

## **Isolation of high yielding, nutritionally improved chickpea mutant lines through induced mutagenesis using gamma rays and EMS**

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### **Abstract:**

Chickpea (*Cicer arietinum* L.) is one of the major pulse crops in India. It is a major source of protein for populations that are vegetarian by choice or unaffordability of animal proteins. Chickpea cultivar 'Vijay' is a very old variety that is still popular among farmers of Maharashtra State. There is a scope to improve this cultivar in terms of yield and nutritional value. The present attempt focused on development of mutant lines with improved yield and nutrition using induced mutation. The Chickpea variety- 'Vijay' was treated with different doses of gamma rays (300Gy, 400 Gy, 500 Gy) and EMS (0.2%, 0.3%, 0.4%) and sown to grow M<sub>1</sub> generation. The M<sub>1</sub> was harvested on single plant basis and sown in next season as plant to row progeny to grow M<sub>2</sub> generation. M<sub>2</sub> generation was visually screened thoroughly for different types of mutations. Additionally the M<sub>2</sub> harvest was also screened for protein content using calorimetric methods. Total 171 mutants were selected based on yield, nutrition and earliness. These mutants were sown as plant to row in M<sub>3</sub> generation to study the breeding behavior. Out of these, 12 mutant lines were found to have higher yield than control Vijay, including 07 lines with bold seeds and one line with earliness in M<sub>3</sub> generation. The selected lines will be forwarded to advance generation (M<sub>4</sub> to M<sub>6</sub>) to stabilize the yield contributing characters and then will be evaluated in various trials.

**Key words:** *Cicer arietinum* L., Germplasm, Gamma rays, EMS, Mutant.

### **Introduction**

Chickpea (*Cicer arietinum* L.) is the second largest pulse crop, grown in over 50 countries, and traded across the globe [1]. Chickpea is valued and accepted globally for its nutritive seed composition and protein content as a substitute for animal protein [2]. Mutation breeding is an

effective tool and playing vital role in crop improvement since its inception in agriculture. Induced mutation technique has proved to be successful for improving different traits in a wide variety of crops especially pulses. To date, more than 3,274 varieties in more than 224 plant species derived from mutagenesis programs have been officially released as listed in the FAO/IAEA Mutant Varieties Database (MVD). Among these, 493 mutant varieties of pulses are registered, with 21 improved chickpea mutants released for cultivation [3, 4].

Mutation breeding is an additional advantage when there is a case of improvement of a good variety as it has to alter just one or two traits [5]. Genetic variability can be effectively induced through mutation and its practical applications are well recognized [6, 7]. Considering the rapidly increasing population and declining per capita pulses consumption in India, while also considering comparatively large area under its cultivation then other pulses, the chickpea production statistics over the last decade is not sufficient to meet the growing demand. Therefore, attempts are needed to crack the stagnation of chickpea productivity by developing high yielding and better adapted varieties. The main purpose of this work was to evaluate the mutagenicity of gamma rays, EMS doses employed and the magnitude of variability induced in various morphological and quantitative traits of the chickpea variety- Vijay for the practicable selection based on yield attributing characters and nutrition.

### **Material and Methods:**

The study was aimed to develop the Chickpea mutant with high yield potential and high nutrition. For this a *Desi* variety- Vijay was selected, and germplasm was procured from Mahatma Phule Agriculture University, Rahuri (MS). After screening of the seed material for purity, they were treated with gamma rays and EMS. Each treatment of mutagen included about 300 seeds. The doses selected were [gamma rays- 300, 400 and 500 Gy and EMS 0.2, 0.3, and 0.4 %]. The treated material was then sown in field during mid of October 2015. Standard agricultural practices were followed throughout the cultivation. After germination, the growing progeny was monitored for germination and survival rate and physiological variation if any. The M<sub>1</sub> population was harvested during January-February 2016. In October 2016, M<sub>2</sub> generation was grown and closely monitored for different morphological variations. The plant types with high yield, bold seeds and other

economic characters were marked and harvested separately from progeny of each treatment. The  $M_2$  progeny was harvested during February- March 2017. The  $M_2$  selections were grown as  $M_3$  progeny on plant to row basis (during October 2017). After germination, each line was monitored closely for the traits under observations and noted. The  $M_3$  progeny was harvested during February – March 2018. After harvest, number of seeds per plant, 100 seed weight was noted and protein content [8] of each seed sample was analyzed.

