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# MUTATION BREEDING FOR YELLOW SIGATOKA DISEASE RESISTANCE AND MOLECULAR CHARACTERIZATION IN BANANA CV RAJAPURI BALE AND RED BANANA

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**ABSTRACT**
Shoot tips of *in vitro*-grown cultures of banana cv. Rajapuri bale and Red banana were treated with various concentrations of the gamma ray irradiation (25, 30,35, 40 and 45 Gy) evaluated their effectiveness in inducing mutations and also with the aim of producing variants tolerant to the fungus *Mycosphaerella musicola* and there molecular characterization were carried out. Based on phenotypic variations in regenerated plants, factors of effectiveness were calculated for each treatment after twelve weeks inoculation. By screening we observed that gamma ray derived mutants are more sensitive to disease in 25, 30 and 35 Gy. Whereas, several mutants obtained from 40 and 45 were resistant as comparable with untreated control. Similar analysis with molecular markers also revealed comparable results in screening. SSR primers showed resistance specific bands and were used for the testing variation among mutants, after confirming its reproducibility. Putative resistant, moderate resistant and susceptible mutants along with untreated control banana plants when amplified with CNPMF 45 and MABN 08 15 in Rajapuri bale showed the presence of a major band at 250 bp and 300 bp and in CNPMF 43 and MABN 08 in Red banana showed the presence 310 bp and 450 bp as major bands, this evidenced the occurrence of random mutations in genome. These plants were considered to be tolerant and were multiplied for field screening.

**KEY WORDS:** Mutation, Screening, *Mycosphaerella musicola*, characterization, SSR, disease resistance.

## INTRODUCTION

Banana (*Musa* sp.) is considered the fourth most important tropical fruit crops in the world.[2] Banana (*Musa paradisiaca* Linn.) is a large herbaceous perennial monocotyledonous plant, belonging to family Musaceae of order Scitamineae. Banana is known for its antiquity and it's interwoven with Indian heritage and culture. It is considered as the symbol of "Prosperity and Fertility". Owing to its greater socio-economic significance and multifaceted uses, banana is referred as 'Kalpatharu' and 'Kalpavriksh'.[17] Long period of domestication under varying growing conditions have created high diversity in Indian bananas. The local cultivar diversity is more conspicuous with groups such as AB, AAA and AAB clones. Rajapuri Bale (AAB) is a popular cultivar grown in Northern parts of Karnataka. It is a dwarf variety growing upto 6-8 feet height with a very thick stem and stands up very well to wind. The leaves are wider than those of most bananas growing upto 3 feet wide. It is the best plant to grow in marginal areas or where a grower does not intend to put much care into cultivation of bananas. The bunches weigh about 10-15 kg with 8-10 hands and 90-100 fingers. Fruits have attractive yellow colour with thick skin and good blend of sweet and acidity. Red banana (AAA) is most relished and highly priced variety of Kerala and Tamil Nadu. Its commercial cultivation is prominent in Kanyakumari and Tirunelveli districts of Tamil Nadu. It is also popular in Karnataka, Andhra Pradesh and to some extent in Western and Central India. In Bihar and other regions, it is popular as Lal Velchi, while in Karnataka as Chandra bale and has geographical indication in Kamalapura. It is a robust plant with bunches weighing 20-30 kg under good management practices. Fruits are sweet, orange yellow colored and with a pleasant aroma. It is highly susceptible to Yellow sigatoka, Bunchy top, *Fusarium* wilt and Nematodes. Bananas are plagued by wide range of fungal diseases resulting in huge loss of fruit loss of fruit production. Among, the fungal diseases, the yellow sigatoka disease is the most important and wide spread in most banana producing regions and cause considerable yield reduction to the tune of 50 %, if uncontrolled.[20]

The progress of banana improvement by conventional breeding methods has been relatively slow or even hindered due to narrow genetic variability resulting from its low female fertility.[16, 13, 3] Conventional breeding methods have been of limited success in *Musa* species due to parthenocarpy, polyploidy in many cultivars, and limited available information on genetics and genomics [6, 4] Mutation breeding combined with tissue culture offer excellent chance for the induction of genetic variation by improving the selection technology and accelerating

breeding time.[11] Thus, the use of shoot tip cultures in mutation breeding offers several benefits over the conventional *in vivo* techniques in improvement of banana.

Microsatellite (SSR) markers have been an increasing trend in crop genetic studies because of the applicability of these markers in breeding programs. SSR markers are highly polymorphic, co-dominant and have got high reproducibility. SSR markers have got potential application for cultivar classification, studies on genetic diversity, mutant identification and molecular breeding of crops.

