



## Assessment of Yield Parameters in M3-Generation of Chickpea (*Cicer arietinum* L.) Treated with Different Concentrations of EMS

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### Abstract

The present study was conducted from February, 2023 to July, 2023 at Centre for Plant Molecular Biology, Osmania University, Hyderabad, Telangana (500007), India. Which involves two chickpea genotypes IC265291(V1) and IC265298 (V2) which were obtained from NBPGR, New Delhi. The aim of the study is effect of different concentrations of EMS (Ethyl Methyl Sulfonate) on the yield parameters of chickpea plants in M3 generation. Uniform and healthy seeds were selected and they were treated with various concentrations (0.1%, to 0.6%) of EMS. The EMS-treated seeds were sown in the field at CPMB (Centre for Plant Molecular Biology), Osmania University, Hyderabad. Various Quantitative traits in the M3 generation were studied. From the ANOVA results, it is evident that the difference in the mean values is highly significant due to the treatment of all the characters which indicates that there is an ample genetic variability in the genotypes. In between (IC265291 & IC265298) the genotypes in consideration, the yield parameters were shown higher by the genotype IC265298 than IC265291 in M3 generation and from the data it is also evident that, as the concentration of EMS is increased, the mean values of yield parameters increased initially but further increase in the EMS concentration leads to decrease in the mean values of quantitative characters. It has been observed that the 0.2% EMS-treated seeds and 0.3% EMS-treated seeds showed the highest mean values for considered quantitative traits in M3 generation. These identified mutants may be used further in crop improvement programmes of chickpeas.

**Keywords:** Chickpea, ethyl methane sulfonate, M3 generation yield parameters

### 1. Introduction

Pulses rank next to food grains and oilseeds in terms of acreage, productivity, and economic worth (Kumari et al., 2019). Chickpea seeds are among the most nutritionally balanced pulses for human consumption because they are high in fiber, protein, carbs, and minerals and low in fat (Kumar et al., 2023). The dry seeds of pulses often contain 20–25% protein, which is roughly 2.5–3.0 times the amount typically found in cereals (Umavathi and Mullainathan, 2018). The vegetarian population depends on pulses for their nutritional security because they are a rich source of protein (Rafiq Wani et al., 2014). It exhibits low genetic variation partly because it is a monophyletic descendant of its wild parent, *Cicer reticulatum* (Journal et al., 2019). It is a good source of vitamins A, C, E, K and Vitamins B1, B3, B6 and B9, Iron, Zinc, Magnesium and Calcium (Koul et al., 2022). Pulses play a crucial role in production systems that can withstand the impact of climate change (Sharma et al., 2020). EMS-induced

mutagenesis is an effective method to find undiscovered genes and characterize gene functions (Chen et al., 2022). One of the most frequently used chemical mutagens in plant is EMS, which has become effective, reliable, and powerful (Serrat et al., 2014). All of the essential amino acids are present in large amounts in chickpeas, making them a good source of protein (Patil et al., 2024). The garbanzo bean was domesticated by humans making it one of the earliest seed legumes to be cultivated in the world (Pipaliya et al., 2020.). Unfortunately, its low productivity renders it uncompetitive when compared to today's high-yielding cereal varieties (Ramesha et al., 2006). Plant breeding techniques to create new types of crops that are more variable and have better biochemical constitutions are needed (Sujatha et al., 2019). Chickpea is known by names like chana (Hindi), garbanzo (Spanish), pois chiche (French), kichar or chicher (German), and gram or Bengal gram in English (Zhang et al., 2024). Due to the self-pollinated nature of chickpeas, mutation



breeding is a useful technique for generating genetic variety in this crop (Dinkar et al., 2020). Chickpea shows numerous health benefits which earned it the title as a 'Functional Food' (Zhang et al., 2024). Chickpea seeds are packed with minerals, dietary fibers, and various vitamins including tocopherol, folic acid, riboflavin, pantothenic acid, pyridoxine, and carotenoids like  $\beta$ -carotene, lutein, cryptoxanthin, and zeaxanthin (Nisa et al., 2020). The growing countries have a predominantly vegetarian population that relies on it as a valuable source of protein and starch (Jain et al., 2013). Chickpea contains 18–22% protein, 52–70% carbohydrates, 4–10% fat, 1.37% crude fibers, 195–205 mg<sup>-1</sup> lysine, 89–94 mg<sup>-1</sup> carotene, 3% fiber, as well as minerals such as calcium, magnesium, phosphorus, iron, and zinc, along with vitamins (Terin et al., 2022). Mutagenesis has been shown to modify genetic characteristics such as variance, mean, heritability, genetic variability, genetic advancement for numerous characters, and character connections in various crops (Vasanthrao et al., 2023). In the genomes of plants and animals can be achieved inexpensively and easily through chemical mutagenesis (Mullins et al., 2021). 80% of its nitrogen requirement is fulfilled through symbiotic nitrogen fixation, leaving a significant amount of residual nitrogen for the crops. (Sarmah et al., 2012). Ethyl methane sulphonate (EMS) is a chemical mutagen because it causes high-density irreversible mutations (Barshile et al., 2021). EMS mutagenesis is a crucial method for producing mutations and finding novel genes for plants (Murugan et al., 2024). It is necessary to artificially treat an organism to physical and chemical mutagens. (Opoku Gyamfi et al., 2022). Natural mutations occur on their own and are one of the fundamental processes that propel evolution (Mullins et al., 2021). Chemical mutagenesis offers a low-cost, uncomplicated method for producing a high density of new nucleotide variants (Jankowicz-Cieslak and Till, 2016). Ethyl Methane Sulphonate (EMS) is a well-known, powerful mutagen that is frequently used to produce genetic diversity (Mahto et al., 2018).

