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Effect of different concentration of EMS on mutations and survival of banana cv. Grand Naine (AAA) and Rajapuri (AAB)

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Abstract
Multiple *in vitro* shoot cultures of two banana cultivars *viz.,* Rajapuri (AAB) and Grand Naine (AAA) were treated with EMS (Ethyl Methane Sulphonate) at different concentration (Control, 0.1%, 0.2%, 0.3%, 0.4% and 0.5%) to create genetic variability through mutation. The interpretation of data pertaining to shoot multiplication ratio revealed that there was significant differences for EMS treatment at I, II and III sub culture stage. The maximum shoot multiplication ratio was observed in water treated plantlets which was found statistically on par with plantlets treated with 0.1, 0.2 and 0.3 per cent of EMS in all the stages of sub culture. However the lowest shoot multiplication ratio was observed in plantlets treated with higher concentration of 0.5 per cent EMS. There was no dwarfing mutants in the plantlets treated with 0.1, 0.2 and 0.3 per cent EMS concentration in all the subculture stages. However, the maximum per cent dwarfing mutants was found in plantlets treated with 0.5 and 0.4 per cent EMS concentration in all the culture cycle. Interactions at II subculture suggested that, Rajapuri plantlet treated with 0.5 per cent EMS concentration had maximum dwarfing mutants. The interpretation of data pertaining to per cent survival among plantlets exposed to different concentration of EMS revealed fewer variations. There was no mortality in plant culture treated with 0.1, 0.2 and 0.3 per cent EMS concentration and control (water treated plant culture). Where as there was marginal reduction in per cent survival of plantlets in plant culture treated with 0.4 and 0.5 per cent EMS.

Keywords: Banana, EMS, mutation, grand Naine and Rajapuri

Introduction
Banana (*Musa* spp.) is the best known tropical fruit popular among masses. It is one of the economically important fruit crop grown in Karnataka both in homestead and commercial farms. Banana culture in India is as old as Indian civilization and is one of the earliest fruit crops grown by mankind at the dawn of civilization. Banana could be considered as one of the cheapest among all other fruits in the country. India has 0.80 million hectare of area under banana cultivation with production of 29.72 million tonnes and productivity of 35.90 metric tonnes per hectare (Anon., 2014). Banana is propagated vegetatively through suckers. Since most of the edible bananas are triploid and are nearly sterile and parthenocarpic, the use of conventional breeding methods for their improvement is difficult and cumbersome. Mutation breeding and biotechnological methods can offer as alternate tools for banana improvement. Mutation breeding *in vitro* is a powerful tool for the induction and isolation of desirable mutants which can be utilized in banana improvement either for higher yields, good quality and resistance to biotic and abiotic factors. The use of *in vitro* cultures in mutation breeding of bananas and plantains offers several advantages over the *in vivo* techniques including obtaining shoot-tips from pre-existing cultures, ease of separating chimera and recovering mutants and rapidly micro-propagating them. Induced mutation by treatment of *in vitro* material with chemical agents, such as ethyl methane-sulphonate, sodium azide, or diethylsulphate, has been applied to banana breeding (Kulkarni *et al.*, 2007) [4] and has been exploited in attempts to compensate for agronomic weaknesses in existing cultivars. In this context the present experiment was conducted to create genetic variability in cv. Rajapuri and Grand Naine through *in vitro* induced mutation by using chemical mutagen (EMS).

Material and Methods
Establishment of aseptic culture (Shoot tip culture)
Healthy and vigorously growing sword suckers cv. Rajapuri (AAB) and Grand Naine (AAA) of 2-3 months age were obtained from field grown mother plants. The suckers were washed thoroughly in running tap water followed by washing in soap water solution for 30 minutes, so as to remove the adhering soil particles.

