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SHORT RESEARCH ARTICLE

In-vitro intervention modifies the outcome of induced mutagenesis using gamma-ray but not ethyl methanesulphonate in cockscomb (*Celosia cristata* L.)

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Abstract

The viability of conventional vis-à-vis *in-vitro* mutagenesis was investigated using EMS and gamma-ray in cockscomb (*Celosia cristata* L.) ethyl methanesulphonate (EMS) was a better mutagen as compared to gamma-ray in terms of mutation frequency, effectiveness, efficiency, factor of effectiveness and mutation rate. The spectrum of mutants induced by EMS and the aforementioned parameters decreased with the increase in its concentration. Moreover, the EMS treatment to dry seed was fructuous compared to the pre-soaked seeds. Although the effect of EMS treatment was similar in conventional and *in-vitro* mutagenesis; the gamma-ray treatment worked differently in these two modes of mutagenesis.

Keywords: Alkylating agent, Effectiveness, Floriculture, Ionizing radiation, Ornamental plants.

Cockscomb (*Celosia cristata* L.) is popular for its unique and vivid-coloured inflorescence, and thus its new varieties are in demand. Induced mutagenesis is a potent way to breed novel varieties of plants. Moreover, coupling it with plant tissue culture to amplify the mutated sector increases the mutation frequency (Nasri et al. 2021). No report mentions the use of *in-vitro* mutagenesis in cockscomb and therefore, a comparative study between the conventional mutagenesis and *in-vitro* mutagenesis in cockscomb was carried out. Thus, the seeds were mutagenized with 0.15, 0.20 and 0.25% (w/v) aqueous EMS and 200, 250 and 300 Gy of GR. The dry, 3-hour pre-soaked in water (PSW) and 6h PSW seeds were treated with EMS in the conventional mutagenesis studies. In the *in-vitro* mutagenesis studies, the shoot-tip explant harvested from mutagenized seedlings was cultured over the media to regenerate the plants according to Rinkey and Badere (2020). The effect of mutagen treatment on pollen fertility was determined in M_1 and RM_1 generations. Individual plants were harvested at maturity and their seeds were sown in the next season on a plant-to-row basis to raise the M_2 and RM_2 population. These populations were screened to isolate various mutants.

Frequency of mutatns

A total of 25 mutants were isolated in the present study. The spectrum and frequency of mutants varied with the mutagen, physiological state of the seeds and mode of treatment. The spectrum of mutants was comparatively wider in M_2 than the RM_2 generation. Moreover, EMS induced a wider mutant spectrum than the GR in M_2 generation. The mutation frequency was also higher in M_2 compared to RM_2 generation. Comparably, the mutation frequency was more with EMS than GR in the M_2 generation (Supplementary Tables S1 and S2). The mutation frequency decreased and the pollen sterility increased with an increase in the dose of mutagen. Contrastingly, the effectiveness, factor of effectiveness and efficiency were inversely related to EMS concentration in M_2 and RM_2 generations. All these

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Table 1. Effect of mutagen treatment on cockscomb in the M_2 generation

Treatment	Pollen sterility (%)	Mutation frequency (%)	Effective-ness	Factor of effective-ness	Efficiency	Mutation rate	
						In terms of effectiveness	In terms of efficiency
Ethyl methanesulphonate (%)							
0h PSW							
0.15	9.49	4.09	1.51	45.26	0.43		
0.20	18.33	2.05	0.57	16.84	0.11	0.84	0.21
0.25	21.18	1.98	0.44	15.79	0.09		
3h PSW							
0.15	7.78	2.57	0.95	18.95	0.33		
0.20	12.96	0.70	0.19	4.21	0.05	0.42	0.14
0.25	12.08	0.53	0.12	3.16	0.04		
6h PSW							
0.15	18.65	0.13	0.05	1.05	0.01		
0.20	23.95	4.47	1.24	8.42	0.19	0.54	0.08
0.25	27.10	1.53	0.34	7.37	0.06		
Gamma-rays (Gy)							
200	22.48	0.66	0.003	6.32	0.03		
250	25.30	1.62	0.006	10.53	0.06	0.003	0.03
300	29.54	0.17	0.001	1.05	0.01		

