International Journal of Pharmacy and Biological Sciences-IJPBS™ (2021) 11 (3): 190-199
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Identification of Molecular Markers to Detect Somaclonal Variations in Banana cv. Grand Naine (AAA) Using Inter Simple Sequence Repeats (ISSR) Molecular Marker Technique

Prathibha K. Y[1], Ravishankar K. V.[2], Shubha.[3], Manjula A.C.[4], and Keshamma E.[5*]

[1]Department of Botany, Maharani's Science College for Women, Bengaluru, Karnataka, India
[2]Department of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru, Karnataka, India
[3]Department of Botany, Government First Grade College, Bengaluru, Karnataka, India
[4]Department of Sericulture, Maharani's Science College for Women, Bengaluru, Karnataka, India
[5]Deaprtment of Biochemistry, Maharani's Science College for Women, Bengaluru, Karnataka, India

Received: 16 Mar 2021 / Accepted: 8 Apr 2021 / Published online: 1 Jul 2021
*Corresponding Author Email: keshamma.blr76@gmail.com

Abstract

The present study was undertaken with the main purpose of detection of variations of somaclones in the banana cv. Grand Naine by using inter simple sequence repeats (ISSR) molecular marker technique. Matured leaves that are free from damage caused by pests or disease were collected in brown covers from different field places like Anekal, Kadur, Hulimavu, Hesaraghatta. Source of these micropropagated planting materials were from Khodays Biotech, MSR Biotech, Green earth, Ramco Biotech companies. Isolation of genomic DNA was carried out by cetyl trimethyl ammonium bromide. The integrity and purity of DNA and visualization of amplified products was done by electrophoresis and quantified. PCR amplification of DNA from banana somaclones using ISSR markers were carried out using Taq DNA polymerase and short stretch of oligonucleotides (primers), which are specific to the DNA to be amplified. Then the ISSR, markers are used to assess the genetic variation in somaclonal variants of banana cv. Grand Naine. The amplified products of ISSR markers were separated using PAGE electrophoresis technique. Out of 98 ISSR primers screened, 68 primers did not yield any amplified DNA profile and only 34 primers showed amplification. In those 34 primers only 9 showed polymorphism. Overall, 85 monomorphic bands were seen, and 15 polymorphic bands could be seen. In conclusion, overall, 17.6% between dwarf off type and true to type normal banana cv. Grand Naine plants.

Keywords
ISSR, Molecular markers, Polymorphism, Banana cv., Grand Naine (AAA).

*****

INTRODUCTION

Banana is most cultivated fruit crop in tropical and subtropical regions [1], and micro-propagated banana planting material offers several advantages over vegetative propagation. Somaclonal variations, either genetic or epigenetic in nature, have been considered an integral part of *in-vitro* regeneration process. Somaclonal variants are an important source of genetic variation especially in vegetatively propagated plants and have been used as a tool for plant improvement as well as development of new varieties [2-4]. Important features for which *in-vitro* propagation are opted is its enormous multiplicative capacity in a relatively short span of time, production of healthy and disease-free plants and its ability to generate propagules throughout the year [5]. These variations are often undesirable in a tissue culture industry where the main aim is production of "true to type" plants. Characterization of off-type variants for traits superior to mother plants in terms of yield, fruit quality, resistance to biotic or abiotic stresses and higher regeneration efficiency in tissue culture media might lead to development of new cultivar. Dwarf Cavendish, Grand Naine Israel, Lancefield, Chinese Cavendish are some of the proven examples where somaclonal variants/mutants have been characterized, selected and released as new variety in different parts of the world.

However, some of the somaclonal variants also show characteristic feature inferior to the mother plants and causes heavy yield and economic loss to farmers as well as tissue culture industry. It is therefore important to characterize, select and assess the somaclonal variants for presence of elite and off types traits observed during *in-vitro* regeneration or in farmers' field. Morphological description, physiological supervision, karyotyping, biochemical estimations and field assessment have been used conventionally to describe and characterize such somaclonal variants. Since most of these traits are governed by many genes having additive or environment interactive effects, they remain highly subjective and difficult to identify. Recently molecular characterization and identification techniques are being used for efficient and effective management of plant genetic resources. Genetic uniformity of *in-vitro* raised plants also a prerequisite for production of quality planting material of banana [6].

