Effect of gamma radiation on growth of explants of banana cv Grand Naine

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ABSTRACT

In the present study survival percentage and regeneration of in vitro shoot tip cultures of banana showed the inverse relation with irradiation doses. Days required for greening were required comparatively more in higher doses of irradiations ie 60 Gy (12.2 days). In case of shoot and root characters significantly maximum number of shoots per explant (3.90), number of leaves per shoot (4.60), average shoot length (5.65 cm), minimum number of days (10.24 days) for first leaf emergence, maximum per cent rooting (100), length of primary roots (6.2 cm), number of roots per shoot (8.1) and minimum days for first root initiation (3.10 days) were recorded in control over rest of the treatments.

Keywords: Irradiation; shoot tip cultures; banana; explants

INTRODUCTION

Banana and plantain (*Musa* sp) are widely grown in India with great socio-economic significance interwoven in the cultural heritage of the country. Conventional breeding methods have been of limited success in *Musa* species due to parthenocarpy and polyploidy in many cultivars and limited available information on genetics and genomics. To produce new banana cultivars breeders should understand the genetic differences of the potential crossing parents for introgression hybridization but extensive genetic information is lacking (Chopra 2005, De Capdeville et al 2009). In plant breeding mutation induction has become an effective way for extending plant genetic germplasm and improvement of cultivars. Induced mutations are similar to spontaneous ones but their frequency is much higher. Mutation induction coupled with in vitro technique causes morphological changes and increases variability in quantitative traits. In *Musa* spp mutation breeding provides opportunities to improve one or a few characteristics in an already established cultivar without affecting the desirable traits (Jain et al 1998, Nybom 2001, Lakshmanan et al 2007). In the present study an effort was made to create variation in cv Grand Naine using physical mutagen and multiplication of mutants.

MATERIAL and METHODS

The sword suckers of the banana cultivar Grand Naine were collected from the BRC, Nanded, Maharashtra. The suckers were initially surface-cleaned with liquid detergent and then with distilled water. After the removal of several ensheathing leaves the explants were treated with 5 per cent chlorine water, 70 per cent ethanol and 0.1 per cent $HgCl_2$ for 15, 8 and 7 minutes respectively under aseptic conditions with a minimum of four rinses with sterile double-distilled water after each treatment. Shoot tip cultures were then maintained on liquid MS medium supplemented with 2 mg/l BAP. After 2 to 3 weeks the established shoots were transferred to semi-solid shoot multiplication medium supplemented with 2 mg/l BAP. The shoot tip cultures of banana var Grand Naine were exposed to ^{60}Co gamma rays doses ranging from 0, 10, 20, 30, 40, 50 and 60 Gy @ 20 Gy/minute. The irradiated explants were sub-cultured (M_1V_0) onto shoot proliferation medium (MS medium containing 2 mg/l BAP). Further sub-culturing was performed at

an interval of 30 days upto M_1V_4 generation and then transferred for rooting media ($1/2^{th}$ strength MS medium supplemented with 2 mg/l IBA and 3 g/l activated charcoal) to obtain rooted plantlets. The pH of the medium was adjusted to 5.8 and all the cultures were maintained under fluorescent light (1000 lux) for 10 h/day at a temperature of 25 $\pm$ 2°C and 55-60 per cent relative humidity.

The experiment was laid out in completely randomized block design with seven treatments (0, 10, 20, 30, 40, 50 and 60 Gy) and replicated thrice. An observation on initial shoot tip explant establishment of in vitro cultures was assessed by recording survival percentage after 28 days, per cent regeneration and days required for greening. The post-irradiation shooting and rooting response of in vitro individual shoots was determined by recording days to root initiation, root number, root length, shoot length and number of leaves in M_1V_4.

RESULTS and DISCUSSION

The perusal of data presented in Table 1 (Plate 1) reveal that shoot tip cultures treated with different doses of gamma irradiation were cultured on MS basal medium supplemented with 2 mg/l BAP. Among the different dosages of gamma irradiation tests maximum survival of shoots and per cent regeneration were observed in unirradiated treatment (100 and 94.41% respectively) while minimum survival of shoots and per cent regeneration were observed in treatment of 60 Gy (33.33 and 30.55% respectively). In general initial establishment of shoot tip cultures exhibited a decreasing trend with increasing levels of gamma irradiations. Minimum days to greening (3.9 days) were recorded in control and maximum in 60 Gy (12.2 days).

Decreased survival rate due to irradiation may be owing to induction in reduced hydrolysis thus resulting in lack of an energy source which may be because of chromosomal and extra chromosomal damage to the cells (Ruprecht 1972). The reduction in regeneration in shoot tips has been attributed to the changes in the level of auxins and ascorbic acid content, physiological and biochemical disturbances, changes in enzymatic activity, chromosomal breakage and DNA synthesis.The effect of gamma rays was more pronounced in increased doses. Similar observations on reduction in survival and regeneration percentage were observed in banana by Pestana et al (2010) and Rayis and Abdallah (2014).