parameters were maximum at 250 and 200Gy of GR in M_2 and RM_2 generation, respectively. Mutation rate in terms of effectiveness was more than that in terms of efficiency with EMS treatment. However, both these parameters were equal with GR treatment in M_2 generation. Contrastingly, the mutation rate in terms of effectiveness was lower than that in terms of efficiency in RM_2 generation (Tables 1 and 2). The action of mutagen depends on its nature, the physiological state of cell during treatment, the availability of loci for mutagen action and active repair mechanisms. Moreover, all the mutations are not perceivable (Balkema 1971; Badere and Choudhary 2005, 2007) and only that mutation is inherited, which occurs in a gamete-forming cell. Additionally, the role played by diplontic selection, which replaces mutated cells with wild types, also cannot be overlooked (Klekowski 2003).

Further, the frequency of mutations induced by EMS in the M_2 and RM_2 generation was similar. However, with GR treatment, the frequency of mutations was higher in RM_2

Table 2. Effect of mutagen treatment on cockscomb in the RM_2 generation

Treatment	Pollen sterility (%)	Mutation frequency (%)	Effective-ness	Factor of effective-ness	Efficiency	Mutation rate	
						In terms of effectiveness	In terms of efficiency
Ethyl methanesulphonate (%)							
0.15	10.07	4.10	1.518	33.33	0.41		
0.20	19.11	2.63	0.731	26.67	0.14	0.85	0.20
0.25	22.24	1.33	0.295	20.00	0.06		
Gamma-rays (Gy)							
200	22.46	2.04	0.010	7.50	0.09		
250	22.86	1.31	0.005	5.00	0.06	0.01	0.07
300	25.30	1.72	0.006	2.86	0.07		

