Journal of Environmental and Applied Bioresearch
Published online Oct 01, 2015 (http://www.scienceresearchlibrary.com)

ISSN: 2319 8745
Vol. 03, No. 04, pp. 234-237

Research Article **Open Access**

EFFECTS OF MUTAGEN TREATMENT ON THE SEED GERMINATION AND CALLUS INDUCTION IN *DENDROCALAMUS HAMILTONII*

Abhishek Kumar[1], Meena Bakshi[2], Manpreet Kaur[2*], Shatakshi Kapruwan[2], Manish Singh[1]

School of Biotechnology, Gautam Budha University, Greater Noida

Plant Physiology Discipline, Forest Research Institute Deemed University, Dehradun

Received: July 06, 2015 / Accepted : September 04, 2015

Abstract

The present study was conducted to determine the effect of different doses of six chemical mutagens i.e. ethyl methane sulphonate (EMS), Colchicine, PEG, Sodium Azide, 2,4-D and Acridine Orange on seed germination and callus induction in *Dendrocalamus hamiltonii*. The aim of this study was to identify the effects of different percentages of mutagen on the seed germination, root and shoot growth and *in vitro* callus induction for determining most effective dose for successful mutagenesis research studies. For this purpose, the seeds of *Dendrocalamus hamiltonii* were treated with 0.001, 0.01, 0.1, 0.2, and 0.3% EMS, Colchicine, PEG, Sodium Azide, 2,4-D and Acridine Orange doses. Germination percentage of seed, survival percentage of seedling, callus induction from seeds and callus growth were evaluated in comparison to the untreated materials. Effect of mutagen treatments showed statistically significant differences among the evaluated characters. The seed germination percentage decreased with increase in the concentration/doses when compared to control. The percent callus induction delayed with the increase in the concentration of mutagen. Maximum percent germination (92 %) was observed in control followed by 0.001% PEG (53.3%), 0.001% 2,4-D (and 0.01% Acridine orange (50%).

Key words: *Dendrocalamus hamiltonii*, EMS, Colchicine, PEG, Sodium Azide, 2,4-D and Acridine Orange

*Corresponding author: manpreetchinna@gmail.com

Background:

For breeding of economically important traits, by means of inducing genetic variations, mutations have been successfully used in several crops. The use of conventional mutation techniques aimed for improvement in yield, disease and pest resistance and quality in crops. Many mutant varieties involving more than 100 plant species have been officially released (Bahar and Akaya, 2008).

The use of chemical mutagens is a very popular way to induce mutation which includes ethyl methane sulphonate (EMS), Colchicine, Polyethylene Glycol, Sodium Azide, 2,4-Dichlorophenoxy acetic acid and Acridine Orange etc. EMS as being alkylating agent, alkylates guanine bases and leads to mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C- to-A/T transitions (Bhat *et al*., 2007). EMS causes point mutations and deletion in some extent in plants (Okagaki *et* al., 1991) whereas, Colchicine is a chromosome doubling agent that possesses antimicrotubular action (Roychowdhary and Tah., 2011). Polyethylene glycols (PEGs) are a wide group of polymers of ethylene oxide, widely used as vehicles or co-solvents in many pharmaceutical and cosmetic preparations, induces chromosomal aberrations (Biondi *et al*., 2002). PEG is used for introduction of a mutagenic nucleobase into plant protoplasts and recommended for enhancing targeted mutagenesis (Bundock *et al*., 2010), whereas Acridine Orange, an aromatic compound, is intercalating agent, favouring insertions and deletions of nucleotide bases upon replication and results in the frame shift (Arshad *et al*., 2005). 2,4-Dichlorophenoxy acetic acid is common systemic herbicide that causes chromatin and chromosome abnormalities in plant cells (Pavlica *et al*., 1991). Sodium Azide (NaN_3), a common bactericide, pesticide is known to be highly mutagenic in several plants and animals. It is metabolized by plant cells to the mutagenic agent presumably azidoalanine, this metabolite enters in nucleus, interacts with DNA and creates point mutation (Al-Qurainy and Khan., 2009).

These mutagens provide an advantage for the plant breeders to obtain useful alleles, over using exotic or wild germplasms in which the group of linked deadly alleles can be present. The most important parameters for inducing mutation with chemical mutagens are concentration and duration of treatment as the high concentrations of the mutagen cause great biological damage. So it is necessary to have knowledge about the various concentrations, effect of time, temperature, pH value, seed soaking for enhancing the seed germination, lethality, pollen sterility and metrical traits, (Khan *et al.*, 2009)

The *Dendrocalamus hamiltonii* belongs to family Poaceae, commonly called 'maggar', is economically important species which is distributed in the North- West Himalaya, Sikkim, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura of India, Bhutan and Bangladesh. It is used as a source of nutritive green fodder for the cattle, especially during winter and is propagated through seed, stem or rhizome cuttings (Bag *et al.*, 2012).