In the light of above information, an investigation was carried out at the Centre for Horticulture Biotechnology, Directorate of Research, University of Horticultural Sciences, Bagalkot with the objective of carrying out induced mutagenesis by gamma irradiation and chemical mutagens to induce genetic variability in regionally important banana cultivar namely Rajapuri bale (AAB) and Red banana (AAA) using SSR molecular marker.

## MATERIALS & METHODS

Rising of shoot buds cultures and induction of mutations were carried out at centre of Horticulture Biotechnology, UHS, Bagalkot. Aseptic culture were subjected to gamma irradiation treatment at 25, 30, 35, 40 and 45 Gy at Baba Atomic Research Centre, Trombay using $^{60}$Co as source of the gamma irradiation. Each treatment was replicated 3 times and about 30 multiple buds used for each treatment.

**Raising of aseptic cultures and regeneration of plantlets**

Aseptic cultures and platelets were raised following the protocol standardized by Kishore (2016) with some modifications. Shoot tip explants were incubated in MS liquid culture media supplemented with 4 mg/l BAP, 75 mg/l of adenine sulphate and 175 mg/l of ascorbic acid, for two weeks maintaining standard culture conditions of 25 ± 2$^0$C temperature, 60% relative humidity and photoperiodic cycle of 16 hours light and 8 hours dark period. After two weeks of incubation, all the explants were evaluated for their ability to establish in liquid media. Healthy and contaminant free explants were excised by removing discolored tissue and transferred to baby jar bottles containing the MS semi-solid media supplemented with 4 mg/l of BAP and 75 mg/l of adenine sulphate for Nanjanagudu Rasabale and 175 mg/l for Ney Poovan concentrations of ascorbic acid and incubated for four weeks maintaining standard culture conditions mentioned above. They were cultured in the same media and incubated for 4 weeks. There aseptic multiple cultures were subjected to gamma irradiation treatments as per experiment.

The proliferated cultures were sub cultured on to fresh multiplication media 25-30 days after incubation for further shoot multiplication. Totally five subculture ($M_1$ $V_1$ to $M_1$ $V_5$) were carried out to minimize the problem of chimeras.

**Induction of variation by gamma irradiation**

Aseptic culture were subjected to gamma irradiation treatment at 25, 30, 35, 40 and 45 Gy at Baba Atomic Research Centre, Trombay using $^{60}$Co as source of the gamma irradiation. Each treatment was replicated 3 times and about 30 multiple buds used for each treatment. Irradiated shoot tips were inoculated on fresh MS-medium supplemented with BAP 4 mg/l within 48 hours.

**Primary and Secondary Hardening**

After rooting, plantlets were taken out from culture bottles and washed thoroughly to remove agar medium adhered to roots. The plantlets were treated with 1% Bavistin for 5 minutes. Later plantlets were transferred to pro-tray containing sterilized cocopeat and kept under green house for 4 weeks. Plants were further transferred to polybags containing Red soil + Sand + FYM (1:1:1 v/v) potting mixture and raised under shade house for six weeks.

**Screening of *in vitro* derived mutants for resistance to yellow sigatoka (*Mycosphaerella musicola*)**.

The variants of Rajapuri bale and Red banana were screened against *Mycosphaerella musicola*. On plants of ~30 cm in height, three leaves were inoculated by fragments of the diseased leaves. After inoculation, the plants were kept at 26 $^0$C with a relative humidity ~85 % plants by covering with polythene sheet. The polythene sheet was moisture two times a day was providing to cover all the plantlets to ensure sporulation and to enhance infection.[14]

**Stages of Symptom development of *in vitro* derived mutant plants inoculated with *Mycosphaerella musicola* leaf fragments in the green house**

| Stage | Description |
|---|---|
| 0 | Leaf symptoms mostly absent |
| 1 | Reddish flecks on lower leaf surface or No symptoms on the upper surface |
| 2 | Regular or irregular reddish circular spots on the lower leaf surface. No symptoms on the upper surface. |
| 3 | Regular or diffused light brown yellow circular spots on the upper leaf surface |
| 4 | Brown circular spots, possibly with a yellow halo or chlorosis of adjacent tissues, on the upper leaf surface. Areas of green tissue sometime present. |
| 5 | Yellow spots with dry centre of grey colour. Leaf completely necrotic, sometimes hanging down |

Resistant: Stages 0-1 (No symptoms in upper side)
Partial resistance: Stages 2-3 (Regular or irregular spots on the upper surface of the leaf)
Susceptible: Stages 4-5 (yellow –brown spots or chlorosis of adjacent tissues)

The resistant/ moderate resistant/ susceptible reaction of the Rajapuri bale and Red banana mutant against *M. musicola* was assessed by the above disease rating scale developed by Alvarado Capo *et al*., (2002).


