

Original Research Article

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## ***In vitro* Inductions of Gamma Ray Mutation for Fusarium Wilt Resistance and Characterization of Mutants in Regionally Grown Banana Cultivars**

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### ABSTRACT

#### Keywords

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Regionally grown banana cultivars are severely infected by several pathogens one such *Fusarium oxysporum* f. sp. *Cubense* which causes significant crop losses in the fields. In this study fusarium wilt screening was carried out on gamma ray induced mutants and molecular characterization was done to assess the acquired resistance to *Fusarium oxysporum* f. sp. *cubense*. From the screening we observed that gamma ray derived mutants are more sensitive 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 Ma 3/2 and Ma 15 in Nanjanagudu Rasabale showed the presence of a major band at 150 bp and 500 bp and in Ma 1/17 and STMS 7 in Ney Poovan showed the presence 500 bp and 250 bp as major bands, this evidenced the occurrence of random mutations in genome.

### Introduction

India being one of the centers of origin and diversity of *Musa*, is rich in its wealth. Natural intra-specific and inter-specific crosses among *M. accuminata* and *M. balbisiana* and 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 and AAB clones.

The Nanjanagudu Rasabale (*Musa* spp., AAB, silk subgroup) known for its unique taste has a huge demand across the country. But, conditions are not favorable enough for

growing the banana and to match the huge demand that it generates (Kishore, 2016). The cultivar Nanjanagudu Rasabale geographical indication tagged and once leading cultivar of then Mysore province is under threat of extinction due to its susceptibility to Panama diseases (Pooja *et al.*, 2013). The cultivar grown in around 600 acres of land in the district of Mysore, Karnataka, now confined to only 30 acres of land in isolated area (Khan, 2015). The variety now which is at the danger of extinct has to strictly bred by potential methodology to save and preserve as a germplasm.

Ney Poovan (AB) is the choicest diploid cultivar of banana, which is under commercial mono cultivation on a large scale especially in Karnataka and Tamil Nadu. Fruits are small, slender having a prominent beak and are arranged around the axis in a way that they bear a wind-blown appearance (Daniells *et al.*, 2001). Fruits are very attractive due to their bright yellow color with ivory white pulp. They retain their firmness upon ripening, thereby improving their overall appeal in the market. Owing to these superior characters, the variety has been included in the list of export suited banana varieties by National Research Center on Banana, Trichy. Recent studies have reported that this variety is able to produce bunches of appreciable size even under low moisture conditions (Ravi *et al.*, 2013).

The *Fusarium* wilt (Panama Diseases) disease is the most important and wide spread in most Nanjanagudu Rasabale and Ney Poovan producing regions and cause considerable yield reduction to the tune of 50 %, if uncontrolled (Stover and Malo, 1972).

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 (Silva *et al.*, 2001; Pua, 2007; Bidabadi *et al.*, 2010). 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 (Chopra, 2005; Capdeville *et al.*, 2009).

## **Materials and 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  $^{60}\text{Co}$  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  $25 \pm 2$  °C temperature, 60 per cent 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  $^{60}\text{Co}$  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 putative mutants for resistance against *FOC***

*Fusarium oxysporum* f.sp. *cubense* (*FOC*) culture was obtained from Indian Type

culture Collection Identification/Culture Supply Services, Division of Plant Pathology, Indian Agriculture Research Institute, New Delhi. For isolation and maintenance of pathogen, potato dextrose agar (PDA) media was used. The haemocytometer was used for counting the fungal spores in liquid suspension.

### **Methodology of *FOC* Race 1 inoculation**

The variants of Nanjanagudu Rasabale and Ney Poovan obtained were screened against *FOC* using the procedure given by the Musoke, *et al.*, (1999). Screening was carried out using *FOC* spore population adjusted to  $3 \times 10^4$  spores ml/l.

Each 3 month old plantlet after hardening was inoculated at 5 to 10 cm soil depth near the root zone with 3 % of the spore suspension. Untreated control plantlets were inoculated with distilled water. The observations were taken at 60 days interval after pathogen inoculation.