Somaclonal variation arising from *in-vitro* production of plantlets is associated with DNA novel and heritable phenotypic variation which could be epigenetic or genetic [7]. The present-day molecular markers have complemented traditional methods to detect genetic variants, monitor genetic fidelity and varietal development of asexually propagated plants. Thus, DNA markers-based characterization of somaclonal variants showing discrete elite or off-type characters followed by identification of markers associated to superior traits (if any) would be extremely beneficial. With this scenario, the present study was undertaken with main aim of detection of variations of somaclones in the banana cv. Grand Naine by using simple sequence repeats (SSR) molecular marker technique.

MATERIALS AND METHODS

Source of Planting Materials

Matured leaves that are free from damage caused by pests or disease were collected in brown covers from different fields places like Anekal, Kadur, Hulimavu, Hesaraghatta. Source of these micropropagated planting materials were from Khodays Biotech, MSR Biotech, Green earth, Ramco Biotech companies.

Isolation of Genomic DNA by CTAB Method (Cetyl Trimethyl Ammonium Bromide)

This is an efficient method for isolating plant genomic DNA from leaf tissues. It provides high quality preparation of high molecular DNA. CTAB is used to liberate the nucleic acid from cell which was further purified by phenol-chloroform to remove proteins and other contaminating plant debris. The protocol for isolation of genomic DNA was carried according to Porebski et al. 1997 [8].

PCR Amplification of DNA

Polymerase chain reaction (PCR) is a very simple method for in-vitro amplification of specific nucleic acids using Taq DNA polymerase and short stretch of oligonucleotides (primers), which are specific to the DNA to be amplified. It is carried out in three steps at discrete temperatures. a) Heat denaturation of template DNA at 94-98°C, b) Annealing of primers to template DNA at 35-55°C and c) Primer extension from their 3' ends at 72°C. These steps are repeated as cycles (30 to 40). The length of the product generated during the PCR is equal to the sum of the length of the primers plus the distance in the target sequence. [9].

ISSR to Detect Genetic Variation in Micropropagated Banana cv. Grand Naine

ISSR are regions found between microsatellite repeats. The technique is based on PCR amplification of inter microsatellite sequences. Because of known abundance of repeat sequences spread all over the genome it targets multiple loci. The ISSR-PCR methodology uses a primer composed of a specific microsatellite sequence anchored at either 3' or 5' end to amplify scorable products and has higher annealing temperature [10]. It has a higher degree of polymorphism and reproducibility [11].

In the present study around 98 ISSR markers have been used to identify the genetic variation in off-type somaclones in banana cv. Grand Naine. The list and sequence of primers used in the present experiment are given in the Table 1.

Table 1: ISSR Primer Sequences Used in Screening

801	ATA TAT ATA TAT ATA TT
802	ATA TAT ATA TAT ATA TG
803	ATA TAT ATA TAT ATA TC
804	TAT ATA TAT ATA TAT AA
805	TAT ATA TAT ATA TAT AC
806	TAT ATA TAT ATA TAT AG
807	AGA GAG AGA GAG AGA GT
808	AGA GAG AGA GAG AGA GC
809	AGA GAG AGA GAG AGA GG
810	GAG AGA GAG AGA GAG AT
811	GAG AGA GAG AGA GAG AC
812	GAG AGA GAG AGA GAG AA
813	CTC TCT CTC TCT CTC TT
814	CTC TCT CTC TCT CTC TA
815	CTC TCT CTC TCT CTC TG
816	CAC ACA CAC ACA CAC AT
817	CAC ACA CAC ACA CAC AA
818	CAC ACA CAC ACA CAC AA
819	GTG TGT GTG TGT GTG TA
820	GTG TGT GTG TGT GTG TC
821	GTG TGT GTG TGT GTG TT
822	TCT CTC TCT CTC TCT CA
823	TCT CTC TCT CTC TCT CC
824	TCT CTC TCT CTC TCT CG
825	ACA CAC ACA CAC ACA CT
826	ACA CAC ACA CAC ACA CC
827	ACA CAC ACA CAC ACA CG
828	TGT GTG TGT GTG TGT GA
829	TGT GTG TGT GTG TGT GC
830	TGT GTG TGT GTG TGT GG
831	ATA TAT ATA TAT ATA TYA
832	ATA TAT ATA TAT ATA TYC
833	ATA TAT ATA TAT ATA TYG
834	AGA GAG AGA GAG AGA GYT
835	AGA GAG AGA GAG AGA GYC
836	AGA GAG AGA GAG AGA GYA
837	TAT ATA TAT ATA TAT ART

