Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7349

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **7 (3):** 311-317 (2019)

Research Article

ISSR Screening of *In vitro* Generated Mutants for Fusarium Wilt Resistance in Regionally Grown Banana Cultivars

Rashmi Hegde[1*], Prabhuling G.[2] and Priya Nagnur[3]

[1]Department of Biotechnology and Crop Improvement,

[2]Centre for Horticulture Biotechnology, Directorate of Research,

[3]Department of Plant Protection,

University of Horticultural Sciences, Bagalkot, India

*Corresponding Author E-mail: rashmihegde2007@gmail.com

Received: 10.03.2019 | Revised: 14.04.2019 | Accepted: 21.04.2019

ABSTRACT

In vitro mutagenesis of regionally grown banana cv Ney Poovan and Nanjanagudu Rasabale were carried out by inducing gamma ray irradiations of different doses 25 Gy, 30 Gy, 35 Gy, 40 Gy and 45 Gy. Regenerated mutants were inoculated with Fusarium oxysoporum f. sp. Cubense and evaluated for Fusarium wilt resistance with untreated control plants using inter-simple sequence repeat (ISSR) marker. Polymorphic fragments were produced by 2 (UBC 818 and UBC 841) of 21 ISSR primers. A total of 109 amplified ISSR fragments varying in size from 150 to 600 bp were obtained, 8 of which were polymorphic. Showed altered genetic profiles compared to control and Susceptible were identified in 25 Gy and 30 Gy, moderately resistance in 35 Gy and 40 Gy and resistant in 40 and 45 Gy mutants. These results suggest that gamma ray irradiation can be effectively utilized to generate mutants in regionally grown banana cultivars and ISSR provides a powerful tool that allows efficient early detection of these mutants. The identified mutants are currently being multiplied for further evaluation of their horticultural characteristics.

Key words: *Inter-simple sequence repeat; Molecular marker; Gamma irradiation; Tissue culture, Fusarium wilt, In vitro.*

INTRODUCTION

Banana belongs to the genus Musa and is the most important of the tropical fruits accounting for the world. Bananas and plantains are large monocotyledonous herbs originated from southern parts of Asia[16]. The evolution of cultivated bananas are the result of two diploid, seeded species of Musa, *M. acuminate Colla* and *M. balbisiana Colla*. Most of the edible cultivars of banana are sterile triploids or tetraploids, propagated mainly by vegetative means. Tissue culture propagation of banana has gained attention due to its potential to provide genetically uniform, pest- and disease- free planting materials. Among the delicious dessert bananas, *Musa acuminata*, var.

Cite this article: Hegde, R., Prabhuling, G. and Nagnur, P., ISSR Screening of *In vitro* Generated Mutants for Fusarium Wilt Resistance in Regionally Grown Banana Cultivars, *Int. J. Pure App. Biosci.* **7(3):** 311-317 (2019). doi: http://dx.doi.org/10.18782/2320-7051.7349

Ney Poovan and Nanjanagudu which are grown in different districts of Karnataka, India, has very high commercial demand due to its inviting aroma and white fluffy sweet pulp. These varieties are highly susceptible to fungal, bacterial and viral diseases, in which Nanjanagudu Rasabale reduced the area of cultivation to few hectares and hence recently considered as an endangered one.

Mutation breeding and biotechnological techniques may provide more possibilities for banana improvement. Mutation breeding *in vitro* is a powerful tool for the induction and selection of desirable mutants which can be utilized in banana improvement either for higher yields, good quality and resistance to biotic and abiotic factors[15]. The mutants so produced facilitate the isolation, identification and cloning of genes used in designing crops with improved yield and quality traits[1].

Molecular technology has been used to investigate the genetic polymorphism because it provides more accurate results as compared to other methods. Molecular techniques have been used to analyze genetic information in fruit crops such as isozyme[4], RFLP[7], RAPD[9,10,6,8] and AFLP[18]. ISSR (inter simple sequence repeat) is another molecular technique developed by Zietkiewicz *et al*.[19] and it can screen 100 to 3,000 bp DNA fragments. ISSR is amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (16-18 bp). The main advantage of ISSR is that it is randomly distributed throughout the genome and results are reproducible. The present study reports the use of ISSR markers in studying the genetic variation among mutants for Fusarium wilt resistance in regenerated through *in-vitro* of regionally grown banana cultivars.

MATERIAL AND METHODS

In-vitro mutation induction:

Explants consist of shoot tips of banana derived micropropagation technique were isolated and irradiated with different doses of gamma rays viz., 25, 30, 35, 40 and 45 Gy at Baba Atomic Research Centre, Trombay using ^{60}Co as source of the gamma irradiation. After irradiation the shoot tips were cultured on MS medium containing 4 mg/l BAP for proliferation to raise M_1V_1. After 30 days the individual plants were dissected and cultured on the same medium up to M_1V_5. Then the plantlets were cultured for rooting on MS medium with 2 mg/l IBA. Each treatment was replicated 3 times and about 30 multiple buds used for each treatment. The well developed plants were primary and secondary hardened. Secondary harden plants were used for Fusarium wilt resistance evaluation.