### **Mutants were classified on the basis of vascular invasion of corm**

The plants with symptom of brown discoloration in the root core of pseudostem and rhizome are as vascular invasion of *FOC* (Bhagwat and Duncan, 1998)

The resistant/ tolerant/susceptible reaction of the Ney Poovan and Nanjanagudu Rasabale mutant against *Fusarium* wilt was assessed by the above disease rating scale developed by Ploetz *et al.*, (1999) (Table 1).

### **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 and Discussion**

Hardened plants obtained after mutagenesis was subjected to early screening for any acquired resistance to *FOC* Race 1.

#### **Screening gamma ray irradiated *in vitro* derived putative mutants of banana cv. Nanjanagudu Rasabale against *FOC* Race 1**

The data pertaining to screening of *in vitro* derived Nanjanagudu Rasabale mutants obtained from gamma ray irradiation mutagens revealed that there was disease development in the mutants due to artificial inoculation (Table 2).

#### **Number of plants inoculated**

A total of 90 Nanjanagudu Rasabale putative mutants (15 plants) from each treatment were selected having three to four leaf stages after secondary hardening plantlets. The plantlets were inoculated with *FOC* Race 1.

#### **Number of plants died after inoculation**

Among the total 90 putative mutant screened, in which 63 plants were died. The highest numbers of plants (15 and 12) were died in doses of gamma irradiation 25 Gy, untreated control, 30 Gy and 35 Gy. The lowest was in (5 and 4) in 40 Gy and 45 Gy, respectively.

#### **Number of plants with external symptoms of *FOC***

The reactions of putative mutants screened against *FOC* inoculated exhibited several peculiar symptoms like yellowing, wilting and stem splitting. The combined effect of the symptoms based on the symptoms (yellowing, wilting and stem splitting) was included among three symptoms based on the severity. The data pertaining to disease symptoms of the mutants are presented in the Table 2.

#### **Yellowing**

Among the different doses of gamma ray irradiated mutants of Nanjanagudu Rasabale, the highest (10 and 9 plants) yellowing was observed in 40, 30, 45 Gy and untreated control. The lowest 8 plants were observed in 25 Gy and 35 Gy. The total 53 plants showed yellowing symptoms among irradiated and untreated control putative mutants.

#### **Wilting**

Results revealed that among the different doses mutagenic treatment in Nanjanagudu Rasabale had highest wilting (5) symptoms was recorded in 35 Gy, followed by 4 plants in 25 Gy, 30 Gy and 45 Gy. The lowest symptoms (2 and 3) were recorded in 40 Gy and untreated control. The total 22 plants showed wilting symptoms among irradiated and untreated control putative mutants of Nanjanagudu Rasabale.

### **Stem splitting**

Among the different doses of gamma ray irradiated mutants, the highest (3 plants) stem splitting was observed in 25 Gy and untreated control, followed by (2 plants) in 30 Gy and lowest was 1 plant was observed in 35 Gy.

The total 9 plants showed stem splitting symptoms among irradiated and untreated control putative mutants of Nanjanagudu Rasabale.

### **Number of plants with no external symptoms of *FOC***

The effect of *FOC* culture on *in vitro* derived mutants of Nanjanagudu Rasabale was found to be effective in inducing resistant plants among the treatments.

The number of plants (3) with no external symptoms was observed in 40 Gy. The treatment 45 Gy had 2 plants and 1 plant in 35 Gy with no external symptoms. The total 6 plants were observed with no external symptoms of *FOC*.

### **Vascular invasion of corm**

The data pertaining to vascular invasion of the corm presented in Table 4. Plants exhibiting vascular invasion are categorized into light, medium and heavy.

Light vascular invasion of corm was the highest in 40 Gy (8.2). The lowest light vascular invasion was observed in 25 Gy (2.9). Medium vascular invasion of corm was more in untreated control (27).

The minimum medium vascular invasion was observed in 40 Gy (13). Heavy vascular invasion of corm was more in untreated control (38). The minimum heavy vascular invasion was observed in 40 Gy and 45 Gy (30) (Fig. 1).

