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Mutagenic Effectiveness and Efficiency of Gamma Rays, Ethyl Methane Sulphonate and their Combination Treatments in Chickpea (*Cicer arietinum* L.)

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Abstract: The present study was undertaken to assess the effectiveness and efficiency of gamma rays, EMS and their combined treatments in chickpea. Seeds of two varieties of chickpea (*Cicer arietinum* L.) were treated with gamma rays (150 Gy, 200G y, 300G y and 400G y), EMS (0.1, 0.2, 0.3 and 0.4%) and their combinations (200 Gy+0.2% EMS, 300 Gy+0.2% EMS, 200 Gy+0.3% EMS and 300 Gy+0.3% EMS). The biological damage was calculated in M_1 generation based on seed lethality (L), seedling injury (I), pollen sterility (S) and meiotic aberrations (M). The M_2 population was carefully screened for various chlorophyll mutations. Mutagenic effectiveness and efficiency was calculated based on biological damage in M_1 and chlorophyll mutations in M_2. Mutagenic effectiveness increased with the increase in dose/treatment. Combination treatments in general proved to be more effective followed by individual treatments of EMS and gamma rays. Mutagenic efficiency varied depending upon the criteria selected for its estimation and the degree of efficiency of various mutagens also showed variation. Intermediate treatments in general were found more efficient in causing less biological damage and inducing maximum amount of mutations. The order of efficiency, however, was gamma rays+EMS>EMS>gamma rays. Among the two varieties, var. Pusa-372 proved to be more sensitive to mutagenic treatment than the var. Pusa-212.

Key words: Induced mutations, biological damage, chlorophyll mutations, mutation rate, *Cicer arietinum* L.

INTRODUCTION

The generation of genetic variability by induced mutagenesis provides a base for strengthening plant improvement programs. Various classes of physical and chemical mutagens differ in their efficiency in inducing mutations and in the spectrum of mutations induced. Combination of different mutagens, if their mutagen induction process is independent and capable of interaction, should increase the mutation frequency and alter the mutation spectrum. While, ionization radiations still remain the most suitable agents for inducing genetic variability (Tah, 2006; Sangsiri *et al*., 2005; Joseph *et al*., 2004; Irfaq and Nawab, 2003; Bhatia *et al*., 2001; Brunner, 1995) a number of chemicals have been found to be equally and even many times more effective and efficient mutagens (Ganapathy *et al*., 2008; Dhanavel *et al*., 2008; Basu *et al*., 2008; Rekha and Langer, 2007; Solanki, 2005; Kharkwal, 1998a; Thakur and Sethi, 1995).

The traditional varieties of chickpea have low potentiality and restricted variability with respect to economic characters. Broadening the genetic base for crop improvement can be quickly achieved through induced mutagenesis. The parameters of M_1 generation help in comparing the effectiveness and efficiency of mutagens, besides identifying the plants with maximum genetic damage that are likely to carry the high frequency of micro mutations in M_2 and M_3 generations. For any mutation-breeding program, selection of effective and efficient mutagen is very essential to recover high frequency of desirable mutations (Solanki and Sharma, 1994). Hence, the basic information on mutagenic sensitivity, efficiency of mutagens, methods of handling the material and treatment methods required to maximize mutation induction is essential for success in any mutation-breeding program. The present study was undertaken to understand the response of chickpea genotypes to physical and chemical mutagens with a view to identify mutagenic treatments inducing maximum frequency of mutations in M_2 generation.