**FIGURE 1.** Establishment and inoculation of *M. musicola* mutant banana plants

**A**. Diseased leaf fragments moisturized, **B.** Diseased leaf fragments placed on the leaf of mutant plants, **C.** Covered with polythene sheet to maintain humidity

**Molecular Characterization**

DNA was extracted from *in vitro* derived mutants using young cigar leaf. Standard protocol for the isolation and extraction of DNA by CTAB method was used. SSR primers were used in this study. PCR was carried out using Model Master Cycle gradient 2551 (Eppendorf, Germany). Agarose gel electrophoresis was used because it gives better separation and visualization of PCR amplified products agarose is polysaccharide derived of agar contains micro pores and hence acts as molecular sieve. Following are the steps involved in setting up of agarose gel electrophoresis for accomplish the visualization of amplified bands

**Gel scoring**

It was done to identify resistant specific band (band which is present in resistant mutant and absent in susceptible individuals) and for testing variation among various morphological mutants, it was expected that the resistant specific band should be present in resistant mutants.

## RESULTS & DISCUSSION

**Screening of putative mutants of banana for resistance against *Mycosphaerella musicola***

The resistant mutants of the banana cultivars of Red banana and Rajapuri bale of secondary harden (2 months old) were compared with untreated or untreated control plants after inoculated with *M. musicola* pathogen. The 60 days were taken for the appearance of the symptoms in Rajapuri bale and Red banana. The mutated plants after two months (60 days) exhibited yellow leaf spot symptoms showing susceptibility to *M. Musicola* in both banana cultivars. However, the mutated plant had fallen into the Susceptible, Moderately resistance and Resistance level category.

**Screening of gamma ray irradiated *in vitro* derived putative mutants of banana cv. Rajapuri bale against *Mycosphaerella musicola***

The data on effect of *M. musicola* on *in vitro* derived mutants of Rajapuri bale were produced. The results pertaining to screening of *in vitro* derived Rajapuri bale mutants obtained from cultures treated with different doses of gamma ray irradiation revealed (Table 1) that there was disease development in the mutants of artificial inoculation of diseased leaf fragments Fig 2.

**Number of plants inoculated**

A total of 90 putative Rajapuri bale mutants plants (each 15 plants) were selected having three to four leaf stages of secondary hardened plantlets. The plantlets were inoculated with *M. musicola*.

**Number of plants died after inoculation**

Among the total 90 putative mutants screened, in which 8 plants were died. The numbers of plants 4 were died in 25 Gy and Untreated (control) treatments in Rajapuri bale.

**Number of lesions**

The result of artificial inoculation of *M. musicola* on Rajapuri bale mutated plantlets produced from the different doses of gamma ray irradiation. The total 90 plants were inoculated. The numbers of lesion was decreased as the dose of gamma ray irradiation was increased. The highest numbers of lesions were recorded in untreated control, followed by 25 Gy. The lowest numbers of lesions was recorded in 45 Gy mutated plantlets. The total number of lesions was 42 in different dose of gamma irradiated Red banana plantlets.

**Number of plants with external symptoms of yellow sigatoka**

The reactions of putative mutants are screened against *M. musicola* inoculated exhibited several peculiar symptoms like yellow or brown spots and chlorosis of adjacent tissues. The combined effect of the symptoms based on the number lesions was included in the severity. The data pertaining to disease symptoms of the mutants are presented in the Table 1. The total 90 mutated plantlets were artificially inoculated, in which total 77 plants showed external symptoms of yellow leaf spot. The highest plants showed external symptoms were 15 plants in 25 Gy, 30 Gy, 35 Gy and untreated control and lowest 8 plants showed symptoms in 45 Gy of Rajapuri bale mutated plantlets.

**Number of plants with no external symptoms of yellow sigatoka**

The effect of *M. musicola* inoculated on *in vitro* derived mutants was found to be effective in inducing resistant plants among the treatments. The number of pants (3) with no external symptoms was observed in 40 Gy and 45 Gy. The total 6 plants were observed with no external symptoms of yellow leaf spot

**Mutants classification according to symptom development**

The effect of *M. musicola* inoculation of diseased leaf fragments on *in vitro* derived mutants of Rajapuri bale was found to be effective among various treatments. The data pertaining to symptom development is presented in Table 45. Plants showing yellow leaf spot symptom development are categorized into susceptible, moderately resistance and resistance.