### **Screening of gamma ray irradiated *in vitro* derived putative mutants of banana cv. Ney Poovan bale against *FOC* Race 1**

The data pertaining to screening of *in vitro* derived Ney Poovan bale mutants obtained from gamma ray irradiation revealed (Table 3) that there was disease development in the mutants due to artificial inoculation

### **Number of plants inoculated**

A total of 90 Ney Poovan putative mutants (15 plants) from each treatment were selected having three to four leaf stages after secondary hardening plantlets. The plantlets were inoculated with *FOC* Race 1.

### **Number of plants died after inoculation**

Among the total 90 putative mutant screened, in which 61 plants were died. The highest number of plants (12, 10, 10 and 15) was died in doses of gamma irradiation 25 Gy, 30 Gy, 35 Gy and untreated control. The lowest was in 40 Gy and 45 Gy treatment (8 & 6), respectively.

### **Number of plants with external symptoms of *FOC***

The reactions of putative mutants were screened against *FOC* inoculated exhibited several peculiar symptoms like yellowing, wilting and stem splitting. The combined effect of the symptoms based on the symptoms (yellowing, wilting and stem splitting) was included among three symptoms based on the severity. The data pertaining to disease symptoms of the mutants are presented in the (Table 3 Fig. 2).

### **Yellowing**

Among the different doses of gamma ray irradiated mutants, the highest (11 plants)

yellowing was observed in 30 Gy and untreated control, followed by 10 plants was observed in 25, 35 and 45 Gy. The lowest (9 plants) yellowing was observed in 40 Gy. The total 61 plants showed yellowing symptoms on irradiated and control plants.

### **Wilting**

Results revealed that among the different doses of mutagenic treatment the highest wilting (3 plants) symptoms was recorded in 25, 30 and 35 Gy followed by untreated control and 40 Gy 2 plants each. The lowest symptom (1 plant) was recorded in 45 Gy. The total 14 plants showed wilting symptoms among irradiated and control plants

### **Stem splitting**

Among the different doses of gamma ray irradiated mutants, highest (2 plants) stem splitting was observed in 25, 35, 40 Gy and untreated control and lowest was 1 plant was observed in 30 and 45 Gy. The total 10 plants showed stem splitting symptoms among irradiated and untreated control putative mutants.

### **Number of plants with no external symptoms of *FOC***

The effect of *FOC* culture on *in vitro* derived mutants was found to be effective in inducing resistant plants among the treatments. The number of plants (3) with no external symptoms was observed in 45 Gy. The treatment 45 Gy had 2 plants with no external symptoms. The total 5 plants were observed with no external symptoms of *FOC* Race 1.

### **Vascular invasion of corm**

The data pertaining to vascular invasion of the corm presented in Table 4. Plants exhibiting vascular invasion are categorized into light, medium and heavy (Fig. 3).

Light vascular invasion of corm was highest in 45 Gy (9.3). The lowest light vascular invasion was observed in untreated control (2.2). Medium vascular invasion of corm was more in untreated control (29). The minimum medium vascular invasion was observed in 40 Gy (12). In the present study, out of the 90 mutants each evaluated from both the banana cultivar 5 and 6 mutants were found to be highly tolerant in Nanjanagudu Rasabale, respectively to *FOC* race 1 pathogen.

External symptoms exhibited by the plants included leaf yellowing, wilting and pseudostem splitting were recorded. The effects of *FOC* culture on various gamma ray irradiated mutants showed varied symptoms. The injured roots of the *in vitro* derived plantlets before inoculation of *FOC* spore suspension in soil, showed symptoms of *FOC* within fifth day of incubation. The leaf veins showed chlorosis gradually entire lamina turned yellow and resulted in the wilting of the leaves as reported by Krishna *et al.*, (2013). The partially resistant plantlets exhibiting the symptoms were recorded. Among the plants inoculated maximum external symptoms were exhibited by unirradiated plants which showed maximum susceptibility to *FOC*. Probably this may be attributed to the difference response of the genomic composition of the cultivar to *FOC* culture.

