ISSN PRINT 2319-1775 Online 2320-7876, www.ijfans.org
Vol.11, Iss.7, Dec- 2022

Research Paper
UGC CARE Listed (Group -I) Journal

Induced physical and chemical mutagenic studies in M_1 generation of Chickpea (*Cicer arietinum* L.)

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ABSTRACT:
The seeds of chickpea (*Cicer arietinum* L.) varieties -Vishal and JAKI-9218 were treated with mutagens EMS, Sodium azide (SA), and Gamma-rays. M_1 generation was raised and studied with respect to different morphological parameters such as germination percentage, plant survival percentage at maturity, plant sterility percentage, and fertility percentage. Germination Percentage was found lesser in both populations treated with chemical mutagens as compared to physical mutagen gamma radiation and control. High sterility is recorded in M_1 plants of both Chickpea varieties treated with EMS and Sodium azide. However, the plant germination, survival and fertility percentage were highest in the gamma-ray-treated M1 population of both varieties. An increasing trend with an increase in mutagenic concentrations/doses is recorded for the percentage of sterility while germination percentage and plant survival percentage at maturity revealed a decreasing trend with increasing mutagenic concentrations/doses.

KEYWORDS: Chickpea, EMS, Gamma Rays, Germination, mutagens, M_1 generation.

INTRODUCTION:
Chickpea botanically described as *Cicer arietinum* L. belongs to the family Fabaceae. It is popularly grown for its edible mature seeds. It is a cheap plant protein, carbohydrates, and minerals source in the human diet, especially in the Indian arena (Jukanti *et.al.*, 2012). This crop plant also plays a key role in the enrichment of soil fertility by fixing atmospheric nitrogen through symbiotic nitrogen fixation. It's a popular grain among people, but the average yield of chickpeas reported in India is far below its potential (Choudhary *et al*., 2013).

Induced mutation breeding has become an established tool in plant breeding to supplement existing germplasm and improve cultivars in certain traits. Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement. This is a highly effective method in enhancing natural genetic resources and have been used in developing improved cultivars of crops (Gaul *et al*., 1972; Lee *et al*., 2002; Jagadeesan and Punniyamoorthy, 2023).

Therefore, it was planned to initiate the work of induced mutation breeding in Chickpea. The present paper deals with the details of the effects of physical mutagen gamma rays and chemical mutagens EMS and SA on different parameters of the M_1 generation of Chickpea varieties Vishal and JAKI. Variety Jaki has its specialized role in the high productivity of the chickpea crop throughout the state while Vishal is an old Desi variety bearing a large pod size. The parameters considered in this work are the percentage of germination, percentage of survival, percentage of sterility, and percentage of fertile mutants.

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MATERIAL AND METHODS:

Seed Material: The seeds of Chickpea cultivars such as JAKI- 9218 and VISHAL were obtained from Pulse Research Station, Dr. P. D. K. V. Akola (MS). Germplasm of Chickpea was irradiated with Gamma Rays (Co-60) and treated with EMS and Sodium Azide in laboratory conditions. The gamma irradiation facility was made available from the Central Instrumentation Facility, Rashtra Sant Tukdoji Maharaj Nagpur University, Nagpur. For the present study, Dry, uniform, and healthy seeds of Chickpeas (Var. Vishal and Jaki- 9218 with a moisture content of 10 to 12% were selected and about 320 seeds of each cultivar and for each dose of mutagen were selected and treated with gamma radiations (100 Gy, 200 Gy, 300 Gy, and 400 Gy) EMS and Sodium Azide (0.1% 0.2% 0.3%).

Treatment Details:

The experiment was conducted to determine the lethal dose for the suitable concentration of EMS, Sodium Azide and duration of seed treatment. The dose of gamma rays 100 Gy, 200 Gy, 300 Gy and 400 Gy, EMS, and sodium Azide 0.1%, 0.2%, and 0.3% were finally selected for the seed treatment and the duration fixed was for 4 hours. Selected seeds were soaked in distilled water for 10 hours and the wet seeds were treated with different concentrations of EMS and Sodium Azide (0.1%, 0.2%, and 0.3%) for 4 hours. The untreated control was sown on either side of each plot.

The seeds treated with different concentrations of Sodium Azide and EMS was washed thoroughly under tap water for 1 hour. The treated seeds from each treatment were used for raising M_1 generation in the field.

