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Studies on genetic variability induced by chemical mutagens on seed characters in winged bean [*Psophocarpus tetragonolobus* (L) DC]

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Abstract
In the present investigation two cultivars/varieties of winged bean (*Psophocarpus tetragonolobus* (L.) DC. namely II-EC-178313 and 2I-EC-38825 were used for mutagenic treatment of Ethyl methane sulfonate (EMS) and Sodium Azide (SA) to study the effect on hundred seed weight, seed coat colour and development of anthocyanin pigment on stem (anthostem mutant) characters. In both the cultivars/varieties of winged bean in M_2 and M_3 generation a mixed trend of positive and negative shift in mean values was observed in all the mutagenic treatments for the number of pods per plant and hundred seed weight. In the present study the anthostem mutant as well as the mutants which shows variation in seed coat colour were observed in both the cultivars/varieties of winged bean. The heritability assessed for days to first pod bearing, number of pods, hundred seed weight and seed yield per plant in M_2 and M_3 generation in both the cultivars/varieties of winged bean. The high estimates of heritability in yield and yield characters have been found to be useful from plant breeders view point as this would enable him to base his selection on the phenotypic performance.

Keywords: winged bean, EMS, SA, genetic variability, anthostem mutant

1. Introduction
Legumes are an essential source of oils and proteins [1]. Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is primarily considered as underutilized, neglected orphan crop though it is known for its high yield potential and nutritional value when compared to soybean [2]. Winged bean is a dicotyledonous legume plant belongs to fabaceae family. It has a diploid genome (2n=2x= 18) consisting of a karyotype with three pairs of short and six pairs of long chromosomes [3]. It is perennial/annual, twining, glabrous, herbaceous plant. The leaves are pinnately trifoliate, petiolate, long deeply grooved at the upper surface with large pulvinus at base, stipules are two parted, small lanceolate inserted above base which are small and sessile. The pods of winged bean are thick, which are square or rectangular in cross section, with four elongated wings running the entire length of the pods. Presence of wings is the characteristic feature of winged bean, hence it is popularly known as winged bean [4]. The individual pods contains 5 to 20 seeds which vary in colour from white, brown to black depending on genotype [5]. It is considered to have a cleistogamous floral system which would usually imply autogamy, with self-pollination having been observed to take place before the large flowers open in the morning hours [6, 7]. From the previous literature it is quite clear that, seed shape, size and variation in seed coat colour seems to be under the control of poly gens. Disruption of any one of the gene might manifest in the form of seed mutations. The observed mutations in seed coat colour, size of pod and seed in the winged bean might be due to disruption of one or few genes controlling these characters. Among the entire mutants high yielding mutant, dwarf mutant, early maturing mutant, long pod mutant, white/dark brown seeded and bold seed mutant exhibits to be the most efficient one for further improvement of the winged bean cultivars and there is scope for utilizing these characters for developing improved varieties of winged bean. It possess immense potential because it can be consumed as tender leaves, flowers, pods green seeds, dried seeds and also tuberous roots are all edible and nutritious[8,9]. It is found to be a potential weapon against food shortage, malnutrition and protein deficiency problems occurs throughout the world. However it possess several positive attributes, but it is not popular among the farmers/growers due to certain negative aspects such as long duration of crop, labour intensive nature of crop and presence of antinutritional factors. To overcome these negative aspects mutation breeding in general is relatively earlier method for crop improvement. It is quite clear that, mutagenic agents can create a wide spectrum of genetic variation. Mostly pulse crops generally lack genetic variation due to their highly autogamous nature. The technique of mutation breeding can be used to create genetic variation.

Material and Methods
The experimental seed material of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) namely II-EC-178313 and 2I-EC-38825 procured from the National Bureau of Plant Genetic Resources, Regional Station, PKV, Akola (M.S.) India were used in the present study. For this experiment two standard chemical mutagens such as Ethyl Methane Sulfonate (EMS) and Sodium Azide (SA) were used. 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 the mutagenic solution for 6 hours with an intermittent shaking. The volume of mutagenic solution used was three times as that of seeds so as to facilitate uniform conditions. 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. The 300 seeds from each treatment were 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. Seeds of each 25 normal looking plants of M_1 were selected and collected on individual plant basis from all the treatments and control. They were used for raising the M_2 generation on the plant to row basis. The study of M2 and M_3 generation comprised of an analysis of the different parameters. Randomly selected plants from each treatment along with control were studied thoroughly for different quantitative characters. The different quantitative traits were analyzed carefully. The weight of seeds were recorded in gm from random samples of hundred seeds from each plant. The present experiment was carried out during kharif season in the botanical garden at Dr. B.A.M.U. Aurangabad.