Heavy vascular invasion of corm was more in untreated control (36). The minimum heavy vascular invasion was observed in 40 Gy and 45 Gy (3). Data analyzed from the mutant plants screened against *FOC* showed varied vascular invasion of corms. Light and medium vascular invasion of corm was less in plant derived from untreated control treatment. Stover and Malo (1972) reported that symptoms of *FOC* often do not develop in banana plants growing in containers. However, the extent of rhizome invasion in

container grown plants is correlated with symptoms in field grown plants are susceptible to the fungus.

**Analysis by SSR markers for gamma ray irradiated mutants**

Simple sequence repeats (SSR) markers were used to detect variation among gamma irradiated mutants of resistant, moderately resistant, susceptible mutants and the mother plant against *FOC*. 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, Nanjanagudu Rasabale (AAB) and Ney Poovan (AB) Table 5.

Among the 15 primers showed 4 primers showed amplification and 4 primers amplified unambiguous, readable and showed polymorphic bands in, Nanjanagudu Rasabale (2) and Ney Poovan (2). A total of 42 amplified products were produced from the selected 4 primers, in which 18 bands were amplified by Nanjanagudu Rasabale and 24 bands were amplified by Ney Poovan mutants.

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. A polymorphism was found among the mutants and mother plant indicating there is a molecular variability among the mutants.

The 4 primers used provided a large number of polymorphism among the resistant, moderately resistant, susceptible mutants and mother plant. Only few of bands observed to be specifically shared either by resistant, moderately resistant, susceptible mutants and mother plant. Nanjanagudu Rasabale putative mutants amplified with Ma 3/2 showed the major bands  $\approx$  150 bp shared by all resistant and moderately resistant mutants and varied from susceptible mutants and mother plant showed bands  $\approx$  180 bp. MA 15 showed the major bands  $\approx$  500 bp shared by all resistant and moderately resistant mutants and varied from susceptible mutants and mother plant showed bands  $\approx$  280 bp (Fig. 4 and 5).

Putative mutants of Ney Poovan bale amplified with Ma 1/17 showed the major band of 500 bp shared by all resistant and moderately resistant mutants and varied from susceptible mutants and mother plant. STMS 7 showed the major band 245 bp shared by all resistant and moderately resistant mutants and varied in susceptible mutants and mother plant  $\approx$  250 bp (Fig. 5 and 6).

Putative resistant and susceptible mutants showed variation in the banding pattern in Ney Poovan and Nanjanagudu Rasabale. 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 (Fig. 7).

**Table.1** Disease rating scale

Scale	Grade	Symptoms
0-1 %	Immune/Resistant	Healthy (No symptoms)
Light (<10%)	Highly Resistant	Slight chlorosis and wilting
Medium (10-30 %)	Moderately resistant	Moderate chlorosis and wilting with of leaf blade
Heavy (> 30 %)	Susceptible	Sever chlorosis, wilting dwarfing of newly emerged leaves or completely dead

**Table.2** The reactions of gamma ray reactions of gamma ray irradiated Nanjanagudu Rasabale to artificial inoculation of *FOC* under greenhouse conditions

Gamma irradiation/ Cultivars	Number of plants inoculated	Number of plants died after inoculation	Number of plants with external symptoms of <i>FOC</i>			Number of plants with no external symptoms
			Yellowing	Wilting	Stem splitting	
25 Gy	15	15	8	4	3	0
30 Gy	15	12	9	4	2	0
35 Gy	15	12	8	5	1	1
40 Gy	15	5	10	2	0	3
45 Gy	15	4	9	4	0	2
Untreated (Control)	15	15	9	3	3	0
<b>Total</b>	90	63	53	22	9	6

**Table.3** The Irradiated putative mutants of banana cv. putative mutants of banana cv. Ney Poovan to artificial inoculation of *FOC* under greenhouse conditions

Gamma irradiation / Cultivars	Number of plants inoculated	Number of plants died after inoculation	Number of plants with external symptoms <i>FOC</i>			Number of plants with no external symptoms
			Yellowing	Wilting	Stem splitting	
25 Gy	15	12	10	3	2	0
30 Gy	15	10	11	3	1	0
35 Gy	15	10	10	3	2	0
40 Gy	15	8	9	2	2	2
45 Gy	15	6	10	1	1	3
Untreated (Control)	15	15	11	2	2	0
<b>Total</b>	90	61	61	14	10	5

