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Biotechnological Comparative Assessment of Mutagenic Agents on Morphological Traits and Phenology in Wild Chickpea

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Abstract: ***The chickpea is the third important legume crop in the world. The wild species of the chickpea is a valuable source of genetic variation for cultigens breeding programme. The few undesirable traits and properties of the wild species constraint the use of the wild species in improvement breeding programme as well as the crossability barriers in interspecific crossbreeding. The mutation breeding is the useful method to bring the desirable traits in the genome and elimination of undesirable character. The suitable and desirable induced mutants could be used in the breeding programme of the cultigens.***

Keywords: Wild chickpea, Phenology, EMS, Gamma radiation, Mutagenesis

1. Introduction

Chickpea (*cicer arietium*) field pea (*pisum sativum*) lentil (*len culineris*) fababean (*vicia faba*) grasspea (*lathyrus sativus*) this three crop has been identified and categorized as cool season food legume[21]. Chickpea has been ranked as the third among the pulses and its production is worldwide with India as single largest producer [12]. The cropping pattern especially rotational with legume crop could offer a basis to break disease cycle thereby improving the soil fertility and weed control [11]. The available genetic variation in chickpea has largely been exploited in the conventional plant breeding approaches which minimized the genetic variation base for this crop [30]. Chickpea breeding programs have limited themselves to a small number of cultivated genotypes with sources of biotic stress resistance and abiotic stress tolerance with little or no use of wild species [25]. Mutation breeding could be used to induct and improve the economically important traits and characters as well as to eliminate the undesirable gene from the elites lines [18]. It is a useful method to extend the genetic variation spectrum of a species within a short time-span and has been reported a significant role in the development of many crop varieties [19] as well as to upgrade the well-adopted plant varieties by altering one or two major traits which enhance the quality [26]. Breeding value of mutants can be improved by uniting different mutant genes in the same genome [13]. The mutants with favorable characters or properties could be utilized into crossbreeding programme in order to transfer specific gene into the genome of well-established cultivar and to improve their breeding values. Mutagenesis was used to develop cultivars with good stability to exogenous factors and with increased productivity [20]. The success rate of crossing cultivated chickpea as the female parent with both *C. reticulatum* and *C. echinospermum* as male parents was more than 75% [24]. The mutagenesis could create many different mutants alleles with varied and different degree of great modification [7]. The EMS and gamma radiation have been reported the important agents employed to increase mutation frequency in plants [6]. The highest efficiency coefficient was obtained in the variant with 40Gy γ rays and 0.2% EMS which correlates with the highest frequency [4]. Wild germplasm contain valuable sources of novel genetic variation for improvement of cultigen traits [9] with respect to limited durability of resistances to many of the major pests and diseases, and limited progress in abiotic stress tolerance breeding. A few undesirable characters constraints the use of wild *Cicer* in chickpea breeding programs [14]. *C. echinospermum and C. reticulatum* are commonly used in chickpea improvement programs this has important ramifications for breeders [5].

2. Material and Method

The seeds of *Cicer reticulatum* of Accession Number ICC 17121 were procured from the ICRISAT, Patancheru, India as shown in Figure 1.The fifteen sets or group of the healthy seeds were treated independently and in combination with chemical and physical mutagenic agents viz. various concentration of EMS 0.1%, 0.2%, 0.3%, 0.4%, combined treatment 0.1% EMS +5KR, 0.2% EMS +10KR, 0.3% EMS +15KR, 0.4% EMS +20KR, various doses of radiation 5KR, 10KR, 15KR, 20KR, 25KR and 30KR and encoded as T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11}, T_{12}, T_{13}, T_{14} and T_{15} respectively while untreated formed T_1.