838	TAT ATA TAT ATA TAT ARC
839	TAT ATA TAT ATA TAT ARG
840	GAG AGA GAG AGA GAG AYT
841	GAG AGA GAG AGA GAG AYC
842	GAG AGA GAG AGA GAG AYG
843	CTC TCT CTC TCT CTC TRA
844	CTC TCT CTC TCT CTC TRC
845	CTC TCT CTC TCT CTC TRG
846	CAC ACA CAC ACA CAC ART
847	CAC ACA CAC ACA CAC ARC
848	CAC ACA CAC ACA CAC ARC
849	GTG TGT GTG TGT GTG TYA
850	GTG TGT GTG TGT GTG TYC
851	GTG TGT GTG TGT GTG TYG
852	TCT CTC TCT CTC TCT CRA
853	TCT CTC TCT CTC TCT CRT
854	TCT CTC TCT CTC TCT CRG
855	ACA CAC ACA CAC ACA CYT
856	ACA CAC ACA CAC ACA CYA
857	ACA CAC ACA CAC ACA CYG
858	TGT GTG TGT GTG TGT GRT
859	TGT GTG TGT GTG TGT GRC
860	TGT GTG TGT GTG TGT GRA
861	ACC ACC ACC ACC ACC ACC
862	AGC AGC AGC AGC AGC AGC
863	AGT AGT AGT AGT AGT AGT
864	ATG ATG ATG ATG ATG ATG
865	CCG CCG CCG CCG CCG CCG
866	CTC CTC CTC CTC CTC CTC
867	GGC GGC GGC GGC GGC GGC
868	GAA GAA GAA GAA GAA GAA
869	GTT GTT GTT GTT GTT GTT
870	TGC TGC TGC TGC TGC TGC
871	TAT TAT TAT TAT TAT TAT
872	GAT AGA TAG ATA GAT A
873	GAC AGA CAG ACA GAC A
874	CCC TCC CTC CCT CCC T
875	CTA GCT AGC TAG CTA G
876	GAT AGA TAG ACA GAC A
877	TGC ATG CAT GCA TGC A

878	GGA TGG ATG GAT GGA T
879	CTT CAC TTC ACT TCA
880	GCA GAG GAG AGG AGA
881	GGG TGG GGT GGG GTG
882	VBV ATA TAT ATA TAT AT
883	BVB TAT ATA TAT ATA TA
884	HBH AGA GAG AGA GAG AG
885	BHB GAG AGA GAG AGA GA
886	VDV CTC TCT CTC TCT CT
887	DVD TCT CTC TCT CTC TC
888	BDB CAC ACA CAC ACA CA
889	DBD ACA CAC ACA CAC AC
890	VHV GTG TGT GTG TGT GT
891	HVH TGT GTG TGT GTG TG
892	TAG ATC TGA TAT CTG AAT TCC C
893	NNN NNN NNN NNN NNN NNN
894	TGG TAG CTC TTG ATC ANN NNN
895	AGA GTT GGT AGC TCT TGA TC
896	AGG TCG CGG CCG CNN NNN NAT G
897	CCG ACT CGA GNN NNN NAT GTG G
898	CAT CAA GCT TNN NNN NAT GTG G
899	CAT CGT GTT GGT CAT TGT TCC A
900	ACT TCC CCA CAG GTT AAC ACA

Resolving DNA Amplification Products Using PAGE

Polyacrylamide Gel Electrophoresis (PAGE) is widely used for the separation of low molecular weight DNA. The amplified products of ISSR markers were having low molecular weight DNA (100 kb 400 kb) and efficient separation was achieved by running the amplified products in PAGE by using standardized technique.