The present study was undertaken to assess effect of chemical mutagens on the seed and seedling characteristics of *Dendrocalamus hamiltonii* apart from callus induction in this species .

Material and Methods:

Plant Material

The experimental material consisted of seeds of *Dendrocalamus hamiltonii* collected from central nursery, Forest Research Institute, Dehradun.

Mutagen Treatment

The chemical mutagen, ethyl methane sulphonate (EMS), Colchicine, Polyethylene Glycol, Sodium Azide (NaN_3), 2,4-Dichlorophenoxy acetic acid and Acridine Orange (Hi media) were used for treating the seeds. For germination studies, Six hundred seeds of *Dendrocalamus hamiltonii* were placed in Six 500ml flasks separately (100 seeds each) and distilled water was added to about 5cm level above the seeds (100ml). Seeds were soaked in distilled water for 24 hours at room temperature. Then twenty presoaked seeds for each treatment were treated with 50ml of 0.001%, 0.01%, 0.1%, 0.2% and 0.3% of ethyl methane sulphonate (EMS), Colchicine, Polyethylene Glycol, Sodium Azide (NaN_3), 2,4- Dichlorophenoxy acetic acid and Acridine Orange solution prepared in distilled water for 30 minutes. One set of seeds was kept untreated to act as control for comparison. Seeds were incubated for 12 hours at room temperature with constant intermittent shaking. The treated seeds were washed under running tap water for 1 hour to remove the excess chemical before planting. Finally seeds were sown for each treatment along with the control in Pots filled with fresh manured soil.

For Callus induction, the seeds were treated with 0.001%, 0.01%, 0.1%, 0.2% and 0.3% of ethyl methane sulphonate (EMS), Colchicine, Polyethylene Glycol, Sodium Azide (NaN_3), 2,4-Dichlorophenoxy acetic acid and Acridine Orange solution for varying period i.e 12 hr and 24 hr at room temperature. The explants were prepared and sterilized with Bavistin (1% w/v) for 3 minutes. Explants were treated with 70% ethanol for 60 seconds and mercuric chloride (0.1% w/v) for 5 minutes followed by washings with sterile distilled water for 3 to 4 times to remove the traces of $HgCl_2$. The explants were inoculated on MS medium fortified with 3% sucrose and supplemented with various concentrations of auxin 2,4-D. The pH of the media was adjusted at 5.8 before solidifying the medium with 0.8% agar (Himedia). The cultures were incubated at 25 ± 1°C with a photoperiod of 16 h at 3000 lux light intensity of cool white fluorescent light. All the experiments were repeated twice with 10 cultures per treatment. Visual observation of culture was made every day and data were recorded after 2 weeks of inoculation.

Results and Discussion

The different mutagen treated seeds of *Dendrocalamus hamiltonii* were sown in pots in the glass house, analysed for germination percentage. It was found that the seeds were slow to germinate in comparison to control. The highest germination percentage i.e. 92.1±0.16 %, was observed under the control on day 10 followed by with 0.001% PEG, 0.001% 2, 4-D and 0. 01% Acridine orange (Fig.1).

A close perusal of Table 1 reveals that though there was initial increase in seed germination percentage but after a span of time , a significant reduction in seed germination with increase in mutagen concentration in most of the cases was observed. The percent survival of seedlings under different treatments is also shown in the table 1. The seed germination percentage was in the range from 26.66±0.47 to 53.33±0.34 % under PEG treated seeds. The increasing concentration of PEG inhibited the seed germination, as polyethylene glycol (PEG) solutions have been used to control water potential in numerous seed germination investigations. Similar findings were observed in other species (Emmerich and Hardegree, 1990).

Colchicine is one of the most commonly used spindle inhibitors in numerous plants (Hancock 1997), including some trees and other woody species (Kadota and Niimi 2002; Shao et al. 2003).The seeds treated with different concentrations of colchicine showed initial increase in seed germination and then continuous decrease up to last treatment i.e. 0.3% colchicine treated seeds. The seed germination was found maximum at 0.001 % colchicine treated seeds i.e. 46.33±0.43%. The Sodium Azide seeds showed similar pattern as observed in seeds treated with PEG. Similar results were observed in pea species (Divanli-Turkan *et al*., 2006). Sodium Azide is least dangerous and the most efficient mutagen as its yields of mutations are achieved at moderate sterility rates (Ali *et al*., 2014). It is known for delay in germination (Fahad and Salim, 2009). The maximum seed germination found in sodium azide treated seeds was 20%, whereas the lowest concentration of sodium azide treated seeds showed 13.33±0.02% germination. Similar decline in % germination was observed in lentil seeds too (Ali *et al*., 2014). In case of EMS, which is known as most effective and powerful mutagen, among the all concentrations, 0.2% and 0.1% EMS treated seeds showed the highest germination percentage. The increase in seed germination in EMS treated seeds, followed with gradual decrease was also observed in seasame also (Anabarsan *et al*., 2013).

