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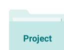
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Improvement of banana cv. Rasthali (Silk, AAB) against *Fusarium oxysporum* f.sp. *cubense* (VCG 0124/5) through induced mutagenesis: Determination of LD_{50} specific to mutagen, explants, toxins and *in vitro* and *in vivo* screening for Fusarium wilt resistance

MS Saraswathi[1,]*, G Kannan[1], S Uma, R Thangavelu & S Backiyarani
Crop improvement, ICAR-National Research Centre for Banana, Tiruchirappalli-620 102, Tamil Nadu, India

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Shoot tips and *in vitro* grown proliferating buds of banana cv. Rasthali (Silk, AAB) were treated with various concentrations and durations of chemical mutagens *viz.,* EMS, NaN_3 and DES. LD_{50} for shoot tips based on 50% reduction in fresh weight was determined as 2% for 3 h, 0.02% for 5 h and 0.15%for 5 h, while for proliferating buds, they were 0.6% for 30 min, 0.01% for 2 h and 0.06% for 2 h for the mutagens EMS, NaN_3 and DES, respectively. Subsequently, the mutated explants were screened *in vitro* against fusarium wilt using selection agents like fusaric acid and culture filtrate. LD_{50} for *in vitro* selection agents calculated based on 50% survival of explants was 0.050 mM and 7% for fusaric acid and culture filtrate, respectively and beyond which a rapid decline in growth was observed. This was followed by pot screening which led to the identification of three putative resistant mutants with an internal disease score of 1 (corm completely clean, no vascular discolouration). The putative mutants identified in the present study have also been mass multiplied *in vitro*.

Keywords: Di ethyl sulphate, Ethyl methane sulfonate, Fusaric acid, Fungal resistance, *Musa* sp., Sodium azide

Bananas and plantains are cultivated in as many as 135 countries worldwide with an annual production of around 144 million metric tonnes[1]. India, with the production of around 29.7 million MT, ranks first in the World banana production[2]. National Horticulture Board, Govt. of India, has estimated banana production of India for the year 2016 at 29.895 million MT from 837000 ha under banana cultivation[3]. Among the 32 states under banana cultivation, Gujarat, Tamil Nadu and Maharashtra rank high in banana production with 43.24, 41.47 and 40.30 million MT, respectively[2]. Currently, banana cultivation is threatened by several pests *viz.* nematodes[4], and diseases such as fusarium wilt, sigatoka leaf spot and viral diseases[5,6]. Fusarium wilt also known as Panama disease is caused by the soil borne fungus *Fusarium oxysporum* f. sp. *cubense* and is recognized as one of the most destructive diseases of banana across the globe. It is causing annual yield loss of 60-90%[5]. The yield loss by the disease has been estimated to be 30-40% in India and it ranged from 2 to 90%, particularly in south India[7]. There are 4 different races of *Fusarium oxysporum*, namely race 1, 2, 3 and 4 which affects Silk, Monthan and Pome, *Heliconium* spp. and Cavendish group of bananas, respectively. However, the wide spread classical race 1 pathogen has been recorded as a serious disease on 'Silk' group of bananas (AAB)[8].

Recently, Shaikh *et al.*[9] have demonstrated antifungal and antibacterial potential of *Pseudomonas aeruginosa* isolated from the banana field rhizopsphere. More effectively, developing and breeding resistant varieties helps in overcoming these challenges. However, development of wilt resistance in a commercially important group of bananas through conventional breeding is handicapped due to their sterile, parthenocarpic and polyploid nature[6,10]. Under such circumstances, use of *in vitro* mutagenesis allows generation of genetic variability and selection of desired mutants with improved agronomic characters like dwarfness, shorter crop duration, high yield and resistance to diseases[5]. Selection of somaclonal variants derived through tissue culture is yet another alternative strategy currently adopted to develop plants with desired characteristics[11].