Results and Discussion

In the present investigation, the mean values showed shift in positive as well as negative directions for various quantitative characters in M2 and M_3 generations. The treatments of EMS/SA succeeded in inducing variability regarding hundred seed weight. The data revealed shift in mean values in negative and positive directions. The hundred seed weight in control of II-EC-178313 was in the range of 33.05 to 33.12 gm. While in 2I-EC-38825 it could be noted as ranging from 35.08 to 35.11 gm. In M_2 generation the maximum mean value for hundred seed weight namely 40.22 and 41.83 gm have been recorded at 0.10 % 0.15 % concentration of EMS in variety II-EC-178313 and 2I-EC-38825 of the winged bean respectively. In M_3 generation, the variety II-EC-178313 demonstrated significantly negative shift in mean values except at 0.05 % of EMS and 0.02 % of SA, while the variety 2I-EC-38825 showed a significantly positive shift in mean values except for the 0.05 % of EMS and 0.01 % of SA as regards the hundred seed weight. The parameter of hundred seed weight is a reliable source of measuring yielding ability in pulses. Similar observations were also made by [10, 11, 12, 13, 14] in winged bean, green gram, chickpea, lentil and urdbean respectively (Table 1, 2, 3 and 4). In the present work one more striking viable mutant such as anthostem was observed which shows the presence of anthocyanin pigment on their stem (purple coloured stem) in both the varieties of winged bean. These anthostem mutant shows variation in certain quantitative parameter such as flowering period, plant height and yield per plant as compared with control in both the varieties of winged bean in M2 and M_3 generations. During this research work the variations in the seed coat colour and seed size were also being observed. The seed coat colour is affected by genetic factors like pigmentation factor (P), pigment complementary factor and modifying factors. In both the varieties of winged bean the seed colour varies from white, brown to black, purple and mottled, light brown to dark brown, reddish brown and Brownish orange As compared with control. Variations in seed coat colour was reported by [15, 16, 17, 18, 19], in different plant systems including winged bean. Variation in seed size has been reported by [20, 21, 22, 19], in Lathyrus, pigeon pea, and in black gram respectively. Positive in mean seed yield per plant may be due to the selection of normal looking plants in M_2 which led to elimination of aberrant plants and also due to changes induced at genetic level. The selection process should be delayed until M_3 or later generations following mutagenic treatments. However the selection of progenies on the basis of desirable mean and greater variance in M_2 was found to be highly useful. Several researchers have also proposed that effective selection for quantitative characters can be done in early generations even in M_2 itself [23, 24, 25]. Variation in seed coat colours were induced due to various mutagenic agents were observed by various workers in pigeon pea. In green gram different seed variants such as bold seed, light black seed, dull green, shining green, light brown and yellow coloured mutants were reported due to induction with gamma rays by [26].

Table 1: The effect of EMS on hundred seed weight in M_2 generation of winged bean.

Variety	Concentration	Mean	Shift in mean
II-EC-178313	Control	33.05	
	0.05 %	32.89	- 0.16
	0.10 %	40.22	7.17
	0.15 %	32.31	- 0.74
	S.E. (Mean)	0.11	
	f (Replication)	3.21	
	f (Treatment)	20.80	
	C.D. at 1 %	0.51	
	C.D. at 5 %	0.30	
2I-EC-38825	Control	35.08	
	0.05 %	31.42	- 3.66
	0.10 %	35.57	0.49
	0.15 %	41.83	6.75
	S.E. (Mean)	0.51	
	f (Replication)	7.29	
	f (Treatment)	13.73	
	C.D. at 1 %	2.37	
	C.D. at 5 %	1.43	

Table 2: The effect of SA on hundred seed weight in M_2 generation of winged bean.

Variety	Concentration	Mean	Shift in mean
II-EC-178313	Control	33.05	
	0.01 %	31.22	- 1.83
	0.02 %	32.28	- 0.77
	0.03 %	31.38	- 1.67
	S.E. (Mean)	0.53	
	f (Replication)	0.15	
	f (Treatment)	0.25	
	C.D. at 1 %	2.47	
	C.D. at 5 %	1.49	
2I-EC-38825	Control	35.08	
	0.01 %	32.07	- 3.01
	0.02 %	35.53	0.45
	0.03 %	35.73	0.65
	S.E. (Mean)	0.45	
	f (Replication)	2.75	
	f (Treatment)	8.91	
	C.D. at 1 %	2.08	
	C.D. at 5 %	1.25	

Table 3: The effect of EMS on hundred seed weight in M_3 generation of winged bean.

Variety	Concentration	Mean	Shift in mean
II-EC-178313	Control	33.12	
	0.05 %	33.30	0.83
	0.10 %	31.60	- 1.52
	0.15 %	32.16	- 0.96
	S.E. (Mean)	0.18	
	f (Replication)	3.06	
	f (Treatment)	36.54	
	C.D. at 1 %	0.83	
	C.D. at 5 %	0.50	
2I-EC-38825	Control	35.11	
	0.05 %	33.13	- 1.98
	0.10 %	35.40	0.29
	0.15 %	41.33	6.22
	S.E. (Mean)	0.73	
	f (Replication)	0.83	
	f (Treatment)	4.47	
	C.D. at 1 %	3.36	
	C.D. at 5 %	2.03	

Table 4: The effect of SA on hundred seed weight in M_3 generation of winged bean.

Variety	Concentration	Mean	Shift in mean
II-EC-178313	Control	33.12	
	0.01 %	32.82	- 0.3
	0.02 %	32.20	- 0.08
	0.03 %	31.03	- 2.09
	S.E. (Mean)	0.38	
	f (Replication)	1.19	
	f (Treatment)	1.38	
	C.D. at 1 %	1.77	
	C.D. at 5 %	1.07	
2I-EC-38825	Control	35.11	
	0.01 %	32.63	- 2.48
	0.02 %	35.53	1.25
	0.03 %	35.80	0.69
	S.E. (Mean)	0.33	
	f (Replication)	3.69	
	f (Treatment)	4.79	
	C.D. at 1 %	1.54	
	C.D. at 5 %	0.93	

Conclusion

From the present experiment, it is clear that both 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 desirable recombinant types.

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