INFLUENCE OF GAMMA RADIATION ON THE PHYSIOCHEMICAL PROPERTIES OF IN VITRO TRIPLOID AND TETRAPLOID BANANA SPECIES

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ABSTRACT

Resistance to different stresses of banana are much needed, therefore, introductions of new and healthy clones are required. Applications of in vitro mutagenesis are considered as the reliable tool to create variations. For that purpose, in vitro cultured multiple shoots of three Musa clones: Giant Cavendish Tissue Culture variant (GCTCV-215), Yangambi KM-5 and FHIA-23 were exposed to different gamma doses (0, 10, 20, 30, 40 and 50 Gy). Radio-sensitivity of in vitro shoots was assessed by counting data on survival % after 40 days of culture, a rate of shoot multiplication, average shoot, fresh weight (g), root number and root length (cm). Different level of radiation sensitivity was viewed in all Musa varieties. Lower dosage 10 Gy produced raising effect compared to control. The present study may be helpful to introduce new Musa germplasm in our region.

Key Words: Musa germplasm, Gamma rays, mutagenesis, Radio-sensitivity

INTRODUCTION

Banana (Musa spp.) is one of the economically important fruit crop of Pakistan, it is a member of the order Zingiberales, belongs to the family Musaceae and genus Musa. Presently, banana is grown in more or less 150 countries of the world, occupying 4.95 million hectares and production is 102 million tons (FAOSTAT 2014). In Pakistan 90% of banana is grown in the province of Sindh, the rest grows in southern areas of Punjab (Khatri et al., 2009). The average area harvested during the last five years is 30 thousand hectares and the average production is 137 thousand tons, which is very low as compared to other banana-producing countries of the world (FAOSTAT 2014). The dominating variety of banana in Sindh is Basrai (dwarf Cavendish) is being planted on 98% of the area, thus, creating a monoculture and almost non-existence of genetic buffering in the field (Mensah et al., 2012). Due to which in 1990 spread of banana bunchy top diseases (BBTD) appear in the form of an epidemic. This situation leads to the major downfall of banana when the harvested area was decreased from 22.7 thousand ha to 11.2 thousand hectares. Whereas about 80% decrease in the production was observed (Hwang et al., 2004). However the area harvested is increased during the last five years, but an increase in the area does not reflect in production because of infected planting material. Besides other factors of low yield are water insufficiency, salinity, frost, tempe-rature, and damages due to high wind velocity also diminish yield and yield stability (Newbury et al., 2000). In terms of genetic expansion, banana is inflexible in nature due to polyploidy, sterility and vegetative mode (Swennen and Rosales, 1994). Therefore, several other approaches like somaclonal variations and mutation induction can be employed for Musa genetic improvement instead of conventional methods (Novak et al., 1990; Ali, 1997; Jankowicz - Cieslak et al., 2012). Induced mutation through gamma rays and in vitro culturing of a desirable mutant population through micropropagation proposed the broad area of genetic variation (Novak, 1992; Nikam et al., 2015). The absorbance of radiation dose depends on the sensitivity of Musa species. Determining the mutagen sensitivity level (LD50) of particular Musa species is the main step to exploit mutagenic treatment (Jain, 2010). The existing situation requires induction of new genetic material, therefore, in this study three exotic Musa clones were selected for in vitro mutagenesis and the multiplication of these mutant clones may conceivably help to reduce disease spread.

MATERIALS AND METHODS

Selection of Musa Clones: Due to the narrow gene pool for most of the economic characters in Musa varieties, three exotic Musa varieties Giant Cavendish Tissue Culture Variant (GCTCV-215), Yangambi KM-5 and FHIA-23 were selected for in vitro mutagenesis. All varieties were grown in experimental field of Nuclear Institute of Agriculture (NIA), Tandojam. After five months, suckers of Musa varieties were collected, removed basal and extra leaves and transported to the tissue culture laboratory of NIA where an experiment was performed.
Sterilization and Culturing Media: For the prevention of microorganisms and dust particles, suckers at first were washed with tap water followed by trimming in a size of about 2.0-3.0 cm in length and 1.5 cm from base width. Isolated explants were then treated with 70 % alcohol and 3 % sodium hypochlorite separately for 1 and 20 minutes, respectively. Then materials were washed four times with distilled sterile water to wash out the remains of sodium hypochlorite. After that explants were trimmed approximately 6-8 mm from the base and shoot apex and dissected into two halves. For the establishment of explants, MS (Murashige and Sakoog, 1962) shooting medium was prepared from the stock solution which comprises of major and micro salts along with shoot promoting hormone benzyl aminopurine (BAP).

