Indian J. Genet., **66**(1): 71-72 (2006)

Short Communication

In vitro approaches for chemical mutagenesis in carnation (*Dianthus caryophyllus*)

Tejaswini, Sona Chowdury, H. Paramesh and Neeraj Tripathi

Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bangalore 560 089

(Received: June 2005; Revised: January 2006; Accepted: February 2006)

Chemical mutagenesis is one of the major approaches for induction of mutation [1]. Subjecting the minimum unit of explant to mutagen can be achieved through induction of mutation under *in vitro* conditions of tissue culture where in pressure of diplontic selection is avoided and the opportunity for the mutated cell to survive gets increased [2]. Carnation (*Dianthus caryophyllus*) is a major cut flower crop grown commercially worldwide and is easily amenable for tissue culture. This study reports the effect of chemical concentration and the incubation duration of explant in mutagen on their survival and growth response. The study is aimed at identifying the right mode of mutagen application under *in vitro* condition. Two experiments were set up of which the first one dealt with incubation of explant in mutagen before culturing where as the second approach was incorporation of the mutagen into culturing media and subjecting the explant for mutageneis through-out the growing period.

Experiment 1: Incubation of explants in ethyl methane sulphonate (EMS): Nodes from *in vitro* shootlets were incubated in EMS at the concentrations of 0.1%, 0.5% and 1%. At each concentration explants were incubated for 15, 30 and 60 minutes. To compare the effect of mutagen, explants were incubated in distill water at various duration and subsequently cultured and observed in comparison with mutagen treated material. All the treatments including control had six replications in each. Controls as well as the treated explants were cultured on Murashige and Skoog (MS) media [3] supplemented with 3% sucrose, 0.25mg/l BAP, 0.25mg/l GA_3, 0.1mg/l NAA, solidified with 0.25% gelrite. Observations on survivability, growth and abnormality percentage were recorded under *in vitro* culture condition after two months of culturing. Formation of callus, occurrence of albino types and any other deviation from normal growth of shootlet were considered as abnormality. Direct effect of EMS concentration and duration of dipping along with their interaction effect was analyzed following factorial design of the experiment.

Experiment 2: Tissue culture on media incorporated with EMS: EMS was incorporated at three different concentrations of 0.01%, 0.05% and 0.1% in the MS media supplemented with 3% sucrose, 0.25mg/l BAP, 0.25mg/l GA_3, 0.1mg/l NAA, and 0.25% gelrite. Nodes from *in vitro* shootlets were cultured on this media. The experiment consisted of six replications in each of the concentration and control. Observations on survivability, growth and abnormality percentage were taken similar to that of experiment 1 and analysed as per Complete Randomised Design.

Results from first experiment are presented in Tables 1, 2 and 3. Survivability ranged from 79.15 % to 100 % across treatments (Table 1). Higher survival percentage indicates the treatment combination of various concentration and duration being not lethal to all cells of the explant used. These results also suggest the possibility of using higher dosage for selecting LD50 dosage. Number of explants showing growth got reduced with increased concentration of EMS. Explants incubated for 30 minutes in EMS exhibited maximum growth. A perusal of interaction effect indicated that treatment duration of 30 minutes could be attempted even at concentration higher than 1% (Table 2). Abnormality was not noticed in case of explants dipped in distilled water. Among various concentrations tried, maximum number of explants showed abnormal response at a concentration of 0.1%. Interaction effect in combination of 0.1 % concentration at 30 minutes resulted in maximum abnormality (Table 3). Surviving ability of treated explant was found to be independent of either EMS concentration or incubation duration or their interaction effect. Growth of the shootlet did change with varying concentration and dipping duration, but the interaction between EMS concentration and incubation duration did not have any significant effect on growth. EMS concentration, treatment duration as well as their interaction were found to have significant influence on abnormality recorded.