RESULTS

Occurrence of somaclonal variation in micropropagated banana is a serious problem facing the tissue culture industry. In the present study, involves identification of field grown dwarf off-types with molecular marker techniques which would help in elimination of such types from micropropagated banana. Experiments were conducted to identify markers specific to the variants using ISSR. Morphological variants like dwarf off-type without bunch, bunch variants were chosen for marker identification as they were causing serious economic loss to the farmers.

ISSR marker was used in the present experiment to bring out genetic variation in micropropagated dwarf off-types with that of normal banana plant. 98 ISSR primers were screened to analyse the genetic diversity between normal and tissue cultured dwarf off-types. Out of 98 primers screened, 68 primers did not yield any amplified DNA profile and only 34 primers showed amplification. In those 34 primers only 9 showed polymorphism. ISSR primers showing polymorphism was represented in Table 2 and 3. Overall 85 monomorphic bands were seen, and 15 polymorphic bands could be seen (Plate 1, Fig. a-d). ISSR primers 841, 846, 829 showed clear polymorphic band between normal and dwarf off-types micropropagated banana (Plate 1, Fig. b-d).

Table 2: Polymorphic and monomorphic bands amplified by ISSR primers for normal and dwarf off-types of banana cultivar Grand Naine

S. No.	ISSR Primers	No. of MB	No. of PB	No. of polymorphic band in true to	No. of polymorphic band in dwarf off types of banana	MB + PB	Polymorphism (%)
1	819	0	1	0	1	1	2.90
2	823	2	1	1	0	3	2.90
3	826	2	1	1	0	3	2.90
4	829	1	1	1	0	2	2.90
5	841	5	2	0	2	7	2.90
6	844	4	4	2	2	8	11.70
7	846	2	1	1	0	3	2.90
8	848	2	2	2	0	4	5.80
9	849	1	3	3	0	4	8.80
	Total	19	15	11	5	35	

MB: Mono-morphic bands; PB: Poly-morphic bands

Table 3: Monomorphic bands by ISSR primers for normal and dwarf off-type banana cv. Grand Naine

S. No.	ISSR Primer	No. of MB
1	807	4
2	808	2
3	814	4
4	815	2
5	816	3
6	817	4
7	818	1
8	825	3
9	826	5
10	827	2
11	840	1
12	843	5
13	845	5
14	847	3
15	854	3
16	855	4
17	856	2
18	857	3
19	881	3
20	888	2
21	889	4
	Total	66