MATERIALS AND METHODS

The materials used for this comparative study of effectiveness and efficiency comprised of two varieties of chickpea (*Cicer arietinum* L.) viz. Pusa-212 and Pusa-372. Dry seeds (10-12% moisture content) of both the varieties were irradiated from a ^{60}Co source at NBRI lucknow with a dose of 150, 200, 300 and 400 Gy. Another set of presoaked seeds in distilled water (12h) was treated with EMS at different concentrations (0.1, 0.2, 0.3 and 0.4%) prepared in sodium phosphate buffer with 7.0 pH for 6 h with constant intermittent shaking. A portion of seeds irradiated at 200 and 300 Gy gamma ray doses were also treated with 0.2% and 0.3% EMS independently for 6 h. A total of 13 treatment combinations (including control) were evaluated separately for each variety in RBD with three replications during the rabi season of 2001-2002 at the Agriculture Field Station, Aligarh Muslim University, Aligarh (India). Each treatment consisted of 300 seeds with 100 seeds in each plot of 3×3 m size. The seed to seed and row to row distance was maintained at 15 and 20 cm, respectively. Data on various biological parameters such as seed lethality (L), seedling injury (I), pollen sterility (S) and meiotic aberrations (M) were recorded in M_1 generation (Wani and Anis, 2002). Each M_1 plant was harvested separately and M_2 generation was raised from a composite sample made by bulking 30 seeds from each M_1 harvested plant of a treatment over all the replications. The M_2 population was also evaluated in RBD with three replications, each replication plot consisting of 500 seeds, with a total of 1500 seeds in each treatment as well as in control in both the varieties. The M_2 population was carefully screened for various chlorophyll mutations (Wani and Anis, 2004). The mutagenic effectiveness and efficiency was calculated on the basis of formula suggested by Konzak *et al*. (1965).

Mutagenic effectiveness:

- $\text{Effectiveness (physical mutagen)} = \dfrac{\text{Mutation rate (Mf)}}{\text{Dose of mutagen (Gy)}}$

- Effectiveness (chemical mutagen) =

$$\frac{\text{Mutation rate (Mf)}}{[\text{Conc. of mutagen in percent}]\times[\text{Time of treatment in h}]}$$

- Effectiveness (combination) =

$$\frac{\text{Mutation rate}}{[\text{Dose of physicalmutagen (Gy)}]\times[\text{Conc.of chemical mutaten (\%)}\times\text{Time(h)}]}$$

Mutagenic efficiency:

$$\frac{\text{Mutation rate (Mf)}}{\text{Percent injury (I) / percent lethality (L) / percent sterility(S) / percent meiotic aberrations(M)}}$$

RESULTS AND DISCUSSION

Perusal of the results (Table 1, 2) revealed that the effectiveness of various mutagens and the response of varieties to different mutagens were varying. In case of gamma rays, the effectiveness increased with an increase in dose from 150 to 400 Gy in the var. Pusa-212, whereas it decreased beyond the dose of 300 Gy in the var. Pusa-372. Among EMS treatments, effectiveness initially increased with an increase in concentration but decreased at higher treatments (0.4% EMS) in both the varieties. Among combination treatments, 200 Gy+0.3% EMS was most effective in both the varieties. In general, EMS proved to be more effective in causing mutations as compared to gamma rays and the combination treatments. The gamma rays were least effective in this regard. Among the two varieties, the response to mutagenic effectiveness was more in the var. pusa-372 than in the var. Pusa-212.

The mutagenic efficiency seemed to vary depending on the criteria selected for its estimation and the degree of efficiency of various mutagens also showed variation (Table 1, 2). The efficiency calculated on the basis of seedling injury was generally higher followed by the efficiency based on meiotic aberrations as compared with that based on lethality and sterility in both the varieties of chickpea. Mutagenic efficiency increased with an

Table 1: Mutagenic effectiveness and efficiency of gamma rays, EMS and their combination treatments in chickpea (*Cicer arietinum* L.) var.Pusa 212