Source of *FOC* culture

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*.[12]. Screening was carried out using *FOC* spore population adjusted to 3 x 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.

DNA isolation:

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

Screening for *FOC* resistance

Banana plants were hardened and inoculated with *FOC* culture, 60 days after inoculation evaluation was carried out. The total 90 putative mutants screened in Ney Poovan, 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. The reactions of putative mutants were screened against *FOC* inoculated exhibited several peculiar symptoms like yellowing, wilting and stem splitting. The number of pants (3) with no external symptoms was observed in 45 Gy. The treatment 45 Gy had 3 plants with no external symptoms. The total 5 plants were observed with no external symptoms of *FOC* Race 1.

The total 90 putative mutants screened in Nanjanagudu rasabale, 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. The reactions of putative mutants were screened against *FOC* inoculated exhibited several peculiar symptoms like yellowing, wilting and stem splitting. The number of pants (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*.

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*.[11]. 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.

ISSR Screening

Inter simple sequence repeats (SSR) markers were used to detect variation among gamma ray irradiated mutants of resistant, moderately resistant, susceptible mutants and the mother plant against *FOC*. 21 ISSR markers were used to determine genetic variation between the various morphological mutants (Table 1) resistant and susceptible mutants along with mother plant are produced in Ney Poovan (AB) and Nanjanagudu Rasabale (AAB).

Table 1: List of ISSR primers used for screening disease resistance in putative banana mutants

Sr. No	Oligo name	Sequences (5'-3')
1	UBC-820	GTGT GTGTGTGTGTGTT
2	UBC-834	AGAGAGAGAGAGAGAGYT
3	ISSR-1	CACACACACACACACARG
4	ISSR-2	CACACACACACACACAA
5	UBC-835	GAGAGAGAGAGAGAGAYC
6	UBC-814	CTC TCT CTC TCT CTC TA

7	UBC-826	ACACACACACACACACC
8	I-840	GAG AGA GAG AGA GAG AYT
9	I-843	CTC TCT CTC TCT CTC TRA
10	I-848	CAC ACA CAC ACA CAC ARG
11	I-854	TCT CTC TCT CTC TCT CRG
12	I-855	ACA CAC ACA CAC ACA CYT
13	I-857	ACA CAC ACA CAC ACA CYG
14	I-868	GAA GAA GAA GAA GAA GAA
15	I-873	GAC AGA CAG ACA GAC A
16	UBC-810	GAGAGAGAGAGAGAGAT
17	UBC-880	GGAGAGGAGAGGAGAGGAGAGT
18	USB-841	AGAGAGAGAGAGAGAGYC
19	UBC-818	CACACACACACACACAG
20	UBC- 812	GAG AGA GAG AGA GAG AA
21	UBC- 811	GAG AGA GAG AGA GAG AC

Among the 21 primers showed 10 primers showed amplification and 6 primers amplified unambiguous, readable and showed polymorphic bands in Ney Poovan (1) and Nanjanagudu Rasabale (1)). A total of 59 amplified products were produced from the selected 2 primers, in which 32 bands were amplified by Ney Poovan mutants and 27 bands were amplified by Nanjanagudu Rasabale. The number of bands varied from 3-4 with an average 3.66 band per primers and the size ranged from 0.1 to 1.5 kb. 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 2 primers used provided 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. Putative mutants of Ney Poovan bale recorded 25 bands were amplified with UBC-818 showed the major bands range of 200 - 800 bp in which 9 were polymorphic and 16 are monomorphic shared by all resistant and moderately resistant mutants and varied from susceptible mutants and mother plant (Fig 1).

