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INDUCED FLOWER COLOUR MUTATIONS IN CARNATION THROUGH *IN VITRO* APPLICATION OF CHEMICAL MUTAGEN

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ABSTRACT

Axillary bud explants of carnation were subjected to mutagenic treatment with ethyl methane sulphonate (EMS) under *in vitro* conditions through media supplementation (MS) (0.025, 0.050, 0.075 and 0.100 per cent) or explant agitation (EA) (0.25, 0.50, 0.75 and 1.00 per cent). EMS, at the dose rates of 0.025 and 0.050 per cent in culture medium and 0.25 per cent used for explant agitation, showed stimulatory effect on sprouting, number of shoots, number of flowers and flower initiation, while higher doses of 0.075 and 0.100 per cent under MS and 0.50, 0.75 and 1.00 per cent employed for EA caused damage to all vegetative and floral characters. Two colour mutants, viz. red with white stripes and pink with white stripes were isolated from the mutagen treated population.

Key Words : Carnation, *in vitro*, EMS, mutant

Carnation occupies unique position among cut flower crops due to its uses as cut flower and planting in beds, pots, borders etc. Due to increased preference of consumers with passage of time, it is imperative to improve the quality of flowers including colour novelties. Mutation breeding, using physical and chemical mutagens has been found to be a suitable tool for the improvement of quality of flowers in carnation[1]. *In vitro* mutagenesis technique has been proved to be an effective method in many vegetatively propagated crops [2] including carnations[3] to induce variability in economically important traits at higher pace. The present experiment was conducted to induce novel colours in carnation using chemical mutagen ethyl methane sulphonate (EMS) under *in vitro* conditions.

MATERIALS AND METHODS

Axillary bud explants of carnation cv. Espana were surface sterilised with 0.1 per cent mercuric chloride ($HgCl_2$). Sterilised explants were treated with ethyl methane

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sulphonate (EMS) either through media supplementation (MS) or through explant agitation (EA). Under media supplementation, half strength Murashige & Skoog (1962) [4] medium supplemented with BAP (0.65 mg/1), NAA (0.01 mg/1) and sucrose (30 g/1) was autoclaved and shifted to laminar air flow chamber. After cooling (about 55°C), the medium was distributed in five conical flasks and required volume of filter sterilised freshly prepared solution of EMS was added in four of these five flasks holding above medium to make four different concentrations of mutagen in medium, i.e., 0.025, 0.050, 0.075 and 0.100 per cent leaving one flask for control. These flasks were then shaken to mix the mutagen uniformly and dispensed in pre-sterilised culture tubes. The surface sterilised explants were inoculated on these media along with control (medium without mutagen). In experiment of explant agitation, surface sterilised nodal explants of carnation were agitated with different concentrations of EMS, viz. 0.25, 0.50, 0.75 and 1.00 per cent for half an hour and cultured on same establishment medium devoiding mutagen along with control. Cultures were maintained at 25 ± 2°C temperature under 16/8 h (light/dark) photoperiod. First sub-culturing for shoot proliferation was done after 21 days of inoculation on the same medium devoiding mutagen followed by second sub-culturing after 15 days. Proliferated shoots were transferred to half strength Murashige & Skoog (1962) [4] medium containing IBA (0.5 mg/1) and sucrose (50 g/1) for rooting. In each case, the pH was adjusted to 5.8 before adding agar (0.8 per cent w/v). Rooted microplants were transferred to plastic pots and hardened. Acclimatized plants were planted in field in the month of October. Data on various characters were recorded.

RESULTS AND DISCUSSION

The data presented in Tables 1 and 2 revealed that EMS applied at lower concentrations, either through MS (0.025 and 0.050 per cent) or through EA (0.25 per cent) had stimulatory effect on most of the parameters. Early sprouting and flowering were observed at these concentrations as compared to control. There was an increase in number of shoots per explant/plant (*in vitro*/*in vivo*), number of flowers per plant and petals per flower at lower concentrations. Stimulatory effect of low doses of EMS (0.025 and 0.050 per cent) was also seen in leaves and plants produced wider leaves with normal colour. Other characteristics like rooting percentage, survival of tissue- cultured plants, internodal length, plant height, length of flowering shoot, duration of flower, flower size were reduced at lower doses also as compared to control.