Using stainless steel knife, outer leaves, the leaf bases and rhizome tissues were trimmed away until the length of the growing shoot was 4 to 6 cm. The explants were immersed in 1% Bavistin solution for 30 minutes, followed by further trimming and treating with 0.5% Bavistin + 0.5% Streptocyclin for eight hours. Bavistin and streptocyclin was discarded and explants were rinsed well with distilled water. Plant material was further treated with 0.05% citrimide for 30 minutes and surface sterilized with 0.1% mercuric chloride for 10 minutes. Later they were washed thoroughly using sterilized double distilled water to remove traces of mercuric chloride. The shoot tips (about 1cm) were excised and inoculated into the culture tubes individually. Established aseptic shoot tips were subcultured for 4 cycles to regenerate sufficient plantlets, which were used for EMS treatment.

EMS treatment

Multiple *in vitro* shoot cultures obtained from 4th sub-culture were subjected to EMS (Ethyl Methane Sulphonate) treatment in the culture room under aseptic condition for 2 hours at 30^0 C. The concentration of EMS were used for inducing mutations were Control, 0.1%, 0.2%, 0.3%, 0.4% and 0.5% respectively. The EMS treated explants were immediately subcultured (M_1V_0) onto shoot proliferation medium. Further sub-culturing was performed at an interval of 30 days up to M_1V_3. At every stage of subculture shoot multiplication ratio (from previous culture stage to the next stage), dwarfing mutants (calculated by dividing number of dwarf plantlets by number of plantlets inoculated and expressed in percentage) and leaf variants were recorded.

Rooting of shoots and Hardening

Individual shoots from EMS cycles (M_1V_3) were transferred to rooting medium [1/4th MS basal medium supplemented with 2 mg/l of IBA] to obtain rooted plantlets (M1V4). After about 4 weeks, the plantlets were subjected to primary hardening. Survival percentage (by dividing number of plantlets survived by number of plantlets inoculated and expressed in percentage) was worked out at primary hardening stage.

Results and Discussion

The results of data pertaining to shoot multiplication ratio revealed significant differences for EMS treatment at I, II and III sub culture stage. The maximum shoot multiplication ratio was observed in water treated plantlets which was found statistically on par with plantlets treated with 0.1, 0.2 and 0.3 per cent of EMS in all the stages of sub culture. However the lowest shoot multiplication ratio was observed in plantlets treated with 0.5 per cent EMS concentration. Interaction revealed, the maximum shoot multiplication ratio was observed in untreated Rajapuri plantlets where as the lowest shoot multiplication ratio was recorded in Rajapuri plantlets treated with 0.4 per cent EMS concentration at I subculture stage (Table 1). The Grand Naine variety recorded maximum shoot multiplication ratio than Rajapuri during III sub culture stage. The higher shoot multiplication ratio at lower concentration may be attributed to maximum number of shoots produced per explant at these concentrations. Lower shoot multiplication ratio at higher concentration may be due to chemo-sensitivity of the cultivars. The similar findings were reported by Xu *et al.* (2011) [9] in banana cv. Yueyoukang-1 (AAA) and Bhagwat and Duncan (1998) [2] in Jahaji (AAA). They also noted shoot multiplication ratio was found decreasing at higher concentration of EMS.

