

Mutagenic sensitivity studies in chickpea employing SA, EMS and gamma rays

J.D. BARSHILE, S.G. AUTI*, S.C. DALVE* and B.J. APPARAO*

Shri Anand College, Pathardi 414 102, Ahmednagar; *P. G. Department of Botany, P. V. P. College, Pravaranagar, Maharashtra

ABSTRACT

Two chickpea cultivars (Vijay and Vishwas) were used for the study of varietal differences in mutagenic sensitivity. The seeds were treated with three concentrations of SA (2, 3 and 4 mM), EMS (8, 12 and 16 mM) and three doses of gamma radiations (400, 500 and 600 Gy). Attempts were made to study mutagenic sensitivity in chickpea through biological parameters such as % germination, seedling injury, pollen sterility and survival at maturity in M_1 generation and the frequency of chlorophyll mutations in M_2 generation on the basis of plant population. There was significant decrease in germination and survival at maturity while seedling injury and pollen sterility increased with increased concentration of mutagen. The frequencies of chlorophyll mutations increased with increase in concentration in Vijay. In Vishwas, frequencies of chlorophyll mutations increased with increase in concentration with exception of 3 mM SA and 600 Gy gamma radiation treatments. EMS 16 mM and 500 Gy gamma radiation treatments induced maximum chlorophyll mutation in variety Vijay and Vishwas, respectively. The 2 mM SA concentration was most effective in both the cultivars.

Key words: Chickpea, Chlorophyll mutation, *Cicer arietinum*, Mutagen, Mutagenic sensitivity

Chickpea (*Cicer arietinum* L.) is the most important pulse crop in India. However, its yield did not witness much appreciation during the past decade. Therefore, development of new plant types for different situations is required. Mutation techniques can be deployed for creating and enlarging the genetically conditioned variability of a species within a short time. Study of mutagenic sensitivity will be helpful for enhancement of genetic variability. Gaul (4) showed that the biological damage caused by mutation to germination, seedling injury, pollen sterility and survival at maturity may be considered as an indication of mutagenic effect. Gregory (6) has reported sensitivity to various mutagens as measured for germination, seedling injury, pollen sterility and survival at maturity in peanut. Although, studies on induced mutations have been undertaken in the past in some pulses, limited reports are available on chickpea (5, 9). Increasing mutation frequency is very important aspect for improvement in the efficiency of the mutation breeding programmes. Hence, the present study was undertaken with an objective of varietal differences of mutagenic sensitivity in chickpea employing sodium azide (SA), ethyl methane sulphonate (EMS) and gamma rays.

MATERIALS AND METHODS

The parent genotypes Vijay and Vishwas taken for the mutation studies, were procured from the Mahatma Phule Krishi Vidyapeeth, Rahuri. Both varieties of chickpea, Vijay and Vishwas were treated separately with chemical (EMS and SA) and physical (γ - radiation) mutagens. For chemical mutagen treatments, seeds were presoaked in distilled water for 6 hours followed by treatment in freshly prepared solutions of mutagens for 12 hours. SA solutions of 2, 3 and 4 mM concentrations were prepared in 1 M phosphate buffer at pH 3.2, while EMS solutions of 8, 12 and 16 mM were prepared in 0.1 M phosphate buffer at pH 7. The chemical mutagen treatments were given at $25 \pm 2^\circ\text{C}$ with intermediate shaking. The volume of mutagenic solutions was about 5 times as that of seed for uniform absorption. The seeds treated with chemical mutagens were thoroughly washed under running tap water for an hour to terminate the reaction of the chemical before sowing. For physical mutagen treatments, dry seeds were irradiated with gamma radiation doses of 400, 500 and 600 Gy from a ^{60}Co source in the Department of Biophysics, Govt. Institute of Science, Aurangabad (M.S.).

