Indian J. Genet., 48(3): 313–316 (1988)

STUDIES ON INDUCED MUTATION FREQUENCY IN *CATHARANTHUS ROSEUS* (L.)G. DON BY GAMMA RAYS AND EMS INDIVIDUALLY AND IN COMBINATION

M. VENKATESWARLU,* B. N. SUSHEELAMMA, P. KUMAR AND K. SUBHASH

Tissue Culture and Mutation Breeding Laboratory, Botany Department, Kakatiya University, Warangal 506009

(Received: January 4, 1988; accepted: January 15, 1988)

ABSTRACT

Seeds of pink flowered (PF) and white flowered (WF) *Catharanthus roseus* were soaked in distilled water for 24 h and treated with gamma rays and 0.1% EMS separately and in combination. Six types of chlorophyll mutations, viz., xantha, albina, chlorina, viridis, maculata and tigrina were recovered in M_2 generation of both forms. The frequency of chlorophyll mutations was found to be dependent on the dose of gamma rays and duration of treatment with EMS. Higher frequency of chlorophyll mutations was noticed in PF, which is mutagenically more sensitive than WF. It was also noticed that the combination treatments of gamma rays and EMS enhanced the frequency of chlorophyll mutations.

Key words: *Catharanthus roseus*, chlorophyll mutations, gamma rays, ethyl methanesulphonate.

The scoring of chlorophyll mutations in M_2 generation has been proved to be the most dependable index for evaluating the genetic effects of mutagenic treatments [1]. In recent years, much emphasis has been laid on the use of combination treatments of physical and chemical mutagens [2–4]. Chemical mutagens are not only mutagenic themselves but also affect mutation in specific ways when combined with radiation [5]. Therefore, it is of interest to find out the mutation frequencies when the physical and chemical mutagens are used in combination. The present investigation deals with the frequency and spectrum of chlorophyll mutations in two forms of *Catharanthus roseus* (L.) G. Don induced by separate as well as combined treatments of gamma rays and ethyl methanesulphonate (EMS).

MATERIALS AND METHODS

Seeds of pink flowered (PF) and white flowered (WF) of *C. roseus*, obtained from Janatha Seeds Corporation, Hyderabad, were used to raise the plants after obtaining pure lines by selfing for three generations. Dry seeds were subjected to 30, 40, 50 and 60 kR gamma irradiation from ^{60}CO source, delivering 200 rads/min

*Present Address: Mulberry Breeding and Genetics Section, Central Sericultural Research and Training Institute, Srirampuram, Mysore 570008.

in a gamma cell installed at the Division of Genetics, IARI, New Delhi. For the combination treatments, 40 kR dose was selected. The seeds were presoaked (24 h) in distilled water and treated with fresh 0.1% EMS solution for 6, 12, 18 and 24 h at 27 ± 2°C. Presoaked (24 h) seeds were also treated with 0.1% EMS solution for the same period. Immediately after the treatments the seeds were thoroughly washed in distilled water and sown in experimental field along with controls. The M_1 plants were harvested separately to raise M_2 generation. Data on chlorophyll mutations in M_2 were scored at seedling stage.

RESULTS

The results on chlorophyll mutation in M_2 following treatment with gamma rays and EMS individually and in combination (Table 1) showed that the chlorophyll mutation frequency increased with increase in the dose of gamma rays and duration of chemical treatment. The chemical EMS was found to be more effective than radiation. However, the combination of gamma rays with EMS gave a higher frequency and wider spectrum of chlorophyll mutations than their individual treatments. Thus, postirradiation treatment with EMS produced additive effects. The 2-flower forms responded differently to mutagenic agents in relation to frequency of chlorophyll mutations; the PF produced more chlorophyll mutations than WF.

FREQUENCY AND SPECTRUM OF CHLOROPHYLL MUTANTS

The various chlorophyll mutations observed and described below, and the frequency of induction presented in Table 1.

Albina. White and relatively smaller than the normal seedlings of same age. These mutants survived for 5–10 days.

Xantha. Straw yellow seedlings with normal growth in the beginning, but started withering after 10 days, died within 15 days.

Chlorina. Yellowish green, majority survived for 30 days. However, only a few, having light yellowish green colour, survived till maturity and set a few seeds.

Viridis. Seedlings with whitish tips of leaves, lethal.

Tigrina. Leaves with transverse whitish bands, lethal.

Maculata. Leaves with whitish dots, survived up to 45 days.

A perusal of Table 1 shows that gamma rays in isolation as well as in combination with EMS produced xantha and albina mutations in large number, followed by chlorina, maculata, viridis and tigrina, while EMS alone produced chlorina in maximum number followed by xantha, albina, viridis, maculata and tigrina in both forms of periwinkle. Albina was more common with gamma rays and in postirradiation treatments with EMS. The frequency of chlorophyll mutations like maculata, viridis, and tigrina was relatively low in all cases when compared to other mutations.