*Correspondence:
Ph.: +91 431 2618125; Fax: +91 0431 2618115
E-mail: saraswathimse@gmail.com
[1]share equal contribution

Several traits have been improved through mutation breeding in different crops like pulses, cereals, vegetables and ornamentals despite their limitations. The advantage of mutation breeding in vegetatively propagated crops is their ability to change one or two characters without changing the basic genetic background, a common phenomena when genetic improvement of banana is attempted through conventional breeding[12,13]. The frequency of occurrence of spontaneous mutation in banana is very low but the same has been enhanced by the use of physical and/or chemical mutagens[14]. For instance, induced mutation through gamma irradiation was successfully used in conferring resistance to some diseases of banana like Fusarium wilt[15], black Sigatoka[16], BBTV and BBMV[17], and abiotic stresses like salinity[18].

Most of the physical mutagens lead to chromosomal changes while chemical mutagens result in point mutations[19,20]. They lead to base pair substitutions especially GC-AT resulting in aminoacid change that alters only the protein function but do not abolish their functions which is common in deletions and frame shift mutations[21]. EMS (Ethyl methane sulfonate), NaN_3 (Sodium azide) and DES (Diethyl sulfate) used in the present study belong to the alkylating group and have been successfully used in banana earlier for creating mutants[22], After induced mutagenesis, the plants are evaluated for Fusarium wilt resistance under field conditions by planting tissue culture plantlets at a closer spacing in a diseased nursery. After 3-5 months, the rhizomes of surviving plants were unearthed, cut and examined for the browning of vascular tissue. But in the recent past, pot evaluation is done in the screen-house through artificial inoculation of the pathogen. After 6-10 wk, the plants are examined for internal symptoms of infection. Plants without symptoms or slightly infected were considered to be putative resistant plants and selected for further evaluation[23].

The most critical part of induced mutagenesis is the selection of an effective mutagen and determination of LD_{50} because it varies with the mutagen and genotype[24]. Higher concentration may lead to tissue damage or increase in per cent mortality. Likewise, the duration of the treatment should be optimum enough to permit hydration and infusion of mutagen into the explants. Therefore, the present study was undertaken to determine the (i) LD_{50} by treating two different explants namely shoot tips and proliferating buds with 3 different chemical mutagens *viz*., EMS, DES and NaN_3 for various time periods; (ii) lethal dose of toxins like fusaric acid and culture filtrate towards the development of fusarium wilt resistance in banana cv. Rasthali (Silk, AAB); and (iii) to identify the putative mutants showing fusarium wilt resistance through pot screening.

Materials and Methods

About 2-3 months old sword suckers of Rasthali weighing 750 g to 1 kg with 90-100 cm height, required for the study were collected from NRCB Farm, Podavur, Trichirappalli, Tamil Nadu. The oldest leaves and corm tissues were carefully removed with a stainless steel knife without damaging the growing tip (shoot meristem) and brought to the laboratory where they were washed with Tween 20 followed by running water for 20 to 30 min and kept soaked in fungicide bavistin 0.1% for 1 h. The shoot tips were then taken to laminar air flow cabinet and surface sterilized with 4% (v/v) sodium hypochlorite for 10 min followed by 0.1% mercuric chloride for 10 min with sterile water rinsing in between. In case of proliferating buds, the buds obtained after 2-3 subcultures were directly subjected for mutagenic treatment.

Preparation of mutagens

The aqueous solutions of EMS, NaN_3 and DES were prepared along with DMSO (Dimethyl sulfoxide) 2%, as carrier using deionized water and stored at 4ºC. Presence of the carrier greatly enhanced uptake of mutagens into the apical meristematic dome, leaf primordia and other tissues[25]. The required concentration of working solution was prepared by diluting the stock with 0.1M sterile phosphate buffer (pH-3). The mutagens were sterilized using 0.2 micron filters. Shoot tips were treated with EMS at 0.5, 1.0, 1.5 and 2.0%, NaN_3 at 0.02, 0.03 and 0.04% and DES at 0.15, 0.22, 0.3 and 0.4 % for 2, 3, 4 and 5 h while the proliferating buds were treated with EMS at 0.2, 0.3, 0.4 and 0.6%, NaN_3 at 0.01, 0.015, 0.02 and 0.03% and DES at 0.04, 0.45, 0.053 and 0.06% for ½, 1, 1½ and 2 h.