Each treatment was carried out for 200 seeds. The treated seeds along with control were sown in a randomized block design (RBD) with three replications at spacings of 15 cm with in row and 45 cm between rows to raise M_1 generation during *rabi* season of 2002. Fifty seeds of each treatment along with control were germinated on moist blotting paper in petri dishes using distilled water for germination and seedling injury study. The germination was recorded on the 5th day after treatment while seedling injury was recorded on the 10th day. The pollen sterility was observed at flowering stage on 10 plants per treatment selected randomly. Survival of plants at maturity in different treatments for each cultivar was recorded in the field. The M_1 plants were harvested individually and M_2 progeny raised in separate row following a RBD with three replications during *rabi* season of 2003. Each treatment comprised 20-21 M_1 plant families and each M_2 progeny row consisted of 10 - 25 plants. Treated as well as control populations were carefully screened from the day of emergence for chlorophyll mutations in M_2 generation up to four weeks. Effectiveness of the mutagenic treatments was calculated following the formula used by Konzak *et al.* (10).

RESULTS AND DISCUSSION

In M_1 generation, germination decreased with increase in concentration or dose of mutagen for both the cultivars (Tables 1 and 2). In the present investigation, it was found that the decrease in germination was more conspicuous with EMS treatments than Gamma rays and SA treatments in both the cultivars. The seed germination was dose dependent. These results corroborated the findings of Farook and Nizam (3) and Toker and Cagirgan (15) in chickpea. The germination for control was 100%. The seed germination decreased 90% to 50% in Vijay and 80% to 42% in Vishwas. Maximum decrease in seed germination was 42% recorded in Vishwas with 16 mM EMS treatment. The result showed that 2 mM SA treatment was less toxic to seed germination in both the varieties. Differential sensitivity between Vijay and Vishwas was observed, the effect being more pronounced on Vishwas. The differential response of these two varieties might be due to difference in their seed size, and genetic, cytological and physiological organizations (4, 12).

Seedling injury is widely used as an index of determining biological effects of various physical and chemical mutagens

in M_1 generation (14). In the present investigation, the seedling injury increased with increase in concentration or dose of mutagenic treatments in both the cultivars. All the treatments resulted in retardation in the height of seedling. The total seedling length was 17.5 cm for Vijay and 18.53 cm for Vishwas. The maximum seedling injury was 60.12% in Vijay and 64.03% in Vishwas with 16 mM EMS treatment as compared to control (Tables 1 and 2). The present results are in agreement with the results obtained in M_1 generation of different species of *Vigna* (7) and in chickpea (1).

Pollen sterility in M_1 generation is the first sign of genetic effectiveness of the treatments. The pollen sterility increased with increase in concentration/doses in both the varieties. There was general trend towards increased pollen sterility with all treatments. The results are in agreement with those of Kharkwal (8). EMS and gamma rays treatments induced higher pollen sterility in the both varieties as compared to SA treatments. Among different mutagens, EMS induced higher pollen sterility (Tables 1 and 2). The pollen sterility was higher in variety Vishwas than Vijay for different treatments, indicating that Vishwas was slightly higher

Table 1. Effect of mutagens on seed germination, seedling injury, pollen sterility and survival at maturity in chickpea variety Vijay in M_1 generation

Mutagen	Concentration/dose	Seed germination (%)	Seedling injury (%)	Pollen sterility (%)	Survival at maturity (%)
Control	--	100	--	00	97.41
SA	2 mM	90	13.13 \pm 0.78	14.94 \pm 0.86	91.17
	3 mM	82	24.23 \pm 1.61	21.09 \pm 1.50	88.67
	4 mM	74	33.47 \pm 1.47	24.13 \pm 0.71	78.21
EMS	8 mM	70	44.4 \pm 1.94	33.00 \pm 1.67	79.79
	12 mM	62	53.36 \pm 0.79	39.53 \pm 1.38	65.38
	16 mM	50	60.12 \pm 0.93	44.18 \pm 1.84	59.00
Gamma rays	400 Gy	88	18.77 \pm 1.20	16.78 \pm 1.50	93.22
	500 Gy	74	38.43 \pm 1.36	27.36 \pm 0.80	88.49
	600 Gy	64	60.06 \pm 1.59	37.47 \pm 2.65	85.59

