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Research Paper

In vitro MUTAGENESIS IN *Dahlia* Cav. USING SODIUM AZIDE

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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. Sodium azide (SA) is one of the potential chemical mutagens being frequently used for improving crop yield and quality. Effect of SA 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 SA. 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*, Sodium Azide (SA).

INTRODUCTION

Genetic variations are the basic tools in the hands of breeders to develop new cultivars with better traits, like tolerance against various environmental stresses, resistance against pests and diseases, and improved yield and quality. Thus, the mutagenesis technology has been applied to plant breeding comprehensively, which allowed crops to produce benefi cial varieties with good traits (Maluszynski *et al.,* 1995; Gu *et al.,* 2003) . Tissue culture techniques have been used to induce genetic variability to improve crop plants (Larkin and Scowcroft 1981). Various *in vitro* techniques are available for most crops, although optimization is still needed for some of them. *In vitro* techniques of protoplast, microspore, anther, ovule, and embryo culture have been used to create somaclonal and gametoclonal variation (Brown and Thorpe 1995). Now, we have better and efficient techniques like *in vitro* mutagenesis by combining both tissue

culture techniques and induced mutation strategy. Tissue culture techniques are utilized to create *in vitro* alterations because they have a number of advantages, like a number of plant materials (e.g., *in vitro* axillary buds, organs, tissues, and cells) can be treated and handled easily. Easy handling of large populations for mutagenic treatment, selection, and cloning of selected variants and the rapid execution of the propagation cycles of subculture aimed to separate mutated from nonmutated sectors (dissolving a chimera to obtain homo-histonts) (Ahloowalia *et al.,* 1998) are further significant contributions of tissue culture techniques.

In recent years, *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 (Arene *et al.,* 2007). *In vitro* culture, especially microspore culture, in combination with induced mutations such as using physical mutagens (UV, gamma, X-ray, and so on), chemical mutagens (EMS, NaN_3 , colchicines, herbicides, salinity, silver nitrate, and so on), and plant growth regulators (GA, IAA, BAP, JA, and so on) has been extensively used to speed up breeding programs, from the generation of variability, through selection and multiplication of the desired genotypes (Maluszynski *et al.,* 1995). The *in vitro* culture of propagated crops in combination with induced mutations has proved to be a valuable method to produce desired variation, and to rapidly multiply the selected mutants and parental material in a disease-free condition (Maluszynski, 2001). *In vitro* culture methods have facilitated the use of mutation technique for improvement of both seeded and vegetatively propagated crops (Jain and Maluszynski, 2004).

Sodium azide (NaN_3) is one of the potential chemical mutagens being frequently used for improving crop yield and quality (Al-Qurainy and Khan, 2009). The mutagenic capability of sodium azide has been reported to occur due to the generation of an organic metabolite of azide compound (Owais and Kleinhofs, 1988) which interacts with cellular enzymes and DNA. Being a powerful mutagen, it can affect different parts of the plants by affecting a variety of metabolic phenomena involved in growth and development (Al-Qurainy and Khan, 2009).

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.

Sodium azide is a colourless and odourless crystal with molecular weight 65.01 gms. The chemical formula is NaN_3. At 275 ^{0}C it decomposes into sodium and nitrogen. It has potential in tissue culture mutagenesis for inducing mutation because it induces single base pair substitution or deletion which results mostly in small point mutation.

Different concentrations of EMS and SA ranging from 0.002% to 0.006% and 0.001% to 0.003% were prepared, respectively.

Effect of SA on shoot formation

The effect of different concentrations of i.e. 0.001%, 0.002% and 0.003% SA on shoot formation in *Dahlia* callus was studied.

Effect of SA on root formation

The effect of different concentrations of i.e. 0.001%, 0.002% and 0.003% SA on root formation on the shoots produced from *Dahlia* callus was studied.

Plant survival in M_1 generation in field condition

Data on plant survival percentage of 3h SA 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 0.001%, 0.002% and 0.003% of SA solutions for three hours separately. The calluses treated with sterilized distilled water served as control.

Effect of SA on shoot formation

The effect of different concentrations of SA on shoot formation in *Dahlia* callus is tabulated in table 1. In control shoot formation was 71.67%. However, incorporation of SA treatment to the callus adversely affected the rate of shoot formation. The rate of shoot formation was decreased by increasing the concentration of SA. Percentages of shoot formation were 59.33%, 50.67% and 43.33% in 0.001%, 0.002% and 0.003% of SA respectively. There was marked decrease in percentage of shoot formation over the control in all concentrations (17.22%, 29.30% and 39.54% in 0.001%, 0.002% and 0.003% SA, respectively).

