Optimal dose rate of gamma irradiation and EMS concentration for mutation induction on shoot tip of Banana cv. Grand Nain

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Abstract

This work was initiated in the year 1997 to develop an *in vitro* technique suitable for mutation induction on banana cultivar Grand Nain using physical and chemical mutagens. The first experiment was established to determine the optimal dose range of gamma source radiation for mutagenic induction on the banana cv. Grand Nain for subsequent mutation breeding in the crop. LD_{50} was established by irradiation of shoot tip explants by 33.6 Gy based on the survival of explants and shoot proliferation. The second experiment was established to test the best method of irradiation comparing acute and split methods with the aim of increasing the chance of variability with a reduced lethal effect on explants. The acute method of irradiation was better than the split one based on the survival of explants and shoot proliferation. Chemical mutagenesis can be induced using .2% ethylmethanesulphonate (EMS) with or without DMSO on in vitro grown shoot tip explants of cv. Grand Nain.

Introduction

Banana and plantains (Musa spp. Colla) are important staple food crops for many people in the tropical zones of the world. Dessert bananas are mainly triploid Cavendish type (Musa acuminate). The breeding of most commercially acceptable banana is handicapped by their parthenocarpic nature and triploidy, so producing hardly any seeds (Shepherd, 1987). Due to the difficulty of improving banana with conventional methods, alternative approaches such as mutation induction have been pursued. The main advantage of mutation induction in vegtatively propagated crops is the ability to change one or a few characters without changing the remaining characters in the genotype (Broertjes and Van Harten, 1978; 1988; Donini and Micke, 1984; Konzak, 1984; and Micke et al., 1987).Two mutation induction systems were used for banana improvement. The first one was based on in vivo sucker irradiation before meristem tip isolation and culture, yielding a low number of mutants (De Guzman, 1975; De Guzman et al., 1976; 1980; 1982). The second one was based on in vitro techniques for mutation induction, recovering mutant plants, avoiding or reducing chimerism and micropropagation of desirable mutants (Novak et al., 1987).

The aim of this work was to determine the optimal dose range of gamma source radiation for mutagenic induction on the banana cv. Grand Nain for subsequent mutation breeding in the crop. The acute and split methods of irradiation were tested to determine means of using a higher dose with the aim of increasing the chance of variability with a reduced lethal effect on explants. Chemical mutagenesis was tested using ethylmethanesulphonate (EMS).

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Materials and methods

The banana cultivars used in this study was Grand Nain 'Cavendish AAA' subgroup. Shoot tips (2-5 mm in size) consisting of the meristematic dome with 2-4 leaf primordial were culture on liquid MS medium (Murashige and Skoog, 1962) supplemented with 10 µM benzlaminopurine (BAP) and 5 µM indoleacetic acid (IAA). The medium pH was adjusted to 5.8 before autoclaving under 1.1 bar at 121 ºC for 20 minutes. Multiple shoot formation was achieved by subculturing shoot tips on MS medium supplemented with 20 µM BAP.

The irradiation procedure involved the transfer of explants, containing the shoot tip and leaf bases obtained from *in vitro* grown plantlets, to Petri dishes which contained a multiplication medium. Cultures were incubated for 7 days before exposure to gamma radiation from a ^{60}Co source.

In the first experiment the banana cultivar Grand Nain was irradiated by the following doses i.e. 0, 10, 20, 30, 40, 60 and 90 Gy at a dose rate of 4770 rad/ min. Plants were then subcultured on solidified MS medium supplemented with 20 µM BAP. Survival of explants, fresh weight and the number of regenerated plantlets per explant were observed after 10, 20, and 40 days from 10 replications per treatment and 5 explants per replication.

In the second experiment shoot tip explants of the banana cultivar Grand Nain were exposed to 30, 60 and 90 Gy as a single dose (called Acute irradiation) or by splitting these doses into 3 (10+10+10, 20+20+20 and 30+30+30 Gy) over a period of three weeks for recurrent irradiation of gamma rays (^{60}Co source). It is expected that with split irradation the injured cells of explants would recover during the 7 days before the treatment is continued thus decreasing lethal effect of high doses.

Shoot tip explants of the banana cv. Grand Nain were treated with varying doses of EMS (0.0, 0.4, 0.8, 1.2, and 1.6%. Dimehtylsulphoxide (DMSO) at 2% and water were used as carriers for the same concentrations of EMS to determine the effect of DMSO. Explants were then subcultured on modified MS multiplication medium. Survival was observed every 2 weeks for a period of 6 weeks. MstatC program was used for analysis of data. Duncan Multiple Range Test (DMRT) was used for mean separation.