Though incubation temperature does not affect the rate of diffusion, lower temperature increases the hydrolysis rate and maintains the stability of the mutagen thereby ensuring their reactivity with the target tissue. Therefore, the sterile shoot tips and *in vitro* proliferating buds were immersed in

mutagens and kept in an orbital shaker maintained at 110 rpm (rotations per minute) and 25°C, for various time intervals. Explants immersed in buffer served as untreated control. After the completion of treatment, they were rinsed 3-5 times with sterile distilled water. Sterile explants were inoculated in MS medium[26] with 3 mg l^{-1} BAP (Benzyl amino purine), 100 mg L^{-1} inositol and 30 g L^{-1} sucrose. The cultures were incubated in a controlled environment at 24±2°C with 2000 light intensity under 16 h photoperiod provided by cool white fluorescent lights. The initial weight of the explants was recorded prior to inoculation in the initiation medium. The final weight was recorded at the time of first subculture. The difference between the initial and final weight was taken as fresh weight gain (FWG). The LD_{50} was calculated as the dose of chemical mutagens which causes 50% reduction in FWG as against untreated control and the same was confirmed using linear regression[27,28].

***In vitro* screening**

Fusaric acid and culture filtrate assay

The individual shoots obtained during third or fourth subculture were transferred to shoot proliferation medium (MS) supplemented with different concentrations of the toxins, fusaric acid (Sigma Aldrich, USA) @ 0.0125, 0.025, 0.0375, 0.05 and 0.0625 mM and culture filtrate (supplied by the Fungal Pathologist) @ 3, 4, 5, 6, 7 and 8% besides the growth regulator. Medium without fusaric acid/culture filtrate served as control. The number of shoots survived after 3 wk of inoculation was recorded and the dose at which 50% of the shoots survived was fixed as the lethal dose. Continuous subculturing was done in the same proliferation medium with toxins for effective screening.

Pot screening

About 203 plants of mutated Rasthali derived from 3 different chemical mutagens, after 3 months of hardening were transferred to earthern pots containing potting mixture (red soil:sand:farm yard manure @ 1:1:1) along with necessary controls. The sand maize meal inoculum of *Foc* race (VCG 0124/5) was applied at the rate of 30 g per pot (12×10^9 cfu/mL). After 6 months of pathogen inoculation, the morphological data on plant height, pseudostem girth, number of leaves, leaf area and the disease severity were recorded. The disease severity was estimated by observing the external and internal symptoms adopting the INIBAP's technical guidelines No. 6 Carlier *et al.*[29] and the descriptions of Ploetz *et* al.[30], respectively. The disease scale of 1-5 was used for the external symptoms and the details are as follows: (1) healthy; (2) slight chlorosis and wilting with no petiole buckling; (3) moderate chlorosis and wilting with some petiole buckling and or no splitting of leaf bases; (4) severe chlorosis, severe wilting, petiole buckling and dwarfing of the newly emerged leaf; and (5) dead. The plants were uprooted and the corm was observed for vascular discoloration and a scale of 1-6 was adopted for disease scoring[11,23]. Internal scoring was done as below: (1) corm completely clean, no vascular discoloration; (2) isolated points of discoloration in vascular tissue; (3) discoloration up to one-third of the vascular tissue; (4) discoloration between one-third and two thirds of the vascular tissue; (5) discoloration of greater than two-thirds of the vascular tissue; and (6) complete discoloration of the vascular tissue.

Statistical analysis

The experiment was designed in a completely randomized design replicated 5 times. The data were analyzed using SPSS (Statistical Package for Social Studies) and the means were compared using DMRT (Duncan's Multiple Range Test) at 5% confidence level.

Results and Discussion

Determination of LD_{50} specific to mutagens and explants

In vitro mutagenesis coupled with toxin based *in vitro* screening has significantly improved the efficiency of mutation in the genetic improvement of vegetatively propagated crops like banana and potato[31,32]. Determination of LD_{50} is a pre-requisite for mutagenesis and 3 mutagens and 2 toxins were used in the current study.