The higher doses of EMS, i.e., 0.075 and 0.100 per cent in culture medium and 0.50, 0.75 and 1.00 per cent under EA proved inhibitory for growth and development

Table 1. Effect of EMS on sprouting, survival and vegetative characters of carnation cv. Espana

Conc. of EMS (%)	Sprouting of axillary bud explants		No. of shoots per explant after 2nd sub-culturing	Rooting (%)	Survival of hardened tissue cultured plants in field (%)	No. of shoots per plant	Inter-nodal length (cm)	Plant height (cm)	Variations in leaf characters (%)
	Days required for sprouting	Sprouting (%)							
Media supplementation									
0.000	6.8	100.0	19.2	99.3	96.7	10.7	7.0	60.5	0.0
0.025	5.4	100.0	22.5	99.1	95.3	13.7	7.0	60.2	4.1
0.050	6.2	100.0	21.8	98.7	90.1	11.9	6.6	59.4	3.0
0.075	8.2	83.4	17.4	98.3	87.1	10.2	6.3	58.3	3.2
0.100	9.1	77.3	16.7	97.8	81.7	9.6	5.6	57.8	3.4
SEm ±	0.1	0.1	0.4	0.7	1.2	0.4	0.1	0.6	-
CD at 5%	0.2	0.3	1.1	2.1	3.9	1.2	0.2	2.0	-
Explant agitation									
0.00	6.8	100.0	19.2	99.3	96.7	10.7	7.0	60.5	0.0
0.25	6.2	100.0	21.1	97.7	94.1	12.5	6.6	59.9	0.0
0.50	8.7	82.3	17.8	96.9	90.2	10.2	6.5	58.9	0.0
0.75	9.1	64.1	17.2	96.5	85.4	9.8	5.9	58.0	5.1
1.00	9.2	58.2	16.6	96.4	79.6	9.4	5.8	57.4	5.7
SEm ±	0.1	0.3	0.3	0.9	1.3	0.3	0.05	0.4	-
CD at 5%	0.19	0.9	1.0	2.8	4.2	0.9	0.2	1.2	-

of plants and delayed sprouting and flower initiation. Among the plants derived from explants treated with higher concentrations, some plants showed chlorophyll deficiency in leaves. Maximum abnormalities in leaves were observed at 1.00 per cent concentration of EMS under EA. Number of shoots per explant/plant (*in vitro/in vivo*), flower per plant and petal per flower were also minimised at higher concentrations of EMS.

Table 2. Effect of EMS on flowering characters of carnation cv. Espana

Conc. of EMS (%)	Days to flower initiation	Length of flowering shoot (cm)	Duration of flowering (days)	Number of flowers per plant	Number of petals per flower	Flower size (cm)	Variations in flower colour (%)
Media supplementation							
0.000	148.69	36.8	28.9	6.3	47.5	6.1	0.0
0.025	144.14	36.6	33.8	6.9	49.4	6.1	0.0
0.050	145.21	36.4	32.6	6.5	49.0	6.0	0.0
0.075	151.34	35.9	26.0	5.9	45.5	6.0	1.6
0.100	153.67	34.9	25.1	5.9	45.2	5.8	1.7
SEm ±	0.1	0.4	0.1	0.1	0.3	0.1	-
CD at 5%	0.4	1.4	0.4	0.2	1.1	0.2	-
Explant agitation							
0.00	148.69	36.8	28.9	6.3	47.5	6.1	0.0
0.25	146.09	35.9	31.4	6.7	49.2	6.1	0.0
0.50	150.11	35.4	26.6	6.0	45.4	6.0	0.0
0.75	152.04	34.9	25.9	5.9	45.1	5.9	1.7
1.00	153.71	34.4	24.8	5.8	44.9	5.6	1.9
SEm ±	0.3	0.5	0.2	0.03	0.4	0.1	-
CD at 5%	0.9	1.5	0.6	0.1	1.2	0.5	-

It was observed from the data presented in Table 2 that there was no induction of colour mutations at lower doses, while higher doses induced variations in flower colour. A red colour mutant with white stripes along with petal length was induced at 0.075 and 0.100 per cent EMS (MS). Another colour variant (pink with white stripes) was also isolated from the population treated with the doses of 0.75 and 1.00 per cent EMS (EA). Frequency of the recovery of both the mutants was recorded more at later concentrations.

In the present experiment, moderate doses of EMS showed stimulatory effect on growth of some vegetative as well as floral characters. It may be due to the increased activity of enzymes involved in biosynthesis of hormones like auxins, cytokinins etc. in cell at lower doses of mutagen[5], which increases the growth of

cell and ultimately whole plant resulting in the increase in number of shoots, petals, advance flowering etc. Stimulatory effects of lower concentrations of chemical mutagens have also been reported by various workers [5, 6]. On the other hand, increase in the dose of chemical mutagens caused damaging effects on biological activities of plants which may be due to inactivation of cells because of mitotic disturbances/chromosomal aberrations at higher doses of EMS, leading to poor growth of the plants[6]. Induction of colour variations in carnation following EMS treatment has been reported earlier also[7], while chlorophyll and morphological variations due to EMS have been found in *Vicia faba* [8].

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