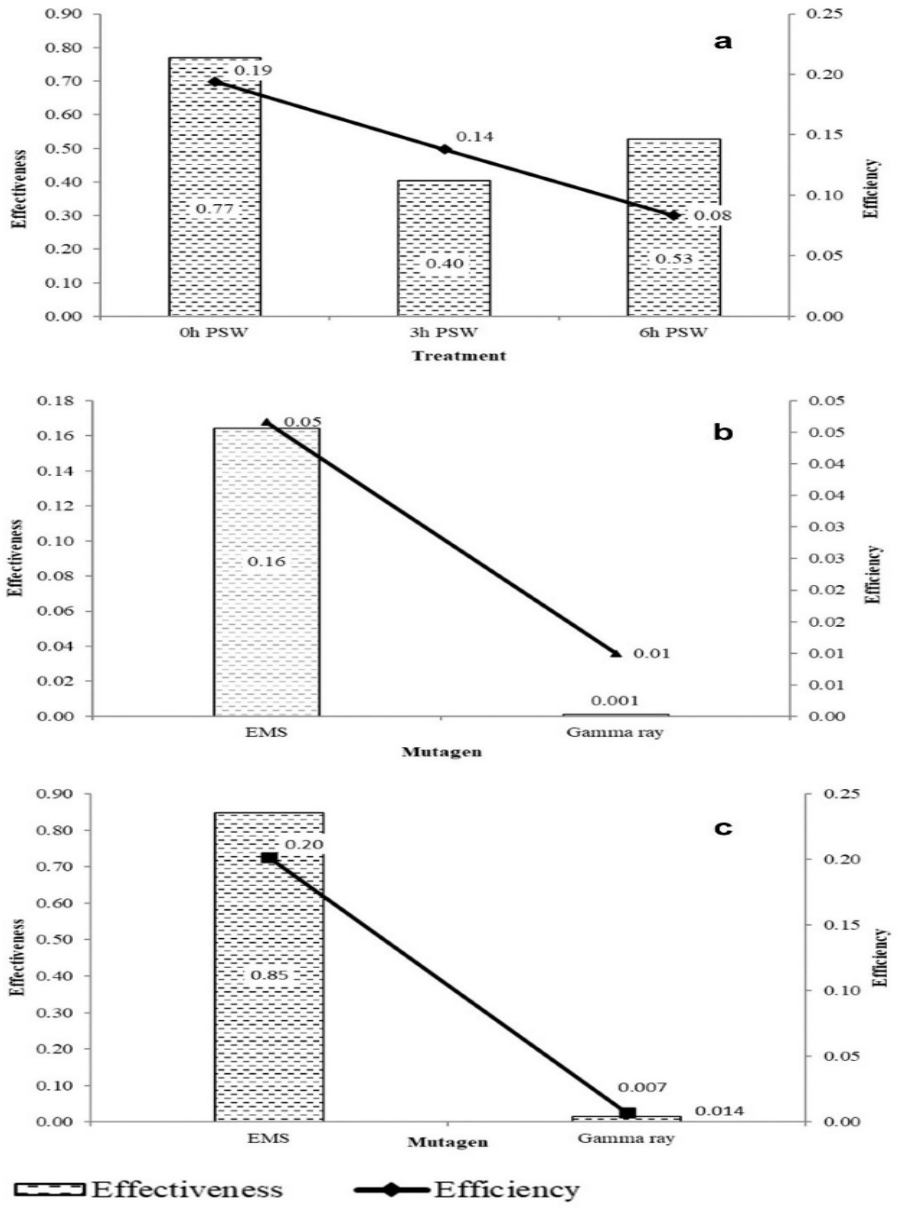

Fig. 1. Effectiveness and efficiency of the mutagens (a) EMS in M_2 generation (treatment pooled over concentration) (b) EMS and gamma ray in M_2 generation (mutagen pooled over treatments) (c) EMS and gamma ray in RM2 generation (mutagen pooled over treatments)

generation compared to the M_2 generation. Notably, we cultured the explant harvested from EMS-treated seedlings over media containing 8.8 µM BAP and those obtained from GR-treated seedlings were cultured over media containing 0.5 and 6.6 µM. Thus, the difference in PGR composition of culture media might have affected the mitotic activity of cells in the explant. Presumably, the media over which GR-treated explants were cultured stimulated the cell division and/or suppressed the diplontic selection. While former increases the availability of mutated cells for regeneration; latter reduces the competition between the mutated and wild-type cells. In effect, both these factors, might have increased the frequency of mutations in the RM_2 generation of GR treatment (Patade and Suprasanna 2008).

Pooling of treatment over concentration displayed maximum effectiveness of EMS in dry seed treatment followed by 6h and 3h PSW treatment. However, the efficiency of EMS decreased with an increase in the duration of treatment from 0 to 6h in M_2 generation (Fig. 1a). EMS was superior to GR with respect to effectiveness and efficiency upon pooling of mutagen over treatment in M_2 and RM_2 generation (Fig. 1b–c). This superiority of EMS over GR might be due to the induction of gene mutations by EMS and the induction of chromosomal aberrations by GR (Rampure et al. 2017). Thus, since the higher doses of mutagens are less fecund to induce mutants and associated

with sterility in the cockscomb; lower doses of EMS and GR seem useful for mutation studies. Hence, the present investigation establishes the potency of low concentration of EMS treatment to the dry seeds for the improvement of cockscomb through induced mutagenesis. The study also demonstrates the influence of cultural conditions on the outcome of *in-vitro* mutagenesis. Thus, it is imperative to decipher the factors responsible for abating the effect of GR in cockscomb to the make most of *in-vitro* mutagenesis.

Supplementary material

Supplementary Tables S1 and S2 are provided and can be accessed only, www.isgpb.org

Authors' Contribution

Conceptualization of research (PKR, RSB); Designing of experiments (PKR, RSB); Contribution of experimental materials (RSB); Execution of field/lab experiments and data collection (PKR); Analysis of data and interpretation (PKR, RSB); Preparation of the manuscript (PKR, RSB).

