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Analysis of Quantitative Traits in M2 Generation of Chick Pea (Cicer Arietinum L.) Seeds Under Induced EMS Mutagenesis

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***Abstract* – In the present investigation, two chickpea genotypes IC265291(V1) and IC265298 (V2) were collected from NBPGR, New Delhi to study the mutagenic effect of EMS (Ethyl Methyl Sulfonate) on the quantitative traits. The chickpea seeds were treated with different (0.1% to 0.6%) concentrations of chemical mutagen EMS and the seeds were sown in the field at CPMB (Centre for Plant Molecular Biology), Osmania University, Hyderabad. The ANOVA results revealed that the difference is highly significant due to the treatment of all the characters indicating that there is a considerable amount of genetic variability in the genotypes. In between the two genotypes (IC265291 & IC265298), the quantitative traits were found to be higher in the genotype IC265298 than IC265291 and it is also evident from the study that when the concentration of EMS increased, the mean values of all the quantitative traits decreased. It is observed that the seeds treated with 0.1% EMS and 0.2% EMS concentrations showed the highest mean values for quantitative characters under consideration. These mutant varieties can be further utilized in chickpea crop improvement programs.**

***Keywords* – Chickpea, Mutant Genotypes, EMS Mutagen, M2 Generation, Yield Parameters.**

I. INTRODUCTION

Chickpeas are an excellent source of functional food ingredients for high-end, health-conscious culinary and nutraceutical products [1]. In addition, chickpeas are a great source of minerals like zinc (Zn), iron (Fe), and selenium (Se). Simultaneously, it is the most important cool-season food legume grown in arid and semi-arid regions of the world under rainfed conditions [2]. The need for sustainably produced proteins is increasing because to the growing global population, which is driving a dietary shift toward plant-based proteins. Vegetable proteins are less biologically valuable and have a lower digestibility than their equivalents generated from animals. Crop improvement requires genetic variability, and it has been discovered that induced mutation is a highly successful method for generating genetic variability in both quantitative and qualitative traits [3]. Chickpeas can be genetically enhanced by a variety of crop improvement techniques, including hybridization, selection, mutation, and more. Chickpea (Cicer arietinum L.) has low genetic variety mainly because of its monophyletic descent from Cicer reticulatum, its wild ancestor [4]. Mutation has been regarded as a powerful tool for crop improvement and evolution among all the adopted breeding approaches [5]. As one of the most often used alkylating agents, ethyl methane sulfonate (EMS) can introduce an active alkyl group that causes base alterations and nucleotide mutations, hence causing chemical modification of nucleotides mutations. EMS mostly causes alterations in guanine residue [6]. Nucleotide substitutions are induced with high frequency in chemical mutagenesis; in EMS-mutated populations, GC to AT base pair transitions account for 70-99% of the alterations. The variations in guanine are mostly caused by the EMS [6]. Because the O6-exhalation pair is more stable than the thymine pair in comparison to cytosine, the O6-GC pair frequently transforms into an AT pair during replication. Numerous research on the genotoxic activity of EMS in vivo and in vitro show unequivocally