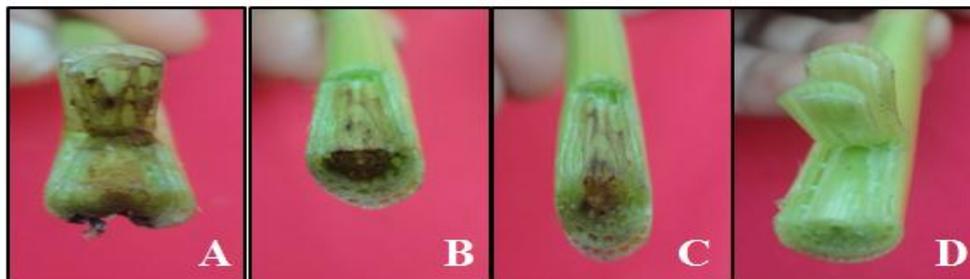
**Table.4** Effect of *FOC* on vascular invasion of corm *in vitro* gamma irradiated mutants of banana cv. Ney Poovan and Nanjanagudu Rasabale under greenhouse condition

Gamma irradiation/ Cultivars	Vascular invasion of corm					
	Nanjanagudu Rasabale			Ney Poovan		
	Light (< 10 %)	Medium (10-20 %)	Heavy (>30 %)	Light (< 10 %)	Medium (10-20 %)	Heavy (>30 %)
25 Gy	2.9	25	35	3.9	28	30.2
30 Gy	4.4	23	34	5.8	26	33
35 Gy	7.2	21	31	5.6	26	31
40 Gy	8.2	13	30	8.2	12	30
45 Gy	8.0	12	30	9.3	14	30
Untreated (Control)	3.6	27	38	2.2	29	36

**Table.5** 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	CAAACACTGTCCCCATCTC
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	TGAGGCCGGGAATCGGTA	GGCGGGAGACAGATGGAGTT
11	MABN 03	TGGTTGTATGTTTGCTGGGA	CAAAGTGCTGGCATGAGAAA
12	MABN 06	GCAACCATCAACCAAAAACC	TTTGCAAGAAAATCGTGCTG
13	CNPMF 41	GCTGCTCTCGCTTGTTATCC	GCTGCTCTCGCTTGTTATCC
14	MABN07	TTTTGATCATCATATGGGTCG	AGAGGGAGAGCCAAAGTGGT
15	MABN13	CCTCAACGAAGCATAACAGCA	CAGTCTGGGCTGACACAGAA

**Fig.1** Response of gamma irradiated Nanjanagudu Rasabale mutants to *FOC* Race 1 on vascular invasion of plantlets



**A.** Heavy >30 %, **B.** Medium 10 -20 %, **C.** Light < 10 %, **D.** 0-1 %

**Fig.2** External symptoms of putative mutants screened against *FOC* inoculation



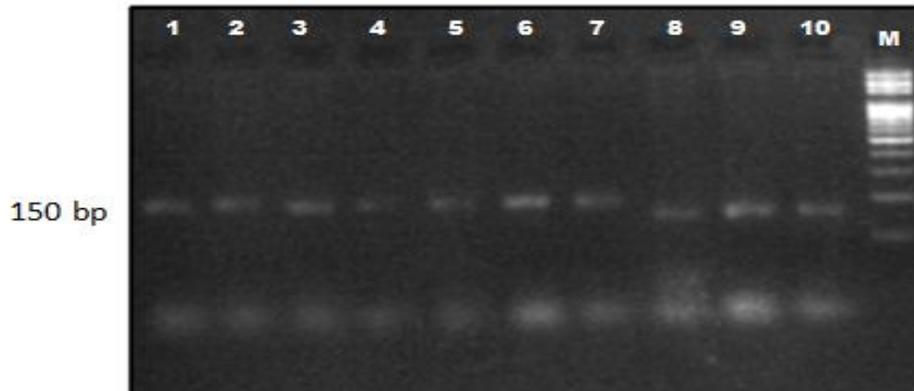
**A.** Yellowing and Wilting in Ney Poovan, **B.** Yellowing and Wilting in Nanjanagudu Rasabale, **C.** Stem splitting in Ney Poovan and **D.** Stem splitting in Nanjanagudu Rasabale