Table No. 1: Effect of SA on shoot induction

Media composition	SA (%)	Duration of Treatment (Hours)	No. of callus culture	Age of culture (Days)	Shoot Induction (%)	Variability in shoot induction (%)
M.S. + BAP (0.5 mg/l)	Control	3	100	15	71.67	0
	0.001	3	100	15	59.33	0
	0.002	3	100	15	50.67	0.33
	0.003	3	100	15	43.33	0

Effect of SA on root formation

The effect of different concentrations of SA on root formation on the shoots produced from *Dahlia* callus is tabulated in table 2. In control the root formation was 78.33%. The rate of root formation was decreased by increasing the concentration of SA treatment during shoot formation. The rate of root formation was 53.33%, 50.67% and 41.67% in 0.001%, 0.002% and 0.003% SA, respectively. There was marked decrease in percentage of root formation over the control in all concentrations (31.91% in 0.001%, 35.31% in 0.002% and 46.80% in 0.003% SA).

Table No. 2: Effect of SA on root formation

Media composition	SA (%)	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
	0.001	100	25	53.33	0
	0.002	100	25	50.67	0.33
	0.003	100	25	41.67	0

Plant survival in M_1 generation in field condition

Data on plant survival percentage of 3h SA treated callus of *Dahlia* in M_1 generation is summarized in table 3. The 56.14% plants were survived in control. In SA treated callus significantly affected survival percentage of *Dahlia* in field condition. The lowest 18.06% plant survival was recorded in 0.003% SA.

Table No. 3: Plant survival in M_1 generation

Treatment	No. of callus culture	Plant survival (%)
Control	300	56.14
3h 0.001% SA	300	31.64
3h 0.002% SA	300	25.67
3h 0.003% SA	300	18.06

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). Maximum

height was recorded in control (68.7 cm) and lowest in 0.002% (54.7 cm). 56.9 total number of branches were recorded to be maximum in 0.003% SA with lowest number of branches in control (24.6). Total number of sub-branches was highest in control (8.7) and lowest 2.4 in 0.001% SA and further increase in no. of sub-branches in 0.002% SA (4.9) and 0.003% SA (8.1). Total number of leaves in control was 151. In 0.001% SA, lowest numbers of leaves (120) were noted. Increasing the concentration of SA, there were marked further increase in total number of leaves i.e. 155 & 206 in 0.002% & 0.003% SA, respectively. Maximum length and breadth of leaves i.e. 9.3 cm and 6.2 was recorded in 0.002% SA, respectively, whereas lowest length and breadth of leaves i.e. 5.3 cm and 3.6 cm, was recorded in 0.003% SA. In control, length (8.2 cm) and breadth (5.8 cm) was recorded. Maximum ten numbers of flowers was recorded in 0.001% SA. In control average six numbers of flowers was recorded. 15.3 cm diameter and 256.9 numbers of florets of flower were noted as maximum in 0.001%. Pink colour flowers were observed in control, light pink in 0.001% and 0.002% and dark pink in 0.003%. The colour of stem was noted as green in control and treated.

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

Plant characters	3h SA treatment			
	Control	0.001%	0.002%	0.003%
Plant height (cm)	68.7	58.9	54.7	57.3
Total no. of branches	24.6	47.3	39.4	56.9
No. of sub-branches	8.7	2.4	4.9	8.1
No. of leaves	151	120	155	206
Length of leaves (cm)	8.2	8.5	9.3	5.3
Breadth of leaves (cm)	5.8	4.4	6.2	3.6
Total no. of flowers	6	10	7	6
Diameter of flower (cm)	12.5	15.3	9.3	5.4
No. of florets	232.4	256.9	207.8	96.3
Flower colour	Pink	Light Pink	Light Pink	Dark Pink
Stem colour	Green	Green	Green	Green

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 SA treatments. The frequency of dwarf plant variation in 0.002% SA (2.60) dwarf plant variation was noted. Good plant type was also recorded in SA treatments. SA treatment showed early flowering. Only single plants were

recorded in 0.001% SA and 0.003% SA treatments with frequencies 1.06 and 1.85, respectively. Striped magenta flower were recorded in lower treatment SA. 0.001% SA showed 1.06, frequencies with single plant variations. Weak plant type was also SA treatment.

Table No. 5: Plant variants in M_1 generation

Plant Variants	SA		
	0.001%	0.002%	0.003%
	No. of plants screened		
	94	77	54
Dwarf plants	-	2 (2.60)	-
Good plant type	-	-	1 (1.85)
Early flowering	1 (1.06)	-	1 (1.85)
Striped magenta flower	1 (1.06)	-	-
Weak plant type	-	-	3 (5.56)

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). Similar type of experiments were carried out using Ethyl methane sulphonate (EMS) as chemical mutagen (Wadankar, 2018)

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