In the present study, LD_{50} (Table 1) was calculated as suggested by Novak *et al.*[33]. In shoot tips treated with EMS, the FWG percent decreased from 100 in untreated control to 77.71, 50.00, 14.57 and 7.42 at 2% EMS for 2, 3, 4 and 5 h, respectively and 50.00% reduction in FWG as against control was observed in 2% EMS for 3h. Similarly, 48.66% reduction in FWG was recorded in proliferating buds at 0.6% EMS for ½ h. In NaN3 treated shoot tips, FWG % decreased from 100 in untreated control to 83.35, 61.87, 44.78 and 17.85 at 0.04% NaN_3 for 2, 3, 4 and 5 h, respectively. The maximum reduction in fresh weight gain (17.85%) was observed in 0.04% NaN_3 for 5 h while 50.83% reduction in FWG was recorded in 0.02%

Table 1—Effect of three different mutagens on fresh weight and no. of shoots produced per explant in various explants of cv. Rasthali

Mutagen Conc.	Explant	Treatment duration (h)	Fresh wt. gain (FWG) (g)	FWG (%) as against control	No. of shoots per explants
EMS (2%)	Shoot tip	2	2.72 ± 0.029^{e}*	77.71**	1.00 ± 0.31^{a}
		3	1.75 ± 0.012^{i}	50.00	1.00 ± 0.00^{b}
		4	0.51 ± 0.017^{k}	14.57	0.20 ± 0.20^{cd}
		5	0.26 ± 0.051^{l}	7.42	1.00 ± 0.00^{d}
		UC	3.50 ± 0.052^{a}	100.0	2.20 ± 0.37^{a}
EMS (0.6%)	Proliferating buds	½	1.46 ± 0.025^{e}	48.66	1.60 ± 0.00^{de}
		1	1.19 ± 0.020^{f}	39.66	1.00 ± 0.00^{e}
		1½	0.86 ± 0.020^{g}	28.66	1.00 ± 0.00^{e}
		2	0.40 ± 0.010^{i}	13.33	1.00 ± 0.00^{e}
		UC	3.00 ± 0.110^{a}	100.0	3.20 ± 0.20^{a}
Sodium azide (0.02%)	Shoot tip	2	6.26 ± 0.03^{b}	94.70	3.00 ± 0.31^{b}
		3	5.74 ± 0.03^{d}	86.83	2.60 ± 0.24^{bc}
		4	4.52 ± 0.04^{f}	68.38	2.00 ± 0.31^{cd}
		5	3.36 ± 0.03^{i}	50.83	1.80 ± 0.20^{cd}
		UC	6.61 ± 0.03^{a}	100.0	5.00 ± 0.44^{a}
Sodium azide (0.01%)	Proliferating buds	½	2.31 ± 0.01^{b}	88.84	2.00 ± 0.31^{b}
		1	2.08 ± 0.03^{d}	79.99	2.00 ± 0.31^{b}
		1½	1.79 ± 0.02^{f}	68.80	1.20 ± 0.20^{cd}
		2	1.32 ± 0.02^{k}	50.76	1.20 ± 0.20^{cd}
		UC	2.60 ± 0.02^{a}	100.0	3.60 ± 0.24^{a}
DES (0.15%)	Shoot tip	2	5.86 ± 0.02^{a}	98.32	4.20 ± 0.20^{ab}
		3	5.25 ± 0.02^{c}	88.53	4.00 ± 0.31^{abc}
		4	4.76 ± 0.03^{d}	80.26	3.40 ± 0.24^{bcd}
		5	2.96 ± 0.02^{h}	49.91	2.80 ± 0.37^{def}
		UC	5.93 ± 0.04^{a}	100.0	5.00 ± 0.31^{a}
DES (0.06%)	Proliferating buds	½	1.71 ± 0.02^{i}	62.18	2.00 ± 0.31^{cde}
		1	1.56 ± 0.02^{k}	56.72	1.60 ± 0.24^{de}
		1½	1.50 ± 0.02^{l}	54.54	1.20 ± 0.20^{e}
		2	1.37 ± 0.02^{m}	49.81	1.00 ± 0.31^{e}
		UC	2.75 ± 0.02^{a}	100.0	4.20 ± 0.37^{a}

UC, Untreated control;

*Mean values within a column having the same alphabet are not significantly different ($P = 0.05$) according to DNMRT.

