RESEARCH ARTICLE

Mutagenic effectiveness and efficiency of individual and combination treatments of EMS and gamma rays in Isabgol (*Plantago ovata* Forsk.)

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ABSTRACT

Mutagenic effectiveness and efficiency of individual and combination treatments of EMS and gamma rays were assessed in Isabgol (*Plantago ovata* Forsk.) var. Mayuri. The experiment included total 16 mutagenic treatments (6 each for individual treatments of EMS and gamma rays and 4 combination treatments), selected on the basis of their LD_{50} values. Effectiveness and efficiency were calculated on the basis of biological damage of M_1 generation and chlorophyll mutation frequency of M_2 generation. While mutagenic effectiveness was found to be decrease with the increasing concentrations/doses of the mutagens, mutagenic efficiency showed variable trend depending upon the mutagen type and the criteria selected for its estimation. EMS treatments showed high mutagenic effectiveness than the gamma rays and combination treatments.

Key words: Isabgol, EMS, gamma rays, mutagenic effectiveness and efficiency, chlorophyll mutations, biological damage.

INTRODUCTION

Plantago ovata Forsk., belongs to family Plantaginaceae, is one of the major medicinal crop having international acceptance and industrial significance. It is grown for its seeds and seed husk which serve as a household medicine for intestinal and stomach disorder in Indian system of medicine. Due to the presence of mucilage, it has diversified uses not only in medicine but also in dyeing, calico printing, cosmetics and in food industry (Brown, 1995). India dominates the world market in the production and export of Isabgol. Thus, it is the main foreign exchange earner crop of India. Therefore, there is a need to increase the productivity by developing new superior varieties for further export promotion. However, narrow genetic base on account of low chromosome number (2n=8) with small chromosome size and a lot of constitutive heterochromatin are the major constraints in undertaking its improvement through conventional breeding methods (Sareen *et al*., 1999). Therefore, mutation breeding holds great potential for inducing genetic variability in this crop.

In order to induce variability and utilize useful mutations for efficient plant breeding, the systematic and comparative study of induced mutagenic effectiveness and efficiency in crop plants and cultivars is essential (Kharkwal, 1998). Effectiveness and efficiency are the two different properties of the mutagens. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of mutagens while mutagenic efficiency gives an idea of the proportions of the mutations in relation to the undesirable changes (Konzak *et al*., 1965; Nerker, 1977). The usefulness of any mutagen in plant breeding depends upon both, the mutagenic effectiveness as well as the mutagenic efficiency.

The present investigation was undertaken to study the effectiveness and efficiency of EMS and gamma rays, applied individually as well as in combination, with a view to identify mutagenic treatments inducing maximum frequency of mutations coupled with least deleterious effects in M_2 generation.

MATERIALS AND METHODS

Seeds of Isabgol var. Mayuri, obtained from CIMAP, Lucknow were used for the present study. For chemical mutagenic treatments seeds were presoaked in distilled water for 6 hours and then treated with six concentrations of EMS i.e., 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0% for 6 hours. For gamma irradiation, seeds were exposed to six doses of gamma rays viz., 15, 30, 45, 60, 75 and 90kR from ^{60}Co source at NBRI, Lucknow following a post soaking period of 6 hours. For the combination treatments, 45kR dose of gamma rays was combined with the first four concentrations of EMS for 6 hours in the same way as in case of individual chemical mutagenic treatments. One set of seeds was kept untreated to act as control and also presoaked in distilled water for the same period in order to exposed them to similar physiological activities as that of treated seeds before sowing. A set of 450 seeds was used for each treatment as well as control, out of which 150 seeds from each treatment along with control were used for BOD experiment to study the seed germination and seedling height. The remaining 300 seeds from each treatment and control were sown in field in 3 replicates in a complete randomized design in a well prepared agriculture farm at F/o Agricultural Sciences, AMU, Aligarh in rabi season to raise M_1 generation. The distance between the seeds along a row was kept 15cm, whereas, the row to row distance was maintained at 30cm in each experimental plot. Recommended agronomic practices were employed for the preparation of field, sowing and subsequent management of populations to raise a good crop. Observations on pollen fertility and plant survival were carefully recorded at the time of flowering and maturity respectively.

Seed germination was counted right from the emergence of first shoot till the maximum germination was attained in each treatment as well as control. Seedling height was measured on 8th day after germination by measuring the root and shoot length of the seedlings. Pollen fertility was assessed by staining the pollen grains in 2% acetocarmine where, stained pollen grains with regular outline were considered as fertile. Data on plant survival was collected at maturity by counting the total number of surviving plants in each treatment and control. Inhibition in seed germination (R), lethality (L), seedling injury (I) and pollen sterility (S) were calculated for each treatment with respect to control in M_1 generation. 15 seeds from 50 normal looking plants from each treatment as well as control were bulked and sown in field in three replicates in the next rabi season to raise M_2 generation. Thus, in M_2 generation, population of each treatment and control consisted of 50 M_1 plant progenies and a total of 750 M_2 plants. Three replicates were maintained in each treatment as well as control. The M_2 population was carefully screened for various chlorophyll mutations. Data on biological damage recorded in M_1 generation and mutation frequency in M_2 generation (calculated on the basis of chlorophyll mutations) were used to determine the effectiveness and efficiency with the help of the following formulae suggested by Konzak *et al*. (1965).