Table 1. Chlorophyll mutation frequency and spectrum in M_2 generation of *C. roseus*

Mutagen	Dose or duration	Form	Total seedlings	Frequency of chlorophyll mutations (%)						
				albina	xantha	chlorina	viridis	tigrina	maculata	total
Control	—	PF	620	—	—	—	—	—	—	—
		WF	528	—	—	—	—	—	—	—
Gamma rays	30 kR	PF	496	—	0.59	—	—	—	—	0.59
		WF	516	—	0.56	—	—	—	—	0.56
	40 kR	PF	385	0.92	2.01	0.72	—	—	0.61	4.26
		WF	495	0.60	1.36	0.79	—	—	—	2.75
	50 kR	PF	392	1.48	3.00	1.07	0.65	0.44	0.86	7.50
		WF	561	1.17	2.60	0.89	0.32	—	0.32	5.20
	60 kR	PF	493	3.03	4.57	2.48	0.85	0.57	0.85	12.35
		WF	485	2.36	4.03	2.03	2.70	0.36	0.70	12.18
EMS 0.1%	6 h	PF	520	—	0.77	1.14	—	—	0.05	1.96
		WF	369	—	0.63	0.84	—	—	—	1.47
	12 h	PF	386	0.85	1.05	2.08	0.65	—	0.70	5.33
		WF	385	0.53	1.03	1.78	0.28	—	0.55	4.17
	18 h	PF	469	1.36	2.25	4.03	0.92	0.47	1.02	10.05
		WF	525	1.06	3.00	3.39	0.55	0.29	0.81	9.10
	24 h	PF	389	2.33	4.11	6.00	1.31	0.80	0.62	15.17
		WF	620	1.75	3.47	4.90	0.89	0.60	—	11.61
Gamma rays 40 kR+EMS 0.1%	6 h	PF	526	1.01	2.38	1.59	0.42	—	0.62	6.02
		WF	484	0.24	1.31	1.10	—	—	—	2.65
	12 h	PF	592	2.24	4.22	3.12	0.91	0.47	1.13	12.09
		WF	486	1.68	3.12	2.92	0.65	0.24	1.06	9.67
	18 h	PF	620	3.16	5.81	4.37	1.48	0.99	1.96	17.77
		WF	495	2.49	4.38	3.23	1.02	0.52	1.51	13.15
	24 h	PF	600	5.78	9.03	6.53	2.28	1.53	3.03	28.18
		WF	386	3.55	7.57	5.31	1.79	1.04	2.29	21.55

PF—pink flowered, WF—white flowered.

DISCUSSION

In the present study, 0.1% EMS induced more chlorophyll mutations than gamma rays. Swaminathan et al. [6] and Mikaelsen et al. [7] reported EMS to be highly superior to gamma rays, inducing a higher frequency and wider spectrum of chlorophyll mutations in M_2 generation. Gaul [8] also showed that EMS induces about five times as many chlorophyll mutations as X rays in barley. However, Chopra and Swaminathan [9] reported negative synergism between EMS and ethylene imine (EI) for chlorophyll mutations in M_2 generation of emmer wheat.

The combination treatments of various mutagenic agents, if their mutation induction process is independent and capable of interaction, may increase mutation frequency and alter the mutation spectrum. Prasad and Das [4] and Aastviet [10] observed that the combination treatments of gamma rays and EMS enhanced frequency of chlorophyll mutations. The combination effects of many chemical mutagens have been demonstrated to be more than additive for chlorophyll mutations [11, 12].

In the present study, the combination treatments of gamma rays and EMS enhanced the frequency of chlorophyll mutations. The two forms of *Catharanthus roseus* also differ in their response to mutagens. The PF form responded better in terms of recovery of chlorophyll mutations than WF.

ACKNOWLEDGEMENT

The award of Research Associateship by UGC, New Delhi, to M. Venkateswarlu is gratefully acknowledged.

REFERENCES

1. A: Gustafsson. 1940. The mutation system of the chlorophyll apparatus. Lunds Univ. Arrskr. N. F. Avd., **30:** 1–40.
2. M. Venkateswarlu. 1986. Mutagenic Studies on *Catharanthus roseus*(L.) G. Don. Ph. D. Thesis, Kakatiya University, Warangal.
3. M. V. Rajam, N. Devadas and K. Subhash. 1984. Increased mutation frequency in *Capsicum* induced by combining X-rays with EMS. Indian Bot. Reptr., **3:** 125–128.
4. A. B. Prasad and A. K. Das. 1980. Studies of induced chlorophyll mutations in *Lathyrus sativus* L. Cytologia, **45:** 335–341.
5. C. S. Reddy and J. D. Smith. 1981. Mutagenic effects of combination treatments of hydrazine, ethyl methanesulfonate and gamma rays in *Sorghum bicolor* L. Moench. Indian J. Bot., **4:** 5–14.
6. M. S. Swaminathan, M. S. Naik, A. K. Kaul and A. Austin. 1970. Improving plant protein by nuclear techniques. *In*: Rice Breeding with Induced Mutations. IAEA Vienna: 165.
7. K. Mikaelsen, K. Karunakaran and I. A. Kiss. 1971. Mutagenic effectiveness and efficiency of gamma rays, fast nuetrons and EMS on rice. *In*: Tech. Rep. Ser. No. 103, IAEA, Vienna: 91–96.
8. H. Gaul. 1980. Critical analysis of the methods for determining the mutation frequency after seed treatment with mutagens. Genet. Agrar., **12:** 297–318.
9. V. L. Chopra and M. S. Swaminathan. 1966. Mutagenic efficiency of individual and combined treatments of EMS and HA in emmer wheat. Indian J. Genet., **26:** 58–62.
10. K. Aastveit. 1968. Effects of combination of mutagens on mutation frequency in barley. Mutations in Plant Breeding. Proc. IAEA Symp. II. Vienna: 5–14.
11. T. J. Arnason, J. L. Miocha and Laila Mohammed El Sadak. 1963. The effects of some combinations of mutagens on mutation frequency in barley. Genetics Today, **1:** 93–94.
12. E. A. Favret. 1963. Genetic effects of single and combined treatment of ionizing radiations and ethyl methanesulfonate on barley seeds. Proc. 1st Intern. Barley Genet. Symp., Wageningen: 68–81.