**Fresh weight gain % as against control has not been statistically analyzed.

NaN_3 for 5 h. Likewise, 50.76% reduction in FWG was recorded in proliferating buds treated with 0.01% NaN_3 for 2 h. In DES, 50% reduction in FWG was observed in both shoot tips and proliferating buds treated with 0.15% DES for 5 h and 0.06% DES for 2 h, respectively.

When the actual LD_{50} was plotted on a graph to predict the LD_{50} (X) in relation to FWG (Y) based on simple linear regression, it was found that both actual and predicted values were tallying with each other in almost all cases with R^2 value nearing one indicating a significant correlation between mutagen and their corresponding FWG (Fig. 1). But in few cases like

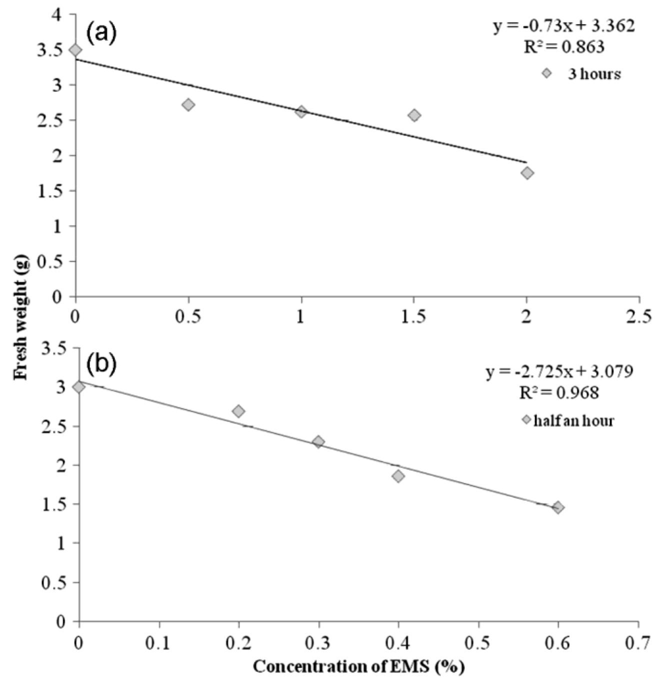

Fig. 1—Determination of LD_{50} for EMS in (a) shoot tip; and (b) proliferating bud explants of cv. Rasthali

NaN_3 treated proliferating buds (0.015% as against 0.01% NaN_3 for 2 h) and DES treated shoot tips (0.24% as against 0.15% DES for 5 h) and proliferating buds (0.073 % as against 0.06% DES for 2 h), the predicted LD_{50} failed to tally with the actual value. However, the predicted dosages were adopted in this study for mutating cv. Rasthali.

Number of shoots produced per explant showed statistically significant differences among the various mutagenic treatments imposed on two different explants. Maximum Number of shoots was produced in untreated control while at LD_{50} dosage the number of shoots produced was almost 50% of the untreated control. Dormancy was more in NaN_3 followed by EMS while no dormancy was recorded in DES treated shoot tips and proliferating buds.

Determination of LD_{50} specific to toxins

Phytotoxins namely fusaric acid and culture filtrate were used in the present study for *in vitro* screening against Fusarium wilt resistance because they appear to be more effective than the use of pathogen itself as they produce disease symptoms and operate at the cellular level[34] Further, these methods are non-destructive, more cost and labour effective and evaluation could be carried out in a short span of time as against field screening[35].

In this study, as the concentration of fusaric acid and culture filtrate increased, the survival percentage of proliferating shoots decreased proportionately and it was statistically significant among the various treatments (Fig. 2). Among the different concentrations of fusaric acid tested to determine the optimal dose (LD_{50}) for *in vitro* screening against Fusarium wilt resistance, 0.0125 and 0.250 mM concentrations recorded 100% survival of the shoots initiated. At higher concentrations from 0.0375 mM to 0.0625 mM, the survival percentage of shoots was drastically reduced from 74 to 14%. Similarly, in case of culture filtrate tested, the shoot survival was 100% up to 4% concentration and thereafter the survival percentage decreased and reached 18% at 8% concentration.