The interpretation of data related to per cent dwarfing mutant obtained revealed significant difference for EMS treatment at I, II and III sub culture stages. The variety and interactions revealed significant differences only at II sub culture. There was no dwarfing mutants in the plantlets treated with 0.1, 0.2 and 0.3 per cent EMS concentration in all the subculture stages. However, the maximum per cent dwarfing mutants was found in plantlets treated with 0.5 and 0.4 per cent EMS concentration in all the culture cycle. Interactions at II subculture suggested that, Rajapuri plantlet treated with 0.5 per cent EMS concentration had maximum dwarfing mutants (Table 2). The per cent dwarfing mutant was found maximum in Rajapuri variety than Grand Naine at II subculture stage. The increase in per cent dwarfing mutants at higher concentration may be attributed to the reason that alkylating agents like EMS brings about extensive cross linking of DNA, chromosomal break up, chromosomal mutation and gene mutation at cellular level (Singh, 2001) [7] in exposed plantlets. This might have resulted in mutations in gene controlling the trait of plant height their by resulting in development of more percentage of dwarfing mutants at higher concentration. The differential response of cultivars may be due to chemo-sensitivity of specific cultivar. The cultivar with genome AAB is more sensitive than cultivar with AAA genome. The results of present investigation are in agreement with the findings of Omar *et al.* (1989) [6] who observed growth reduction in *in vitro* banana plantlets of SH-3362 and GN-60 at higher concentration of EMS. Musoke *et al.* (1999) [5] observed that, concentration of EMS beyond 0.4% retarded growth in all the cultivars (Sukalindizi (AB), Gros Michel (AAA), French Plantain (ABB), Kayinja (AAB) and Mbwazirume (AAA-EA).

The interpretation of data pertaining to per cent survival among plantlets exposed to different concentration of EMS revealed less variations. There was no mortality in plant culture treated with 0.1, 0.2 and 0.3 per cent EMS concentration and control (water treated plant culture). Where as there was marginal reduction in per cent survival of plantlets in plant culture treated with 0.4 and 0.5 per cent EMS (Table 3). Bhagwat and Duncan (1998) [2] reported that, there was decline in survival rate with the increase in EMS concentration in banana cv. Jahaji (AAA). On the contrary Cieslak *et al.* (2012) [3] reported that, regardless of concentration of EMS all explants incubated for 2 hr. survived the treatment. The present investigation matches with observation of Cieslak *et al.* (2012) [3] in banana cv. Kayinja.

The plants treated with 0.5 per cent EMS showed more leaf variants like narrower yellow colour leaves, leaf with uneven lamina and had small brownish spot in cv. Rajapuri and downward rolling of leaf lamina, slightly yellow colour leaves with uneven leaf lamina in cv. Grand Naine (Table 4). The plants treated with 0.4 per cent EMS also exhibited considerable leaf variants in Rajapuri (Leaves with uneven leaf lamina, light green in color having small spot on the apex) and cv. Grand Naine (Light green colour leaves partially rolled with small brownish spot on leaf blade). The plants treated with EMS at 0.1, 0.2 and 0.3 per cent concentration showed lesser leaf variants. It was clearly observed from the present investigation that, there was considerable increase in occurance of leaf variants with increased concentration of EMS. The present investigation results are in conformity with the findings of Bhagwat and Duncan (1998) [2] who observed phenotypic variations like leaf colour changes, aberrant morphology of the laminae, leaf curling and unsymmetrical leaves in generated plants of

banana treated with higher concentration of EMS. A considerable phenotypic variation was observed and reported by Valerin *et al.* (1998) [8] in banana cv. Kayinja produced *in vitro* from EMS treated shoot tips.

Table 1: Effect of different concentration of EMS on shoot multiplication ratio of banana cv. Rajapuri (AAB) and Grand Naine (AAA)

Treatment	Shoot multiplication Ratio								
	I sub-culture			II sub-culture			III sub-culture		
	Rajapuri (V_1)	Grand Naine (V_2)	Mean	Rajapuri (V_1)	Grand Naine (V_2)	Mean	Rajapuri (V_1)	Grand Naine (V_2)	Mean
E_1 : Control	2.37	2.12	2.24	2.11	2.12	2.12	2.25	2.75	2.50
E_2 : 0.1% EMS	2.28	2.22	2.25	2.07	2.06	2.07	2.33	2.67	2.50
E_3 : 0.2% EMS	2.25	2.13	2.19	2.03	2.04	2.04	2.42	2.67	2.54
E_4 : 0.3% EMS	2.23	2.06	2.15	2.08	2.06	2.07	2.17	2.75	2.46
E_5 : 0.4% EMS	1.72	1.89	1.81	1.85	1.87	1.86	2.08	2.50	2.29
E_6 : 0.5% EMS	1.73	1.74	1.74	1.75	1.79	1.77	1.83	2.25	2.04
Mean	2.10	2.03		1.98	1.99		2.18	2.60	
	S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%	
Variety	0.01	NS		0.02	NS		0.04	0.15	
EMS	0.03	0.12		0.03	0.13		0.06	0.27	
Variety x EMS	0.04	0.17		0.05	NS		0.09	NS	
CV (%)	3.81			4.09			5.08		