that EMS is a carcinogenic substance and should be used with extreme caution. Mutant populations have been created by the application of physical mutagens. On the other hand, greater DNA inversions and deletions caused by gamma radiation and rapid neutrons can make it more difficult to identify the genes responsible for a mutant phenotype. Alternatively, a chemical substance called EMS has been widely used to cause mutations in seeds. Random point mutations are frequently induced by EMS, and some of these mutations can result in the creation of unique stop codons in target genes [7]. With the world's population continuing to rise, hunger ghosts are haunting millions of individuals worldwide. In these cases, improving crop varieties with better-than-average proteins, minerals, and large yields is the first step in salvaging the situation [8]. When a good variety needs to be improved, mutation breeding is an additional benefit because it only needs to change one or two features [9]. The mutagen dose ought to be strong enough to enhance the likelihood of triggering a mutation, but not so high as to harm cells or tissues to the point of fatality. To get good results in seed treatment, the dose known as LD 50 is typically utilized, which is adequate to block roughly 50% germination. Irradiated populations are typically produced at a dose below LD 50 and with an LD 50 dose therapy [10]. To create the greatest number of viable mutants with the least amount of plant harm, the LD50 value for each mutagen must be determined [11]. One of the traditional breeding techniques used in plant breeding is mutation breeding. It is pertinent to a number of disciplines, including molecular biology, biotechnology, morphology, and cytogenetics. Chickpeas come in two different varieties: Kabuli (macrosperma) and Desi (microsperma). The two varieties can be identified by variations in the seed's size, shape, and color. Large, spherical seeds that are white or cream in color are typical of the Kabuli variety. The Desi variety often has angular, gritty seeds that range in hue from pale brown to black and all shades in between [12]. Ethyl Methane Sulphonate (EMS) is an alkylating chemical that induces high frequency of base pair substitutions and is a well-potential mutagen that is frequently used to produce genetic diversity [13] In Chickpeas, Seeds are the ediple part that can be eaten which is a great source of nutrients, carbs, and protein for vegetarians in particular [14].

II. Materials and Methods

Two chickpea varieties (IC265291 & IC265298) were obtained from NBPGR New Delhi. Seventy healthy and uniform seeds were selected and initially soaked in water for nearly 3-4 hours then cleaned with tissue paper and dried. Different concentrations of EMS mutagen are prepared to start from 0.1% to 0.6% as per mutagenesis protocol. Fifteen seeds each were soaked in each concentration of EMS mutagen for 6 hours in a rotary shaker at 180 rpm at 27±1°C of room temperature. For the effective and uniform absorption of EMS, the volume of EMS solution should be 10 times the proportion of seed volume. Untreated fifteen seeds were used as the control along with the JG11 genotype. The JG11, Untreated seeds and EMS-treated seeds were sown in the field in an RBD (Randomised Block Design) with three replications each. Quantitative characters under consideration were studied in the M1 generation. The genotypes showing the highest quantitative characters in the M1 generation were selected and sown to get the M2 generation. In M2 generation, different yield parameters were studied.

III. Results and Discussions

The ANOVA results revealed that the difference in the means of different parameters were highly significant due to the treatment of all the characters indicating there is ample genetic variability in the genotypes.

Table 1. Analysis of variance of chickpea mutant IC265291 under EMS mutagenesis for quantitative characters in M2 generation.

"Source of variations	DF	MSSQ										
		Characters										
		Pl.ht (cm)	P. Br/pl	Days for Ini Flower	Days 50% Flower	Days to P.M.	No. of P/pl	Pod wt/pl(g)	No. of S/pod	No. of S/pl	Seed wt (g/pl)	100 S. wt(g)
Replications		0.762	0.104	0.003	0.006	0.132	1.269	0.480	0.012	32.246	2.395	0.414
Treatments		60.010*	21.007*	24.447*	31.933*	48.540*	325.511*	120.736*	0.230*	1411.767*	67.881*	6.349*
Error		0.248	0.097	0.285	0.175	0.018	0.570	0.114	0.013	23.572	1.120	0.168
St E		0.287	0.180	0.308	0.241	0.245	0.436	0.195	0.067	2.803	0.611	0.237
SEd		0.406	0.255	0.436	0.341	0.346	0.616	0.275	0.095	3.964	0.864	0.335
CV(%)		1.604	3.182	1.443	1.017	0.489	2.424	2.015	7.625	10.103	10.039	2.024

Table 2. Analysis of variance of chickpea mutant IC265298 under EMS mutagenesis for quantitative characters in M2 generation.