Culture Condition and Maintenance: Explants were transferred to the media (Table 1). Before and after culturing of explants fresh weight was taken to find out the actual weight of each explant and then shifted to growth room. For the establishment of culture, growth room maintained at 25±1°C under 16/8 hours photoperiod with light intensity 2000 Lux. After 25 to 30 days, shoot formation was started. Initially, a bulbous type structure was developed and then it converted into a complete shoot.

Application of Mutagen: To induce variations, micro shoots were treated with physical mutagen, i.e., Gamma rays (0, 10, 20, 30, 40, 50 Gy). For Gamma irradiation, control treatment system (Cobalt 60) at a dose rate of 5.30 Gy per second was used.

Subculturing and Data Collection: After irradiation, the shoots were transferred into same fresh media (Table 1) and maintained in growth room under described light and temperature condition. After culturing of 40 days (M1V0), survival percentage was calculated by counting the number of surviving shoots. For multiplication of the shoot, single shoot was separated from the irradiated surviving cluster (M1V0) and subcultures into fresh medium up to two multiplication cycles (M1V1 to M1V2) to reduce the possibilities of chimeras and stability of mutant traits were carried out. After four weeks of each multiplication cycles the number of shoots per inoculated shoot (M1V0) and fresh weight (g) were counted.

Rooting: For rooting purpose, MS half strength media supplemented with 1.0 mgL⁻¹ were used for in vitro regenerated mutant shoots and maintained the culture in the growth room at the same physical environment as provided for in vitro shoot generations. Data was recorded after four weeks (M1V3), including mean root per irradiated shoot and root length (cm). After that, all plantlet shifted from growth room to greenhouse in plastic bags rich with humus and garden soil.

Statistical Analyzes: Statistical analyzes of all treatment mean were accomplished by the linear model analyses of variance (ANOVA) suggested by Fisher (1921). An experiment was conducted in Completely Randomized Design (CRD) with four replications. Results significance was measured by least significance difference (LSD) at 0.05. STATISTIX (10.0 version) was used as statistical software.

RESULTS AND DISCUSSION
After mutagenic treatment, considerable phenotypic differences were noticed among all varieties for most of the variables. Data was found to significant (p ≤ 0.05) (Table 1a, b, c and d).

![Table -1a: ANOVA showing mean square for survival % (SP), number of shoots per inoculated shoot (NS) and fresh weight (FW) of GCTCVE-215](image)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SP</th>
<th>NS</th>
<th>FW</th>
</tr>
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<tbody>
<tr>
<td>Replications</td>
<td>3</td>
<td>1.06056</td>
<td>0.16667</td>
<td>0.30810</td>
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<tr>
<td>Treatments</td>
<td>5</td>
<td>3.70800*</td>
<td>2.82400*</td>
<td>3.89442*</td>
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<tr>
<td>Error</td>
<td>15</td>
<td>0.46956</td>
<td>0.12000</td>
<td>0.38019</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
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</table>

Significant at p ≤ 0.05, DF= Degree of freedom

![Table -1b: ANOVA showing mean square for survival % (SP), number of shoots per inoculated shoot (NS) and fresh weight (FW) of KM-5](image)

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SP</th>
<th>NS</th>
<th>FW</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>Treatments</td>
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<td>3.36542*</td>
<td>2.32042*</td>
<td>4.73375*</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>0.38008</td>
<td>0.20975</td>
<td>0.16486</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Significant at p ≤ 0.05, DF= Degree of freedom
Survival percent after 40 Days ($M_1 V_0$): Data for survival percent is presented in Table 2. Control show highest survival percent in GCTCV-215 and ‘Yangambi’ KM-5 compared to the mutants. In GCTCV-215 and ‘Yangambi’ KM-5, minimum decrease percent in survival was observed at 10 Gy and maximum at 40 Gy. Among all the doses of gamma rays, 20 Gy showed a moderate reduction, whereas doses higher than 20 Gy found lethal for both GCTCV-215 and ‘Yangambi’ KM-5. In FHIA-23, 10 Gy showed a positive response than that of control and the moderate reduction was noticed at 30 Gy. Shoot survival decline in all varieties with increase in the irradiation dose representing the inverse relationship between two variables. Actually it is accepted the thing that higher mutation frequency achieved at higher dosage rate but on the other hand surviving ability of treated shoot to become decline (Bhagwat and Duncan, 1998; Novak et al., 1990; Dizon et al., 2012). As for concern with the specific varietal response to gamma rays, almost similar level of radiosensitivity was observed in GCTCV-215 and ‘Yangambi’ KM-5 (Figure 1). Both belong to same AAA genomic group. Similar response of AAA genome to gamma radiations was reported by Roux (2004). On the other hand, the post-irradiation recovery of FHIA-23 showed higher tolerance to gamma rays than that of ‘Yangambi’ KM-5 and GCTCV-215. FHIA-23 belongs to AAAA genomic group. Results of present experiment and previous literature indicated that radiosensitivity of treated in vitro shoot is dependent on the mitotic rate of actively growing region, genetic constitution of species and irradiation dose and its rate (Coggle, 1983; Karmarkar et al., 2001).