It is evident from Table 2 (Plate 2) that in case of shoot characters significantly maximum number of shoots per explant (3.90), number of leaves per shoot (4.60), average shoot length (5.65 cm) and minimum number of days (10.24) for first leaf emergence were recorded in the treatment T_1 over rest of the treatments under study. Significantly minimum number of shoots per explant (1.0), number of leaves per shoot (2.4), average shoot length (2.77 cm) and maximum number of days for first leaf emergence (23.05) were recorded in the treatment 60 Gy gamma irradiations followed by T_6. Reduced shoot characters may be due to irradiation that caused cell damage and inhibited growth that may have resulted in drastic chromosomal aberration in addition to genetic mutations. At higher doses too many mutational events may be induced with increased risk of favorable mutation being accomplished with one or more unfavorable genetic changes viz deletion, duplication, aneuploidy, breakage of linkage association and translocation as opined by Donini and Micke (1984). Similar effects were obtained at lower doses by Rathod (2013) and Mishra et al (2007) in banana.

In case of root characters as depicted in Table 3 significantly maximum per cent rooting (100), length of primary roots (6.2 cm), number of roots per shoot (8.1), minimum days for first root initiation (3.10) and days to complete rooting (21.45) were recorded in the shoot tip cultures applied with 0 Gy gamma irradiation which were found statistically superior over rest of the treatments of irradiations applied. The treatment T_7 recorded significantly minimum rooting (85.33%), length of primary roots (2.92 cm), number of roots per shoot (2.9), maximum days to first root initiation (27.8) and days for complete rooting (53.58). The root initiation was more at higher doses (>40 Gy) and this might be due to the recovery of solid homohistonts as a result of repeated subculture and absence of chimeric tissue.

Post-irradiation subculture facilitates separation of chimeras and diplontic selection which further lead to elimination of mutated cells because of competition between mutated and non-mutated cells and enhance the recuperation of solid mutants which are mostly periclinal chimeras in the tissue as suggested by Broerjtes and van Harten (1988). The results of present study are in accordance with the reports of Kulkarni et al (1997) who studied gamma irradiation effects in banana and observed rooting even at 40 Gy and Karmarkar et al (2001) who recorded rooting at 50 Gy in banana var Basrai.

Table 1. Effect of gamma irradiation on radio-sensitivity of shoot-tip cultures of banana var Grand Naine