Table 1. Impact of EMS concentration and incubation duration on survival percentage of explants

Concentration of EMS	Incubation duration			Average due to concentration
	15 min.	30 min.	60 min.	
0.0%	80.84 (64.02)	100 (90.00)	100 (90.00)	86.95 (68.80)
0.1%	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
0.5%	100 (90.00)	85.00 (67.19)	100 (90.00)	(70.00) 86.38
1.0%	100 (90.00)	100 (90.00)	79.15 (62.81)	86.38 (68.32)
Average due to inucbation duration	87.71 (69.45)	88.75 (70.37)	87.29 (69.09)	

CD @5%: EMS concentrations - 5.78, Dip. Timings - 5.01, Interaction - 10.01 (Arc-sine transformed values are given in the parenthesis)

Table 2. Impact of EMS concentration and incubation duration on percentage of explants showing growth response

Concentration of EMS	Incubation duration			Average due to concentration
	15 min.	30 min.	60 min.	
0.0%	73.94 (59.28)	72.35 (58.25)	72.36 (58.26)	72.88 (58.59)
0.1%	65.16 (53.80)	82.49 (65.24)	52.04 (46.15)	66.56 (54.65)
0.5%	39.13 (38.71)	58.93 (50.12)	53.80 (47.16)	50.62 (45.34)
1.0%	58.93 (50.12)	59.99 (50.74)	39.13 (38.71)	52.68 (46.52)
Average due to incubation duration	59.29 (50.33)	68.44 (55.80)	54.33 (47.46)	

CD @ 5% - EMS concentrations -12.39, Dip. Timings - 10.73, Interaction - 21.45. (Arc-sine transformed values are given in the parenthesis)

Table 3. Impact of EMS concentration and incubation duration on abnormality observed during *in vitro* regeneration

Concentration of EMS	Incubation duration			Average due to concentration
	15 min.	30 min.	60 min.	
0.0%	0.00 (1.13)	0.00 (1.13)	0.00 (1.13)	0.00 (1.13)
0.1%	59.98 (50.74)	100 (90.00)	40.02 (39.23)	63.33 (52.71)
0.5%	0.00 (1.13)	59.98 (50.74)	48.29 (44.00)	36.09 (36.91)
1.0%	62.52 (52.23)	51.67 (45.94)	37.03 (37.47)	50.41 (45.22)
Average due to incubation duration	30.63 (33.59)	50.41 (45.22)	31.34 (34.03)	

CD @ 5% - EMS concentrations - 7.17, Dip. Timings- 6.21, Interaction -12.41 (Arc-sine transformed values are given in the parenthesis)

Survival percentage and percentage of explants showing growth was reduced with the increased concentration of EMS in media. Survival percentage had decreased to a minimal of 3.13 % at 0.1 % of EMS compared to 59.26% at 0.01% (Table 4). On the contrary, percentage of explants exhibiting abnormal response increased with the increased EMS concentration in media. Because of such a low survival rate, no abnormal response could be detected at higher concentration. Even at a minimum concentration of 0.01%, survival was very low indicating the possibility of getting more variation with the incorporation of lower concentration of mutagen into media.

Table 4. Effect of EMS concentration incorporated into culture media on percentage of explants survived and on percentage of explants exhibiting abnormal response

Concentration of EMS incorporated into media	Percentage of explants survived	Percentage of explants exhibiting abnormal response
0.01%	51.74 (59.26)	0.64 (0.11)
0.05%	42.35 (45.83)	2.51 (0.83)
0.10%	4.46 (3.13)	0.01 (1.13)

CD @ 5% - Percentage of explants survived - 7.01, Percentage of explants exhibiting abnormal response 0.83 (Arc-sine transformed values are given in the parenthesis)

Comparison of results considering survival, growth and abnormal response observed in both the experiments gives a clear indication of selecting the right method of treatment as well as mutagen concentration that can be used for mutagenesis. A lower concentration of EMS is sufficient for incorporation, whereas comparatively a higher concentration need to be used in case of explants being dipped in the mutagen.

Acknowledgements

Funding support received from DBT, India for the present work is thankfully acknowledged.

References

1. **Kharkwal M. C., Pandey R. N. and Pawar S. E.** 2004. Mutation Breeding for Crop Improvement. 601-646. *In*: "Plant Breeding — Mendelian to Molecular Approaches", H.K. Jain and M.C. Kharkwal (eds.), Narosa Publishing House (P) Ltd., New Delhi.
2. **Broertjes C. and Van Hasten A. M.** 1976. Ornamental crops. Development in crop science (2): Application of Mutation Breeding Methods in the Improvement of Vegetatively Propogated Crops. Elsevier Scientific Publishing Company, Amsterdam, pp.76.
3. **Murashige T. and Skoog F.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiology Plantarum, **15**: 473-497.