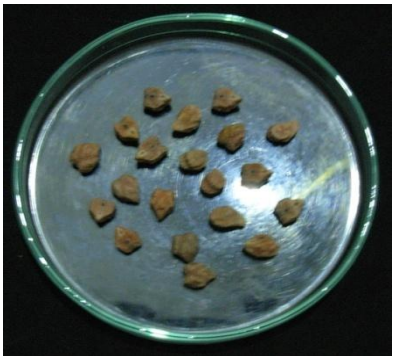

Figure 1: The Seeds of *Cicer reticulatum*

The sowing of pretreated *Cicer* seeds was done in first week of September. The cultivation period of *Cicer* in India has been reported from September to December [11]. The treated seeds alongwith the control were sown in the field following randomized block design (RBD) to raise M_1 generation in 3 replicates [8]. The seed-to-seed and row-to-row distance was maintained at 15 cm and 50 cm, respectively. Data for various phenological quantitative and qualitative traits were recorded at the interval of 20 days from the day of sowing. Data analyzed to deduce mean, standard error (SE), standard deviation (SD) and coefficient of variability (CV) using standard statistical procedure and ANOVA [27].

3. Result and Discussion

The independent and combined mutagenic effect on stem length and plant length of M_1 generation are represented in Table 1. The stem length and plant length were observed at regular interval of 20 days after sowing (DAS Days After Sowing). The maximum mean plant length 28.74 cm was observed in T_4 treatment and minimum 8.83 cm in T_{15} treatment of M_1 generation at 20 DAS, found to be significant at 0.05%.The mean maximum stem length 3.27 cm was observed in T_{14} and minimum 2.76 cm was observed in T_{15} treatment in M_1 generation at 40 DAS and was observed significant and depicted in Table 1.

Table 1: Effect of Mutagens on stem length and plant length in M_1 Generation

Sr. No	*Treatment*	*Mean stem length in cm 40DAS*	*Mean plant length in cm 20DAS*
1	T_1	2.92	18.54
2	T_2	3.11	24.04
3	T_3	3.09	23.08
4	T_4	3.24	28.74
5	T_5	3.24	21.60
6	T_6	3.03	18.27
7	T_7	3.07	19.46
8	T_8	3.2	21.11
9	T_9	3.05	19.0
10	T_{10}	2.89	12.07
11	T_{11}	2.96	17.23
12	T_{12}	3.06	19.28
13	T_{13}	3.14	16.9
14	T_{14}	3.27	15.26
15	T_{15}	2.76	8.83
	F-test	Significant	Significant
	SE(m±)	0.03	0.36
	CD at 5%	0.1	1.03

The effect of mutagen on the primary and secondary branching pattern was recorded at regular interval of 20 days and depicted in the Table 2 and Table 3 for M_1 generation. The delayed primary branching was observed in all the treatments over the control except in T_{11} treatment at 20 DAS. The maximum number of primary branches i. e. 5.06 in T_{11} treatment and minimum 1.53 in T_5 treatment was observed at 40 DAS, while maximum 6.8 in T_{13} treatment and minimum 3.06 in T_5 and T_6 treatment were observed at the interval of 60 DAS. The maximum 6.8 in T_{13} treatment and minimum 3.46 in T_1treatment were observed at the interval of 80 DAS. The length of primary branches showed variation at different stage of vegetative growth and development. The maximum length 29.76 cm in T_8 and minimum length 15.46 cm in T_{15} was observed at 40 DAS and at 60 DAS and 80 DAS, the maximum length 35.03 cm in T_{13} and minimum 23.93 cm, 24.1 cm in T_5 were observed, the data analyzed and depicted in Table 2.

The number and length of secondary branches revealed the variation and represented in Table 3 The maximum number of secondary branches 4.26 in T_{12} treatment and minimum 3.66 in control T_1 treatment were observed at 40 DAS, whereas maximum 6.93 in T_{13} treatment was recorded at 60 and 80 DAS. The minimum number of secondary branches was observed in T_6 and T_1 at 60 and 80 DAS. The maximum length of secondary branches 13.3 cm was observed in T_{11} and minimum 7.06 cm in T_1 at 40 DAS. The minimum length 4.83 and 7.0 cm in T_4 and maximum length 17.2 cm in T_{13} at 60 and 80 DAS respectively. The data are tabularized in Table 3 for M_1 generation.

