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Mutation Induction for Improvement of Banana *(Musa Spp.)* "Berangan Cv. Intan-AAA"

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Abstract: **In vitro mutation induction by using gamma ray at 20, 30, 40 and 60 Gy was used to generate variability in triploid banana "Berangan cv. Intan (AAA), so as to provide the opportunity to select plants with desirable characters such as early fruiting and short stature. Mutation frequency increased with increased dosage whereas, survival and capacity to regenerate decreased with increased doses. Time to initiation varied from 4-8 weeks for gamma-irradiated materials compared to 2-3 weeks in the control. It appeared that the higher the dose, the longer it took for shoot initiation. The exposure of shoot-tip meristem pieces to radiation doses produced wide variation in growth and morphogenetic performance. Mutagenic treatments induced 2 to 3- fold increases in variability in both quantitative and qualitative traits at different stages, in vitro; at nursery and field. For the field-grown plants; the proportion varied from 2.9% for the control plants to 16.8% for 60 Gy and 20.1% for 40 Gy-treatments, while for treatments at 20 and 30 Gy variations was at 18% and 19.2%, respectively. The frequency of variants was highest in 40 Gy followed by 30 Gy and 20 Gy, while it was very low in 60 Gy except for plant stature (dwarfism or stunted growth). Earliness to flowering variants were recorded at 20, 30 and 40 Gy at low frequencies (0.6%, 0.7% and 1.7%) respectively, while none was observed for 60 Gy treatments. As in vitro mutation induction could create genetic variability as well as many undesirable variants, it is highly desirable to integrate in vitro mutation with a selection system that can screen for large mutagen treated population. The useful variants recorded for earliness to flowering were selected for 30 and 40 Gy treatments. 40 Gy showed high frequency in earliness as compared with 20 and 30 Gy., which came to flowering as early as 6 – 6.5 months compared to 7-8 months for control. The useful dwarf, which considered as desirable traits, showed a balance of height and girth.**

Keywords: **Musa spp.- mutation breeding- gamma irradiation (Gy)**

I. INTRODUCTION

Banana (*Musa spp.*) is one of the most important fruit crops in the world both as a major export income as well as a staple food for millions in the developing countries. Banana and plantain are among the worlds major food crops, and considered as the poor man fruit crop in tropical and subtropical countries. The world total banana and plantain production ranks 5th after cereals, and there is still much scope for yield improvement (Jain *et al,* 2004). Berangan (cv. Intan) has good quality, color, texture, size and shelf-life in addition to its ease of ripening without cold treatment. As Berangan are sterile triploid bananas, genetic recombination in parthenocarpic triploids is extremely difficult due to high sterility and meiotic irregularities. Generally, the genetic system of *Musa* is extremely complicated to study because of the serious problems of sterility, interspecific hybridity, heterozygosity, and polyploidy that common in most of the clones (Novak *et al.* 1993). Therefore, the complexity of *Musa* genetics needs a more effective conventional breeding programs, and prospects of using biotechnology in bananas are very high (Lakshmanan, *et al.* 2007). During the last decade, many *in vitro* techniques of plant tissue culture and cell culture have been developed and refined as an aid to conventional plant breeding (Razdan, 2002; Takayama, 2002 and Manickam 2007). These include efficiency in obtaining variation, selection and multiplication of the desired genotypes. Some of these biotechnologies applied to *Musa* include *in vitro* mutation induction (Ho *et al.* 1994), cell protoplast culture (Lakshmanan, *et al.* 2007) and somatic embryogenesis (Escalant *et al.* 1994; Wieczorek, 2003; Lopes, *et al.* 2006). In plant breeding, mutation induction has become an effective way for

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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 cause morphological changes and increase variability in quantitative traits. Physical mutagens such as gamma ray are tools for enhancing and generating genetic variation by inducing mutations at the gene, chromosome and genome levels, in nuclear and cytoplasmic organelle DNA (Larkin, 1998; Nybom, 2001; Korzun, 2002; Lakshmanan, *et al.* 2007). In *Musa* spp., mutation breeding provides opportunities to improve one or a few characteristics in an already established cultivar without affecting the desirable traits.

