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Research Paper

In vitro MUTAGENESIS IN *Dahlia* Cav. USING ETHYL METHANE SULPHONATE

Wadankar, G. D.

Department of Botany,
R. A. Arts, Shri M. K. Commerce and Shri S. R. Rathi Science Mahavidyala,
Washim- 444505, Maharashtra State,
India.

Abstract

In vitro mutagenesis technology has been applied more frequently to the development of quality and to improve resistance traits, which has accelerated crop improvement and germplasm innovation. Ethyl methane sulphonate (EMS) is one of the potential chemical mutagens being frequently used for improving crop yield and quality. Effect of EMS on shoot formation, root formation, Plant survival in M_1 generation in field condition, Plant morphology in M_1 generation and M_1 plant variants was carried out. The rate of shoot and root formation and plant survival in M_1 generation was found to be decreased with the increasing concentrations of EMS. Data on plant variants in M_1 generation observed were dwarf plant, good plant type, early flowering, and striped magenta flower and weak plants were also recorded..

Key words: *In vitro* mutagenesis, *Dahlia*, Ethyl methane sulphonate (EMS).

INTRODUCTION

A survey of literature indicates that tissue culture of ornamental plants has been studied by various scientists using different types of explants (Zhang, 2002; Modh *et al.,* 2002; Tyagi and Kothari, 2004; Aswath and Wanzeen, 2004; Thakur *et al.,* 2004; Kumar and Kanwar, 2005, 2006; Sharma and Srivastava, 2005 and Ray *et al.,* 2005). The techniques for *in vitro* propagation of ornamental plants and tissue culture laboratory equipment are being continuously improved to meet the demand of the floriculture breeding and industry (Rout *et al.,* 2006). Tissue culture has become a routine technique in agricultural and horticultural development which has revolutionized the

ornamental industry and most popular application of this technique is micropropagation (Maira *et al.,* 2010; Bhattacharya and Bhattacharyya, 2010).

Mutagenic treatments increase the genetic variability, which can be utilized for selection and improvement of plants. Induced mutations have great potentials and serve as a complimentary approach in genetic improvement of crops (Mahandjiev *et al*., 2001). Induction of viable mutations by chemical mutagens has been reported by several workers e.g. Khanolkar (1977) in *Carum copticum*, Girhe (1999) in *Lathyrus sativus*, Landge (2000) in *Brassica napus*. Among the chemical mutagens, EMS is reported to be the most effective and powerful mutagen (Minocha and Arnason, 1962, Hajra, 1979).

MATERIALS AND METHODS

Dahlia accessions were procured from National Bureau of Plant Genetic Resources, New Dehi namely IC318964, IC318972, IC318973 and IC 318978. For the present study Acession-IC318964, was subcultured and mutiplied in laboratory of Post Graduate Department of Botany, Government Vidarbha institute of Humanities and Science, Amravati, was used for the present investigation.

Dahlia is a genus of bushy, tuberous, herbaceous perennial plants native mainly in Mexico, but also Central America, and Colombia. A member of the Asteraceae, dicotyledonous plants, related species include the sunflower, daisy, C*hrysanthemum* and *zinnia*. There are at least 36 species of *dahlia*, with hybrids commonly grown as garden plants.

Twenty test tubes with thirty days old callus produced by the protocol of Wadankar and Malode, 2012, were used for each mutagenic treatment to raise M_1 plant population. Different concentrations of EMS i.e. 0.002%, 0.004% and 0.006% were prepared,

Effect of EMS on shoot formation

The effect of different concentrations i.e. 0.002%, 0.004% and 0.006% EMS on shoot formation in *Dahlia* callus was studied.

Effect of EMS on root formation

The effect of different concentrations of i.e. 0.002%, 0.004% and 0.006% EMS on root formation on the shoots produced from *Dahlia* callus was studied.

Plant survival in M_1 generation in field condition

In vitro plants obtained from callus, they were gradually acclimatized in field condition. Data on plant survival percentage of 3h EMS treated callus of *Dahlia* in M_1 generation was recorded.