### **Results and Discussion:**

Total 12 high yielding mutants were selected from  $M_2$  population of variety Vijay for their yield performance to observe and evaluate their breeding nature in  $M_3$ . All of them were found segregating in  $M_3$  for their yield character. However, few of them showed interesting results in terms of their average yield, 100 seed weight and boldness of seeds. The data of performance of high yielding mutant lines is presented in table-1.

Mutant 1(14-03) with total 20 plants showed segregation in yield and yield contributing characters but observed to be early flowering and early maturing as compare to control (by 8 days) (Fig. 2). Mutant 11(04-03) with 10 plants respectively in  $M_3$  showed segregation in yield but the average yield and seed weight was found to be slight higher than control. Mutant 17(03-01) does not showed any stability in selected characters. Mutant 17(04-01) with 10 plants in  $M_3$  showed segregation in  $M_3$  generation for yield, but interestingly the average seed yield was double than control with slight increase in 100 seed weight. Mutant 16(06-22) was found to be true breeding for yield characters with average seed yield nearly 3 times than control. 100 seed weight and protein content was also found to slight higher than control.

Mutant 16(07-01) showed segregation in yield character in terms of seed number, however most of these plants showed higher seed weight and bold size. Mutant 16(04-11) plants also showed segregation in yield, with average seed yield less than control but average seed weigh (24.78g) per 100 seeds and increased protein content (18.45 mg/g to 21.82 mg/g) was seen.

Mutant 11(04-11) with 26  $M_3$  plants showed segregation in yield as compare to its  $M_2$  performances. But their average yield was nearly same as that of control. Moreover, seeds of all

these plants were bolder than control (100 seed weight 25.38g; Fig. 1) with sizable increase in protein content. Mutants 11(04-12) A, B & C with about 50 plants in M<sub>3</sub> showed segregation in M<sub>3</sub> for yield but seeds of entire progeny were found to be bold, where 100 seed weight were found to be nearly 04 to 06 gm more than control. Mutant 16(06-09) also showed segregation in yield characters averaging less than control in terms of number of seed grains but its seed size was bold (100 seed weight 23.81g) with increase in protein content (table-1).

The use of induced mutations has played a key role in the improvement of superior plant varieties especially pulses. A large number of improved mutant varieties have been released for commercial cultivation [9,10]. In releasing mutant varieties of Chickpea, India is contributing immensely through the efforts of Indian Agriculture research Institute, its sub-centers and collaboration with several State Agriculture Universities [11]. In some earlier reports [12] similar results in chickpea breeding were noted that reported segregating lines till M<sub>4</sub> generation but showed stability from M<sub>5</sub> onwards to develop a superior line with high yield and high protein content. Few workers [13] reported the isolation of high yielding bold seeded mutant lines of chickpea using gamma rays and EMS. Development of bold seeded Kabuli cultivar “CM2008” also revealed the same procedure [14,15]. Some workers have used EMS and SA as mutagen and isolated the desired mutant lines especially high yielding and yield contributing characters [16, 17]. Thus, results of the present study are in line, however, needs evaluation at least up to M<sub>6</sub> or M<sub>7</sub> to observe the stability of mutants for yield and yield contributing characters. From the results discussed, it is clear that the lower doses (300 Gy) are found to be more effective inducing high degree of defined mutations as compare to their counter higher doses of gamma radiations. No selections were recorded from EMS progeny. Further on the basis of yield performances and protein content analysis it could be stated that, the selected mutant lines are potential sources for developing the new improved variety.

**Conflict of Interest:**

Authors have no Conflict of interest.

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**Table- 1: M<sub>3</sub> Performance of high yielding mutants isolated from M<sub>2</sub> progeny of Var- Vijay**

Variety	Treatment/ Dose	M <sub>2</sub> plant no. (Seed plant <sup>-1</sup> / 100 Seed wt.	M <sub>3</sub> Plant no.	Seeds/ plant	100 Seed wt. (gm)	Protein content
Vijay	Control			74.89	18.12	18.28 ± 0.53
	<b>Gamma rays</b>					
	<b>300 Gy</b>	01 (14-03) [680/17.32]	1(14-03)-1	58	18.22	18.56
			1(14-03)-2	89	18.54	18.48
			1(14-03)-3	102	18.52	18.39
			1(14-03)-4	150	18.78	18.62
			1(14-03)- 5	130	18.52	18.56
			1(14-03)-6	111	18.38	18.42
			1(14-03)- 7	124	18.70	18.42
			1(14-03)-8	78	18.23	18.45
			1(14-03)-9	57	18.52	18.65
			1(14-03)-10	65	18.50	18.38
			1(14-03)-11	79	18.25	18.48
			1(14-03)- 12	164	18.63	18.58
			1(14-03)- 13	162	18.65	18.65
			1(14-03)- 18	215	17.80	18.45
			1(14-03)-19	110	18.25	18.42
			1(14-03)-20	108	17.89	18.28