The objectives of this study are to generate genetic variability in Berangan cultivar through mutation induction by gamma irradiation, to study mutagenic effect at morphological and agronomical levels and to provide the opportunity to select plants with desirable characters such as early fruiting and short stature.

II. MATERIALS AND METHOD

Mutation induction:

The shoot-tip meristem pieces (about 1cm x 2 mm) were aseptically excised from micropropagated plantlets of (Berangan cv. Intan). Each shoot-tip was cut longitudinally into two pieces and then transferred to sterile petri dishes, which contained 10 meristem pieces and was sealed with parafilm. The petri dishes, which contained the meristem pieces, were irradiated in a gamma cell with a Cobalt-60 (^{60}Co) source (GC 400A, 10 Kci). A total of four batches were irradiated at 20 Gy, 30 Gy, 40Gy and 60Gy. The irradiated meristem pieces were washed thoroughly with sterile distilled water and cultured on modified MS solid medium. The explants were cultured in jar-jar bottles each containing five explants. Sub culturing was carried out at 4 – 6 weeks interval until generation M_1V_4 i.e. three sub-cultures. The explants after M_1V_3 were allowed to root in rooting medium containing MS salts supplemented with 2 mg/l IAA and 5 mg/l kinetin. After three to four weeks in rooting medium, the plantlets were deflasked and transplanted in the nursery for acclimatization or hardening. The healthy, vigorous and well-rooted plantlets were transplanted into the polybags (5″ x 7″) filled with peat and washed sand at 1:2 ratio. The plantlets were maintained under 70% - 80% shade (using shading net) and high humidity. The plantlets were irrigated twice a day. Shade was removed completely after 5 weeks. From the 4th week after transplanting until plants were ready for planting, foliar fertilizer "GROFAS" was applied twice weekly to the plants at a rate of 10 g in 4.5 liters of water. The nursery plants were ready for field planting after 8 – 9 weeks of hardening.

Evaluation of gamma irradiated plants:

Five batches of materials were generated for field evaluation trials (Table 1). The dimensions of the planting hole were 30 cm^2. The plants were grown in rows spaced at 2.5m x 2.5 m in a triangular pattern in the field.

The five treatments (0, 20, 30, 40 and 60 Gy) were completely randomized in the field. Ground Magnesium Limestone (GML) was broadcasted and mixed into soil a few days before planting.

Organic fertilizer Supergro Green (CIRP) - with a 5: 5: 5:1 nutrient composition – 75 grams per point – was mixed with soil in the planting hole. CCM65 and ccm44 were applied after that around the base of the stem at doses of 220 and 200 g/point, respectively at monthly interval. Weeding was done at monthly intervals. Unwanted young suckers were pruned 2-3 months after field planting and then at regular interval of 6-8 weeks.

Table 1: The number of generated banana plants (irradiated and control) for field evaluation

Dose (Gy)	Batches					Total
Treatment	1	2	3	4	5	
0 (control)	260	345	361	362	202	1540
20	170	170	170	170	210	890
30	180	180	180	180	188	908
40	274	280	668	466	400	2288
60	71	148	-	-	-	219

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Morphological variation evaluated in the greenhouse and the field:

The nursery plants were examined for any mutagenic affects on growth and morphological characteristics. The observation was made at the final stage of hardening (7-8 weeks old) before transplanting to the field. Additional observations were made in the field at the vegetative stage and after flowering.

Statistical analysis:

In numerical identification, analysis of variance (ANOVA) of each character was compared. Means and coefficients of variation were also calculated. Statistical analysis used the SPSS (Norusis, 1990) Base 9.0, which is a comprehensive system for analyzing data.