Pot screening against Foc race 1 (VCG 0124/5) through challenge inoculation

In the present investigation, the morphological data were observed at the time of disease scoring for all the 203 plants of mutated Rasthali and those for the resistant mutants alone have been tabulated (Table 2). Results indicated that the plant height, pseudostem girth, number of leaves, leaf area were higher as compared to the susceptible mutant. The external scoring was uniformly 1 in all the resistant mutants as against 4 in the susceptible mutant. Results of internal scoring indicated that in EMS treatment, out of 32 shoot tip derived plants observed, none of them were free from the disease. But in the case of plants derived from proliferating buds, 3 (namely NRCBRM – 7, 15 and 17) out of 43 exhibited no vascular discoloration (Score 1- healthy) (Fig. 3) and 6 plants recorded the disease score of 2 on the disease scale (Table 3). While in the case of other two mutagens tested, namely DES and NaN_3, all the 128 plants screened showed severe vascular discoloration in the corm (disease score from 3 to 6).

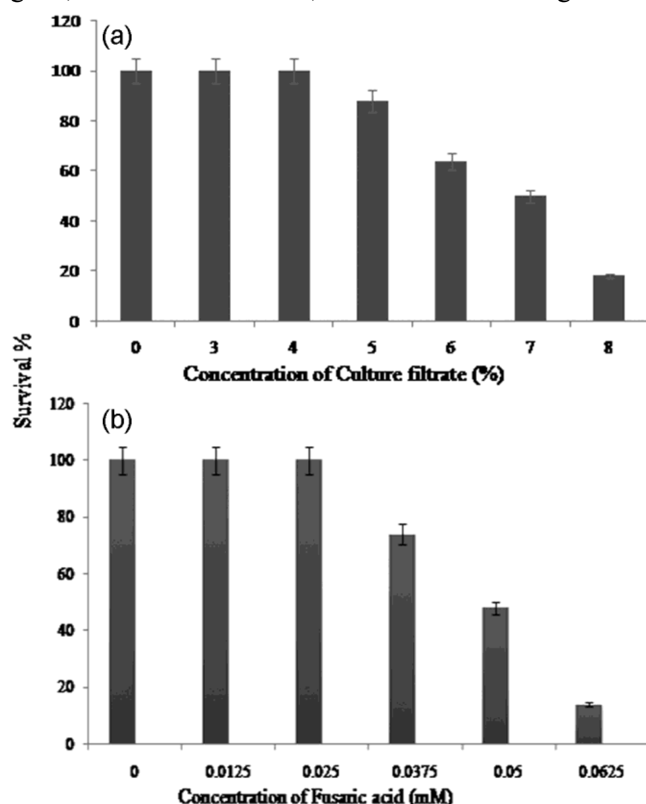

Fig. 2—Effect of culture filtrate and Fusaric acid on survival of proliferating shoots of cv. Rasthali.

Chemical mutagens have been previously used in banana by several workers. for the development of disease resistance through *in vitro* mutagenesis and selection of Fusarium wilt resistant clones[22,36-38]. The FWG in untreated control was significantly higher than the mutagen treated explants. After 3 wk of initiation, the FWG decreased significantly with an increase in both the concentration of chemical mutagens and the duration of treatment. This might be due to the enhanced reactivity of the mutagen with the target tissues at higher concentrations and prolonged

Table 2—Morphological data of resistant mutants of cv. Rasthali *vs*. susceptible mutant

Plant No.	Plant height	Pseudostem irth	No. of leaves	Leaf area	External Score
NRCBRM7	53	16	7	1122	1
NRCBRM15	60	16	7	1166	1
NRCBRM17	61	17	7	1122	1
Susceptible mutant	41	14	6	798	4

Table 3—Disease scoring for Fusarium wilt resistance after six months of pathogen inoculation (*Foc* race 1 – VCG 0124/5)