Table 2: Effect of different concentration of EMS on per cent dwarfing mutant in banana cv. Rajapuri (AAB) and Grand Naine (AAA)

Treatment	Per cent dwarfing mutants (%)								
	I sub-culture			II sub-culture			III sub-culture		
	Rajapuri (V_1)	Grand Naine (V_2)	Mean	Rajapuri (V_1)	Grand Naine (V_2)	Mean	Rajapuri (V_1)	Grand Naine (V_2)	Mean
E_1 : Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
E_2 : 0.1% EMS	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
E_3 : 0.2% EMS	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
E_4 : 0.3% EMS	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
E_5 : 0.4% EMS	14.30 (12.34)	10.53 (9.85)	12.42 (11.30)	24.74 (22.21)	15.27 (14.21)	20.00 (18.10)	25.89 (23.12)	17.39 (16.21)	21.64 (20.12)
E_6 : 0.5% EMS	23.63 (21.34)	17.20 (16.23)	20. (18.23)	34.21 (30.12)	22.27 (20.12)	28.24 (26.21)	36.28 (31.21)	26.49 (23.12)	31.39 (30.12)
Mean	6.32 (5.82)	4.62 (4.10)		9.82 (8.23)	6.26 (5.80)		10.36 (9.81)	7.31 (6.88)	
	S.Em±	CD at 1%		S.Em±	CD at 1%		S.Em±	CD at 1%	
Variety	0.43	NS		0.68	2.70		0.80	NS	
EMS	0.75	2.96		1.18	4.67		1.39	5.51	
Variety x EMS	1.06	NS		1.67	6.61		1.97	NS	
CV (%)	9.54			9.82			9.33		

* Figures in parenthesis indicates arcsine transformed values

Table 3: Effect of different concentration of EMS on per cent survival of banana cv. Rajapuri (AAB) and Grand Naine (AAA)

Treatment	Per cent survival		
	Rajapuri (V_1)	Grand Naine (V_2)	Mean
E_1 : Control	100.00 (90.0)	100.00 (90.0)	100.00 (90.0)
E_2 : 0.1% EMS	100.00 (90.0)	100.00 (90.0)	100.00 (90.0)
E_3: 0.2% EMS	100.00 (90.0)	100.00 (90.0)	100.00 (90.0)
E_4 : 0.3% EMS	100.00 (90.0)	100.00 (90.0)	100.00 (90.0)
E_5 : 0.4% EMS	97.00 (93.10)	98.37 (96.12)	97.68 (94.21)
E_6 : 0.5% EMS	87.97 (85.21)	95.33 (91.20)	91.65 (90.10)
Mean	97.49 (96.21)	98.95 (96.12)	
	S.Em±	CD at 1%	
Variety	0.75	NS	
EMS	1.30	5.14	
Variety x EMS	1.85	NS	
CV (%)	3.24		

* Figures in parenthesis indicates arcsine transformed values

Table 4: Effect of different doses of EMS on leaf variants in banana cv. Rajapuri (AAB) and Grand Naine (AAA)