III. RESULTS

Mutation Induction, Post-irradiation recovery and radio sensitivity:

The irradiation meristems resumed growth 35-40 days after gamma-irradiation, while the control (non-irradiated) produced shoot/buds within 2-3 weeks. Browning and blackening were observed 15-17 days after irradiation. Time to initiation varied from 4-8 weeks for gamma-irradiated materials compared to 2-3 weeks in the control. It appeared that the higher the dose, the longer it took for shoot initiation. Adventitious buds were differentiated from single or a few superficial cells of an irradiated rhizome. No callus formation was observed in bud regeneration. Four sub culturing at monthly interval for irradiated explant (M_1) were carried out (i.e. M_1V_4) to reduce chimerism Post-irradiation recovery was estimated as survival rate of clean explants, which showed bud/shoot proliferation. The time required for proliferation in gamma-irradiated explants was twice that required during the usual proliferation cycle. The treated plants showed 0% - 50% survival rate, while the control showed vigorous growth. The rooted plants were transplanted into soil foe acclimatization and hardening, the transplanted in the field. The irradiated explants showed some unusual foliar features and other abnormalities.

Variability induced by gamma irradiation:

Mutation induction caused morphological changes in qualitative as well as in quantitative traits. However, mutagenic treatments increased the proportion of variant plants at different stages, *in vitro*; at nursery and field. For the field-grown plants; the proportion varied from 2.9% for the control plants to 16.8% for 60 Gy and 20.1% for 40 Gy-treatments, while for treatments at 20 and 30 Gy variations was at 18% and 19.2%, respectively.

Frequency of mutagenic changes:

The results showed that frequency of variants increased as gamma doses increased. However, the frequency of variants was highest in 40 Gy followed by 30 Gy and 20 Gy, while it was very low in 60 Gy except for plant stature (dwarfism or stunted growth) (Table 2). It was also noted that the frequency of variant plants in the 60 Gy treatments was not highest in both nursery and field-grown plants. Many lethal or abnormal mutants at this dose might not survival at all. For the control plants, 2.1% variant plants were observed at the nursery stage and 2.9% for the field grown plants. Such phenomenon of occasional phenotypic variants known as somaclonal variant is ubiquitous in banana tissue cultured plants. Table 2, showed the types and frequency of variants in gamma irradiated population at the field. The total frequency of leaf characters was evident in (12.5), 1.79 % of 20 Gy treatments, in about 2.9 % of 30 Gy, 5.4 of 40 Gy and 2.4 in 60 Gy. Also, the total frequency of: pseudostem characters; bunch characters; plant stature and flowering was 8.5, 0.7, 7.6 and 3.0 respectively (Table 2). Earliness to flowering (plants which flowered at 5 -6 months or earlier after planting were considered an early in comparison to those which flowered late in 9 -11 months) variants were recorded at 20, 30 and 40 Gy at low frequencies (0.6%, 0.7% and 1.7%) respectively, while non was observed for 60 Gy treatment. The response for flowering time was skewed to lateness for high dose of gamma-irradiated plants, while earliness to flowering was recorded for about 3.0% of the whole population. The variants came to shooting as early as 0.6 – 6.5 months, compared with 7 -8 months for the control. Weeks to flowering time, however, remained the same for both low dose irradiated (20 Gy and 30 Gy) and non irradiated plants (50 -60 days), while it differed significantly for plant treated with the high doses (40 Gy and 60 Gy) which ranged from 65 to 95 days. The variability observed for all quantitative traits resulted from mutagenic treatments as well as environmental factors. Many of these changes were undesirable, nevertheless, some were desirable. Plants of short stature (extra dwarf) were undesirable because they produced small fruit bunch, Berangan (cv. Intan) is taller cultivar where a shorter stature is desirable but not dwarfism. A wide variation

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in plant height was also observed among gamma-irradiated plants (Table, 3). In control plants the frequency distribution appeared normal with coefficient of variation (CV) of 10.8%. Plant height for 20 Gy showed frequency distribution very similar to control plants. At 30 Gy showed smaller range of variability in plant height. The reduced variability was reflected in the (CV) value of 7.9%. The frequency of plant exceeding 300 cm was 20% while those of less than 290 cm was 10% of the population. At 40 Gy treatments, variation in plant height ranged from 202 to 390 cm with C.V. of 10.9%. On the other hand extremely short plants (<200 cm) occurred at a frequency of 5% at 60 Gy. The variability increased based on C.V. values of 14.4%. Many plants showed stunted growth, with frequency of 10% for plants ranging from 150 – 180 cm.