## 2. Materials and Methods

The present study was conducted from February, 2023 to July, 2023. Two types of chickpeas (IC265291 and IC265298) were collected from NBPGR, New Delhi. Seventy uniform, healthy seeds were chosen, and after being washed with tissue paper and immersed in water for around three to four hours, they were dried. As per the mutagenesis protocol, varying doses of EMS mutagen are prepared, ranging from 0.1% to 0.6%. Each group of fifteen seeds was immersed in a different concentration of EMS mutagen for six hours at 180 rpm and 27±1°C room temperature on a rotary shaker. The volume of EMS solution needed for uniform and efficient absorption needs to be ten times that of the seed volume. Fifteen chickpea seeds that are not treated with any concentration of EMS and along with the JG11 genotype were considered as the untreated and the control respectively. In the field, three replications of each of the JG11, untreated,

and different concentration EMS-treated seeds were planted using an RBD (Randomized Block Design). They form the M0 generation. The plants showing the highest mean value for quantitative characters under consideration were studied in the M1 generation and plants showing the highest mean value for quantitative traits were selected and their 30 uniform and healthy seeds were sown to get the M2 generation. In the M2 generation, plants showing the highest mean value for quantitative characters were selected and their 50 uniform and healthy seeds were sown to get the M3 generation. In M3 generation, yield parameters under consideration were studied.

## 3. Results and Discussion

From the ANOVA results (Table 1 and 2), it is evident that there is ample genetic variability among selected genotypes due to the treatment of all the characters except the number of seeds pod<sup>-1</sup>.

The quantitative traits observed for IC265291 (V1) genotype (Table 3) are as follows: The height of the plant ranged from 36.58 cm to 22.52 cm. The highest value for the height of the plant is observed for IC-265291-M3 (0.2) at 35.16 cm followed by the mutant plant IC-265291-M3 (0.1) at 35.16 cm. The number of branches plant<sup>-1</sup> is highest for IC-265291-M3 (0.3) which is 12.88 followed by the plant IC-265291-M3 (0.2) which is 11.72. The duration of time taken is lower for the plant IC-265291-M3 (0.2) by 33.66 days and the least number of days taken for initial flowering is 33.42 days by IC-265291-M3 (0.3). The time taken for 50% flowering is showed by IC-265291-M3 (0.1) at 38.50 days followed by IC-265291-M3 (0.2) at 36.72 days and the least number of days for 50% flowering is 36.42 days by IC-265291-M3 (0.3). Number of days taken for pod maturity is lower for the plant IC-265291-M3 (0.2) by 82.90 days. and the least number of days taken for pod maturity is IC-265291-M3 with 82.04 days. The number of pods plant<sup>-1</sup> is highest for IC-265291-M3 (0.3) which is 41.20 pods followed by IC-265291-M3 (0.2) with 40.52 pods plant<sup>-1</sup>. The pod weight plant<sup>-1</sup> is observed highest for IC-265291-M3 (0.1) which is 21.73 g followed by the mutant IC-265291-M3 (0.2) showing 21.47 g pod weight plant<sup>-1</sup> followed by IC-265291-M3 (0.3) weighing 21.30 g. The number of seeds pod<sup>-1</sup> is seen highest in IC-265291-M3 (0.2) which is 1.74 seeds followed by IC-265291-M3 (0.3) which is 1.72 seeds. The number of seeds plant<sup>-1</sup> is shown highest by the plant IC-265291-M3 (0.3) with 69.56 seeds followed by the mutant IC-265291-M3 (0.2) with 68.04 seeds. The total seed weight plant<sup>-1</sup> ranged from 17.50 g to 1.90 g. The highest value for total seed weight plant<sup>-1</sup> is 17.50 g shown by the mutant IC-265291-M3 (0.3) followed by the variety IC-265291-M3 (0.2) which is 17.06 g. The 100 seeds weight is observed to be highest for the mutant plant IC-265291-M3 (0.3) with 23.04 g followed by the mutant IC-265291-M3 (0.2) with 22.77 g. The quantitative traits observed for IC265298 (V2) genotype (Table 4) are as follows: The plant height was observed to