A. (i) Mutagenic effectiveness (Physical mutagen) $= \dfrac{\text{Mutation rate (Mf)}}{\text{Dose in kilo Roentgen (kR)}}$

(ii) Mutagenic effectiveness (Chemical mutagen) $= \dfrac{\text{Mutation rate (Mf)}}{\text{Conc. of Mutagen (\%) X duration of treatment (hrs)}}$

(iii) Mutagenic effectiveness (Combination) $= \dfrac{\text{Mutation rate (Mf)}}{\text{Dose of Phy. Mutagen (kR) X Conc. of Chem. mutagen(\%) X duration of treatment (hrs)}}$

B. Mutagenic efficiency $= \dfrac{\text{Mutation rate (Mf)}}{\text{*Biological damage in M1generation}}$

*Biological damage: The following criteria were used for calculating the biological damage:

i. Inhibition (R): % reduction in seed germination (Mf/R)
ii. Injury (I): % reduction in seedling height (Mf/I)
iii. Lethality (L): % reduction in plant survival (Mf/L)
iv. Sterility (S): % reduction in pollen fertility (Mf/S)

RESULTS AND DISCUSSION

Perusal of the results presented in Table 1 reveals the differential behavior of Isabgol variety to the different mutagens with regard to mutagenic effectiveness and the efficiency as well. Similar differences in mutagenic response have also been reported by Dhanavel *et al.* (2008); Kharkwal (1998); Mishra and Singh (2014) and Sharma and Sharma (1981). Kundi *et al.* (1997) reported differential sensitivity within crop and even genotype. It was opined that the sensitivity depends upon the genetic architecture and mutagens employed (Blixt, 1970) besides the amount of DNA, its replication time in initial stage and degree of heterochromatin.

Mutagenic effectiveness showed a decreasing trend with the increase in concentrations/doses of mutagens in individual as well as in combination treatments with a single exception at 1.0% EMS. The highest effectiveness was noticed at the lower treatments of both the mutagens applied individually as well as in combination. Among all the mutagenic treatments, the highest effectiveness value was recorded at the 0.1% EMS (1.857) and the lowest at 45kR+0.4% and 45kR+0.6% combination (0.021 for each). Our findings are in close agreement with the results of other workers as Badere and Chaudhary (2007) in linseed; Dixit and Dubey (1986) in lentil; Gupta and Yashvir (1975) in fox millet; Girija and Apparao (2011) in pigeon pea; Kumar and Ratnam (2010) in sunflower; Sharma *et al.* (2005) in urdbean and Shah *et al.* (2008) in chickpea who also reported dose dependent decrease in mutagenic effectiveness. The decrease in effectiveness at higher treatments may be attributed to the failure in proportional increase of mutation frequency with the increase in concentrations/doses of the mutagens. Many workers have been reported the higher effectiveness values at the lower concentrations/doses of EMS and gamma rays (Desai and Bhatia, 1975; Khan and Tyagi, 2010 and Pavadai *et al.*, 2009).

In general, EMS treatments showed very high mutagenic effectiveness as compared to gamma rays and combination treatments. The greater effectiveness of chemical mutagens over the physical ones has also been reported by Shah *et al.*, 2008 and Satpute and Fultambkar, 2012.

Mutagenic efficiency varied depending upon the criteria selected for its estimation. The degree of efficiency of mutagens in individual and in combination treatments also showed variations. It did not follow any particular (increasing or decreasing) trend in the mutagenic treatments. Similar results were obtained by Bhosle and Kothekar (2010) in cluster bean and Gaikwad and Kothekar (2004) in lentil.

Generally, lower concentrations/doses of mutagens in individual and in combination treatments showed high mutagenic efficiency with a single exception in combination treatments when efficiency was estimated on the basis of lethality, higher efficiency was showed by the higher combination treatments. Sharma *et al.* (2005) in urdbean reported that the lower doses of mutagens were more efficient than the higher doses. Higher efficiency at lower and intermediate doses of mutagens as observed in the present study might be due to the fact that biological damage (injury, lethality, sterility etc.) increased with an increase in dose at a rate faster than the frequency of mutations (Cheema and Atta, 2003; Konzak *et al.*, 1965). In other words, lower or intermediate concentrations/doses cause relatively less damage enabling the organism to express the induced mutations successfully. Higher efficiency at lower concentrations/doses of the mutagens could be ascribed to the lesser percentage of damage at such doses (Khadke, 2005; Panchabhaye, 1997).