Table 2: Types and frequency of variants for Gamma-irradiated Berangan at the field stage

Types of variation	**Frequency (%)**				**Total frequency**
	20 Gy	30 Gy	40 Gy	60 Gy	
Leaf characters	1.7	2.9	5.4	2.4	12.5
Pseudostem characters	1.7	2.0	3.5	1.3	8.5
Bunch characters	0.0	0.0	0.3	0.4	0.7
Plant stature	0.7	0.9	1.4	4.6	7.6
Earliness to flowering (5-7 months)	0.6	0.7	1.7	0.0	3.0

The control plants showed significant difference from gamma-treated plants at the different doses with respect to pseudostem circumference (girth) measurements. As showed in (Table, 3) the C.V. was very high for all doses as compared with control in particular for 40 and 60 Gy. The results showed wide variation in days to flower in all treated plants and their control. However, inflorescence emergence (spiking) occurred 189 days (27 weeds) after field planting and 55% of the plants flowered after 245 days (35 weeks).

Table 3: Performance of Gamma irradiation plants (cv. Intan)

Character	**Statistics**	**20 Gy**	**30 Gy**	**40 Gy**	**60 Gy**	**Con. (0Gy)**
	N	**113**	**87**	**200**	**200**	**113**
Height at flowering@ (cm)	Mean± SE	306.6±31.1	310 ±24.4	287± 31.3	218±31.5	302.6±32.7
	C.V.	10.2%	7.9 %	10.9 %	14.4%	10.8 %
	Min-Max	221-381	240 -374	202 -390	150- 324	209 -380
Girth at flowering# (cm)	Mean± SE	66.4± 7.89	66.5 ± 7.4	70.5±10.6	69.5±11.1	67.1± 8.14
	C.V.	11.8 %	11.1 %	15.1 %	16.0%	8.2 %
	Min-Max	46 -91	54 -84	45 -93	36-96	60 -70
Planting to flower (Dayes)	N*	100	100	100	100	100
	Mean± SE	274 ± 44.1	290± 33.3	274± 44.0	310.2±34	248.2± 10.1
	C.V.	16 %	11.5%	16 %	11 %	4.06 %
	Min-Max	192 -364	217-364	189 -350	250-380	

*REF: @ Height from ground level to curve of peduncle (bunch stalk);# Girth at 30 cm above ground level; N*t the number of plants examined at planting to flowering which differ from other sample sizes; SE = (std error) Standard deviation of mean; C.V. Coefficient of variation*

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Mean values of days to flowering showed significant difference between 40 Gy treatments and control, while plants treated at 20 Gy and 30 Gy were later by 26 and 32 days from control, respectively. Plants which flowered in less than 231 days (189, 217 and 224 days) were selected for further evaluation and considered as early flowering candidate plants.