Results and discussion

Radio sensitivity test on banana cv. Grande Nine

Survival of the shoot tip explants of the banana cv. Grand Nain decreased significantly with the increase of radiation dose after 40 days (Table 1). The survival of the untreated shoot tips was significantly higher than the irradiated ones. Among the irradiated explants, the effect of low levels of irradiation (10 and 20 Gy) on the survival of explants was similar, but it decreased significantly on higher doses (30 to 90 Gy) 40days after irradiation. Based on the survival and shoot proliferation of Grand Nain LD_{50} was 30.6 Gy (Figure 1). The number of shoots regenerated per explant also decreased significantly with the increase of radiation dose (Table 1).

Fresh weight of explants irradiated by 10 Gy was similar to the untreated shoot tip explants, but it was significantly higher than that of explants irradiated by doses higher than 20 Gy (Table 2).

Effect of Acute and split irradiation on Musa AAA (Cavendish subgroup) "Grand Nain"

The survival of shoot tip explants treated with 90 Gy in a single dose was significantly lower than other treatments after 20 and 30 days (Table 3). The survival of explants decreased significantly with the increase in irradiation dose in both acute and split irradiation compared to the control after 40 days. Shoot proliferation was induced on explants irradiated by all doses except 90 Gy as a single or split dose and 60 Gy in split dose. Survival of explants irradiated by a single dose was higher on any dose compared to that on the same dose when applied in split doses (Table 3).

Mutation induction on banana cv. Grand Nain by ethylmethanesulphonate (EMS)

Survival of shoot tip explants decreased significantly with the increase of EMS and time of incubation (Table 4). Survival of explants was significantly higher on the low concentration of EMS (0-0.8%) compared with high concentration in the presence and absence of DMSO after 2 weeks. However, the survival of explants was similar on the same EMS concentration in presence and absence of DMSO after 2 weeks. The survival shoot tip explant on control treatments was significantly higher compared to all treatments after 4 and 6 weeks. The survival of explants was comparable on EMS concentrations from 0.4 to 1.2% with and without DMSO, however, no survival on 1.6% EMS. Shoot regeneration was induced on explants cultured on 0.2% EMS. Chemical mutagenesis of banana cv. Grand Nain can be induced by 0.2% with and without DMSO.

In vitro shoot-tip culture has been used as a system for mutation induction in banana and plantain (Novak *et al.*, 1987). They also stated that the differences in radio sensitivity were dependent on ploidy level the hybrid constitution of the A and B genome. The diploid clone 'SH- 3142' (AA) was the most sensitive to gamma irradiation while the tetraploid 'SH – 3436' (AAAA) expressed the lowest level of radiation damage among seven clones tested. The suitable doses for exposure of *Musa* shoot-tips cultured *in vitro* were calculated on the base of 50% of growth reduction and on morphogenetic performance in reference to the non-irradiated control. The recommended doses were 25 Gy for diploids, 35 Gy for triploids AAA, 40 Gy for AAB and, and 50 Gy for tetraploids AAAA. Yang *et al.* (1995) reported that LD_{50} for mutation induction of shoot tip explants of banana cv. Williams was 40 Gy. Yang *et al*. (1995) found that the LD_{50} dose for intact shoot tips of banana was 42.5 Gy.

Omer *et al*. (1989) found that the optimal response of cultured shoot-tips to the chemical mutagen ethyl methane sulphonate (EMS) in both diploid (SH-3362) and triploid (Grand Nain) clones was achieved with 24.67 mM (0.2%) concentration following 3 h incubation in the mutagen solution. Presence of dimethylsulfoxide (DMSO) in combination with EMS greatly enhanced uptake of EMS into the apical meristematic dome, leaf primordial and tissue. Accumulation of the mutagenic component in the shoot- tip explant is an essential step towards mutation induction, since many adventitious buds are initiated from the meristematic tissue. Novak *et al.* (1987) used 0.5% EMS for two hours for mutation induction of Cavendish banana (AAA).

Recommendations

The following are recommendations for *in vitro* mutation induction on shoot tip explants of the Banana cv. Grand Nain:

1. The dose rate recommended for LD_{50} using gamma radiations from ^{60}C source is 33.6 Gy.
2. Chemical mutagenesis can be induced by EMS at 0.2% (v/v) with or without DMSO on shoot tip explants.

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Table 1. Survival and number of shoot regenerated per explants of banana cv. Grand Nain after 6 weeks of irradiation by different doses of gamma rays

Irradiation dose Gy	Survival of explants %	No. of shoots per explant
0	100.0a	3.1a
10	84.0b	2.8a
20	78.0b	1.1b
30	46.0c	1.1b
40	16.0d	0.7c
60	12.0d	0.5c
90	2.0d	0.1d

Means followed by the same letter within each column are not significantly different according to Duncan's Multiple Range Test at 5%.