Plant morphology in M_1 generation

Plant morphology of *Dahlia* in M_1 generation i.e. plant height, total number of branches, number of sub-branches, number of leaves, length of leaves, breadth of leaves, total number of flowers, diameter of flower, number of florets, flower colour and stem colour per plant was recorded at the time of flowering stage.

M_1 plant variants

Data on plant variants in M_1 generation were observed were dwarf plant, good plant type, early flowering, and striped magenta flower, and weak plants is recorded at the time of flowering stage of plant.

RESULTS

Thirty days old calluses were treated with 0.002%, 0.004% and 0.006% EMS solutions for three hours separately. The calluses treated with sterilized distilled water served as control.

Effect of EMS on shoot formation

The effect of different concentrations of EMS on shoot formation in *Dahlia* callus is tabulated in table. 1. In control shoot formation was 71.67%. However, incorporation of EMS treatment to the callus adversely affected the rate of shoot formation. The rate of shoot formation was decreased by increasing the concentration of EMS. Percentages of shoot formation were 61.67%, 55.00% and 48.33% in 0.002%, 0.004% and 0.006% EMS, respectively. There was marked decrease in percentage of shoot formation over the control in all concentrations (13.95%, 23.26% and 32.57% in 0.002%, 0.004% and 0.006% EMS, respectively).

Table No. 1: Effect of EMS on shoot formation

Media composition	EMS (%)	Duration of Treatment (Hours)	No. of callus culture	Age of culture (Days)	Shoot formation (%)	Variability in shoot induction (%)
M.S. + BAP (0.5 mg/l)	Control	3	100	15	71.67	0
	0.002	3	100	15	61.67	1.33
	0.004	3	100	15	55	0.67
	0.006	3	100	15	48.33	0

Effect of EMS on root formation

The effect of different concentrations of EMS on root formation on the shoots produced from *Dahlia* callus is tabulated in table. 2. In control root formation was 78.33%. The rate of root formation was decreased by increasing the concentration of EMS treatment during shoot formation. The rate of root formation was 58.33%, 56.67% and 43.33% in 0.002%, 0.004% and 0.006% EMS, respectively. There was marked decrease in percentage of root formation over the control in all concentrations (25.53%, 27.65% and 44.68% in 0.002%, 0.004% and 0.006% EMS, respectively).

Table No. 2: Effect of EMS on root formation

Media composition	EMS (%)	No. of shoot culture	Age of culture (Days)	Root formation (%)	Variability in root induction (%)
M.S. + NAA (2.0 mg/l)	Control	100	25	78.33	0
	0.002	100	25	58.33	1.33
	0.004	100	25	56.67	0.67
	0.006	100	25	43.33	0

Plant survival in M_1 generation in field condition

In vitro plants obtained from callus, they were gradually acclimatized in field condition. Data on plant survival percentage of 3h EMS treated callus of *Dahlia* in M_1 generation is summarized in table. 3. The 56.14% plant survival was recorded in control. Increasing the concentration of EMS badly affected the plant survival percentage of *Dahlia*. The lowest 20.94% plant survival was recorded in 0.006% EMS.

Table No. 3: Plant survival in M_1 generation

Treatment	No. of callus culture	Plant survival (%)
Control	300	56.14
3h 0.002% EMS	300	35.97
3h 0.004% EMS	300	31.17
3h 0.006% EMS	300	20.94