Table 1: Analysis of variance of mutant variety of chickpea IC265291 under EMS mutagenesis for quantitative traits in M3 generation

“Source of variations	DF	MSSQ				
		Characters				
		Plant height (cm)	Primary branches plant <sup>-1</sup>	Days initial flower	Days 50% Flower	Days to pod maturity
Replications		1.065	0.485	0.898	1.313	0.191
Treatments		169.729**	42.759**	122.467**	133.804**	159.613**
Error		0.307	0.189	0.176	0.180	0.172
St E		0.248	0.194	0.188	0.190	0.186
Sed		0.350	0.275	0.265	0.268	0.262
CV (%)		1.757	4.396	1.115	1.022	0.476

Table 1: Continue...

Source of variations	DF	MSSQ					
		Characters					
		No. of Pods plant <sup>-1</sup>	Pod wt plant <sup>-1</sup> (g)	No. of Seedpod <sup>-1</sup>	No. of Seed plant <sup>-1</sup>	Seed wt (g plant <sup>-1</sup> )	100 Seed wt (g)
Replications		3.088	0.962	0.009	1.900	0.803	3.284
Treatments		319.761**	179.368**	6.532	3539.270**	108.648**	286.251**
Error		0.186	0.067	0.008	5.737	0.444	1.448
St E		0.193	0.116	0.039	1.071	0.298	0.538
Sed		0.273	0.103	0.055	1.515	0.422	0.761
CV(%)		1.196	1.440	4.394	5.061	5.221	6.613

\*Significant at: ( $p=0.05$ ) and \*\*: ( $p=0.01$ ) level

be highest in IC-265298-M3 (0.3) showing 37.18 cm which is followed by IC-265298-M3 (0.2) with 36.96 cm. The number of branches plant<sup>-1</sup> is observed to be highest for the mutant IC-265298-M3 (0.2) with 12.64 followed by the mutant IC-265298-M3 (0.3) with 12.56 branches. The time taken for initial flowering is observed to be lower by the plant IC-265298-M3 (0.2) with 33.60 days followed by IC-265298-M3

(0.3) with 32.90 days. The time duration (in days) taken for 50% flowering is observed to be lower by IC-265298-M3 (0.2) with 36.60 days and the least number of days taken for 50% flowering is IC-265298-M3 (0.3) which is 35.92 days. The number of days taken for pod maturity is observed to be less by the mutant (0.1) with 83.14 days and the least is observed for IC-265298-M3 (0.2) with 82.82 days. The number of pods

Table 2: Analysis of variance of mutant variety of chickpea IC265298 under EMS mutagenesis for quantitative traits in M3 generation

“Source of variations	DF	MSSQ				
		Characters				
		Plant height (cm)	Primary branches plant <sup>-1</sup>	Days initial flower	Days 50% Flower	Days to pod maturity
Replications		0.778	0.333	0.778	0.539	1.167
Treatments		206.303**	46.154**	99.346**	153.052**	180.361**
Error		0.246	0.102	0.252	0.159	0.117
St E		0.222	0.143	0.225	0.178	0.153
Sed		0.314	0.202	0.318	0.252	0.216
CV (%)		1.535	3.118	1.348	0.965	0.394

Table 2: Continue...



Source of variations	DF	MSSQ					
		Characters					
		No.of Pods plant <sup>-1</sup>	Pod wt plant <sup>-1</sup> (g)	No. of Seedpod <sup>-1</sup>	No.of Seed plant <sup>-1</sup>	Seed wt (g plant <sup>-1</sup> )	100 Seed wt (g)
Replications		2.019	1.012	0.030	20.073	1.812	1.295
Treatments		933.330**	286.456**	0.466	3432.330**	199.597**	70.858**
Error		0.378	0.154	0.004	4.312	0.278	0.189
St E		0.275	0.176	0.028	0.929	0.236	0.195
Sed		0.389	0.248	0.040	1.313	0.333	0.275
CV(%)		1.854	2.187	4.263	4.141	4.377	2.176

\*Significant at: ( $p=0.05$ ) and \*\*: ( $p=0.01$ ) level

Table 3: Showing the effect of induced EMS mutagenesis on yield parameters in M3 generation of mutant chickpea variety (V1)- IC265291

IC265291 (V1)	Plant height (cm)	Primary branches plant <sup>-1</sup>	Days initial flower	Days 50% flower	Days to pod maturity
0.1% EMS	35.16	10.90	34.50	38.50	84.50
0.2% EMS	36.58	11.72	33.66	36.72	82.90
0.3% EMS	33.68	12.88	33.42	36.42	82.04
0.4% EMS	30.54	8.52	36.18	40.60	83.46
0.5% EMS	24.88	7.18	43.38	46.96	93.48
0.6% EMS	22.52	5.32	45.80	50.00	96.46

Table 3: Continue...