The shoot length was decreased with the increase in the concentration of mutagenic treatment in all the treatments T_2 to T_{15}. The branching pattern has been reported unaltered in the guar [28] and also the height of treated plant was found to be increased. No variation was observed in size of leaves.

The plant height were significantly higher in T_2 to T_5 and T_8 in M_1 generation and maximum mean plant height 28.74 cm in T_4 of M_1. The maximum height induced in combination mutagenic treatments of EMS and gamma rays has been reported in chickpea [30]. The plant height significantly higher in 10KR, 15KR, 20KR and 0.5 % EMS in M_1 has been reported in grasspea [29]. No dose dependant relation however, Das and Prasad [10] reported to achieve dose dependant increased mean value from M_2 and M_3 using NMU as mutagen.

Kulshreshtha and Singh [17] reported the increased plant height at 10 KR, of gamma rays in green gram. The increase of branching resulted into an increase in the number of fruit in a mutant of *Brassica juncea* [22]. Whereas decrease in plant height was observed in 25 KR and those of 30 KR, which offer resistance against the lodging and has a considerable important agronomic trait to some extent. The reduction of the length in internodes may be due to the reduction of cell length or the reduction of cell number [31]. However, according to Arumugam et *al.* [3], the chromosomal damage might be the major factor in growth inhibition.

Similar findings that is the reduction in plant-height has been reported in *Solanum melanogena* (L.) treated with chemical mutagen [1], in brinjal treated by chemical mutagen [16], in Rhodes grass followed by gamma rays treatment [15], in Mungbean treated with the chemical mutagen MMS [2] in *Ammi majus* L. by EMS.

The number of primary branches was observed higher than control in M_1 and maximum mean 6.8 and 6.73 in T_{13} of M_1 and M_2 generation. The number of more primary branches per plants than control has been reported in 15KR, 20KR, 25KR treatment in grasspea [29].

Number of secondary branches were recorded at maximum in T_{13} treatment 6.93 in M_1 generation in the present investigation 80 DAS. An increase in plant height and number of primary branches per plants has been reported in chickpea followed by the treatment with EMS, gamma rays separately and both in combination [30].

The mutation inducing many traits could be attributed to the mutation of pleiotropic gene or mutation of gene cluster or chromosomal arrangement as has been reported in chickpea [30]. The observations in present investigation revealed the conformity as reported in chickpea [30].

4. Conclusion

The chickpea is cool season legume crop and improving the soil fertility. The genetic variability of the crop narrowed considerably and the mutation breeding could serve the basis for variation in the crop. The wild species of the chickpea is important owing to having the resistance to various biotic and abiotic stresses.
The useful and desirable morphological and reproductive traits and characters present in wild annual species of chickpea could be tapped and brought into the cultigens for the betterment and improvement of the cultivated chickpea. The wild chickpea could offer promising and prospective traits to the cultigens. The interspecific cross between the cultigens and wild could improve the quality of the cultigens however, there is crossability barrier and success is very low. The mutagenesis brings the variation in the wild species and such mutant might be appeared suitable for interspecific cross between cultigens and wild towards improvement of the cultivated chickpea.

The overall comparative study with respect to phenological parameter the T_{12} treatment appeared the fairly good treatment over all other treatments as it shows maximum qualitative traits and characteristics over all other treatments.

ANOVA for all the treatments were observed significant for all phenotypic characters ($p<0.05$). The treatment with desirable character could be used in breeding programme. Similarly, ANOVA for genotypes were significant for all the characters ($p<0.05$). The genotypes possessed desirable characters that could be directly produced after release and they could used indirectly in breeding programme. The comparative result on overall variability in M_1 was observed significant in present investigation.