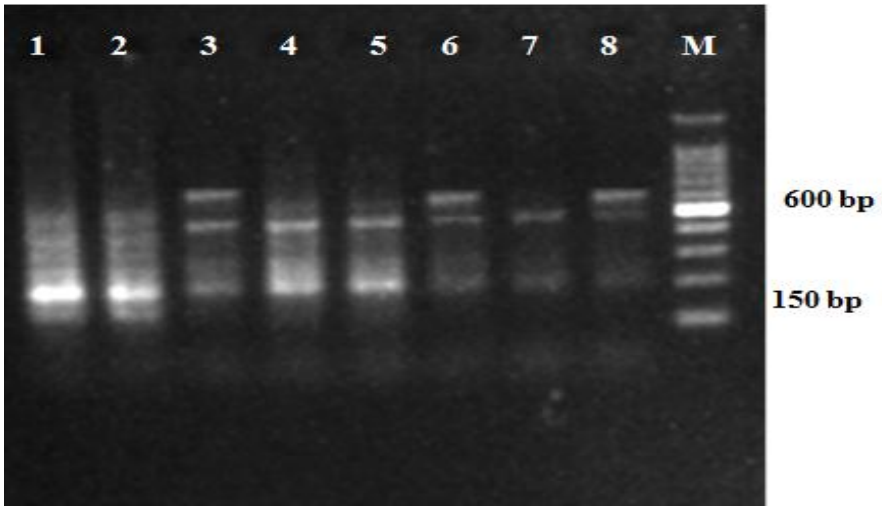

Fig. 1: ISSR profile of mother plant and gamma irradiated mutants of Ney Poovan obtained with UBC-818

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

Nanjanagudu Rasabale putative mutants recorded 44 bands were amplified with UBC-841, showed the major bands range of 260-700 bp in which 6 were polymorphic (Fig 3) and 20 were monomorphic, shared by all resistant and moderately resistant mutants and varied from susceptible mutants and mother plant.

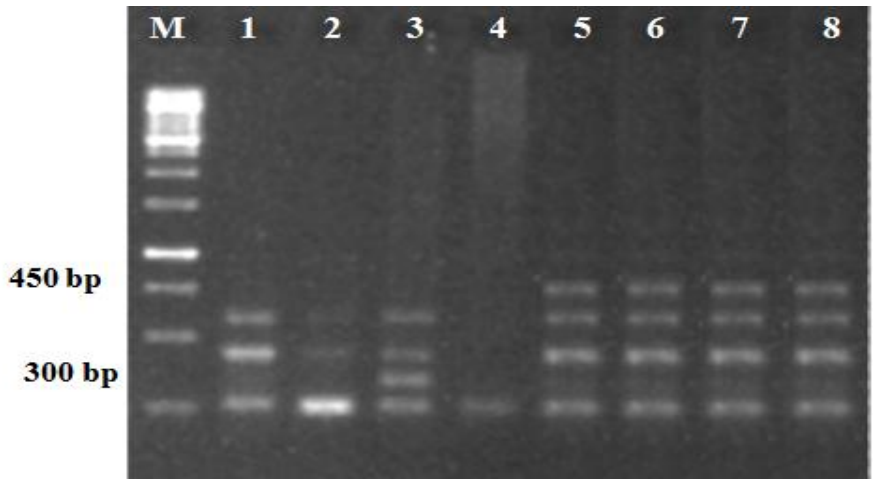

Fig. 3: ISSR profile of mother plant and gamma irradiated mutants of Nanjanagudu rasabale obtained with UBC-841

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

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.

Similar investigation was carried out by Azzam *et al*.[3] Seven ISSR primers produced storable banding patterns for the eight peanut varieties i.e., Giza-5, Giza-6, Ismailia-1, Sohag-104, Sohag-110, R-92, Virginia and Gregory and three newly developed gamma ray irradiated mutants resistant to aflatoxigenic fungi named CMPM2, CMPM6 and CMPM8. All primers succeeded to generate reproducible polymorphic DNA products. Results recorded are in harmony with the usage of ISSRs to distinguish between the resistant peanut mutants to pod rots and aflatoxin and their original parent Giza 5

Araujo *et al*.[2] investigated on the nature of blast resistance was monogenic dominant. The inheritance of the marker identified by primer OPC02 was monogenic and dominant, indicating its viability as a genetic marker. DNA from the resistant and susceptible parents was screened using 240 RAPD primers to identify RAPD fragments linked to the resistance gene in the somaclone SC09. Of the 240 primers, 203 produced amplification products. By this they identified 48 RAPD primers that differentiated resistant and susceptible parents, of which eight were specific for the resistant parent and resistant bulk, or for the susceptible parent and susceptible bulk. This was the first to report on identification of a RAPD marker linked to resistance gene Pi-ar in a somaclone derived from a rice cultivar susceptible to blast.

Even single base change at the primer annealing site is manifested as appearance or disappearance of ISSR bands[5], it could be suggested that tissue culture conditions have induced varied amount of genetic changes in different regenerated plants[13]. Some of these changes appeared identical in different plants as represented by appearance of non-parental bands. The reason for such commonness of genetic variation in these plants could be because they were all derived from the same callus[17]. The variations observed in the ISSR

pattern may be due to different causes including loss/ gain of a primer annealing, due to point mutations or by the insertion or deletion of sequence or transposable elements[14].