Yellow leaf spot 4-5 per plant was fallen into susceptible category in Rajapuri bale. The highest yellow leaf spot

was recorded in untreated control and 25 Gy (10 spots and 9 spots) per plant. The minimum yellow leaf spot was observed in 30 Gy and 35 Gy (8 and 7 spots)

Moderately resistance category had 2-3 leaf spot per plant in Rajapuri bale. In 40 Gy and 45 Gy treatment plants showed 2 yellow leaf spot per plant was recorded. In Resistance category had 0-1 leaf spot per plant in Rajapuri bale. In 40 Gy and 45 Gy treatment plants showed 1 yellow spot each was recorded.

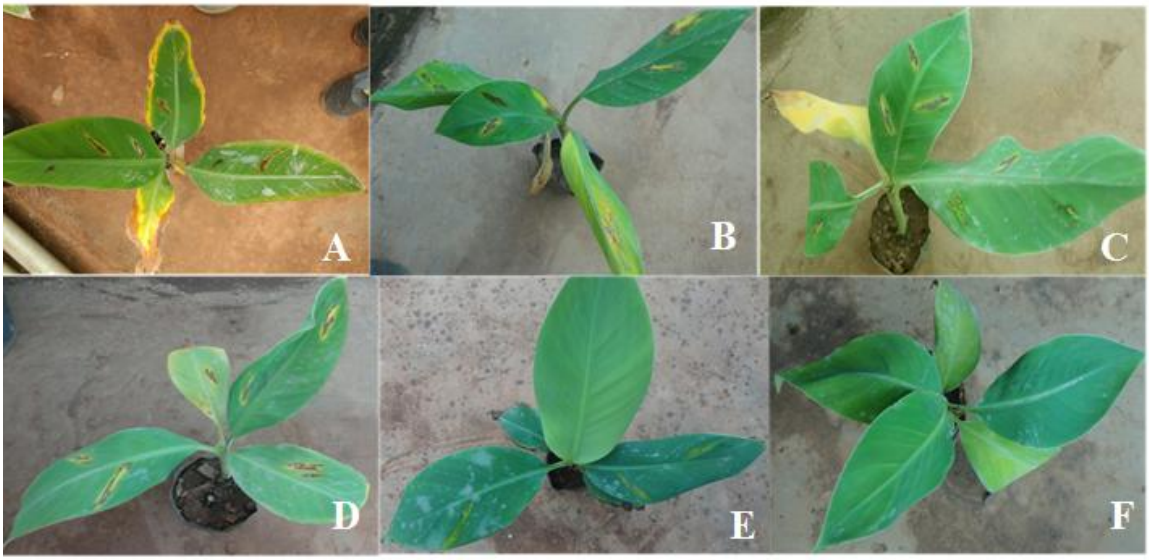


**FIGURE 2.** Response of Rajapuri bale gamma ray irradiated mutants to *Mycospherulla musicola*
**A.** Control, **B**. 25 Gy, **C**. 30 Gy, **E**. 40 Gy, **F**. 45 Gy

**TABLE 1.** Effect of *Mycospherulla musicola* (yellow sigatoka) *in vitro* gamma ray irradiated banana cv. Rajapuri bale under greenhouse conditions

| Gamma irradiation/ Cultivars | Rajapuri bale (AAB) | | | | |
|---|---|---|---|---|---|
| | Number of plant inoculated | Number of plants died after inoculated | Number of lesions | Number of plants with external symptoms of yellow leaf spot | Number of plants with external no symptoms |
| 25 Gy | 15 | 4 | 10 | 15 | 0 |
| 30 Gy | 15 | 0 | 7 | 15 | 0 |
| 35 Gy | 15 | 0 | 7 | 15 | 0 |
| 40 Gy | 15 | 0 | 3 | 9 | 3 |
| 45 Gy | 15 | 0 | 2 | 8 | 3 |
| Untreated Control | 15 | 4 | 12 | 15 | 0 |
| Total | 90 | 8 | 42 | 77 | 6 |

**Screening of gamma ray irradiated *in vitro* derived putative mutants of banana cv. Red banana against *Mycosphaerella musicola***

The results of effect of *M. musicola* on *in vitro* derived mutants of Red banana were produced. The data pertaining to screening of *in vitro* derived Red banana mutants obtained from cultures treated with different doses of gamma ray irradiation revealed (Table 2) that there was disease development in the mutants of artificial inoculation of diseased leaf fragments Fig 3.