As indicate by the pooled mean values (Table 2) EMS was found to be more efficient when efficiency was calculated on the basis of seedling injury and pollen fertility, while with respect to inhibition in seed germination and lethality gamma rays were found to be more efficient in this crop. The overall pooled mean value for all the mutagens showed that the mutagenic efficiency estimated on the basis of seedling injury was the highest followed by inhibition in seed germination, lethality and pollen sterility. The low efficiency of some mutagens has been attributed to the use of low concentrations/doses corresponding to their mutation induction. The higher efficiency of the mutagen indicates relatively less biological damage (seedling injury, sterility etc.) in relation to the mutations induced (Gaikwad and Kothekar, 2004; Kharkwal, 1998). Higher mutagenic effectiveness and efficiency at lower concentrations/doses of EMS and gamma rays were observed by Kumar *et al.* (2003) in *Phaseolus lunatus* and Waghmare and Mehra (2001) *Lathyrus sativus*. Greater effectiveness and efficiency of lower or intermediate treatments of chemical mutagens alone or in

Table 1: Mutagenic effectiveness and efficiency of EMS, γ rays and their combination treatments in Isabgol var. Mayuri

Mutagen	Treatment	% Inhibition in seed germination (R)	% Seedling injury (I)	% Lethality (L)	% Pollen sterility (S)	% M_2 seedlings mutated (Mf)	Mutagenic effectiveness	Mutagenic efficiency			
								Mf/R	Mf/I	Mf/L	Mf/S
	Control	-	-	-	-	-	-	-	-	-	-
EMS	0.1%	4.62	2.88	7.02	7.37	1.114	1.857	0.241	0.387	0.159	0.151
	0.2%	6.92	3.75	10.73	8.07	1.306	1.089	0.189	0.349	0.122	0.162
	0.4%	10.77	7.49	12.55	9.88	1.713	0.714	0.159	0.229	0.137	0.173
	0.6%	16.92	11.82	20.60	20.51	2.208	0.613	0.130	0.187	0.107	0.108
	0.8%	21.54	14.99	22.81	21.09	2.801	0.583	0.130	0.187	0.123	0.133
	1.0%	32.31	19.02	36.73	24.35	3.671	0.612	0.114	0.193	0.100	0.151
γ rays	15kR	3.08	5.76	3.67	11.74	0.843	0.056	0.274	0.146	0.230	0.072
	30kR	6.15	9.22	6.27	15.22	1.299	0.043	0.211	0.141	0.207	0.085
	45kR	10.77	14.12	12.09	16.49	1.788	0.040	0.166	0.127	0.148	0.108
	60kR	14.62	16.14	17.56	16.87	2.147	0.036	0.147	0.133	0.122	0.127
	75kR	20.77	17.58	24.35	22.04	2.669	0.036	0.128	0.152	0.110	0.121
	90kR	30.77	23.63	33.04	30.26	3.141	0.035	0.102	0.133	0.095	0.104
γ rays + EMS	45kR+0.1%	8.46	12.68	7.52	9.34	1.437	0.053	0.170	0.113	0.191	0.154
	45kR+0.2%	12.31	16.43	14.78	15.47	1.846	0.034	0.150	0.112	0.125	0.119
	45kR+0.4%	20.00	20.17	22.39	25.54	2.226	0.021	0.111	0.110	0.099	0.087
	45kR+0.6%	31.54	25.65	32.94	27.64	3.411	0.021	0.108	0.133	0.104	0.123

Table 2: Average mutagenic effectiveness and efficiency of EMS, γ rays and their combination treatments in Isabgol var. Mayuri

Mutagen	Mutagenic effectiveness	Mutagenic efficiency			
		Mf/R	Mf/I	Mf/L	Mf/S
EMS	0.911	0.161	0.255	0.124	0.146
γ rays	0.041	0.171	0.139	0.152	0.103
γ rays+EMS	0.032	0.135	0.117	0.130	0.121
overall pooled mean	**0.984**	**0.467**	**0.511**	**0.406**	**0.370**

*Data based on pooled values of 6 treatments (in EMS and γ rays individually) and 4 treatments (in combinations) combination with gamma rays has also been reported earlier (Dhanavel *et al*., 2008; Kharkwal, 1998; Singh and Singh, 2007; Thakur and Sethi, 1995).

CONCLUSION

From the data on mutagenic effectiveness and efficiency, it could be concluded that 0.1% treatment of EMS and 15kR dose of gamma rays were the most effective treatments. While in case of mutagenic efficiency 0.1% EMS treatment was found to be more efficient among all the treatments of EMS with respect to inhibition in seed germination, seedling injury and lethality, whereas, with regard to pollen sterility 0.4% EMS treatment was proved to be more efficient. Among the gamma rays treatments, 15kR dose was the more efficient with regard to inhibition in seed germination and lethality, while in case of injury and pollen sterility 75kR and 60kR doses respectively, were noticed to be more efficient. In case of combinations, 45kR+0.1% treatment was noticed to be more effective as well as efficient with respect to all the selected criteria. Efficient mutagenesis and their treatments are indispensable for the cost effective use of mutagen as a tool for the induction of mutations and their direct and indirect utilization in successful breeding programmes (Shah *et al*., 2008).

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