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Table 2: Effect of Mutagens on number and length of primary branches in M_1 Generation

Sr No.	Treatment	Number of Primary Branches Mean				Length of PrimaryBranches Mean (In cm)			
		No. of Primary Branches 20DAS	No. of Primary Branches 40DAS	No. of Primary Branches 60DAS	No. of Primary Branches 80DAS	Length of Primary Branches 20DAS	Length of Primary Branches 40DAS	Length of Primary Branches 60DAS	Length of Primary Branches 80DAS
1	T_1	2.34	3.13	3.46	3.46	12.67	18.8	29.9	29.9
2	T_2	--	3.13	4.66	4.86	--	24.2	26.86	33.2
3	T_3	--	3.06	3.73	4.93	--	23.8	24.03	26.56
4	T_4	--	2.26	4.66	5.6	--	21.6	24.43	24.54
5	T_5	--	1.53	3.06	5.73	--	20.4	23.93	24.1
6	T_6	--	2.16	3.06	4.66	--	25.6	24.73	25.36
7	T_7	--	2.53	3.26	4.53	--	26.16	26.43	25.8
8	T_8	--	2.8	4.73	6.00	--	29.76	29.56	29.73
9	T_9	--	3.0	3.53	5.46	--	25.2	25.46	27.43
10	T_{10}	--	3.4	4.66	4.66	--	23.1	34.93	34.93
11	T_{11}	0.2	5.06	5.33	5.33	0.5	26.6	32.33	32.33
12	T_{12}	--	4.0	5.8	5.8	--	22.63	32.33	32.33
13	T_{13}	--	3.86	6.8	6.8	--	23.93	35.03	35.03
14	T_{14}	--	4.06	5.4	5.4	--	23.03	33.43	33.43
15	T_{15}	--	3.06	3.53	3.53	--	15.46	27.4	27.4
F-test		Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant
SE(m±)		0.01	0.18	0.06	0.25	0.06	0.62	0.42	0.49
CD at 5%		0.04	0.52	0.18	0.72	0.18	1.79	1.19	1.42

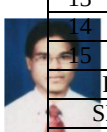

Table 3: Effect of Mutagens on number and length of secondary branches in M_1 Generation

Sr. No.	Treatment	Number of Secondary Branches Mean			Length of Secondary Branches Mean (In cm)		
		No of Secondary Branches 40DAS	No of Secondary Branches 60DAS	No of Secondary Branches 80DAS	Length of Secondary Branches 40DAS	Length of Secondary Branches 60DAS	Length of Secondary Branches 80DAS
1	T_1	3.66	4.33	4.33	7.06	11.7	11.7
2	T_2	--	3.8	5.66	--	7.26	9.63
3	T_3	--	2.2	6.4	--	5.36	8.26
4	T_4	--	1.86	4.8	--	4.83	7.0
5	T_5	--	2.06	4.6	--	6.46	7.06
6	T_6	--	1.80	4.66	--	5.30	9.33
7	T_7	--	2.80	5.06	--	6.23	9.03
8	T_8	--	3.06	5.73	--	9.03	14.33
9	T_9	--	2.73	4.46	--	6.53	8.8
10	T_{10}	3.86	4.6	4.6	10.86	15.23	15.23
11	T_{11}	3.86	4.73	4.73	13.3	16.93	16.93
12	T_{12}	4.26	5.33	5.33	9.13	15.86	15.86
13	T_{13}	4.0	6.93	6.93	10.06	17.2	17.2
14	T_{14}	4.0	5.46	5.46	9.83	17.0	17.0
15	T_{15}	--	5.00	5.00	--	15.6	15.6
F-test		Significant	Significant	Significant	Significant	Significant	Significant
SE(m±)		0.17	0.27	0.38	0.32	0.53	0.55
CD at 5%		0.49	0.78	1.1	0.93	1.52	1.59