**Number of plants inoculated**

A total of 90 putative Red banana mutants (each 15 plants) were selected having three to four leaf stages of secondary hardened plantlets. The plantlets were inoculated with *M. musicola*.

**Number of plants died after inoculation**

Among the total 90 putative mutants screened, in which 7 plants were died. The highest numbers of plants 4 were died in 25 Gy. The lowest death was observed in untreated control.

**Number of lesions**

The result of artificial inoculation of *M. musicola* on Red banana mutated plantlets produced from the different doses of gamma ray irradiation. The total 90 plants were inoculated. The numbers of lesions were decreased as the dose of gamma ray irradiation was increased. The highest numbers of lesions were recorded in untreated control followed by 25 Gy. The lowest number of lesions was recorded in 45 Gy mutated plantlets. The total number of lesions was 37 in different dose of gamma irradiated Red banana plantlets.

**Number of plants with external symptoms of yellow leaf spot**

The reactions of putative mutants are screened against *M. musicola* inoculated exhibited several peculiar symptoms like yellow or brown spots and chlorosis of adjacent tissues. The combined effect of the symptoms based on the

number lesions was included in the severity. The data pertaining to disease symptoms of the mutants are presented in the Table 2. The total 90 mutated plantlets were artificially inoculated, in which total 74 plants showed external symptoms of yellow leaf spot. The highest plants showed external symptoms were 15 plants in 25 Gy, 30 Gy, 35 Gy and untreated control and lowest 6 plants showed symptoms in 45 Gy Red banana mutated plantlets.

**Number of plants with no external symptoms of yellow leaf spot**

The effect of *M. musicola* inoculated on *in vitro* derived mutants was found to be effective in inducing resistant plants among the treatments. The number of pants (3) with no external symptoms was observed in 40 Gy and 45 Gy. The total 5 plants were observed with no external symptoms of yellow leaf spot

**Mutants classification according to symptom development**

The effect of *M. musicola* inoculation of disease leaf fragments on *in vitro* derived mutants of Red banana was found to be effective among various treatments. The data pertaining to symptom development is presented in Table 45. Plants showing yellow leaf spot symptom development are categorized into susceptible, moderately resistance and resistance.

Yellow leaf spot 4-5 per plant was fallen into susceptible category in red banana. Highest yellow leaf spot was recorded in untreated control and 25 Gy (12 spots and 10 spots) per plant. The minimum yellow leaf spot was observed in 30 Gy and 35 Gy (8 spots).

Moderately resistance category had 2-3 leaf spot per plant in Red banana. In 40 Gy and 45 Gy treatment plants showed 3 yellow leaf spot per plant was recorded. In Resistance category had 0-1 leaf spot per plant in Red banana. In 40 Gy and 45 Gy treatment plants showed 1 yellow spot each was recorded.

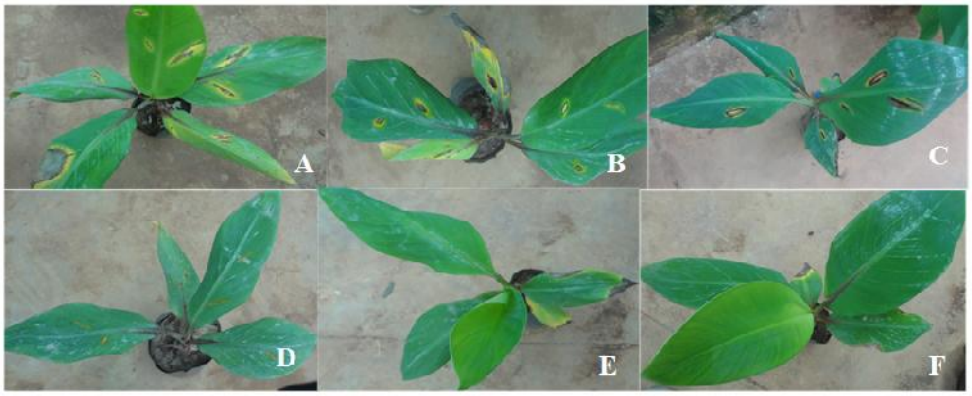


**FIGURE 3.** Response of Red banana gamma ray irradiated mutants to *Mycospherulla musicola*
**A.** Uuntreated control, **B.** 25 Gy, **C.** 30 Gy, **D.** 35 Gy, **E.** 40 Gy, **F.** 45 Gy