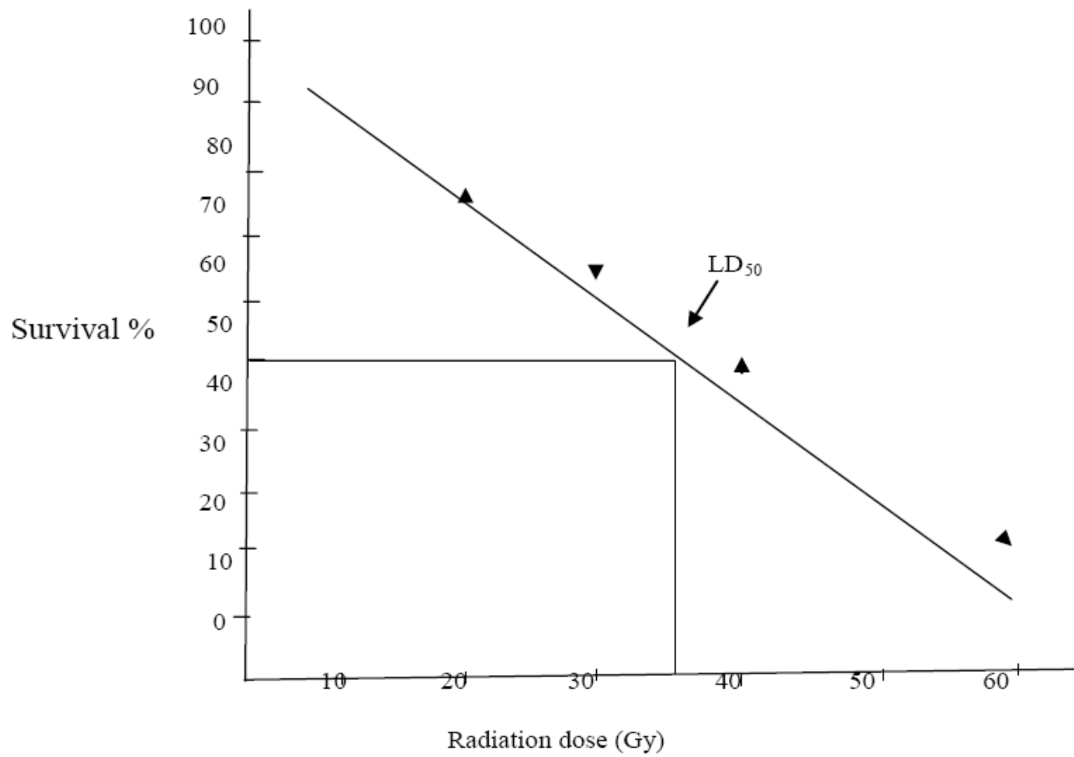

Fig. (1): Radio sensitivity test curve of shoot tip from Berangan cv. Int Radiation dose (Gy)

IV. DISCUSSION

Exploitation of *in vitro* mutation for banana improvement by using gamma ray:

The advantages of the system (*in vitro* shoot-tip culture) included rapid propagation of the explants prior to mutagenic treatment (Manickam, 2007), and the recovery of preclinical and/or homohistonic structures through repeated subculture by formation of axillary and/or adventitious shoots from the treated apex. Prior to any bulk mutagenic treatment, the optimal doses of mutagens to be determined due to the clone x mutagen dose/concentration interactions (Novak *et al.* 1990; Smith *et al.* 1990). Generally, a radiation dose that reduces the growth to 50% et *al*1990). Therefore, for mutation induction of *in vitro* cultured shoot-tip meristems of Berangan by gamma irradiation, the LD_{50} dose of about 38 Gy (Make, *et al.* 1995) and 30 to 45 Gy by Novak, *et al.* (1990), were used as guide for the present study. The gamma-irradiated explants showed variable response. Control plants showed earlier initiation while the latest was for explants treated at high dose of 60 Gy. This indicates that high dose of irradiation could cause cell damage and inhibit growth. Similar response was reported by Mak and Ho (1997). The number of buds/shoot produced per explant decreased with an increase of irradiation dose. The multiplication rate was low for the first two subcultures but gradually increased after M_1V_4. This indicates that ionizing radiation affected some cells while the others resumed their growth after 2 subcultures. Van Harten (1998) reported that most meristem pieces contain several initial cells in their growing points; however, a distinct mutation would only occur in one of them. The generally regularity is that the higher the number of surviving initial cells, the smaller the mutant sector of the M_1 plant, the lower the proportion of mutant plants in the respective generation (Manickam, 2007).

Post-irradiation recovery and radio-sensitivity:

The 50% growth reduction occurred between 30 -40 Gy as reflected by the number of shoots produced, agreed very well with that reported by Novak *et al.* (1990) that gamma-dose of 35 - 40 Gy was suitable for triploids (AAA). Furthermore,

Mak *et al.* (1997) and Jain (2004) recorded similar results with Berangan (AAA). Radio sensitivity varies between plants and depends mainly on the nuclear volume, the greater the DNA contents, the more sensitive; and the ploidy level, the higher it is, the less radio sensitivity (Broertjes & Van Harten, 1988). However, Novak *et al.* (1990) reported that diploid clones were most sensitive to gamma irradiation while the tetraploids expressed the lowest level of irradiation damage. Therefore, Berangan which is a triploid (AAA), exhibited moderate sensitivity to gamma irradiation. The technique of repeated *in vitro* subculture of irradiated meristems may provide good opportunities for cells carrying mutants to reach phenotypic expression.