Treatment	Explant	No. of plants screened	No. of plants with disease score 1-6					
			1	2	3	4	5	6
EMS (2%)	ST	32	-	-	3	-	4	25
EMS (0.6%)	PB	43	3	6	3	-	4	27
DES (0.16%)	ST	48	-	-	4	2	12	30
DES (0.06%)	PB	32	-		1	1	9	21
NaN_3 (0.02%)	ST	24	-	-	-	-	2	22
NaN_3 (0.01%)	PB	24	-	-	-	1	4	19
Control		20	-	-	-	-	-	20
Total No. of plants screened		223	3	6	11	4	35	164

ST, Shoot tip; PB, Proliferating bud

Fig. 3—(a) Challenging with spores of *Foc* race1 @ 30g/pot; (b) Disease scoring after 6 months of inoculation; (c) Resistant mutants of Rasthali (NRCBRM 5& 15) with the susceptible mutant; and (d) Resistant (NRCBRM 17) mutant of Rasthali with the susceptible mutant.

duration of the treatment. Prolonged treatments with higher doses of mutagen are likely to result in a relatively higher physiological damage in relation to the mutation rate. So, it was ensured that the explants are always treated with a lower dosage of the mutagen for longer duration which besides improving the mutagen efficiency[39] is likely to minimize the chromosomal damage and other negative effects[40]. This is in line with the findings of Omar *et al.*[36] and Musoke *et al.*[28]. The LD_{50} values obtained for the 3 chemical mutagens in cv. Rasthali was different from those determined earlier for Cavendish clones. This could be attributed to the varietal difference and varied genomic composition which might have affected the accumulation of mutagenic compounds has been reported[41]. LD_{50} concentration for the 3 mutagens also varied with explants, and it was comparatively lesser for proliferating buds. This might be because the proliferating buds are actively dividing explants with very few leaf sheaths which would have made it more sensitive to mutagens than shoot tip explants. The shoot tip explants require a higher concentration of mutagen to facilitate its penetration into the deep seated meristem enclosed by several protective leaf sheaths. This is in conformity with the findings of Kantoglu *et al.*[38] who have also reported the differential sensitivity of explants to mutagens and their concentrations[42].

In the present study, the regeneration rate decreased with an increase in concentration and duration of treatment which is quite evident from the reduction in fresh weight gain, proliferation rate and the number of shoots which remained dormant after the mutagenic treatment (Fig. 4). Similar results have been documented earlier[15]. Increased dormancy was recorded in NaN_3 (Fig. 4e) followed by EMS while DES treated shoot tips and proliferating buds exhibited no dormancy (Fig. 4b). Dormancy, is a common phenomena in mutated explants and it is attributed to the excess secretion of phenols that inhibits the uptake of nutrients and thereby growth[17]. Generally, the phenol secretion depends on the genetic makeup of the cultivar but in the present study, it varied with the mutagen[15]. Phenol is not a major limiting factor during *in vitro* multiplication of cv. Rasthali. Thus, the varied response of mutagen treated cv. Rasthali to phenolic exudation could be correlated with the extent of stress imposed by different mutagens.

It is evident from the present study that the survival percent is less than 20% when concentrations are increased beyond 0.05 mM and 7% fusaric acid and culture filtrate, respectively. Our findings are in accordance with the findings of Morpurgo *et al*[43] which reported significant reduction in growth of two diploid clones, namely SH 3362 and Pisang Mas at