			<b>Total Plant=</b> <b>20</b>	<b>Avr= 87.02</b>	<b>Avr= 18.40</b>	<b>Avr= 18.26</b>
		11 (04-03) [465/18.02]	11 (04-03)-1	85	18.52	18.52
			11 (04-03)-2	48	18.28	18.33
			11 (04-03)-3	69	18.39	18.45
			11 (04-03)-4	55	18.25	18.29
			11 (04-03)-5	89	17.88	18.33
			11 (04-03)-6	92	18.62	18.42
			11 (04-03)-7	167	18.72	18.39
			11 (04-03)-8	152	18.80	18.45
			11 (04-03)-9	160	18.93	18.52
			11 (04-03)-10	101	18.38	18.45
			<b>Total Plant=</b> <b>20</b>	<b>Avr= 87.40</b>	<b>Avr= 18.33</b>	<b>Avr= 18.41</b>
		17(03-01) [1026/17.30]	17(03-01)-1	115	19.30	18.58
			17(03-01)-2	108	18.45	18.46
			17(03-01)-3	40	18.00	18.45
			17(03-01)-4	46	17.90	18.42
			17(03-01)-5	168	18.50	18.42
			17(03-01)-6	108	16.80	18.66
			17(03-01)-7	62	17.85	18.36
			17(03-01)-8	118	18.00	18.64
			17(03-01)-9	102	18.22	18.22
			17(03-01)-10	70	19.40	18.45
			17(03-01)-11	135	18.50	18.98
			17(03-01)-12	98	17.80	18.43
			17(03-01)-13	50	19.20	18.52
			17(03-01)-14	102	20.70	18.54
			17(03-01)-15	175	19.60	18.28
			17(03-01)-16	115	18.95	18.33

			17(03-01)-17	165	19.45	18.46
			17(03-01)-18	65	18.60	18.36
			17(03-01)-19	142	19.70	18.78
			17(03-01)-20	40	20.00	18.48
			17(03-01)-21	85	18.20	18.46
			17(03-01)-22	85	20.18	18.48
			17(03-01)-23	52	19.00	18.58
			17(03-01)-24	82	19.05	18.96
			17(03-01)-25	25	19.08	18.56
			<b>Total Plants= 25</b>	<b>Avr= 94.12</b>	<b>Avr= 18.81</b>	<b>Avr= 18.51</b>
		17(04 – 01) [305/18.80]	17 (04- 01)- 1	102	18.03	18.38
			17 (04- 01)- 2	120	18.50	18.40
			17 (04- 01)- 3	325	19.02	18.42
			17 (04- 01)- 4	107	18.60	18.55
			17 (04- 01)- 5	109	18.30	18.54
			17 (04- 01)- 6	212	18.20	18.87
			17 (04- 01)- 7	305	19.50	18.48
			17 (04- 01)- 8	98	18.30	18.79
			17 (04- 01)- 9	268	17.90	18.44
			17 (04- 01)- 10	102	18.30	18.38
			<b>Total plants= 10</b>	<b>Avr= 164</b>	<b>Avr= 18.46</b>	<b>Avr= 18.52</b>
		16( 06- 22) [320/18.08]	16( 06- 22)- 01	105	18.30	18.44
			16( 06- 22)- 02	98	18.06	18.48
			16( 06- 22)- 03	97	18.00	18.58
			16( 06- 22)- 04	432	20.27	18.85
			16( 06- 22)-05	105	18.70	18.53
			16( 06- 22)- 06	303	18.50	18.32
			16( 06- 22)- 07	412	18.60	18.38