Treatment	Leaf variants in banana	
	Rajapuri (V_1)	Grand Naine (V_2)
E_1 : Control	No variants	No variants
E_2 : 0.1% EMS	Broad thicker leaves with dark green in colour	Dark green coloured shiny leaves
E_3: 0.2% EMS	Green coloured broad leaves	Green coloured broad symmetrical leaves
E_4 : 0.3% EMS	Light green coloured broader leaves and symmetrical	Broad leaves with uneven leaf lamina slightly rolled downwards
E_5 : 0.4% EMS	Leaves with uneven leaf lamina, light green in colur having small spot on the apex	Light green colour leaves partially rolled with small brownish spot on leaf blade
E_6 : 0.5% EMS	Narrower leaves turned yellow colour. Uneven leaf lamina with small brownish spot	Leaves rolling downwards. Slightly yellow in colour with uneven lamina

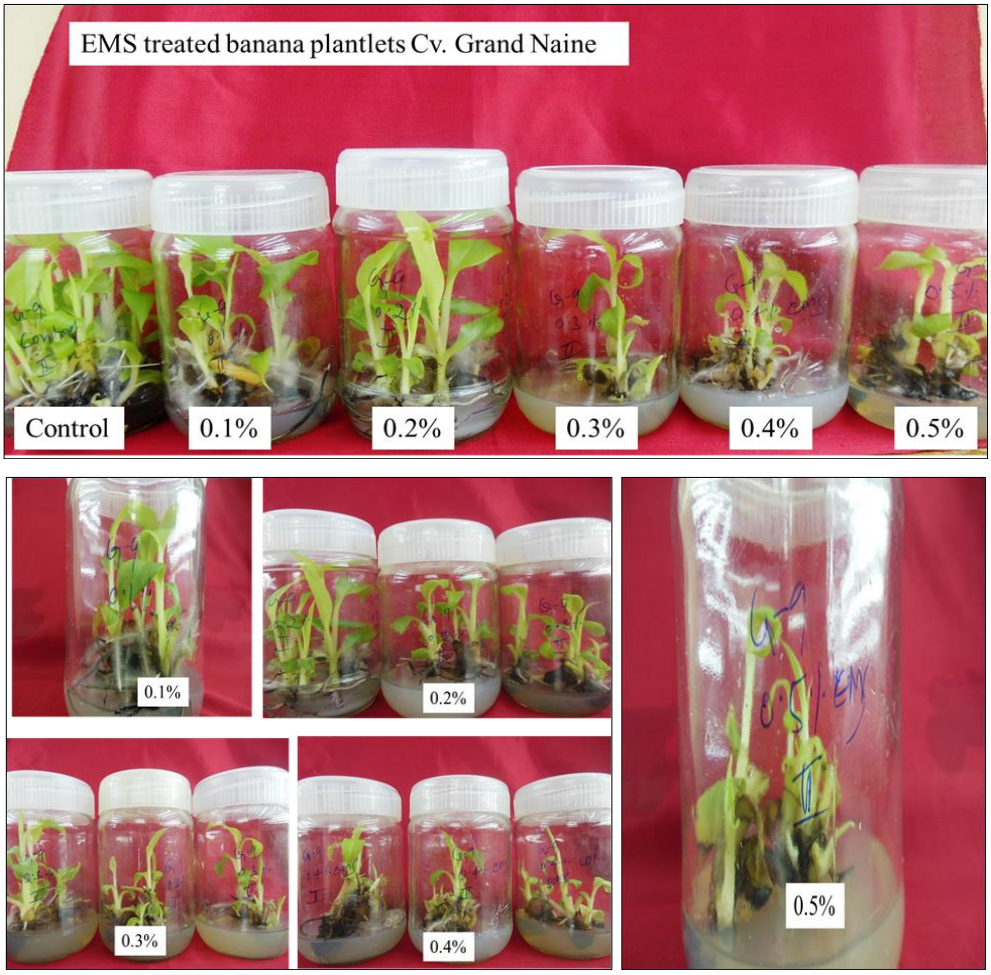

Plate 1: Effect of EMS on shoot multiplication ratio and dwarfing mutants in banana

Plate 2: Effect of EMS on leaf variants, shoot length and per cent survival in banana

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