IC265291 (V1)	No. of Pods plant <sup>-1</sup>	Pod wt plant <sup>-1</sup> (g)	No. of seed pod <sup>-1</sup>	No. of seed plant <sup>-1</sup>	Seed wt (g plant <sup>-1</sup> )	100 Seed wt (g)
0.1% EMS	38.24	21.73	1.64	59.86	15.18	21.24
0.2% EMS	40.52	21.47	1.74	68.04	17.06	22.77
0.3% EMS	41.20	21.30	1.72	69.56	27.83	23.46
0.4% EMS	34.64	20.68	1.42	47.76	11.90	20.91
0.5% EMS	18.60	9.58	1.22	20.36	5.21	16.06
0.6% EMS	8.84	4.55	1.00	7.84	1.90	14.78

plant<sup>-1</sup> is observed to be highest for the mutant IC-265298-M3 (0.3) with 44.40 pods plant<sup>-1</sup> which is followed by the mutant IC-265298-M3 (0.2) with 43.26 pods. The weight of the pods plant<sup>-1</sup> (in g) is highest for IC-265298-M3 (0.1) Showing 23.17 g followed by the mutant IC-265298-M3 (0.3) Showing 22.92

g. The highest number of seeds pod<sup>-1</sup> is shown by the mutant IC-265298-M3 (0.2) with 1.78 seeds pod<sup>-1</sup> followed by the mutant IC-265298-M3 (0.3) with 1.68 seeds. The number of seeds plant<sup>-1</sup> is ranged from 74.98 seeds to 7.64 seeds. The highest mean value for the number of seeds plant<sup>-1</sup> is seen

Table 4: Showing the effect of induced EMS mutagenesis on yield parameters in M3 generation of mutant chickpea variety (V2)- IC265298

IC265291 (V1)	Plant height (cm)	Primary branches plant <sup>-1</sup>	Days initial flower	Days 50% flower	Days to pod maturity
0.1% EMS	36.64	12.40	35.14	38.14	83.14
0.2% EMS	36.96	12.64	33.60	36.60	82.82
0.3% EMS	37.18	12.56	32.90	35.92	81.98
0.4% EMS	31.04	8.80	36.24	40.38	84.08
0.5% EMS	24.82	7.28	41.88	47.10	93.66
0.6% EMS	22.3	5.46	44.92	50.62	97.14

Table 4: Continue...



IC265291 (V1)	No.of Pods plant <sup>-1</sup>	Pod wt plant <sup>-1</sup> (g)	No. of Seedpod <sup>-1</sup>	No.of seed plant <sup>-1</sup>	Seed wt (g plant <sup>-1</sup> )	100 Seed wt (g)
0.1% EMS	39.52	23.17	1.60	63.56	14.39	21.21
0.2% EMS	43.26	22.79	1.78	74.98	18.66	23.76
0.3% EMS	44.40	22.92	1.68	71.04	17.40	23.32
0.4% EMS	36.10	19.92	1.48	53.46	12.57	20.88
0.5% EMS	19.50	10.04	1.08	19.32	4.91	15.25
0.6% EMS	8.64	4.45	1.00	7.64	1.83	14.24

in IC-265298-M3 (0.2) showing 74.98 seeds plant<sup>-1</sup> which is followed by IC-265298-M3 (0.3) with 71.04 seeds. The weight of the seeds plant<sup>-1</sup> (in g) is highest for IC-265298-M3 (0.2) with 18.66 g followed by IC-265298-M3 (0.3) with 17.40 g. The 100 seeds weight is observed highest for the mutant IC-265298-M3 (0.2) with 23.76 g followed by the mutant IC-265298-M3 (0.3) with 23.32 g.

#### 4. Conclusion

It is observed that among the seeds treated with different concentrations of EMS (0.1% to 0.6%). The chickpea genotypes treated with 0.2% EMS and 0.3% EMS were shown highest mean value for quantitative characters in the M3 generation. In between variety-1 (IC2652910) and variety-2 (IC265298), it is clear that variety-2 (IC265298) showed higher mean values when compared with variety-V1 (IC265291). These identified mutants may be further used in chickpeas breeding programmes.

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