CONCLUSION

Our results indicate the effectiveness of ISSR markers for the identification Fusarium wilt resistance in regionally grown banana mutants obtained from gamma ray irradiation that could allow earlier selection and reduction of the mutant population size. The combined utilization of ISSR analysis together with *in vitro* mutagenesis as a source for inducing genetic variation provides a useful tool for future improvement of banana. The putative mutants of banana obtained after mutagenesis needs to be screened in the field for its performance related to morphological, agronomic, and economic traits before commercial exploitation.

REFERENCES

1. Ahloowalia, B. S. and Maluszynski, M.., Induced mutations: A new paradigm in plant breeding. *Euphytica*, **118:** 167-173 (2001).
2. Araújo, L. G., Prabhu, A. S. and Filippi, M. C., Identification of rapid marker linked to blast resistance gene in a somaclone of rice cultivar Araguaia, *Fitopatolo Bras.* **27:** 181-185 (2002).
3. Azzam, C. R., Azer, S. A., Khaleifa, M. M. A. and Abol-Ela, M. F., Characterization of peanut mutants and molecular markers associated with resistance to pod rot diseases and aflatoxin contamination by RAPD and ISSR, *Arab J. Biotech.* **10(2):** 301-320 (2007).
4. Bhat, K. V., Bhat. S. R., Chandel, K. P. S., Survey of isozyme polymorphism for clonal identification in Musa. I. Esterase, acid phosphatase and catalase, *J Hort Sci.* **67:** 501–507 (1992).
5. Creste, S., Tulmann-Neto, A., Silva, S. O. and Figueira, A., Genetic characterization of banana cultivars (Musa spp.) from Brazil using microsatellite markers. *Euphytica* **132:** 259–268 (2003).
6. Eiadthong, W., Yonemori, K., Sugiura, A., Utsunomiya, N. and Subhadrabandhu, S., Identification of mango cultivars of Thailand and evaluation of their genetic variation using the amplified fragments by simple sequence repeat- (SRR-) anchored primers. *Sci Hortic-Amsterdam*, **82:** 57-66 (1999).
7. Gawel, N. J. and Jarret, R. L., A modified CTAB DNA extraction procedure for Musa and Ipomoea. *Plant Mol Biol Rep* **9:** 262–266 (1991).
8. Grapin, A., Noyer, J. L., Carreel, F., Dambler, D., Baurens, F. C., Lanaud, C. and Lagoda. P. J. L., Diploid *Musa acuminate* genetic diversity assayed with sequence tagged microsatellite sites. *Electrophoresis*, **19:** 1374-1380 (1998).
9. Howell, E. C., Newbury, H. T., Swennan, R. L., Withers, L. A., Ford-Lioyd BV The use of RAPD for identifying and classifying *Musa* germplasm. *Genome* **37:** 328-32 (1994).
10. Khatri, A., Dahot, M. U., Khan, I. A., Raza, S., Bibi, S., Yasmin, S. and Nizamani, G. S., Use of RAPD for the assessment of genetic diversity among exotic and commercial banana clones. *Pak. J. Bot*., **41(6):** 2995-2999 (2009).
11. Krishna, V. V., Kumar, K. G., Pradeepa, K., Kumar, S. R. S. and Kumar, R. S., Biochemical markers assisted screening of Fusarium wilt resistant *Musa paradisiacal* (L) cv. Puttabale micropropagated clones. *Indian J. Exp. Biol.*, **51:** 531-541 (2013).
12. Musoke, C., Rubaihayo, R. P. and Magambo, M., Gamma rays and ethyl methane sulphonate *in vitro* induced *Fusarium* wilt resistant mutants in bananas, *Afr. Crop Sci. J.* **7(4):** 313-320 (1999).
13. Newbury, H. J., Howell, E. C., Crouch, J. H. and Lioyd, B.V. F., Natural and culture-induced genetic variation in plantains (*Musa* spp. AAB group), *Aust. J. Bot*. **48:** 493- 500 (2000).

14. Peschke, V. M., Philip R. L. and Gengenbach B. G., Genetic and molecular analysis of tissue culture derived Ac elements. *Theor. Appl. Genet*, **82:** 121-129 (1991).
15. Purseglove, J. W., Tropical crops. Monocotyledons. Longman Scientific and Technical. Longman Group U.K. Ltd. pp. 607 (1998).
16. Simmonds, N. W., Evolution of the bananas. Longmans, London, (1962).
17. Soniya, E. V., Banerjee, N. S. and Das, M. R., Genetic analysis of somaclonal variation among callus derived plants of tomato. *Curr Sci*, **80:** 1213-1215 (2001).
18. Wong, C., Kiew, R., Argent, G., Set, O., Lee, S. K. and Gan, Y. Y., Assessment of the validity of the sections in Musa (Musaceae) using AFLP. *Ann Bot.* **90:** 231–238 (2002).
19. Zietkiewicz, E., Rafalski, A. and Labuda, D., Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics* **20(2):**176–183 (1994).