**TABLE 2.** Effect of *Mycospherulla musicola* (yellow sigatoka) *in vitro* gamma ray irradiated banana cv. Red banana under greenhouse conditions

| | Red banana (AAA) | | | | |
|---|---|---|---|---|---|
| Gamma irradiation/ Cultivars | Number. of plant inoculated | Number. of plants died after inoculated | Number. of lesions (per plants) | Number. of plants with external symptoms of Yellow leaf spot | Number. of plants with no external symptoms |
| 25 Gy | 15 | 4 | 9 | 15 | 0 |
| 30 Gy | 15 | 0 | 7 | 15 | 0 |
| 35 Gy | 15 | 0 | 6 | 15 | 0 |
| 40 Gy | 15 | 0 | 3 | 8 | 1 |
| 45 Gy | 15 | 0 | 2 | 6 | 4 |
| Untreated (Control) | 15 | 3 | 10 | 15 | 0 |
| Total | 90 | 7 | 37 | 74 | 5 |

Out of the 90 putative of Rajapuri bale mutants evaluated 6 mutants were found to be highly tolerant in Rajapuri bale to *M. musicola* pathogen. Similarly, in case of Red banana, out of the 90 mutants screened for against *M. musicola* pathogen, 5 tolerant mutants showed resistance.

The appearance of fewer symptoms following infection is closely related to a response by the plant defense mechanism. By crossing two susceptible triploid plantain cultivars (Bobby Tannap and Obino 1 Ewai) as female parents with the resistant wild, diploid banana Calcutta 4[12] obtained segregated progeny with a durable horizontal resistance. In the case of the progeny with a partially resistant response, slow lesion development and, ultimately, reduced sporulation were observed. Yellow Sigatoka disease caused by *M. musicola* reduces the photosynthetic capability of the plant by the formation of symptomatic necrotic lesions on leaves resulting in the reduced crop yield and premature ripening of the fruit.[7] Romero and Sutton (1997) reported similar results when they examined the response of FHIA-01 and FHIA-02

inoculated with conidia. They pointed out that although the mechanism of resistance to black leaf streak is not known, a low density of stomata, and increased production of cuticular wax, phytoalexin, suberin and lignin, or resistance to phytotoxins may be associated with partial resistance.

**Molecular characterization by SSR markers**

Simple sequence repeats (SSR) markers were used to detect variation among gamma irradiated mutants and the mother plant against *M. musicola*. 15 SSR markers were used to determine genetic variation between the various morphological mutants, resistant and susceptible mutants along with mother plant are produced in Rajapuri bale (AAB) and Red banana (AAA).

**TABLE 3.** List of SSR primers used for screening disease resistance in putative banana mutants

| Sr.No | Primer | Forward | Reverse |
|---|---|---|---|
| 1 | Ma 15 | AGGCGGGGAATCGGTAGA | GGCGGGAGACAGATGGAGT |
| 2 | Ma1 | TGAATCCCAATTTGGTCAAG | CAAAACACTGTCCCCATCTC |
| 3 | Ma 1/17 | TCGGCTGGCTAATAGAAGGAA | TCTCGAGGGATGGTGAAAGA |
| 4 | MABN 34 | TAGGTGAGAATGGGACGGAG | CAGTAGCCAGCAACCTGGTGA |
| 5 | CNPMF 43 | GTTTGGTGCTCATTGCTGTG | GTTTGGTGCTCATTGCTGTG |
| 6 | MABN08 | TTACCGTAAACGGAGCCAAC | GAAATCGAGGAAAACCGACA |
| 7 | Ma 3/2 | TGCGCGTCACACACACACA | GGCGATACGCAACAAATAGACTTAGG |
| 8 | STMS 7 | AAGAAGGCACGAGGGTAG | CGAACCAAGTGAAATAGCG |
| 9 | CNPMF 26 | TGGAGATGAAGAAGATCGTC | TCATCAAGTGCGTTGCATTC |
| 10 | STMS 1 | TGAGGCGGGGAATCGGTA | GGCGGGAGACAGATGGAGTT |
| 11 | MABN 03 | TGGTTGTATGTTTGCTGGGA | CAAAGTGCTGGCATGAGAAA |
| 12 | MABN 06 | GCAACCATCAACCAAAAACC | TTTGCAAGAAAATCGTGCTG |
| 13 | CNPMF 41 | GCTGCTCTCGCTTGTTATCC | GCTGCTCTCGCTTGTTATCC |
| 14 | MABN07 | TTTTGATCATCATATGGGTCG | AGAGGGAGAGCCAAAGTGGT |
| 15 | MABN13 | CCTCAACGAAGCATACAGCA | CAGTCTGGGCTGACACAGAA |