MB: Mono-morphic bands

Total No. of Monomorphic bands obtained through ISSR primers= 85

Total No. of Polymorphic bands obtained through ISSR primers= 15

% Of Polymorphism observed by using ISSR primers = 17.6

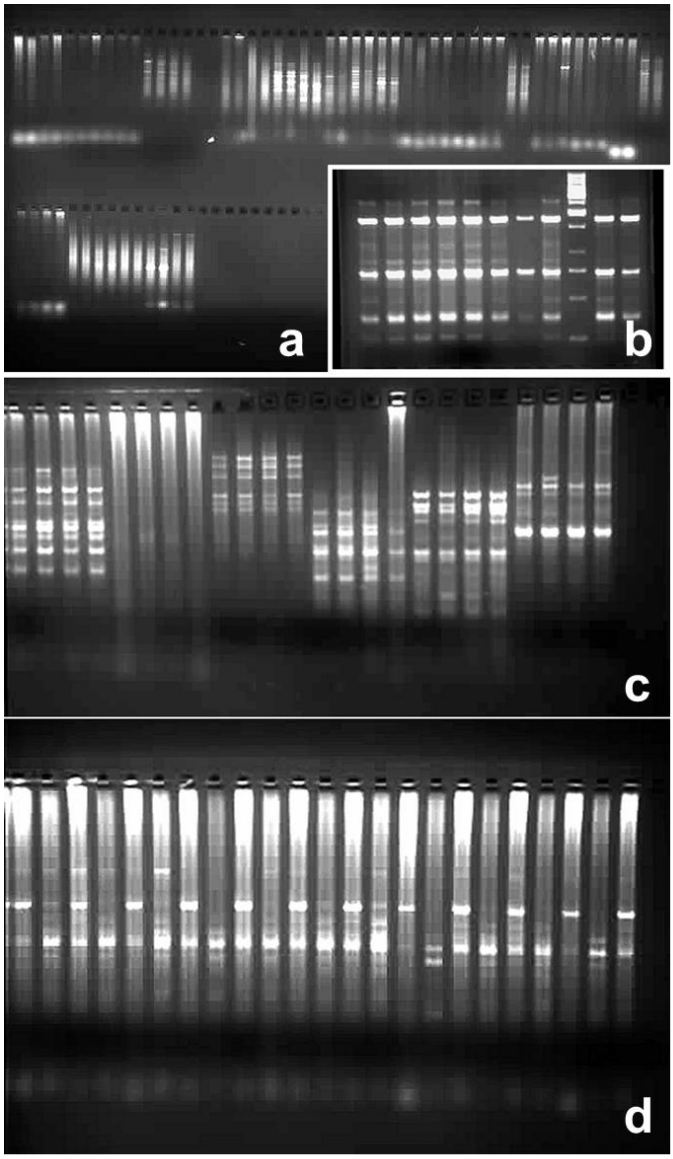

Plate-1

Figs. a-d Gels showing ISSR primers DNA amplification in true-to-type and off-type *in vitro* banana plants cv. Grand Naine.

a. Gel showing initial screening with 801-900 ISSR primers to detect polymorphism in amplification between normal and dwarf off-type plants.
b. Gel showing polymorphism generated by 841 ISSR primer. 2 polymorphic bands in dwarf off-types can be seen which is absent in normal plant.
c. Agarose gel showing bands created by ISSR primers 857, 852, 841, 844, 845, primer 846 showing an extra band in dwarf off-type plants.
d. Gel showing amplification done by 829 ISSR primer. A polymorphic band can be seen in true-to-type which is absent in dwarf off-type.

Polyacrylamide gel was used to separate ISSR amplified bands since the bands formed were of smaller kb in length and ISSR amplify 25 to 40 ISSR products in one position. On PAGE very good polymorphism was seen with primers 823, 841, 844, 848, 849. A clear intense polymorphism could be seen with 844, 848, 849 primers indicating the percentage of variations between dwarf off-types variants and normal banana plants (Plate 2, Table 2).

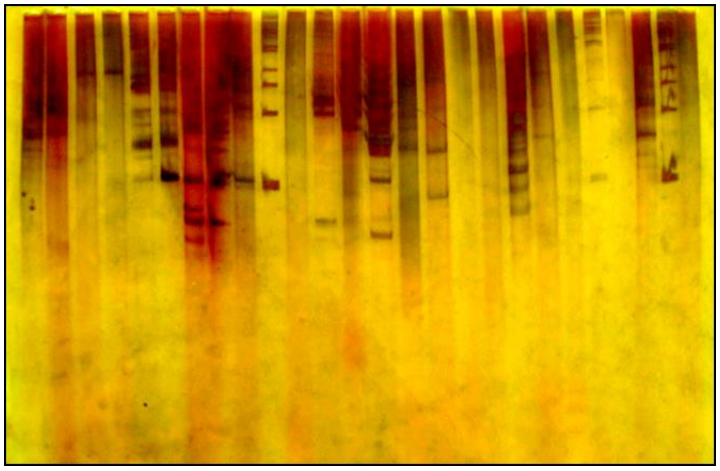

Plate-2: PAGE showing DNA amplification by ISSR primers 823, 829, 841, 844, 846, 849, 851, 856, 858, 876, 857 in true-to-type and dwarf off-type banana plants.
ISSR primers 823, 829, 841, 844, 846, 849 showing polymorphism