Table 2. Effect of mutagens on seed germination, seedling injury, pollen sterility and survival at maturity in chickpea variety Vishwas for M_1 generation

Mutagen	Concentration / dose	Seed germination (%)	Seedling injury (%)	Pollen sterility (%)	Survival at maturity (%)
Control	--	100	--	00	97.90
SA	2 mM	80	11.34 \pm 1.47	22.93 \pm 2.80	88.00
	3 mM	66	23.56 \pm 1.86	29.40 \pm 2.43	79.08
	4 mM	60	34.44 \pm 0.94	31.05 \pm 2.16	70.83
EMS	8 mM	72	41.08 \pm 0.79	20.93 \pm 1.58	65.55
	12 mM	56	58.12 \pm 1.26	40.27 \pm 1.07	55.55
	16 mM	42	64.03 \pm 0.82	44.87 \pm 1.94	47.36
Gamma rays	400 Gy	78	32.50 \pm 0.69	24.47 \pm 2.48	84.76
	500 Gy	62	48.47 \pm 1.17	29.39 \pm 1.05	78.65
	600 Gy	56	60.61 \pm 0.76	44.00 \pm 1.92	71.08

Table 3. Mutagenic sensitivity of chickpea cultivars in M₂ generation on the basis of chlorophyll mutation

Treatment	Vijay					Vishwas				
	Population size		Chlorophyll mutation frequency (%)		Mutagenic effectiveness	Population size		Chlorophyll mutation frequency (%)		Mutagenic effectiveness
	M ₁	M ₂	M ₁	M ₂		M ₁	M ₂	M ₁	M ₂	
Control	21	483	00	00	--	21	452	00	00	--
SA 2 mM	21	431	9.52	0.93	0.0383	21	532	4.76	1.13	0.0470
SA 3 mM	21	582	14.28	1.03	0.0286	21	548	14.28	1.09	0.0302
SA 4 mM	21	615	23.81	1.14	0.0237	21	495	23.80	1.21	0.0252
EMS 8 mM	21	525	19.04	1.90	0.0197	21	518	33.33	2.32	0.0241
EMS 12 mM	21	550	28.57	2.36	0.0207	21	573	28.57	2.97	0.0206
EMS 16 mM	21	541	33.33	2.77	0.0144	00	--	--	--	--
γ Rays 400Gy	20	596	15.00	1.68	0.0042	21	513	14.28	1.17	0.0029
γ Rays 500Gy	21	539	19.04	2.04	0.0040	21	535	33.33	3.18	0.0063
γ Rays 600Gy	21	519	33.33	2.31	0.0038	21	564	23.80	2.30	0.0038

sensitive to mutagen than Vijay. The highest pollen sterility in the present investigation was 44.18% in Vijay and 44.87% in Vishwas with 16 mM EMS treatment. Lower pollen sterility in Vijay (14.94 % to 24.13%) was observed for SA treatments.

The percentage of survival at maturity decreased with increased concentration or dose of the mutagens (Tables 1 and 2). The percentage of survival was more pronounced in variety Vishwas than Vijay. The EMS was more lethal than the SA and gamma rays. The highest percentage of survival (93.22%) at maturity was observed in variety Vijay for 400 Gy gamma treatment. The lowest survival (47.36%) at maturity was recorded in Vishwas with 16 mM EMS treatment. However, the relative sensitivity difference was observed in both the varieties. The decrease in survival of plants at maturity might be due to the rapid infusion of chemical mutagens and the mutagens are known for their ability to produce chromosomal aberration (11, 14).

In M₂ generation, the frequency of chlorophyll mutation increased with increase in concentration/dose of mutagen, with the exception of 3 mM SA and 600 Gy gamma rays treatments in Vishwas (Table 3). The maximum frequency of chlorophyll mutation for Vijay was 2.77% in 16 mM EMS. Similarly, the highest frequency of chlorophyll mutation for Vishwas was 3.18 % in 500 Gy gamma treatment. Similar trend of concentration/dose dependence relationship was reported by Gaul (4) and Nerker (12). Swaminathan (13) attributed this decline at higher dose due to rigor of diplontic and haplontic selections in irradiated materials. It is observed that EMS and gamma rays were found to be more effective in inducing chlorophyll mutation than SA.