Treatments	Lethality (%L)	Pollen sterility (%I)	Seeding injury (%M)	Chromosomal aberrations (%M)	Percent M_2 effectiveness mutated (Mf)	Mutagenic plants (Mf/dose)	Mutagenic efficiency Mf/L	Mf/S	Mf/I	Mf/M
γ- rays (Gy)										
150	3.92	9.18	3.46	5.33	0.309	0.021	0.079	0.034	0.089	0.058
200	12.85	12.51	8.33	9.43	0.820	0.041	0.064	0.066	0.098	0.087
300	10.31	18.47	7.38	17.37	1.751	0.058	0.170	0.095	0.237	0.101
400	26.31	27.35	11.07	23.84	3.570	0.089	0.136	0.131	0.322	0.150
EMS (%)										
0.10	6.32	8.28	5.60	3.82	0.864	1.440	0.137	0.104	0.154	0.226
0.20	15.17	14.31	8.48	7.82	2.290	1.908	0.151	0.160	0.270	0.293
0.30	23.62	23.06	15.42	13.43	3.802	2.112	0.161	0.165	0.246	0.283
0.40	32.90	34.43	23.39	25.49	3.659	1.525	0.111	0.106	0.156	0.143
γ-rays +EMS (%)										
200 Gy+0.2	13.73	16.40	10.84	12.65	2.962	0.123	0.216	0.181	0.273	0.234
300 Gy+0.2	20.10	24.15	5.16	16.5	3.300	0.092	0.164	0.137	0.639	0.200
200 Gy+0.3	32.83	30.96	14.31	19.27	4.737	0.132	0.144	0.153	0.331	0.246
300 Gy+0.3	37.15	39.33	21.25	26.36	4.281	0.079	0.115	0.109	0.201	0.162

Mf: Total chlorophyll mutation frequency in M_2 generation. Dose: Gray (Gy) for gamma rays, time×conc. for EMS and Gy×time×conc. for combined treatments

Table 2: Mutagenic effectiveness and efficiency of gamma rays, EMS and their combination treatments in chickpea (*Cicer arietinum* L.) var.Pusa 372

Treatments	Lethality (%L)	Pollen sterility (% I)	Seeding injury (% M)	Chromosomal aberrations (% M)	Percent M_2 effectiveness mutated (Mf)	Mutagenic plants (Mf / dose)	Mutagenic efficiency Mf/L	Mf/S	Mf/I	Mf/M
γ- rays (Gy)										
150	5.30	12.48	4.17	4.73	0.893	0.059	0.168	0.071	0.214	0.189
200	15.26	15.75	6.66	11.45	1.248	0.062	0.082	0.079	0.187	0.109
300	19.49	24.32	9.42	19.16	4.445	0.148	0.228	0.183	0.472	0.232
400	28.58	29.36	14.13	24.72	4.788	0.120	0.167	0.163	0.339	0.194
EMS (%)										
0.10	5.43	10.61	5.77	5.56	1.305	2.175	0.240	0.123	0.226	0.235
0.20	15.38	18.59	12.31	10.18	3.845	3.204	0.250	0.207	0.312	0.378
0.30	24.65	30.66	14.05	16.81	5.116	2.842	0.207	0.167	0.364	0.304
0.40	37.77	36.66	19.60	22.91	6.436	2.682	0.170	0.175	0.328	0.281
γ-rays +EMS (%)										
200 Gy + 0.2	17.83	23.21	8.58	10.37	3.422	0.143	0.192	0.147	0.399	0.330
300 Gy + 0.2	22.24	28.40	10.94	17.51	5.731	0.159	0.258	0.202	0.524	0.327
200 Gy + 0.3	32.17	35.10	19.07	23.52	7.416	0.206	0.230	0.211	0.389	0.315
300 Gy + 0.3	36.17	42.17	24.46	28.63	7.028	0.130	0.194	0.167	0.287	0.245

Mf: Total chlorophyll mutation frequency in M_2 generation.Dose: Gray (Gy) for gamma rays, time×conc. for EMS and Gy×time×conc. for combined treatments

Table 3: Mutation rate of different mutagens in relation to biological effects such as lethality, sterility, injury and chromosomal aberrations in chickpea (*Cicer arietinum* L.)

