was exclusively in negative direction in the variety II-EC-178313 in M_3 generation. The parameter of seed yield per plant exhibited a significant positive shift in mean values at 0.05 %, 0.10 % of EMS and 0.03 % of SA in M_3 generation of variety 2I-EC-38825. The earlier researchers such as [22 & 23] have observed similar type of results in urdbean.

Table – 2: Effect of Ethyl Methane Sulphonate (EMS) on some quantitative parameters of winged bean in M_3 generation.

Variety	EMS Concentration %	Days to flowering		Days to first pod bearing		Days to maturity		Yield per plant	
		Mean	Shift in mean	Mean	Shift in mean	Mean	Shift in mean	Mean	Shift in mean
II-EC-178313	Control	105.00		114.00		145.60		139.70	
	0.05	104.52	-0.48	112.00	-2.00	143.66	-1.94	134.83	-4.87
	0.10	103.81	-1.19	110.66	-3.34	140.48	-5.12	133.30	-6.40
	0.15	102.12	-2.88	111.66	-2.34	137.55	-8.05	127.00	-12.70
	S.E. (Mean)	0.41		0.42		0.29		0.92	
	F(Replication)	0.37		0.35		14.33		1.14	
	F(Treatment)	1.19		3.75		24.66		2.78	
	C.D. at 1 %	1.89		1.96		1.35		2.58	
	C.D. at 5 %	1.14		1.18		0.80		4.27	
2I-EC-38825	Control	102.30		113.30		139.90		142.00	
	0.05	97.42	-4.88	104.00	-9.30	129.91	-9.99	143.00	1.00
	0.10	96.74	-5.56	107.00	-6.30	132.12	-7.78	144.33	2.33
	0.15	95.66	-6.64	106.33	-6.97	129.18	-10.72	141.66	-0.34
	S.E. (Mean)	0.82		0.73		1.73		0.86	
	F(Replication)	0.15		0.61		0.10		0.31	
	F(Treatment)	1.16		3.31		0.21		4.51	
	C.D. at 1 %	3.79		3.37		7.96		3.97	
	C.D. at 5 %	2.29		2.03		4.80		2.40	

Table – 3: Effect of Sodium Azide (SA) on some quantitative parameters of winged bean in M_2 generation.

Variety	SA Concentration %	Days to flowering		Days to first pod bearing		Days to maturity		Yield per plant	
		Mean	Shift in mean	Mean	Shift in mean	Mean	Shift in mean	Mean	Shift in mean
II-EC-178313	Control	105.80		113.50		142.20		139.20	
	0.01	104.33	-1.47	113.33	-0.17	141.00	-1.20	134.15	-5.04
	0.02	100.33	-5.47	109.00	-4.50	138.00	-4.20	136.04	-3.16
	0.03	99.66	-6.14	107.85	-5.65	137.03	-5.17	132.33	-6.87
	S.E. (Mean)	0.34		0.09		0.12		0.38	
	F(Replication)	2.10		22.60		35.45		0.80	
	F(Treatment)	7.29		24.36		91.00		3.05	
	C.D. at 1 %	1.57		0.43		0.58		1.78	
	C.D. at 5 %	0.94		0.26		0.35		1.07	
2I-EC-38825	Control	102.10		110.10		138.80		142.20	
	0.01	98.66	-3.44	107.33	-2.77	137.00	-1.80	139.66	-2.54
	0.02	97.88	-4.22	106.66	-3.44	134.67	-4.13	136.52	-5.68
	0.03	96.66	-5.44	110.41	-0.31	135.99	-2.81	142.48	0.28
	S.E. (Mean)	0.34		0.17		0.40		0.45	
	F(Replication)	1.11		16.26		1.13		5.69	
	F(Treatment)	6.36		23.56		18.36		20.91	
	C.D. at 1 %	1.59		0.82		1.84		2.10	
	C.D. at 5 %	0.96		0.49		1.11		1.26	

Table – 4: Effect of Sodium Azide (SA) on some quantitative parameters of winged bean in M_3 generation.

Variety	SA Concentration %	Days to flowering		Days to first pod bearing		Days to maturity		Yield per plant	
		Mean	Shift in mean	Mean	Shift in mean	Mean	Shift in mean	Mean	Shift in mean
II-EC-178313	Control	105.00		114.00		145.60		139.70	
	0.01	102.00	-3.00	109.66	-4.34	137.32	-8.28	132.88	-6.82
	0.02	103.00	-2.00	111.00	-3.00	139.18	-6.42	134.21	-5.49
	0.03	103.33	-1.67	111.33	-2.67	135.52	-10.08	130.77	-8.93
	S.E. (Mean)	0.44		0.12		0.42		0.63	
	F(Replication)	32.00		7.00		2.43		1.01	
	F(Treatment)	1.22		39.00		15.35		16.12	
	C.D. at 1 %	2.04		0.55		1.97		2.89	
	C.D. at 5 %	1..23		0.33		1.19		1.75	
2I-EC-38825	Control	102.30		113.30		139.90		142.00	
	0.01	97.00	-5.30	104.66	-8.64	126.10	-13.80	139.81	-2.19
	0.02	98.21	-4.09	108.66	-4.64	133.82	-6.08	136.41	-5.59
	0.03	99.66	-2.64	109.07	-4.23	136.61	-3.29	143.73	1.73
	S.E. (Mean)	0.48		0.46		1.43		0.85	
	F(Replication)	0.61		2.14		0.01		5.56	
	F(Treatment)	2.06		3.62		1.91		2.47	
	C.D. at 1 %	2.21		2.14		6.60		3.91	
	C.D. at 5 %	1.33		1.29		3.98		2.36	

CONCLUSION

From the present investigation, it could be inferred that the mutagens EMS and SA used in the present study although acted differentially have definitely proved successful in broadening the genetic base to an appreciable extent. This can very much create additional opportunities for making effective selection and for incorporating the useful variability in conventional breeding programme of winged bean for developing its recombinant types.

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