**Fig.3** Response of gamma irradiated Ney Poovan mutants to *FOC* Race 1 on vascular invasion of plantlets



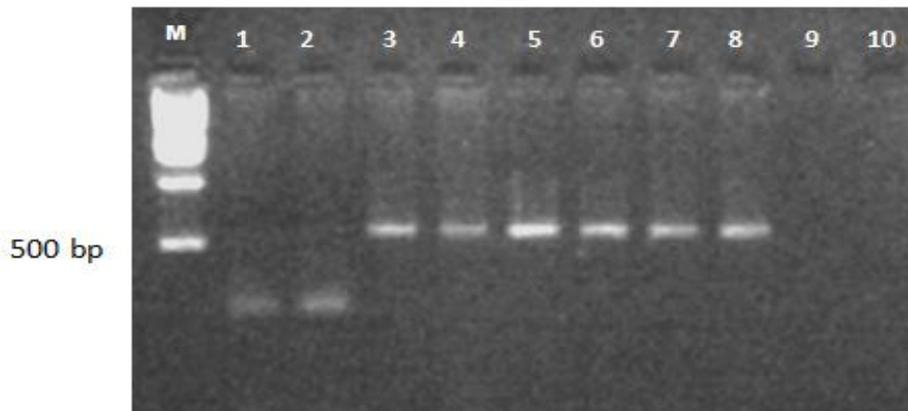
**A.** Heavy >30 %, **B.** Medium 10 -20 %, **C.** Light < 10 %, **D.** 0-1 %

**Fig.4** SSR profile of control and gamma ray irradiated mutants of Nanjanagudu Rasabale obtained with Ma 3/2



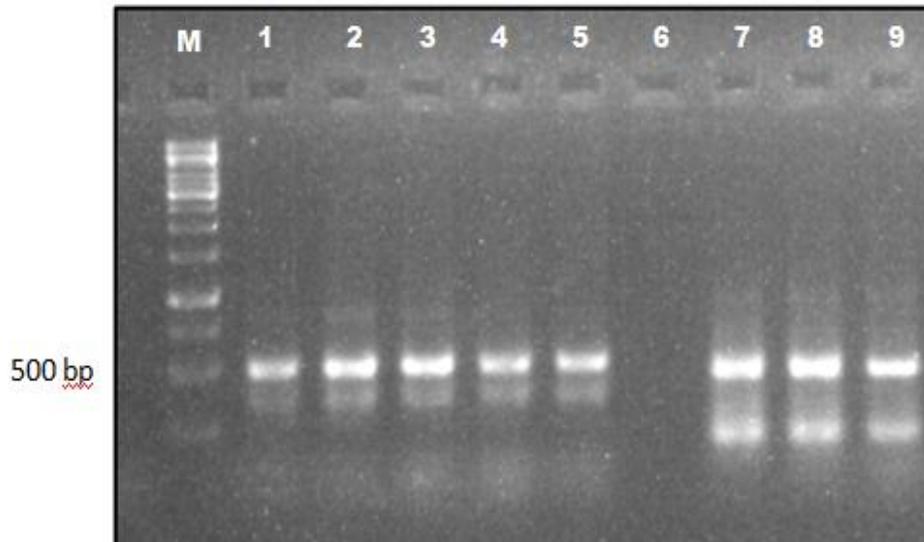
M- 100 bp Ladder, Moderately resistance: 1-35 Gy, 2-35 gy, 3-35 Gy, 4- 35 Gy, 5-40 Gy, 6-40 Gy, Resistance: 6-45 Gy, 7-45 Gy, Susceptible: 8-25 Gy, 9-30 Gy, 10 – Untreated (Control)