Mutagens	MRL	MRS	MRI	MRM
var. pusa-212				
γ-rays	0.112	0.082	0.187	0.099
EMS	0.140	0.134	0.207	0.236
γ-rays +EMS	0.160	0.145	0.361	0.211
var. pusa-372				
γ-rays	0.161	0.124	0.303	0.181
EMS	0.217	0.168	0.308	0.300
γ-rays +EMS	0.218	0.182	0.400	0.304

MRL: Mutation rate base on lethality, MRS: Mutation rate based on sterility, MRI: Mutation rate based on injury, MRM: Mutation rate based on chromosomal aberrations

increase in the dose of gamma rays in the var. Pusa-212 except in case of lethality, where it decreased at 400 Gy.

Contrarily, in the var. Pusa-372, mutagenic efficiency increased up to 300 Gy dose but decreased at highest dose (400 Gy) for all the criteria used. In case of EMS, 0.2 and 0.3% treatments were more efficient than the lower and higher treatments in both the varieties, with a few exceptions.

Combination treatments did not show any regular trend, however, 300 Gy+0.2% EMS proved to be most efficient based on lethality, sterility and seedling injury, whereas, 200 Gy+0.2% EMS was the most efficient treatment based on meiotic aberrations.

Mutation rate (Table 3) based on lethality, sterility and injury was highest among the combination treatments in both the varieties. The EMS in turn was more efficient than gamma rays. However, EMS turned out to be most efficient on the basis of meiotic aberrations in the var. Pusa-212 and was comparable to combination treatments in the var. pusa-372. Gamma rays appeared to be least efficient for all the criteria used.

The usefulness of a mutagen depends both on its effectiveness and efficiency, efficient mutagenesis being production of maximum desirable changes accompanied by the least possible undesirable changes. Mutagenic effectiveness is a measure of frequency of mutations induced by unit dose of mutagen, where as mutagenic efficiency is indicative of proportion of mutations as against undesirable biological effects such as gross chromosomal aberrations, lethality and sterility (Konzak *et al.*,1965). A common observation in the present study revealed that the degree of effectiveness and efficiency varied between different mutagens and also between the two varieties. Similar differences in mutagenic response have also been reported by many workers (Dhanavel *et al.*, 2008; Bhat *et al.*, 2007; Kharkwal, 1998 a). In the present study, lower or intermediate dose treatments proved to be more effective and efficient. The decrease in effectiveness at higher dose treatments may be attributed to the failure in proportional increase of mutation frequency with the increase in dose/conc. of the mutagens. The higher efficiency at lower and intermediate doses of mutagens as observed in the present study might be due to the fact that the biological damage (injury, lethality, sterility etc.) increased with an increase in dose at a rate greater than the frequency of mutations (Konzak, 1965). Greater effectiveness and efficiency of lower or intermediate treatments of chemical mutagens alone or in combination with gamma rays has also been reported earlier (Dhanavel *et al.*, 2008; Kharkwal, 1998a; Singh and Singh, 2007; Khan *et al.*, 2005; Thakur and Sethi, 1995).

Besides, many workers as mentioned above have also observed variations in mutagenic efficiency based on different criteria used as observed in the present study. According to Konzak *et al.* (1965), the reason for greater efficiency of lower doses/treatments is due to the fact that the biological damage such as injury, lethality and sterility increases with the increase in mutagenic treatments at a faster rate than the mutations. In other words lower or intermediate doses/concentrations cause relatively less damage enabling the organism to express the induced mutations successfully. It could be well stated here that while physical mutagens have been exploited to a greater extent for inducing mutations in crop plants and majority of the varieties released through induced mutations belong to physical mutagens. However, some genotypes in crop plants respond more to chemical mutagens than physical ones and in such genotypes appropriate dose/concentration followed by efficient handling of the mutagenised population could yield better results in terms of economic traits like yield, adaptability, protein content etc.

An overview of the above discussion reveals that the two varieties of chickpea used in the present study have proved to be highly responsive to chemical mutagens alone as well as in combination with gamma rays. The intermediate doses of gamma rays (200 and 300 Gy), EMS (0.2 and 0.3%) and lower combination treatments (200 Gy+0.2% EMS, 300 Gy+0.2% EMS) are here recommended for exploiting variability and isolating promising mutants in chickpea in general and the present two varieties in particular.

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