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Supplementary Table S1. Frequency of mutations in the M_2 generation

Mutant \ Treatment	Control	0h PSW EMS (%)			3h PSW EMS (%)			6h PSW EMS (%)			Gamma ray (Gy)		
		0.15	0.20	0.25	0.15	0.20	0.25	0.15	0.20	0.25	200	250	300
Robust	-	0.19	0.13	-	-	0.17		-	-	-	-	-	-
Tall	-	0.10	0.13	-	-	-	-	-	-	-	-	-	-
Dwarf	-	0.19	0.13	-	0.41	-	0.18	-	0.56	-	-	0.16	-
Bushy habit	-	0.10	-	-	0.14	-	-	-	0.56	-	-	-	-
Highly branched	-	0.10	-	0.17	-	-	-	-	-	-	-	-	-
Less branched	-	0.10	-	-	-	-	-	-	-	-	-	-	-
Unbranched	-	0.29	-	-	-	-	-	-	-	-	-	0.16	-
Branching pattern	-	-	-	-	0.27	0.17	-	-	-	-	-	-	-
Branched inflorescence	-	0.19	0.38	0.83	-	-	-	-	0.56	-	-	-	-
Asymmetric leaf lamina	-	-	-	-	0.27	-	-	-	-	-	-	-	-
Large leaf area	-	0.19	-	-	0.14	-	-	-	-	-	-	-	-
Thick stem	-	-	-	-	0.14	-	-	-	-	-	0.11	0.16	-
Long internode	-	0.10	-	-	-	-	-	-	-	-	-	-	-
Short internode	-	0.38	-	0.17	-	-	-	-	-	0.22	-	-	-
Multiple comb	-	0.86	0.13	0.17	-	0.17	-	-	0.56		-	-	-
Branched comb	-	-	-	-	0.41	-	-	-	-	-	-	0.16	-
Sterile	-	-	-	0.17	-	-	-	-	0.56	0.44	-	0.16	-
Long fertile comb	-	0.10	-	-	-	-	-	-	-	-	-	-	-
Long inflorescence	-	0.38	0.38	-	-	-	-	-	-	0.22	-	-	-
Broad comb	-	-	0.13	0.17	-	-	-	-	-	-	0.11	-	-
Combless	-	0.10	-	-	0.41	0.17	-	0.13	1.12	-	0.11	0.49	-
Plumose type inflorescence	-	0.38	-	0.17	0.14	-	-	-	-	0.22	-	-	-
Comb colour	-	0.29	0.64	0.17	0.27	-	0.35	-	0.56	-	0.33	0.32	0.17
Early flowering	-	-	-	-	-	-	-	-	-	0.22	-	-	-
Late flowering	-	0.10	-	-	-	-	-	-	-	0.22	-	-	-
Total Frequency	0	4.09	2.05	1.98	2.57	0.70	0.53	0.13	4.47	1.53	0.66	1.62	0.17

Supplementary Table S2. Frequency of mutations in RM_2 generation

Treatment / Mutant	Control	EMS (%)			Gamma ray (Gy)		
		0.15	0.20	0.25	200	250	*300*
Robust	-	1.64	-	-	0.68	-	-
Tall	-	-	-	-	-	-	-
Dwarf	-	-	-	-	0.68	-	-
Bushy habit	-	-	1.32	0.44	-	-	-
Highly branched	-	-	-	-	-	-	-
Less branched	-	-	-	-	-	-	-
Unbranched	-	-	-	-	-	-	-
Branching pattern	-	-	-	-	-	-	-
Branched inflorescence	-	-	-	-	-	-	-
Asymmetric leaf lamina	-	-	-	-	-	-	-
Large leaf	-	-	-	-	-	-	-
Thick stem	-	-	-	-	-	-	-
Long internode	-	-	-	-	-	-	-
Short internode	-	-	-	-	-	-	-
Multiple comb	-	-	-	-	-	-	-
Branched comb	-	1.64	0.66	-	0.68	0.65	-
Sterile	-	-	-	-	-	-	-
Long fertile comb	-	-	-	-	-	-	-
Long inflorescence	-	-	-	-	-	-	-
Broad comb	-	-	-	-	-	-	-
Combless	-	-	-	-	-	0.65	-
Plumose type inflorescence	-	-	-	-	-	-	-
Comb colour	-	0.82	0.66	0.88	-	-	1.72
Early flowering	-	-	-	-	-	-	-
Late flowering	-	-	-	-	-	-	-
Total frequency (%)	0.00	4.10	2.63	1.33	2.04	1.31	1.72