**Fig.5** SSR profile of control and gamma ray irradiated mutants of Nanjanagudu Rasabale obtained with Ma 15



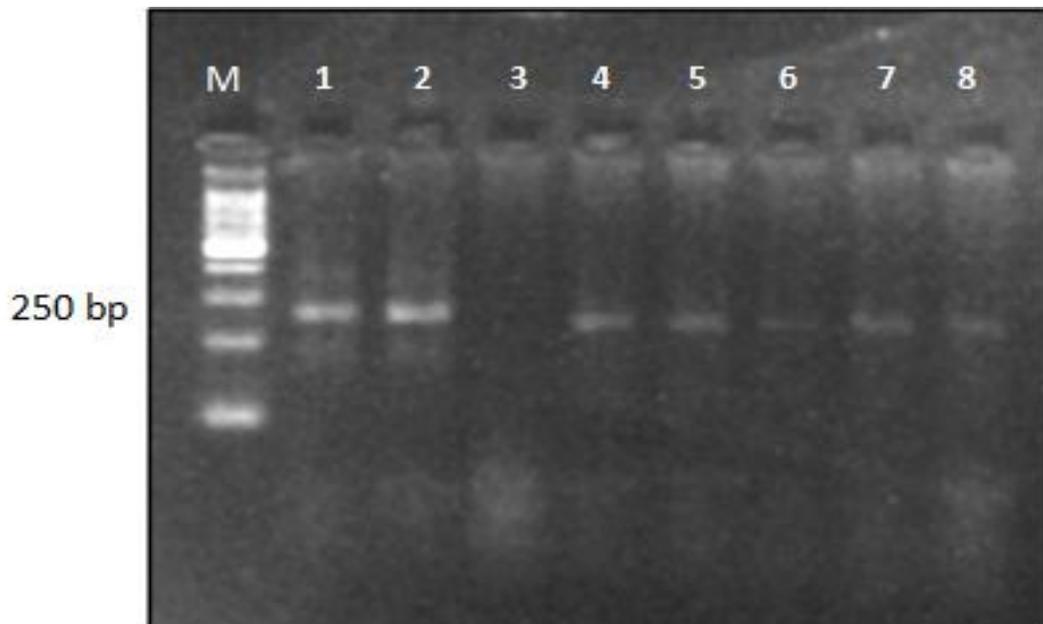
M- Ladder, 1- Untreated, Susceptible: 2- 25 Gy, Moderately resistance: 3-35 Gy, 4-35 Gy, 5-40 Gy, 6-40 Gy, 7-40 Gy, 8-40 Gy, Resistance: 9- 45 Gy, 10-45 Gy

**Fig.6** SSR profile of control and gamma ray irradiated mutants of Ney Poovan obtained with Ma 1/17



M- Ladder, 9- Untreated, Susceptible: 7- 25 Gy, 8- 25 Gy, Moderately resistance: 1-35 Gy, 2-35 Gy, 3-30 Gy, 4-30 Gy, 5-35 Gy, Resistance: 6-45 Gy

**Fig.7** SSR profile of control and gamma ray irradiated mutants of Ney Poovan obtained with STMS 7



M-Ladder, 1- Untreated, Susceptible: 2-25 Gy, Moderately resistance: 4- 30 Gy, 5-40 Gy, 6-30 Gy, 7-35 Gy, 8-35 Gy, Resistance: 3-45 Gy

It was reported that SSR marker GWM533 is closely linked to Sr2 gene Malik *et al.*, 2013. 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 (Spielmeyer *et al.*, 2003; Mago *et al.*, 2011). 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.

Development of Fusarium wilt resistant in regionally grown banana crop is necessary to reduction of considerable yield losses. The conventional breeding method takes several years to develop cultivars from wild species. In the present study, the disease tolerance or susceptibility was based on the external symptoms observed after two months of *FOC* inoculation under green house conditions. Gamma ray irradiation is the potential tool and being highly used for crop to improve disease resistance. Gamma ray irradiation, a potent mutagen in plants and create point mutations easily. 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 tolerate Fusarium wilt in increased doses.

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