Table 1. Mutagens and Doses used:

Sr. No.	Mutagens	Dosages			
1.	Gamma rays	100 Gy	200 Gy	300 Gy	400 Gy
2.	Sodium Azide (S.A.)	0.1%	0.2%	0.3%	
3.	EMS	0.1%	0.2%	0.3%	

Experimental Site:

The present investigation was carried out in an experimental field located at Jaipur, Taluka Sengaon District Hingoli (M.S.). The soil type of the experimental field was medium-deep to deep black soil with good drainage. The average annual minimum temperature recorded was 18°C and that of maximum was 31°C during winter with average rainfall of 596.5 mm. (Source: World Weather Online).

The M_1 generation was thoroughly studied for the following parameters.

1. **Percentage of germination.**
   The germination percentage is recorded directly at the field from the number of seeds germinated, 7 to 14 days after seen sowing. The number of germinated plants is recorded from each treatment/dose of mutagen and for control.
2. **Percentage of survival.**
   The plant's survival rate is recorded at the complete maturity level. Generally, at the time of flowering and pod setting. The number is denoted in the form of percentage.
3. **Percentage of sterile and fertile mutants.**
   From the raised progeny from each treatment, few plants were found completely sterile at the time of flowering and pod setting. The number of sterile plants is noted and their percentage values are calculated. The sterile plant flowers were observed, and pollens are tested under the microscope on a slide by adding a few drops of 1.5% acetocarmine. Fully stained pollen grains were considered as fertile while empty, partially stained ones were considered sterile. The remaining plants were counted as fertile and their percentage was

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calculated for each dose.

RESULTS AND DISCUSSION:

The results of the morphological parameters recorded in M_1 population of gamma rays, EMS and SA treated progenies of variety Vishal and variety JAKI are given in table 2 and table 3 respectively. This includes seed germination %, Plant survival, and percentage of sterile and fertile plants in given populations.

Table 2. Effect of mutagens on different M_1 parameters in chickpea variety Vishal.

Treatment	Concentration (%)/Dose	Seed Sown	Seed germinated	Seed germination %	Plants survived	Plant survival %	Plant sterile	Plant sterility %	Plants fertile	Plant fertility %
Control	--	320	318	99.37	318	100	00	00	318	100
Gamma rays	100 Gy	320	316	98.75	310	98.10	02	0.64	308	99.35
	200 Gy	320	306	95.62	289	94.44	03	1.03	297	99.00
	300 Gy	320	313	97.81	308	98.40	01	0.32	307	99.67
	400 Gy	320	306	95.62	285	93.13	06	2.10	279	97.89
Sodium Azide (S.A.)	0.1 %	320	208	65.00	186	89.42	27	14.51	159	69.35
	0.2 %	320	184	57.50	152	82.60	42	21.05	110	72.36
	0.3 %	320	100	31.25	64	64.00	38	59.37	26	40.62
E.M.S.	0.1 %	320	205	64.06	166	80.97	12	0.07	154	92.77
	0.2 %	320	151	47.18	105	69.53	18	17.14	87	82.85
	0.3 %	320	101	31.56	57	56.43	13	22.80	44	77.19

Table 3. Effect of mutagens on different M_1 parameters in chickpea variety Jaki.

Treatment	Concentration (%)/Dose	Seed Sow	Seed germinated	Seed germination %	Plants survived	Plant survival %	Plant sterile	Plant sterility %	Plants fertile	Plant fertility %
Control	--	320	318	99.37	319	99.68	00	0.00	319	100
Gamma rays	100 Gy	320	319	99.68	318	99.68	02	0.62	316	98.70
	200 Gy	320	314	98.12	309	98.40	04	1.29	305	98.70
	300 Gy	320	309	96.56	304	98.38	07	2.30	297	97.69
	400 Gy	320	308	96.25	295	95.77	05	1.69	290	98.30
Sodium Azide (S.A.)	0.1 %	320	313	97.81	225	71.88	15	6.66	210	93.33
	0.2 %	320	258	80.62	177	68.60	22	12.42	155	87.57
	0.3 %	320	230	71.87	154	66.95	21	13.63	123	79.87
E.M.S.	0.1 %	320	293	91.56	230	78.49	12	5.21	218	94.78
	0.2 %	320	251	78.43	195	77.68	27	13.84	168	86.15
	0.3 %	320	203	63.43	155	76.38	32	20.64	123	79.35