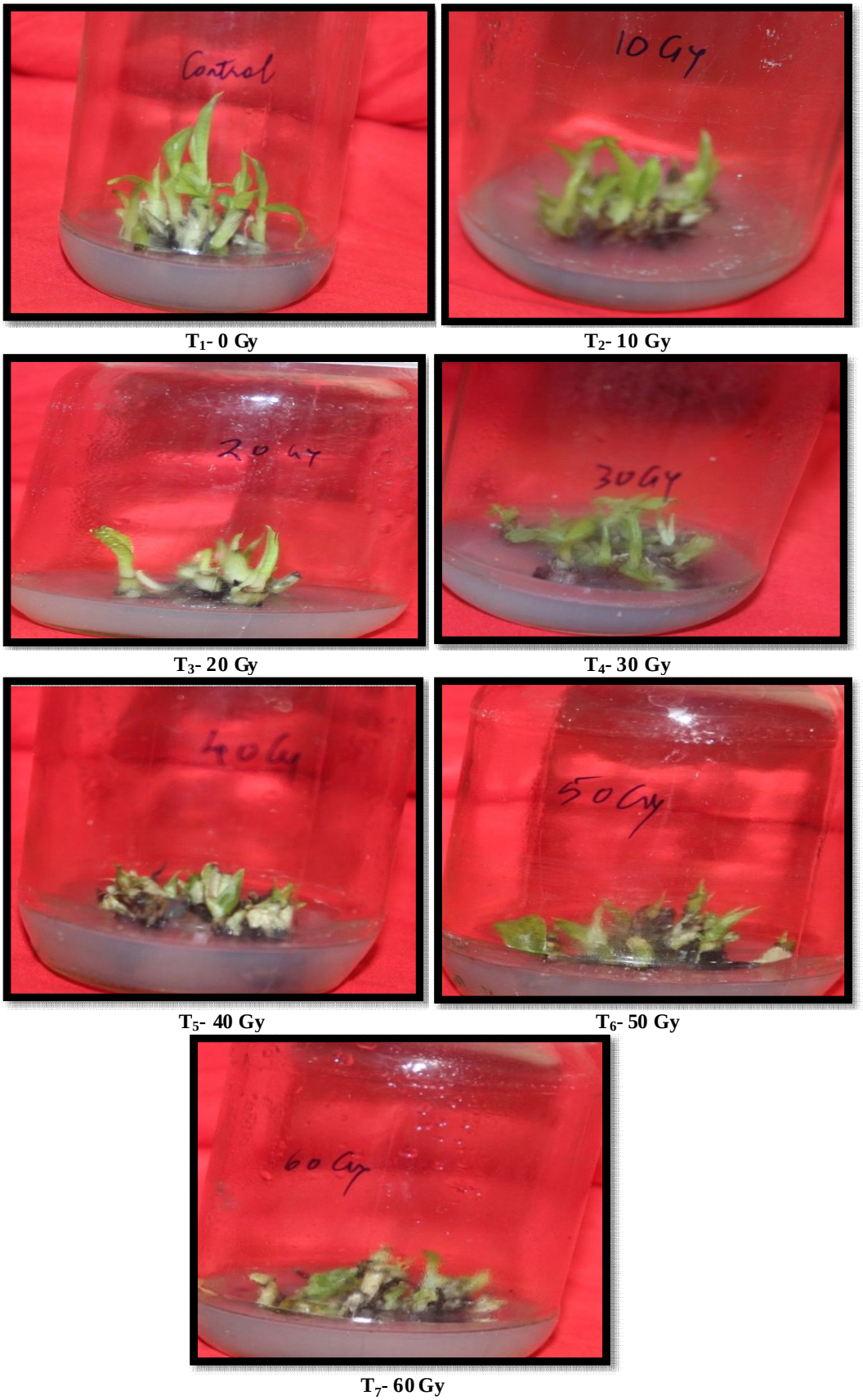
Treatment	Dosage	Per cent survival (28 days after irradiation)	Per cent regeneration	Days required for greening
T_1	0	100.00 (89.71)	94.41 (77.33)	3.90
T_2	10	85.41 (67.73)	80.55 (64.43)	4.80
T_3	20	72.01 (58.74)	69.44 (54.06)	6.10
T_4	30	60.41 (51.02)	52.77 (46.19)	7.82
T_5	40	54.66 (47.66)	41.66 (41.858)	9.35
T_6	50	45.66 (40.19)	35.83 (36.76)	11.10
T_7	60	33.33 (35.26)	30.55 (33.555)	12.20
Mean		55.75 (48.30)	50.79	7.89
SE±		1.42	0.26	2.38
$CD_{0.01}$		4.27	0.78	7.16

Table 2. Effect of gamma irradiation on shoot characters of shoot-tip cultures of banana var Grand Naine

Treatment	Dosage (Gy)	Shoot character			
		Days for first leaf emergence	Number of shoots/explant	Number of leaves/shoot	Shoot length (cm)
T_1	0	10.24	3.90	4.60	5.65
T_2	10	14.90	3.10	4.32	5.20
T_3	20	17.45	2.80	4.03	4.57
T_4	30	19.51	1.85	3.66	4.30
T_5	40	21.20	1.35	3.45	3.68
T_6	50	21.97	1.00	3.05	3.46
T_7	60	23.05	1.00	2.40	2.77
Mean		18.33	2.14	3.62	4.23
SE±		0.89	0.04	0.14	0.22
$CD_{0.01}$		2.68	0.12	0.42	0.66

Table 3. Effect of gamma irradiation on in vitro rooting attributes of an individual shoot of banana

Treatment	Dosage (Gy)	Rooting character			
		Per cent rooting	Days to first root initiation	Number of roots/shoot	Length of primary root (cm)
T_1	0	100 (89.71)	3.10	8.10	6.20
T_2	10	100 (89.71)	7.40	6.05	5.70
T_3	20	95.66 (77.97)	9.65	5.65	5.10
T_4	30	95.66 (77.97)	13.40	4.89	4.55
T_5	40	95.66 (77.97)	17.55	4.27	3.82
T_6	50	90.00 (71.56)	22.30	3.40	3.60
T_7	60	85.33 (71.56)	27.80	2.90	2.92
Mean		94.61 (76.57)	15.88	36.28	4.51
SE±		1.35	0.24	0.47	0.17
$CD_{0.01}$		4.05	0.72	1.46	0.58



T_1- 0 Gy T_2- 10 Gy

T_3- 20 Gy T_4- 30 Gy

T_5- 40 Gy T_6- 50 Gy

T_7- 60 Gy

Plate 1. Effect of doses of gamma irradiation on survival of shoot tips of banana

Control, 10 Gy

Control, 20 Gy

Control, 30 Gy

Control, 40 Gy

Control, 50 Gy

Control, 60 Gy

Plate 2. Effect of doses of gamma irradiation on shoot characters of shoot tips of banana

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