			16( 06- 22)- 08	93	17.80	18.42
			16( 06- 22)- 09	103	18.02	18.47
			16( 06- 22)- 10	100	18.21	18.42
			<b>Total plants 10</b>	<b>Avr= 214.80</b>	<b>Avr= 18.46</b>	<b>Aver= 18.48</b>
		16(07-01) [382/20.94]	16(07-01)-01	70	24.60	18.87
			16(07-01)-02	75	20.00	18.47
			16(07-01)-03	77	22.10	18.56
			16(07-01)-04	170	20.25	19.87
			16(07-01)-05	65	22.06	18.42
			16(07-01)-06	70	20.24	18.57
			16(07-01)-07	89	20.16	18.78
			16(07-01)-08	59	20.60	18.45
			16(07-01)-09	85	20.18	18.47
			16(07-01)-10	130	19.08	19.20
			16(07-01)-11	58	17.60	18.97
			16(07-01)-12	58	17.80	18.78
			16(07-01)-13	74	21.50	18.58
			16(07-01)-14	97	18.78	18.48
			16(07-01)-15	88	19.20	18.45
			16(07-01)-16	66	19.80	18.59
			16(07-01)-17	126	21.31	19.56
			16(07-01)-18	154	18.51	19.47
			16(07-01)-19	201	17.99	19.78
			16(07-01)-20	216	18.50	19.48
			16(07-01)-21	93	19.30	18.87
			16(07-01)-22	83	20.82	18.65
			16(07-01)-23	100	18.30	18.63
			16(07-01)-24	168	23.47	19.20
			16(07-01)-25	168	19.79	19.46



			<b>Total plants 25</b>	<b>Avr= 105.6</b>	<b>Avr= 20.15</b>	<b>Avr= 18.90</b>
		16(04-11) [258/23.76]	16(04-11)6-1	32	26.96	18.97
			16(04-11)6-2	68	25.02	18.85
			16(04-11)6-3	31	25.16	18.59
			16(04-11)6-4	40	27.20	19.27
			16(04-11)6-5	45	23.34	18.98
			16(04-11)6-6	60	25.52	19.23
			16(04-11)6-7	74	26.98	21.52
			16(04-11)6-8	87	25.74	21.45
			16(04-11)6-9	91	24.52	21.42
			16(04-11)6-10	32	25.44	19.85
			16(04-11)6-11	48	26.18	19.54
			16(04-11)6-12	89	26.18	21.85
			16(04-11)6-13	48	23.12	18.45
			16(04-11)6-14	100	25.90	21.82
			16(04-11)6-15	67	22.89	18.59
			16(04-11)6-16	65	25.68	19.23
			16(04-11)6-17	45	26.84	20.17
			16(04-11)6-18	49	25.54	20.05
			16(04-11)6-19	39	26.40	21.42
			16(04-11)6-20	59	22.40	18.97
			<b>Total plants=20</b>	<b>Avr= 60.80</b>	<b>Avr= 24.78</b>	<b>Avr= 19.91</b>
		11(04-11) [258/23.76]	11(04-11)01-1	107	25.63	21.45
			11(04-11)01-2	72	28.60	21.83
			11(04-11)01-3	134	26.85	21.48
			11(04-11)01-4	145	27.40	21.89
			11(04-11)01-5	106	25.48	21.26
			11(04-11)01-6	52	29.10	22.82
			11(04-11)01-7	42	25.32	21.42
			11(04-11)01-8	43	26.00	21.30

			11(04-11)01-9	116	27.21	21.66
			11(04-11)01-10	36	28.00	22.28
			11(04-11)01-11	57	27.54	22.18
			11(04-11)01-12	20	24.50	20.88
			11(04-11)01-13	26	25.20	21.20
			11(04-11)01-14	37	25.20	20.85
			11(04-11)01-15	47	25.40	21.17
			11(04-11)01-16	111	24.40	21.55
			11(04-11)01-17	78	24.28	20.84
			11(04-11)01-18	73	27.12	21.08
			11(04-11)01-19	67	26.00	20.85
			11(04-11)01-20	123	27.94	21.46
			11(04-11)01-21	72	26.25	20.98
			11(04-11)01-22	121	27.45	21.58
			11(04-11)01-23	33	27.30	21.15
			11(04-11)01-24	34	27.20	21.10
			11(04-11)01-25	39	27.30	20.97
			11(04-11)01-26	68	23.48	20.85
			<b>Total plants=26</b>	<b>Avr= 71.11</b>	<b>Avr=25.38</b>	<b>Avr= 21.38</b>
		11(04-12) A [320/22.2]	11(04-12)02-1	66	21.72	20.17
			11(04-12)02-2	42	22.80	20.02
			11(04-12)02-3	26	20.30	19.18
			11(04-12)02-4	102	23.41	21.48
			11(04-12)02-5	38	26.38	21.25
			11(04-12)02-6	22	20.18	19.85
			11(04-12)02-7	110	27.20	22.02
			11(04-12)02-8	69	24.40	19.85
			11(04-12)02-9	147	25.60	21.98
			11(04-12)02-10	221	26.90	21.48
			<b>Total plants=10</b>	<b>Avr=84.3</b>	<b>Avr= 23.88</b>	<b>Avr= 20.72</b>