The mutagen effectiveness is defined as the frequency of chlorophyll mutation in relation to concentration/irradiation

dose of mutagen. In M₂ generation, mutagenic effectiveness decreased with increase in concentration/dose of mutagen in both the cultivars of chickpea (except 500 and 600 Gy gamma rays treatment in variety Vishwas). Similar observations were made by different workers in different crops (2, 10). In the present investigation, 2 mM SA treatment was found to be the most effective for mutagenic treatment in both the cultivars. Chemical mutagens were found to be more effective than gamma rays. It is observed that variety Vishwas of chickpea have slightly higher mutagenic sensitivity than Vijay.

LITERATURE CITED

1. Bhamburkar, S. and Bhall, J.K. 1985. Effect of differential and combined treatments of gamma rays, hydrazine and mitomycin-c in blackgram. *Indian Journal of Botany* 8(1): 82-87.
2. Blix, S. 1964. Studies of induced mutations in peas. VIII. Ethylene imine and gamma ray treatments of the variety Witham Wonder. *Agriculture, Horticulture and Genetics* 22: 171-183.
3. Farook, Fazal, S.A. and Nizam, J. 1979. Mutagenic sensitivity of specific chemicals in chickpea. *Indian Journal of Botany* 2(1): 12-16.
4. Gaul, H. 1964. Mutation in plant breeding. *Radiation Botany* 4(3): 155-232.
5. Gaur, P.M. and Gour, V.R. 1999. An induced fasciated mutants of Chickpea (*Cicer arietinum* L.). *Indian Journal of Genetics and Plant Breeding* 99(3): 325-330.
6. Gregory, W.C. 1968. A radiation breeding experiment with peanut. *Radiation Botany* 8: 81-147.
7. Ignacimuthu, S. and Babu, C.R. 1988. Radiosensitivity of the wild and cultivated urd and mung beans. *Indian Journal of Genetics and Plant Breeding* 48(3): 331-342.
8. Kharkwal, M.C. 1998. Induced mutations in chickpea (*Cicer arietinum* L.). *Indian Journal of Genetics and Plant Breeding* 58(4): 465-474.

9. Kharkwal, M.C. 2001. Induced mutations in chickpea. (*Cicer arietium* L.) V. Evaluation of micromutations. *Indian Journal of Genetics and Plant Breeding* 61(2): 115-124.
10. Konzak, C.F., Nilam, R.A., Wagner, J. and Foster, R.J. 1965. Efficient chemical mutagenesis. Pp.49-70, In: *The Use of Induced Mutation in Plant Breeding*. Report of FAO/IAEA Technical Meeting, Rome, Italy. Pergamon Press, New York.
11. Mutschen-Dahmen, J. 1965. Cytogenetic effects and sterility brought about by ethyl methane sulphonate in barley. *Review of Cytology, Biology and Vegetables* 28: 34-42.
12. Nerker, Y.S. 1977. Mutagenic effectiveness and efficiency of rays, EMS and NMU in *Lathyrus sativus*. *Indian Journal of Genetics and Plant Breeding* 37(1): 137-141.
13. Swaminathan, M.S. 1961. Effect of diplontic selection on the frequency and spectrum of mutations induced in polyploids following seed irradiation. Pp.279-288. In: *Effects of Ionizing Radiations on Seeds*, IAEA. Vienna.
14. Swaminathan M.S., Chopra, V.L. and Bhaskaran. S. 1962. Chromosome aberrations, frequency and spectrum of mutations induced by ethyl methane sulphonate in barley and wheat. *Indian Journal of Genetics and Plant Breeding* 22:192-207.
15. Toker, C. and Cagiran, M.I. 2004. Spectrums and frequency of induced mutation in chickpea. *International Chickpea and Pigeonpea Newsletter* 11: 8-10.