DISCUSSION

Somaclonal variants are usually inferior and can result in serious economic losses to the farmers through poor yields, compounded by wasted investment of time, field space and other resources in cultivation of propagated plants [12]. The largescale production of commercially elite plants by in vitro micropropagation is technically available for a number of species. A common problem encountered when growing plants by tissue culture is the development of tissue cultured induced genomic polymorphisms in which genetic changes in the nuclear, mitochondria, chloroplast genomes result in the lack of homogenecity among the regenerants and the production of inferior plants that are not true-to-type (off-types) with little commercial value. Many characteristics are only expressed in more mature stage of plant development and not in young stages. Off-types such as dwarfs, thicker leaves are difficult to identify at both tissue culture and nursery stage [13].

Present requirement for confronting somaclonal variation is the early-stage markers that could be applied in-vitro to identify variation as soon as it occurs. In this direction molecular markers have particularly been suggested to be useful for early-stage confirmation of genetic fidelity in micropropagated elite species [14]. The aim of the present study was to provide polymorphic markers for detection of somaclonal variations in tissue cultured banana cv. Grand Naine. Although there are many references dealing with banana tissue culture [15-17], the analysis of tissue culture derived plants for somaclonal variations of Indian cvs. are minimum [18,19]. Therefore, study was undertaken to isolate molecular markers for early detection of plants that are not true-to-type. More particularly the work relates to the use of such genetic markers as a diagnostic and quality control tool for monitoring the development of genetic polymorphisms arising during tissue culture regeneration of plants.

Our morphological study of variants in the field revealed dwarf off-type was the most common somaclonal variant in the field, and economically affecting variants like dwarf non-flowering and malformed fingers (small fingers with bitter taste) were also in more numbers and studied for molecular characterization. Many authors have also reported dwarf off-type variant to be the common variant in micropropagated bananas [20,21]. In oil palm non-flowering micropropagated variant has caused enormous loss to the producers [22], along with 10% of regenerants showing mantled flowering in oil palm [23]. Similar non-flowering variant has been recorded in this study and isolation of molecular markers were also tried for such variant along with malformed fingered bunch and bunch variants. Early detection of these variants would help to discard these inferior variants from the nursery fields.

ISSR are the regions found between microsatellite repeats. Relatively novel technique has proven to be a powerful, simple, inexpensive, faster and reproducible [24]. In our study we have used 98 ISSR primers, out of which 21 primers showed monomorphic bands and only 9 primers showed polymorphic bands and the rest 68 did not amplify. The percentage of polymorphism was 17.6% between dwarf off type and true to type normal banana cv. Grand Naine plants. Similarly, the genetic fidelity in micropropagated banana cvs. Robusta (AAA), Giant Governor (AAA), Martaman (AAB) was assessed by ISSR by Ray et al. They observed 5% polymorphism generated by ISSR primer for Robusta

and Giant Governor; whereas Martaman was genetically stable [19]. Further, using ISSR marker, Osipova et al observed 23% of polymorphism between normal and regenerated plants from callus cultures of maize [25]. According to Devarumath et al., ISSR finger printing detected more (12.8%) polymorphic bands loci than RAPD (4.28%) finger printing in micropropagated tea. Even in our report ISSR detected 17% polymorphic loci in dwarf off type micropropagated Grand Naine banana [26]

We have used acrylamide gel for resolving many bands and primers 841, 844, 848, 849 showed good resolution on the gel. Venkatachalam et al reported the genetic analysis of micropropagated plantlets of Rasthali (AAB) wherein they have used 50 RAPD and 12 ISSR primers and obtained 625 monomorphic bands without genetic variability [27]. Furthermore, Podwyszynska et al used ISSR markers and detected genetic variation which gave rise to phenotypic variation in micropropagated tulips derived from 4-7 year cultures [28]. Moreover, genetic stability in micropropagated almond plants were analysed through ISSR marker by Martins et al and reported that 10 ISSR primers formed 326 monomorphic bands in almond micropropagated plant. Hence ISSR can be used as a good marker to assess genetic fidelity in micropropagated plants [29]