Table 1. Effect of Different mutagens on seed germination and percent survival of seedlings in *Dendrocalamus hamiltonii*

Mutagens	Concentration %	Germination Percentage (%)	Survival seedlings Percentage (%)
Control	NA	92.1±0.16	90.17±0.23
PEG	0.001	53.33±0.43	42.23±0.23
PEG	0.01	40.00±0.12	38.24±0.74
PEG	0.1	36.66±0.33	31.27±0.63
PEG	0.2	30.00±0.42	29.85±0.69
PEG	0.3	26.66±0.21	19.43±0.43
Col	0.001	46.66±0.32	36.58±0.64
Col	0.01	33.33±0.95	29.96±0.44
Col	0.1	36.66±0.10	34.84±0.11
Col	0.2	26.66±0.31	23.75±0.83
Col	0.3	16.66±0.34	10.47±0.72
SA	0.001	20.00±0.19	15.85±0.62
SA	0.01	20.00±0.43	17.74±0.82
SA	0.1	16.66±0.87	11.83±0.19
SA	0.2	16.66±0.49	15.73±0.10
SA	0.3	13.33±0.32	10.34±0.52
EMS	0.001	23.33±0.65	21.43±0.73
EMS	0.01	16.66±0.78	15.33±0.52
EMS	0.1	26.66±0.09	25.35±0.83
EMS	0.2	26.66±0.35	25.42±0.63
EMS	0.3	23.33±0.91	19.34±0.42
2,4-D	0.001	50.00±0.63	48.37±0.62
2,4-D	0.01	36.00±0.69	34.38±0.72
2,4-D	0.1	40.00±0.21	37.72±0.83
2,4-D	0.2	20.00±0.16	17.42±0.62
2,4-D	0.3	20.00±0.48	14.85±0.t2
AO	0.001	36.66±0.38	30.33±0.73
AO	0.01	50.00±0.72	43.69±0.43
AO	0.1	23.33±0.71	19.83±0.10
AO	0.2	16.66±0.83	10.66±0.19
AO	0.3	16.66±0.14	14.83±0.47

Germination percent and percent survival of seedlings results are shown as mean±SD

Acridine orange is intercalating agent that favours deletion and addition of nucleotide bases during replication. Acridine orange treated seeds showed the same pattern as shown by the seeds treated with different concentrations of EMS with a maximum of 50±0.22% germination under 0. 01% Acridine orange treatment, whereas the maximum germination of 50.0±0.43% was noticed in 2,4-D treated seeds that decrease upto 36.66±0.24% and showed a sharp decline. Negative effect of 2,4-D on seed germination was reported earlier (Kamble *et al*., 2006).

The mutagenic effect of different concentrations of ethyl methane sulphonate (EMS), Colchicine, Polyethylene Glycol, Sodium Azide, 2,4- Dichlorophenoxy acetic acid and Acridine Orange on callus induction and callus growth were examined. Fig 2, shows the graphical representation of percent callus formation in *Dendrocalamus hamiltonii*. Callus mostly introduces somaclonal variation induction and the high regenerative potential of seeds make them a good candidate for plays important role in mutagenesis.

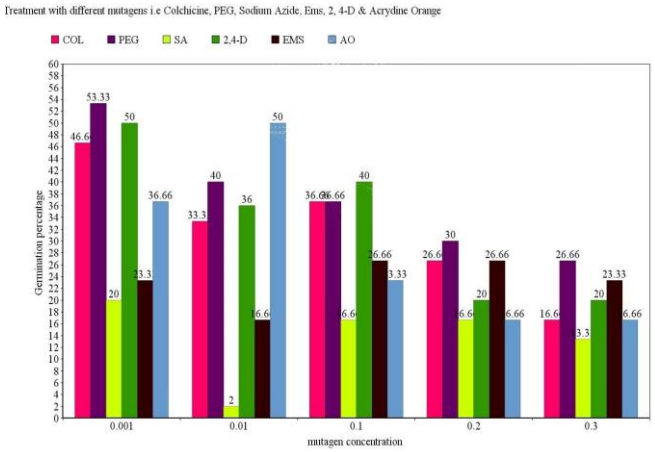

Fig 1. Effect of different mutagens on seed germination of *Dendrocalamus hamiltonii*