Table -2: Mean performance and percent increase or decrease for survival rate of irradiated shoot from respective control

<table>
<thead>
<tr>
<th>Doses (Gy)</th>
<th>GCTCV-215</th>
<th>KM-5</th>
<th>FHIA-23</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survival relative to control (%)</td>
<td>Survival relative to control (%)</td>
<td>Survival relative to control (%)</td>
</tr>
<tr>
<td>0</td>
<td>2.50 a</td>
<td>2.37 a</td>
<td>2.00 ab</td>
</tr>
<tr>
<td>10</td>
<td>1.80 ab</td>
<td>-28</td>
<td>1.75 ab</td>
</tr>
<tr>
<td>20</td>
<td>1.27 bc</td>
<td>-49</td>
<td>1.22 bc</td>
</tr>
<tr>
<td>30</td>
<td>0.62 cd</td>
<td>-75</td>
<td>0.80 cd</td>
</tr>
<tr>
<td>40</td>
<td>0.25 cd</td>
<td>-90</td>
<td>0.17 d</td>
</tr>
<tr>
<td>50</td>
<td>0.00 d</td>
<td>0</td>
<td>0.00 d</td>
</tr>
</tbody>
</table>

LSD = p ≤ 0.05, Rate of survival relative to control (sum of survival – value to control*100/value to control)
Number of Shoots per Inoculated Shoot: Data for the number of shoots per inoculated shoot (M₁V₀) is presented in Table 3. As compare to control there is no variations were observed in GCTCV-215 (Figure 2a,b,c and d) and ‘Yangambi’ KM-5 in 1st subculture (M₁V₁) generation (Figure 3a, b, c and d). However, on multiplication stimulatory effect of lower dose 10 Gy within the 2nd subculture (M₁V₂) gene-ration were observed in both varieties (Figure 2e and 3e). In the case of FHIA-23, enhancing an effect of lower dose 10Gy observed in both generations M₁V₁ and M₁V₂ (Figure 4b and 4e). At 30 Gy decline in shoot multiplication was observed while higher doses 40 and 50 Gy were completely lethal for all varieties. Present results are in accordance with the earlier finding Karmarkar et al., (2001) wherein 10 and 15 Gy showed stimulatory affects on multiplication rate. All varieties showed higher shoot multiplication at lower dose 10 Gy and decline in shoot multiplication at higher doses (30 to 50 Gy). However, the response of FHIA-23, to produced shoot was better as compared to other two Musa varieties. In the present research work influence of genomic composition has been observed on multiplication. Earlier researchers have also been reported strong genotypic influence on the rate of multiplication (Novak et al., 1990; Mishra et al., 2007). Another reason for low multiplication suggested by Kulkarni et al. (2007), that apical dominance of a single shoot was also the main cause to reduced multiplication as due to apical dominance inhibit the growth of lateral shoot. It should be observed that multiplication of irradiated shoots was better in 2nd subculture cycle than that of 1st subculture, after mutagenic treatment all varieties required time to become stable. Mutation in the shoot tip containing meristematic zone provided greater mutation frequency that after repetitive in vitro culturing appear as phenotypic expression (Harten, 1998; Mishra et al., 2007). Novak et al. (1990) suggested that by repeated culturing of mutant may help full for the development of homohistant structure and selection of desired characters. Some morphological changes were also observed in Musa varieties during subculturing of mutants. Such as formation of albino shoots were observed in GCTCV-215 and ‘Yangambi’ KM-5 during second subculture cycle treated with 10 and 20 Gy, respectively (Figure 2e and f). In many plant, these morphological changes were reported earlier (Girija and Dhanavel, 2009; Akhar et al., 2011). Radiations definitely alter the genetic architecture of treated explant (Ikram et al., 2010) which upset the mechanism of photosynthesis and brings changes in various cytological and physiological aspects that expresses through morphological changes (Kim et al., 2004; Wi et al., 2005).