Among the 15 primers (Table 3) showed 13 primers showed amplification and 10 primers amplified unambiguous, readable and showed polymorphic bands in Rajapuri bale (3), and Red banana (3). A total of 104 amplified products were produced from the selected 10 primers 33 bands were amplified by Rajapuri bale and 21 bands were produced by Red banana. The number of bands varied from 1-2 with an average 1.33 bands per primers and the size ranged from 0.2 to 1.5 kb. The bands which are more than 100 kb are selected for analysis. The details are presented in Table 11. Each and every individual could be identified using gel profiles. A polymorphism was found among the mutants and mother plant indicating there is a molecular variability among the mutants.

The putative mutants of Rajapuri bale amplified with CNPMF 45 showed the major bands ≈ 250 bp shared by all resistant and moderately resistant mutants and varied from susceptible mutants and mother plant showed bands ≈ 260 bp (Plate 24). MABN 08 showed the major bands ≈ 230 bp shared by all resistant and moderately resistant mutants and varied from susceptible mutants and mother plant showed bands ≈ 280 bp Fig 4 & 5.

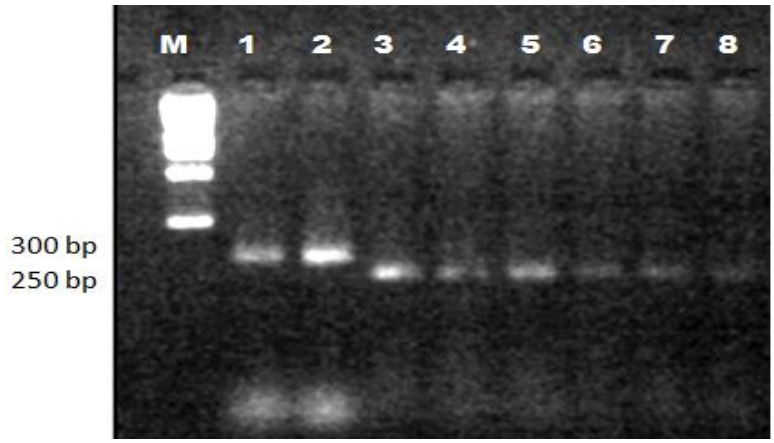


**FIGURE 4.** SSR profile of gamma ray irradiated mutants and untreated control plants of Rajapuri bale obtained with CNPMF 45

**M**-Ladder, **Moderately resistance**: **3**-35 Gy, **4**-35, **5**-35 Gy, **6**-40 Gy**, Resistance: 7**-45 Gy, **8**-45 Gy **Susceptible**: **2**-25 Gy, **1**-untreated control

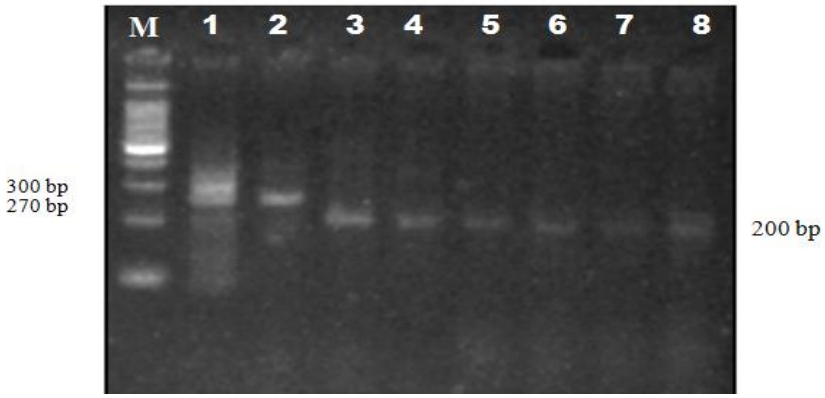


**FIGURE 5**. SSR profile of gamma ray irradiated mutants and untreated control plants of Rajapuri bale obtained with MABN 08

**M**-Ladder, **Moderately resistance**: **3**-35 Gy, **4**-35, **5**-35 Gy, **6**-40 Gy**, Resistance: 7**-45 Gy, **8**-45 Gy **Susceptible**: **2**-25 Gy, **1**-untreated control

The putative mutants of Red banana amplified with MABN 08 showed the major band ≈ 250 bp shared by all resistant and moderately resistant mutants and varied from susceptible mutants and mother plant showed ≈ 300 bp (Plate 32). CNPMF 43 08 showed the major band ≈ 230 bp shared by all resistant and moderately resistant mutants and varied from susceptible mutants and mother plant showed ≈ 220 bp Fig 6 & 7.