"Source of variations	DF	MSSQ										
		Characters										
		Pl.ht (cm)	P. Br/pl	Days for Ini Flower	Days 50% Flower	Days to P. M.	No. of P/pl	Pod wt/pl(g)	No. of S/pod	No. of S/pl	Seed wt (g/pl)	100 S. wt(g)
Replications		0.674	0.027	1.220	0.644	0.808	0.687	0.122	0.007	83.097	2.199	0.043
Treatments		79.749*	20.694*	19.675*	36.915*	71.514*	449.586*	161.219*	0.244*	1749.834*	99.373*	9.610*
Error		0.293	0.058	0.360	0.266	0.276	1.019	0.174	0.030	32.146	1.482	0.052
St E		0.312	0.139	0.346	0.298	0.303	0.583	0.241	0.100	3.273	0.703	0.132
SEd		0.442	0.196	0.490	0.421	0.429	0.824	0.341	0.141	4.629	0.994	0.186
CV(%)		1.648	2.253	1.653	1.271	0.607	3.010	2.275	11.511	11.174	10.457	1.116

Table 3. Effects of EMS on quantitative characters in M2 generation of chickpea variety (V1)-IC265291.

V1	Pl. ht (cm)	P. Br/pl	Days F. Ini	Days 50% F	Days to P.M.	No. of P/pl	Pod wt/pl(g)	No. of Seeds/pod	No. of Seed/pl	Seed wt (g/pl)	100 Seed wt(g)
0.1% EMS	32.33	10.80	36.30	40.50	86.30	36.07	21.67	1.67	60.83	13.16	21.23
0.2% EMS	33.20	10.40	35.67	39.60	85.47	36.17	18.92	1.70	59.37	12.58	20.39
0.3% EMS	32.97	11.10	35.70	39.70	84.70	36.43	18.53	1.73	61.90	13.21	21.13
0.4% EMS	32.17	10.87	34.83	38.97	82.97	36.20	18.51	1.63	57.50	13.96	21.26
0.5% EMS	26.23	6.73	40.07	44.13	90.13	22.20	10.61	1.27	25.00	5.60	19.47
0.6% EMS	23.40	5.43	42.10	47.10	94.10	11.20	5.55	1.00	9.20	2.07	17.32

The quantitative traits observed for variety (V1) IC-265291 in M2 generation is as follows: The highest plant height is observed in the mutant IC-265291-M2 (0.2) which is 33.20cm and the lowest plant height is observed

for the plant IC-265291-M2 (0.6) which is 23.40cm. The number of branches per each plant is highest for the mutant IC-265291-M2 (0.3) and the mutant plant with least number of branches is IC-265291-M2 (0.6) with 5.43branches per plant. The duration (in days) taken for initial flowering is highest for IC-265291-M2 (0.6) which is 42.10days and least number of days taken for initial flowering is IC-265291-M2 (0.4) which is 34.83days. The highest number of days taken for 50% flowering is by the plant IC-265291-M2 (0.6) which is 47.10days and the least number of days taken for 50% flowering is IC-265291-M2 which is 38.97days. The highest number of days taken for pod maturity by IC-265291-M2 (0.6) is 94.10days and the mutant plant showing least number of days for pod maturity is IC-265291-M2 (0.4) and the number of days taken for 50% flowering is 82.97days. The number of pods per plant is highest for the mutant plant IC-265291-M2 (0.3) which is 36.43pods and the plant with least number of pods per plant is IC-265291-M2 (0.6) showing 11.20pods. The plant with the highest pod weight per plant is IC-265291-M2 (0.1) with 22.67grams per plant and the plant with least pod weight per plant is IC-265291-M2 (0.6) with 5.55grams of pod weight per plant. The number of seeds per pod is shown highest by the plant IC-265291-M2 (0.3) which is 1.73 seeds per pod and the least number of seeds per pod is IC-265291-M2 (0.6) with 1.00 seed per pod. The number of seeds per plant is observed to be highest for the plant IC-265291-M2 (0.3) which is 61.90 and the plant showing the least number of seeds per plant is IC-265291-M2 (0.6) with 9.20seeds per plant. The highest seed weight per plant is 13.96 grams shown by the mutant plant IC-265291-M2 (0.4) and then the mutant plant with the least seed weight per plant is IC-265291-M2 (0.6) showing 2.07 grams. The 100 seeds weight is highest for the mutant plant IC-265291-M2 (0.4) which is 21.26 grams and the mutant plant with the least value of 100 seeds weight is IC-265291-M2 (0.6) showing 17.32 grams.