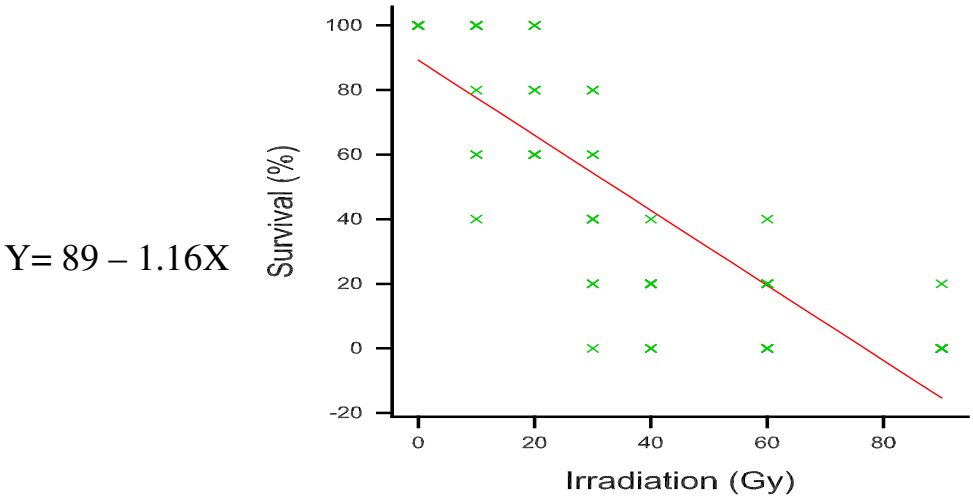

Fig.1. Response of percentage survival of shoot tip explants of banana cv. Grand Nain to irradiation dose after 40 days.

Table 2. Fresh weight of shoot tip explants of banana cv. Grand Nain irradiated by gamma rays.

Irradiation dose (Gy)	Fresh weight per explant (mg)		
	10 days	20 days	40 days
0	153a	609 a LD_{50}	1607 a
10	165a	572 a	1288 b
20	52b	155 b	398 c
30	65b	169 b	298 c
40	60b	156 b	194 d
60	62b	127 b	148 de
90	73b	111 b	115 e

Means followed by the same letter within each column are not significantly different according to Duncan's Multiple Range Test at 5%.

Table 3. Effect of acute and split irradiation on percentage of survival and number of shoots regenerated per shoot tip explant of banana cv. Grand Nain .

Irradiation dose (Gy)	Survival of shoot tip explants after			Shoot per explant
	20 days	30 days	40 days	
0 (control)	100.0a	100.0 a	93.3a	2.4a
30 acute dose	100.0a	100.0 a	46.7b	1.4b
60 acute dose	100.0a	93.3a	40.5bc	1.0b
90 acute dose	86.7b	53.3b	33.3c	0.0c
30 split dose	100.0a	100.0a	53.3b	1.0b
60 split dose	100.0a	96.7a	0.0e	0.0c
90 split dose	100.0a	50.0b	0.0e	0.0c

Means followed by the same letter within each column are not significantly different according to Duncan's Multiple Range Test at 5%.

Table 4. Effect of ethylmethanesulphonate concentration on survival and morphogenesis of banana cv. Grand Nain.

Concentration of EMS[a] (%)	Survival of explants (%)			Explants with shoots (%)	Shoots Per explants
	2 weeks	4 weeks	6 weeks		
0 EMS+D	86.7a	93.3a	93.3a	30	1.6
0.2 EMS+D	83.3a	40.0b	40.0bc	20	1.0
0.4 EMS+D	73.3a	33.3b	26.0c	0	0
0.8 EMS+D	43.3b	6.7c	16.7c	0	0
1.2 EMS+D	20.0c	20.0c	23.3c	0	0
1.6 EMS+D	0.0d	0.0d	0.0d	0	0
0 EMS	93.3a	94.7a	60.0b	28	1.0
0.2 EMS	70.0a	50.0b	40.0bc	10	1.0
0.4 EMS	73.3a	6.7c	6.7d	0	0
0.8 EMS	36.7bc	40.0b	36.7bc	0	0
1.2 EMS	16.7c	13.3c	13.3c	0	0
1.6 EMS	0.0d	0.0a	0.0d	0	0

a =EMS = Ethymethanesulphonate.

b= EMS + D = Ethymethanesulphonate + 2% Dimethyl sulphoxide.