Percentage of germination:

The germination rate of treated seeds is found to be lesser than the control in each

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dose of treatment of all mutagens used (table-2 and 3). Among treated 100 Gy dose of gamma radiations showed the highest percentage of germination for both varieties. In general gamma-ray treated, seeds showed higher germination rates followed by SA-treated seeds and EMS-treated seeds respectively. Further in each case, the rate of percentage of germination in each mutagen treatment was found to decrease with an increase in dose or concentration of mutagen (table-2 and 3). Figures 1 and 2 are representative photographs of control and treated plots of chickpea varieties Vijay and JAKI. In both of the selected varieties the process of germination in EMS treated seeds is found to be very slow, it took more 6-8 days extra for germination than control seeds in the field. But after germination the growth rate in same mutant plants found to rapid and it recovers the gap of long duration.

Figure 1. Germination in control seeds plots of both selected varieties.

Figure 2. Germination in gamma ray (100Gy) plots of variety JAKI.

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Percentage of survival:

After germination, all the germinated plants do not survive till maturity. There might be different reasons for their deaths like microbial infections, morpho-physiological abnormalities, injury etc. In controls of both varieties, the survival was 100%. In both varieties, gamma irradiation 100Gy showed the highest survival among all. The survival rate of gamma treated population was noted as 97.89 to 99.35 in variety Vishal and 97.69 to 98.70 in JAKI. Further SA and EMS-treated populations showed lesser survival as compared to gamma ray treated populations (Table-2 and 3).

Percentage of sterile mutants:

The highest percentage of sterility was recorded in the progeny of EMS-treated seeds in both varieties. In variety JAKI its percentage ranges from 5.21 to 20.64%, while that of SA treated seeds was ranging from 6.66 to 13.63% and of gamma-treated seeds progeny was ranging from 0.62 to 2.30% (Table-2 and 3). In case of variety Vijay similar trend was observed. In sterile mutants generally petals are found to be absent and so that the flower bud directly enclosed by calyx. Because of absence of petals the anthers can directly see by outside of flower buds. (Figure 3)

Percentage of fertile mutants:

When the percentage of fertile plant types calculated in M1 generation of variety Vijay, gamma radiation treated progeny showed the highest percentage ranging from 97.89 to 99.35% followed by that of SA (40.62 to 72.36%) and EMS (72.19 to 92.77) (table-2) and a similar trend was observed in Variety JAKI. Figure 3 shows a sterile M1 plant type variety Vishal treated with EMS and Figure 4 represents a gamma radiation (300Gy) treated normal fertile plant type of the same variety. Fig. 5 and Fig. 6 represents Control normal fertile plant type of variety Vishal and variety Jaki respectively. Fertile plant types bears a good viable pollens.

Figure.3. Sterile mutant from 0.1% EMS-treated seeds of variety Vishal.

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Figure. 4. Normal fertile plant type from Gamma 300 treatment variety- Vishal.

Figure. 5. Normal fertile plant type from Control variety- Vishal.

Figure. 6. Normal fertile plant type from Control variety- Jaki.

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CONCLUSIONS:

The mutagen directly affects various plant traits directly or indirectly. As our results indicate that their gamma rays showed the highest germination percentage, high survival rate, and higher plant fertility in both varieties of chickpea. Similar studies were reported by Swaminathan *et al*., (1962) in Barley, Avan *et al*., (1980) in rice, Wongpiyasatid *et al*., (2000) in mungbean, Wani *et al*., (2009) in Chickpea, Mahamune and Kothekar (2012) in *Phaseolus* sp., Bogawar *et al*., (2017) in Chickpea varieties, Sofia *et al*., (2019) in Mungbean varieties and Kondapalli and Lavanya (2021) in Chickpea varieties.

From the study, it could be concluded that the mutagenic doses affect the germination, survival and plant fertility percentage. In general, the treated progeny has low rate of germination, low survival higher sterility in chickpeas. Further, as we increase the dose or concentration of mutagen, it increases its effect and further lowers the rate of germination, survival, and fertility.

The lowers in the rate of germination, survival, and fertility it might be because of the random chromosomal aberrations in the treated seeds of mutants. As we increase the concentration of doses there may more chromosomal breakage and can cause more mutations that can disturb or loss the functions within the genome of plant.

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