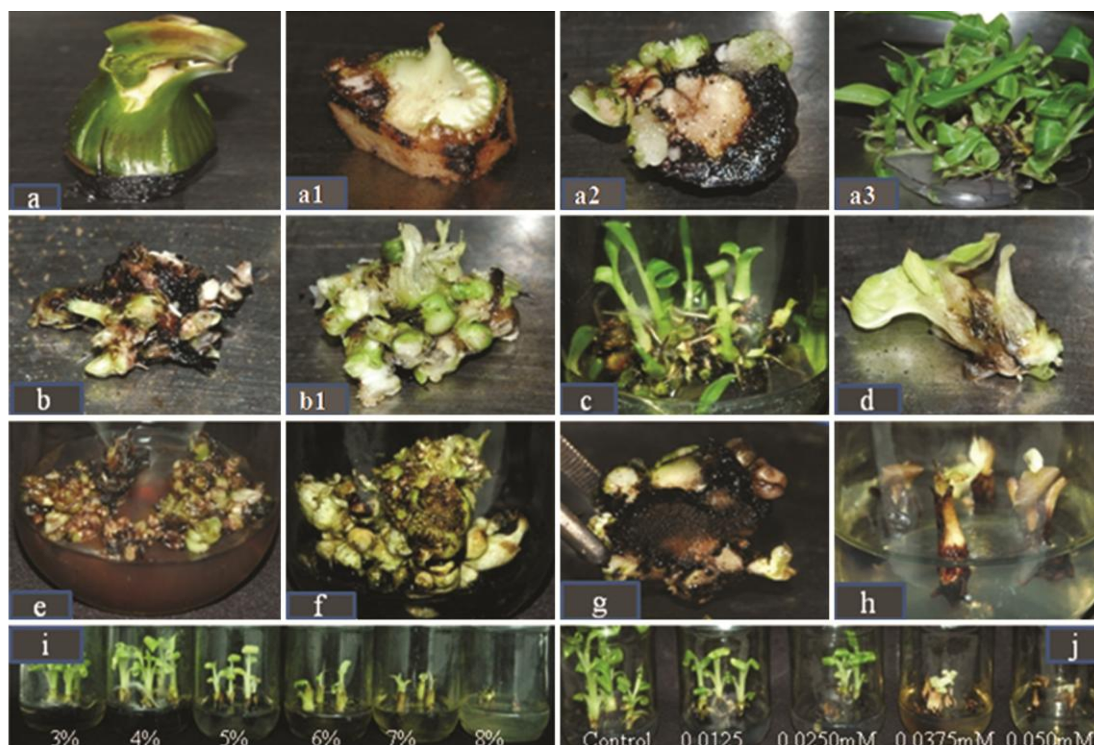

Fig. 4—Establishment of shoot tips and proliferating buds during *in vitro* mutagenesis and screening. [(Panel a): Chemical mutagen treated shoot tip after 3 wk of initiation; (Panel a1): Shoot tip after 4 wk of initiation; (Panel a2): Shoot tip after 8 wk of initiation; (Panel a3): Proliferating shoots after 16 wk of initiation; (Panel b): Chemical mutagen tre; (Panel d): Shoot abnormality (variant) encountered during chemical mutagenesis; (Panel e): Chemically mutated proliferating bud showing browning at supra-optimal concentration; (Panel f): Shoot buds showing abnormality at higher concentration of NaN_3;. (Panel g, h): Dried and decayed proliferating buds and shoots at higher concentrations of toxins in the culture media; and (Panel i, j): Effect of varied concentrations of culture filtrate and fusaric acid on the survival and growth of proliferating shoots]

higher concentrations of fusaric acid (0.09 mM) and culture filtrate (9%). This could be attributed to the induced excess secretion of phenols due to the stress imposed at higher concentrations of toxins. Release of excess phenols due to toxins normally leads to the browning of media and subsequent inhibition of growth of proliferating shoots[44].

Pot screening against Foc race 1(VCG 0124/5) has resulted in the identification of 3 putative resistant mutants (NRCBRM – 7, 15 and 17) which are free from both the external and internal symptoms of fusarium wilt disease. This is quite evident from the other morphological data observed in the mutated plants of cv. Rasthali. Similar screening strategies have been adopted by TBRI, Taiwan resulting in the successful release of TR 4 resistant Giant Cavendish clones like GCTCV - 218 (Formosona), GCTCV – 215 (Thai Chiao No.1) and GCTCV – 105 (Pei Chiao)[45]. The propagules of these mutants have been taken for large scale *in vitro* multiplication for further evaluation and confirmation in both sick plot and hot spots for *Foc* race 1 (VCG 0124/5).

Conclusion

Determination of LD_{50} is a pre-requisite for mutation breeding and it seemed to vary with the mutagen efficiency, genotype and explant type. Similarly, the lethal doses of toxin seem to vary with agent, genotype and race of the pathogen.

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