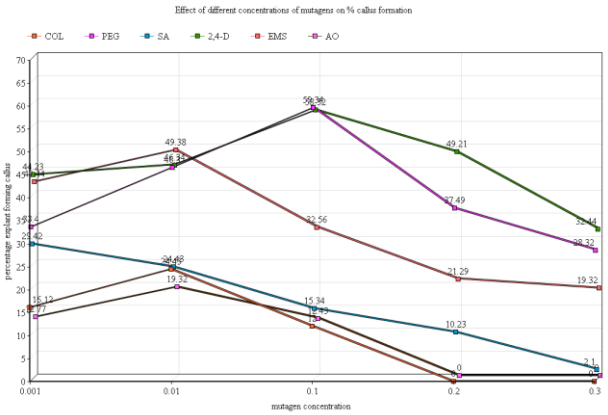

Fig 2. Callus formation response (%) of *Dendrocalamus hamiltonii* under different mutagen treatment

In the present study the maximum callus induction (%) was noticed 59.34±0.1% in the seeds treated with 0.01% PEG followed by 58.32±0.21% in 0.1% 2,4-D treated seeds and 49.38±0.52% in 0.01% EMS treated seeds (Table 2). It was observed that at higher concentration of Sodium azide significantly suppressed the growth of callus. In high concentrations of mutagen colchicine and Acridine orange i.e. more than 0.1% , there was no callus formation observed in *Dendrocalamus hamitonii*. It is supported by the fact that higher dose of mutagens can lead to death of cells (Kleinhoff's *et al*., 1978). The EMS treated seeds showed increase in callus formation up to 0.1% concentration, beyond this, there was gradual decline was observed in the percent callus induction. The callus induction in seed explants treated with different concentration of sodium azide and colchicine was delayed with the increase in the concentration of the mutagens. Similar results were reported in leaf explants of *Stevia rebudiana* (Pande and Khetmalas, 2012). The use of mutagens is common in breeding programmes as to improve the plant traits. These results indicate that 2,4-D is more effective in the range of 0.001% to 0.2% for callus induction, where the % percent callus induction is about 44% to 58% noticed as compared to other mutagens. The seeds treated with PEG also showed good results in callus induction ranging from 28.32±0.3% in 0.3% PEG to 59.34±0.1 in 0.1% PEG treated seeds.

Table 2. Effect of different mutagen on % Callus formation in *Dendrocalamus hamiltonii*

Mutagen	Concentration %	% explant forming callus
Colchicine	0.001	16.12±0.21
	0.01	24.43±0.32
	0.1	12.0±0.12
	0.2	0
	0.3	0
Polyethylene Glycol(A)	0.001	33.4±0.71
	0.01	46.3±0.54
	0.1	59.34±0.1
	0.2	37.49±0.22
	0.3	28.32±0.3
Sodium Azide	0.001	29.42±0.18
	0.01	24.43±0.72
	0.1	15.34±0.30
	0.2	10.23±0.51
	0.3	2.1±0.41
2,4-D	0.001	44.23±0.29
	0.01	46.34±0.19
	0.1	58.32±0.21
	0.2	49.21±0.80
	0.3	32.44±0.73
EMS	0.001	42.44±0.58
	0.01	49.38±0.52
	0.1	32.56±0.69
	0.2	21.29±0.26
	0.3	19.32±0.32
Acridine Orange	0.001	12.77±0.15
	0.01	19.32±0.19
	0.1	12.43±0.36
	0.2	0
	0.3	0

Conclusion:

The present investigation was carried out to study the effect of various mutagens on seed germination and callus induction in *Dendrocalamus hamiltonii*. The effect of (EMS), Colchicine, Polyethylene Glycol, Sodium Azide (NaN_3), 2,4-Dichlorophenoxy acetic acid and Acridine were determined by observing the germination percentage, survival percentage and callus induction under field and laboratory conditions.

A significant reduction in seed germination, and survival percentage was observed in seedlings with increasing concentration of mutagen. The germination percentage was high in seeds treated with different concentrations of PEG and callus induction studies also showed the same results. The mutagens are known for inducing changes in populations on genetic level. The effective dose of mutagens determined from this study can be used further to study the somaclonal variations in *Dendrocalamus hamiltonii*.

Acknowledgement: Authors are thankful to Director, Forest Research Institute, Dehradun for providing laboratory facility.

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