Table 4. Effects of EMS on quantitative characters in M2 generation of chickpea variety (V2)-IC265298.

V2	Pl.ht (cm)	P. Br /pl	Days F.Ini	Days 50% F	Days to P.M.	No. of P/pl	Pod wt/pl(g)	No. of Seeds/pod	No.of Seed/pl	Seed wt (g/pl)	100 Seed wt(g)
0.1% EMS	35.93	12.27	35.17	38.23	83.40	39.17	23.14	1.63	62.00	13.26	21.14
0.2% EMS	36.53	12.23	34.77	38.27	83.27	40.43	22.47	1.63	62.00	12.97	21.14
0.3% EMS	35.87	11.87	35.00	38.33	83.33	41.03	21.24	1.70	67.80	17.02	21.68
0.4% EMS	33.90	11.23	35.13	38.93	82.93	39.60	20.73	1.73	66.80	16.54	21.66
0.5% EMS	26.27	7.33	37.03	42.03	90.03	23.67	11.63	1.20	24.93	5.60	19.79
0.6% EMS	24.60	6.53	41.87	47.87	95.87	9.67	4.69	1.00	7.67	1.76	16.66

The quantitative traits observed for variety (V2) IC-265298 in M2 generation is as follows: The plant height is observed to be highest in the mutant plant IC-265298-M2 (0.2) at 36.53 and the mutant plant showing the least height is IC-265298-M2 (0.6) with 24.60cm height. The number of branches per plant is shown highest in the mutant IC-265298-M2 (0.1) which is 12.27 and the mutant plant with the least number of branches IC-265298-M2 (0.6) by 6.58. The highest number of days taken for initial flowering is IC-265298-M2 (0.6) with 41.87 days least number of days taken by the mutant IC-265298-M2 (0.2) with 34.77 days for initial flowering. The highest number of days taken by the mutant for 50% flowering is IC-265298-M2 (0.6) with 47.87 days and the mutant taking the least number of days for initial flowering is IC-265298-M2 (0.1) which is 38.23 days. The highest number of days taken for pod maturity is 95.87 days followed by IC-265298-M2 (0.5) and the lowest number of days taken by the mutant plant IC-265298-M2 (0.2) with 83.27 days for pod maturity. The highest

number of pods per plant is for IC-265298-M2 (0.3) with 41.03 pods and the mutant with the least number of pods per plant is IC-265298-M2 (0.6) with 9.67 pods per plant. The weight of the pods per plant is highest for IC-265298-M2 (0.1) weighing 23.14 grams and the mutant showing the least pod weight per plant is IC-265298-M2 (0.6) showing 4.69 grams pod weight per plant. The number of seeds per pod is observed to be highest in the mutant IC-265298-M2 (0.4) at 1.73 seeds per pod and the least number of seeds per pod is IC-265298-M2 (0.6) showing 1.00 seeds per pod. The highest number of seeds per plant is shown by the mutant plant IC-265298-M2 (0.3) showing 67.80 seeds per plant and the least number of seeds per plant is shown by the mutant IC-265298-M2 (0.6) which is 7.67 seeds per plant. The highest value for seed weight per plant is observed for the mutant IC-265298-M2 (0.3) which is 17.02 grams and the least seed weight per plant is observed for the mutant IC-265298-M2 (0.6) which is 1.76 grams per plant. The 100 seed weight in grams is observed to be highest for the mutant IC-265298-M2 (0.3) which is 21.68 and the mutant IC-265298-M2 (0.6) showed 16.66 grams seed weight for 100 seeds.

IV. Conclusion

Seeds treated with 0.1% and 0.2% EMS concentrations have been shown the highest value for quantitative characteristics in M2 generation. These mutant chickpea genotypes can be used in future agricultural developmental programs.

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