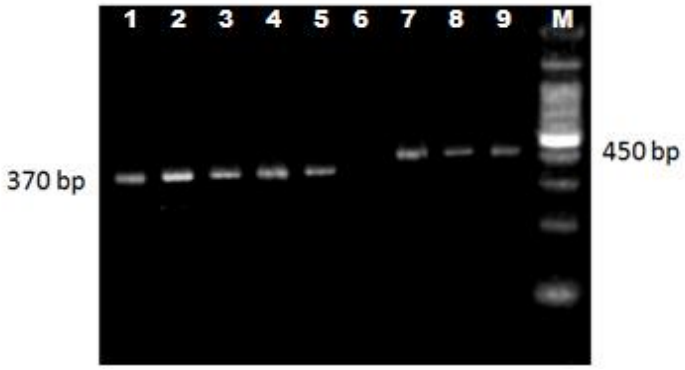


**FIGURE 6.** SSR profile of gamma ray irradiated mutants and untreated control plants of Red banana obtained with MABN 08

**M**-Ladder, **Moderately resistance**: **1**-35 Gy, **2**-35, **3**-35 Gy, **4**-40 Gy**, 5**-40 Gy, **Resistance:** 6-45 Gy, **Susceptible**: **8**-25 Gy, **9**-untreated control

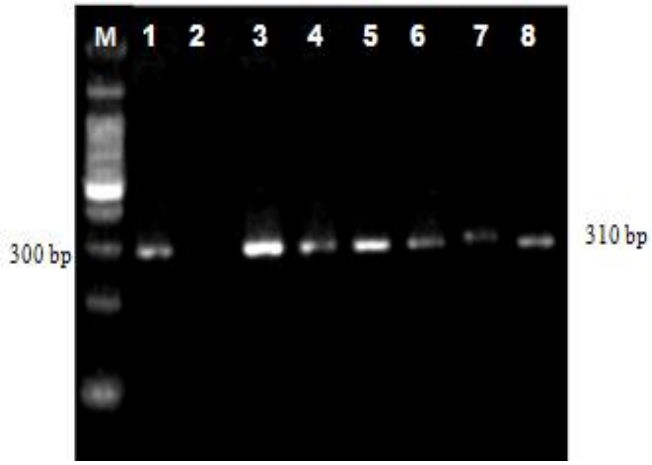


**FIGURE 7.** SSR profile of gamma ray irradiated mutants and untreated control plants of Red banana obtained with CNPMF 43

**M**-Ladder, **Moderately Resistance: 3**- 35 Gy, **4**- 35 Gy, **5**-40 Gy, **6**-40 Gy, **Resistance: 7**-45 Gy, **8**-45 Gy, **Susceptible: 2**-25 Gy, **1**-Untreated control

Putative resistant and susceptible mutants showed variation in the banding pattern in Rajapuri bale and Red banana. This clearly indicates the changes under molecular level. These bands may be associated with the resistant character of putative mutants. SSR marker screening in four banana cultivar confirms that they are linked to disease resistance genes. It was reported that SSR marker GWM533 is closely linked to Sr2 gene.[10] Simple sequence repeat (SSR) based GWM533 and CAPS based CsSr2 markers reported for Sr2 gene were found promising for molecular confirmation of gene and have been used in stem rust breeding programmes in Australian, US and CIMMYT, Mexico.[18,8] Chakraborty *et al*. (2015) identified the two sets of SSR markers, namely rbcL and matK were used to detect the presence or absence of PLB gene present in the shorter arm of chromosome 8 in the s1/s2 locus which confers resistance to purple blotch of onion. The presence band of PLB gene inferred the resistance.
Putative resistant and susceptible mutants showed variation in the banding pattern in Rajapuri bale, and Red banana. This clearly indicates the changes under molecular level. These bands may be associated with the resistant character of putative mutants.

## CONCLUSION

In the present study, the disease tolerance or susceptibility was based on the external symptoms observed after two months of *M. musicola* inoculation under green house conditions. Gamma ray irradiation is the potential tool and being highly used for crop to improve disease resistance. The mutant plant species can be easily selected from banana plants by SSR. This marker is reproducible and consistent as compared to morphological. The mutant plants produced by gamma ray irradiation are capable to produce tolerate *M. musicola* in increased doses.

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