Plant morphology in M_1 generation

Plant morphology of *Dahlia* in M_1 generation i.e. plant height, total number of branches, number of sub-branches, number of leaves, length of leaves, breadth of leaves, total number of flowers, diameter of flower, number of florets, flower colour and stem colour per plant was recorded at the time of flowering stage (Table No. 4). Decrease in plant height was recorded in treated EMS callus (69.7 cm in 0.002%, 73.1 cm in 0.004% and 69.3 cm in 0.006% EMS) as compared to control (79.9 cm). However, increase in total number of branches and sub-branches were found in the treated over the control. Total average number of leaves in control was 72. Whereas, increase in no. of leaves in 0.002% (79) and decreased in 0.004% (65) > 0.006% (47) over the control. Length of the leaves was also highest in 0.002% i.e. 10.6 cm followed by 0.004% i.e. 10.1 cm. In 0.006% (8.9 cm) smallest length of leaves were recorded. Breadth of leaves was smallest in 0.002% (4.1 cm) whereas largest in control (7.2 cm). Maximum fourteen numbers of flowers were recorded in 0.002% EMS. There were marked decreases in number of flowers with increase in concentration i.e. 12 in 0.004% and 10 in 0.006%. Diameters of flowers were increased in EMS treated plants. In 0.002% concentration showed maximum diameter (16.4 cm) over the control (12.3 cm). Maximum numbers of florets (233.3) were observed in 0.004% EMS. Control as well as treated showed red colour flower only. Brown colour stem was marked in the control and 0.006%, green in 0.002% and greenish brown in 0.004%.

Table No. 4: Plant morphology of *Dahlia* in M_1 generation

Plant characters	3h EMS treatment			
	Control	0.002%	0.004%	0.006%
Plant height (cm)	79.9	69.7	73.1	69.3
Total no. of branches	14.4	19.5	14.7	15.3
No. of sub-branches	2.3	2.8	8.1	3.8
No. of leaves	72	79	65	47
Length of leaves (cm)	9.5	10.6	10.1	8.9
Breadth of leaves (cm)	7.2	4.1	6.3	5.4
Total no. of flowers	6	14	12	10
Diameter of flower (cm)	12.3	16.4	14.3	5.7
No. of florets	176.9	204.5	233.3	160.7
Flower colour	Red	Red	Red	Red
Stem colour	Brown	Green	Greenish brown	Brown

M_1 plant variants

Data on plant variants in M_1 generation observed were dwarf plant, good plant type, early flowering, and striped magenta flower and weak plants is summarized in table 5. Plants obtained in M_1 generation were screened for plant variations. Dwarf plant variations were observed in EMS treatments. The frequency of dwarf plant variation in 0.002% EMS was 0.93 and in 0.006% EMS was 1.61. Good plant type was also recorded. It was observed in lower concentration of EMS i.e. 0.002% (3.74). EMS treatments showed early flowering. The frequency of early flowering in 0.004% EMS was 2.15 and 0.006% EMS was 1.61. Striped magenta flower were recorded in lower treatments of EMS. 0.002% EMS showed 0.93 frequencies with single plant variations. Weak plant type was also found. A couple of plants were observed in 0.004% EMS frequencies of weak plants.

Table No. 5: Plant variants in M_1 generation

Plant Variants	EMS		
	0.002%	0.004%	0.006%
	No. of plants screened		
	107	93	62
Dwarf plants	1 (0.93)	-	1 (1.61)
Good plant type	4 (3.74)	-	-
Early flowering	-	2 (2.15)	1 (1.61)
Striped magenta flower	1 (0.93)	-	-
Weak plant type	-	2 (2.15)	-

Figure in parenthesis denote frequency

DISCUSSION:

In general the most common manifestation of induced mutagenesis is the reduction in plant height (Malode, 1995). Mutants with much lesser plant height were recorded as dwarf mutants**.** Bolbhat *et al.,* (2012) recorded in *Horsegram* (*Macrotyloma uniflorum*) dwarf mutants which ranged from 15 to 20 cm and had profuse branching at the base which formed a dense umbrella like canopy. Induction of early flowering/maturity is one of the most frequent characters modified in the mutation experiments in all the crops. In oil seed *Brassica* crops, several early flowering/maturity have been reported (Pawar *et al.,* 1991).

Plant height was also found to be significantly reduced at higher doses of mutagenic treatment but some of the plants at lower doses respond positively to mutagen and recorded a slight increase in plant height (Kumar and Yadav, 2010). The successful utilization of sodium azide to generate genetic variability in plant breeding has been reported in barley (Kleinhofs and Sander, 1975) and other crops (Micke, 1988; Routaray, *et al.,* 1995).

Mutations affecting the plant height have been reported by Karpate and Choudhary (1997a, 1997b), Girhe and Choudhary (2002).

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