		11(04-12) B [320/22.2]	11(04-12)07-1	86	23.80	20.47
			11(04-12)07-2	70	27.00	20.85
			11(04-12)07-3	55	28.12	20.58
			11(04-12)07-4	66	25.20	20.15
			11(04-12)07-5	46	27.20	20.65
			11(04-12)07-6	42	25.75	20.27
			11(04-12)07-7	60	27.00	21.12
			11(04-12)07-8	50	25.10	20.17
			11(04-12)07-9	135	26.80	21.85
			11(04-12)07-10	38	26.40	21.25
			11(04-12)07-11	90	27.05	21.45
			11(04-12)07-12	37	27.10	21.62
			11(04-12)07-13	40	24.20	20.42
			11(04-12)07-14	40	27.00	21.72
			11(04-12)07-15	90	30.26	21.83
			11(04-12)07-16	108	24.58	21.48
			11(04-12)07-17	80	25.10	20.45
			11(04-12)07-18	50	25.00	20.45
			11(04-12)07-19	120	25.70	21.95
			11(04-12)07-20	40	24.50	20.48
			<b>Total plants= 20</b>	<b>Avr= 65.85</b>	<b>Avr=26.15</b>	<b>Avr= 21.98</b>
		11(04-12) C [320/22.20]	11(04-12)08-1	25	20.20	18.98
			11(04-12)08-2	34	25.60	20.85
			11(04-12)08-3	30	25.25	18.87
			11(04-12)08-4	17	25.00	18.96
			11(04-12)08-5	44	25.45	19.05
			11(04-12)08-6	41	27.20	19.45
			11(04-12)08-7	90	26.30	21.46
			11(04-12)08-8	25	23.40	18.87
			11(04-12)08-9	75	24.20	20.12

			11(04-12)08-10	40	23.80	19.86
			11(04-12)08-11	36	24.50	20.23
			11(04-12)08-12	45	25.25	20.27
			11(04-12)08-13	70	25.50	20.17
			11(04-12)08-14	60	28.20	20.22
			11(04-12)08-15	90	26.50	20.15
			11(04-12)08-16	115	26.29	22.05
			11(04-12)08-17	67	26.34	21.18
			11(04-12)08-18	40	25.40	20.27
			11(04-12)08-19	82	24.00	19.82
			11(04-12)08-20	97	25.46	20.15
			11(04-12)08-21	60	26.96	21.25
			11(04-12)08-22	60	26.40	20.26
			11(04-12)08-23	20	25.30	20.75
			11(04-12)08-24	62	24.48	20.02
			11(04-12)08-25	45	26.00	21.12
			11(04-12)08-26	48	25.30	20.47
			11(04-12)08-27	60	25.32	20.17
			11(04-12)08-28	45	25.20	20.32
			11(04-12)08-29	110	26.20	22.06
			11(04-12)08-30	33	23.00	19.85
			<b>Total plants 30</b>	<b>Avr= 54.86</b>	<b>Avr= 25.26</b>	<b>Avr= 20.85</b>
		16 (06-09) [301/21.43]	16(06-09)01-1	57	23.01	19.83
			16(06-09)01-2	51	24.72	20.12
			16(06-09)01-3	41	24.24	20.15
			16(06-09)01-4	--	--	--
			16(06-09)01-5	81	22.86	18.98
			16(06-09)01-6	52	23.52	19.35
			16(06-09)01-7	96	23.10	19.23
			16(06-09)01-8	87	26.28	19.85
			16(06-09)01-9	60	22.48	18.45

			16(06-09)01-10	26	24.12	20.25
			16(06-09)01-11	147	24.23	21.46
			16(06-09)01-12	--	--	--
			16(06-09)01-13	46	22.80	19.58
			16(06-09)01-14	32	25.20	20.46
			16(06-09)01-15	33	24.44	20.24
			16(06-09)01-16	25	27.20	21.23
			16(06-09)01-17	32	24.08	20.58
			16(06-09)01-18	122	24.17	21.45
			16(06-09)01-19	74	26.10	20.84
			<b>Total plants=19</b>	<b>Avr= 60.36</b>	<b>Avr= 23.81</b>	<b>Avr= 20.11</b>



Fig. 1: High yielding bold seeded plant type in M<sub>3</sub>



Fig. 2: Early flowering mutants of Variety Vijay (M<sub>3</sub> Population)

### References:

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