Mutant Evaluation:

Generally, the micropropagated banana plants have high survival rate in the field of about 90%. At high doses of irradiation, too many mutational events per cell may be induced, with increased risk that a favorable mutation is accompanied by one or more unfavorable genetic changes. However, in vegetatively propagated crops (bananas), it is impossible to separate favorable from unfavorable mutations by cross-breeding mutants among each other or by back-crossing with the original material. Therefore, the evaluation of the mutants is very difficult genetically unless the use of other techniques such as Flow cytometry for detection of ploidy level and DNA content or RAPD analysis (Nybom, 2001) and gibberellic acid for detection of dwarfism. But phenotypically the evaluation of mutants can be possible depending upon certain characteristics when compared with control plants. Applied mutagenesis is particularly important for the sterile triploid Berangan. Manickam (2007) stated that mutation frequency of 31 -37% was achieved, including both morphological and agronomical traits. Novak *et al.* (1990) recorded 7- 42% mutation frequency in their studies on several banana genotypes treated with 30 Gy gamma rays, while Ponce & Orellana (1994) obtained 25% for Cavendish types treated with 40 Gy gamma rays. The mutations of individual genes and their phenotypic contribution to the agronomic and morphological characters were influenced by both their genetic background and environmental factors. Increased variability is indicated by higher of coefficient of variation (CV). This difference indicated that there was indeed genotype x mutation rate interaction and as such direct comparison should only be made within the same genotype group (Siti Hawa, 1996). Quantitative traits showed continuous variation; the short plant stature variants induced mainly by 30 and 40 Gy-treatments (about 5%) which showed desirable agronomic traits might be selected for confirmation trails. The useful dwarf, which considered as desirable traits, showed a balance of height and girth. As girth size is positively correlated with days to flowering and also plant height at flowering, plants of great girth size also tended to be tall and late in flowering. 40 Gy-plants showed high frequency in earliness (1.7%) as compared with 20 and 30 Gy. These variants came to flowering as early as 6 – 6.5 months compared to 7-8 months for the control. This finding is quite in agreement with that recorded by Mak *et al.* (1998). Earliness and short plant statures are two of the main objectives for the improvement of Intan cultivar. The variations evident in the control plants were very low frequency. These variations ranged from 2.1% to 4.4% for all stages of growth in this study. Such phenomenon of occasional phenotypic variants called somaclonal variation is common in tissue cultured bananas (Jain, 2004; Mak *et al.*, 1995; Larkin, 1998; and Cote *et al.* 1993). But not all variations observed were genetic and only a part of the observed variation could be heritable as reported by Van Harten (1998) as epigenetic changes (Manickam, 2007; Rieget, *et al.* , 1991).

V. CONCLUSIONS

In vitro culture technique facilitates induction, selection and multiplication of mutants. In vitro culture in combination with mutation techniques offers several advantages to overcome some of problems of conventional breeding. Mutation frequency increased with increased dosage. The time required for proliferation in gamma-irradiation explants was twice that required during the usual proliferation cycle. The treated plants showed (0%-50%) survival rate, while the control showed vigorous growth. Mutagenic treatments increase the proportion of variant plants at different stages: in vitro, at nursery and field. The frequency of variants was highest in 40 Gy followed 3o Gy and 20 Gy, while it was very low in 60Gy expected for plants stature (dwarfism stunted growth). The response for flowering time was skewed to lateness for high dose of gamma-irradiated plants, while earliness to flowering was recorded for about 3.0% of the whole population. Not all variations observed were genetic but only a part of these variations could be heritable. The suitable irradiation dose was between 30 Gy and 40 Gy.

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