Table -3: Number of shoots per inoculated shoot (M₁V₀) measured in M₁V₁ and M₁V₂ generation after irradiation

<table>
<thead>
<tr>
<th>Doses (Gy)</th>
<th>No. of inoculated shoot (M₁V₀)</th>
<th>GCTCV-215</th>
<th>KM-5</th>
<th>FHIA-23</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>1.95 ab</td>
<td>1.75 a</td>
<td>1.85 b</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>1.75 a</td>
<td>2.50 a</td>
<td>2.50 a</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>1.20 b</td>
<td>1.22 b</td>
<td>1.17 ab</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>0.80 b</td>
<td>0.85 bc</td>
<td>0.50 bc</td>
</tr>
<tr>
<td>40</td>
<td>10</td>
<td>0.00 c</td>
<td>0.00 c</td>
<td>0.00 c</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>0.00 c</td>
<td>0.00 c</td>
<td>0.00 c</td>
</tr>
</tbody>
</table>

SE 0.24 0.43 0.32 0.29 0.28 0.42
CV (5%) 0.52 0.92 0.69 0.62 0.61 0.91

LSD= p≤ 0.05, Mx= mutant of the generation, Vx= vegetative generation cycle
Figure 2: Effect of different doses of gamma rays on the number of shoots of GCTCV-215 in 1st generation (M1V1) (a) Control (b) 10 Gy (c) 20 Gy & (d) 30 Gy and in 2nd (M1V2) generation (e) 10 Gy (f) 20 Gy & (g) 30 Gy.

Figure 3: Effect of different doses of gamma rays on the number of shoots of KM-5 in 1st generation (M1V1) (a) Control (b) 10 Gy (c) 20 Gy & (d) 30 Gy and in 2nd (M1V2) generation (e) 10 Gy (f) 20 Gy & (g) 30 Gy.

Figure 4: Effect of different doses of gamma rays on the number of shoots of FHIA-23 in 1st generation (M1V1) (a) Control (b) 10 Gy (c) 20 Gy & (d) 30 Gy and in 2nd (M1V2) generation (e) 10 Gy (f) 20 Gy & (g) 30 Gy.
Fresh Weight (g) of Irradiated Shoots: In GCT CV-215 and ‘Yangambi’ KM-5, lower dose 10 Gy showed significant maximum fresh weight than that of higher doses in second subculture (M₁V₂) generation (Figure 5). Whereas in FHIA-23 enhancing effect of lower dose 10 Gy was observed in both subculture cycles (M₁V₁, M₁V₂). Among all Musa varieties, FHIA-23 tolerated and gained fresh weight up to 40 Gy. All varieties exhibited increased fresh weight within subculture cycle. At increased dosages the capacity to gained fresh weight decreased. This was observed in the studies of Bhagwat and Duncan (1998), Novak et al., (1990) and Dizon et al., (2012).

Mean Root per Irradiated Shoot: In all varieties control produced maximum rooting. In GCTCV-215, Yangambi KM-5 and FHIA-23 among various doses 10Gy gave maximum rooting response respectively (Figure 6a). Effect of 20 and 30 Gy was similar in both triploid varieties. FHIA-23 show significant reduction in root number with increase in gamma doses. It is obvious from the results that the number of roots is inversely proportional to different doses of gamma rays in Musa varieties. Furthermore, no morphological differences were noticed in rooting in all varieties. All varieties produced dark and thick roots as produced by control.

Root Length (cm) of Irradiated Shoot: In all Musa varieties, untreated shoot produced maximum root length (Figure 6b). In GCTCV-215, among the various doses of gamma rays 10 Gy show the significant (p ≤ 0.05) maximum root length whereas the effects of 20 and 30 Gy was similar. ‘Yangambi’ KM-5 also showed maximum root length at 10 Gy while an effect of higher doses (20, 30 Gy) almost gave a similar response. In FHIA-23, maximum root length was observed at 20 Gy and the effect of 10 and 30 Gy found to similar. In case of root length ‘Yangambi’ KM-5 and GCTCV-215 showed decreased significantly with high in gamma doses. Present result agreed with the judgment of Karmarkar et al., (2001). In FHIA-23, such correlation between gamma doses and parameter was not found.
CONCLUSION: For in vitro mutagenesis, it is concluded that all the phenotypic characters affected by different doses of gamma rays, 10 Gy produce enhancing the effect on in vitro shoot culture. Semi lethal dose (LD_{50}) for triploid varieties established at 20 Gy and 30 Gy for tetraploids varieties. Polyploidy level had an important role during post-irradiation recovery in Musa varieties. Genetic diversity generated in present work for high yield with the broad range of resistance can be exploited to introduce new Musa varieties to explode monoculture of indigenous variety Basrai in Pakistan.

ACKNOWLEDGMENTS: The author would like to acknowledge Nuclear Institute of Medicine and Radiotherapy Jamshoro, (NIMRA) for providing irradiation facility and Nuclear Institute of Agriculture (NIA) for lab and chemicals facilities.

CONFLICT OF INTEREST
We (authors) have declared that there is no conflict of interests in the study.

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