CONCLUSIONS

The potential of molecular markers has been used for testing the genetic fidelity of micropropagated cv. Grand Naine (AAA) plants in the fields. Molecular marker like ISSR were used to identify the genetic variations that has resulted in banana cv. Grand Naine. SSR markers tried clearly showed polymorphism between the dwarf off types and the normal banana plants. Out of 98 ISSR primers screened, 68 primers did not yield any amplified DNA profile and only 34 primers showed amplification. In those 34 primers only 9 showed polymorphism. Overall, 85 monomorphic bands were seen and 15 polymorphic bands could be seen. The percentage of polymorphism was 17.6% between dwarf off type and true to type normal banana cv. Grand Naine plants. Moreover, this is a preliminary report on limited number of individuals, and hence a large number of individuals needs to be tested before confirming the association between these markers to a specific genotype.

REFERENCES

1. Kalloo G. Banana and plantation research in India–a perspective. Global Conf. on Banana and Plantain. 2002.
2. Cullis CA. The molecular biology of plant cells and cultures. *Plant biotechnol*. Comprehensive biotechnology, second supplement. 1992:19-32.
3. Karp A. Are your plants normal? Genetic instability in regenerated and transgenic plants. Agro-Food-Industry Hi-Tech May/June. 1993:7-12.
4. Cassells AC, Joyce SM, Curry RF, McCarthy TF. Detection of economically important variability in micropropagation. InPlant Biotechnology and in vitro Biology in the 21st Century 1999:241-244). Springer, Dordrecht.
5. Dhawan VI, Bhojwani SS. Micropropagation in crop plants. Glimpses Plant Res. 1986; 7:1-75.
6. Rout GR, Senapati SK, Aparajita S, Palai SK. Studies on genetic identification and genetic fidelity of cultivated banana using ISSR markers. *Plant Omics*. 2009;2(6):250-85.
7. Lukens LN, Zhan S. The plant genome's methylation status and response to stress: implications for plant improvement. *Curr opin plant biol.* 2007;10(3):317-22.
8. Porebski S, Bailey LG, Baum BR. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol Biol Rep*. 1997;15(1):8-15.
9. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *InCold Spring Harbor symposia on quantitative biology* 1986;51:263-273. Cold Spring Harbor Laboratory Press.
10. Gupta M, Chyi YS, Romero-Severson J, Owen JL. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theor Appl Genet.* 1994;89(7):998-1006.
11. Fang DQ, Roose ML. Identification of closely related citrus cultivars with inter-simple sequence repeat markers. *Theor Appl Genet.* 1997;95(3):408-17.
12. Krikorian AD. In vitro culture of bananas and plantains: background, update and call for information. *Trop Agric*. (Trin.). 1989; 66:194-200.
13. Vuylsteke D, Swennen R, Wilson GF, De Langhe E. Phenotypic variation among *in-vitro* propagated plantain *(Musa sp.* cultivar 'AAB'). *Sci, Hortis*. 1988;36(1-2):79-88.
14. Gupta PK, Varshney RK. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*. 2000;113(3):163-85.
15. Banerjee, N., Vuylsteke, D. and De Langhe, E. Meristem tip culture of musa in: withers LA and Anderson PS (eds.) Butterworths, London.! 86:139-147.
16. Smith MK, Hamill SD. Early detection of dwarf off-types from micropropagated Cavendish bananas. *Aust J Exp Agric*. 1993;33(5):639-44.
17. Zaffari GR, Peres LE, Tcacenco FA, Kerbauy GB. Indole-3-acetic acid metabolism in normal and dwarf micropropagated banana plants (Musa spp. AAA). *Braz. J. Plant Physiol*. 2002; 14:211-7.
18. Martin KP, Pachathundikandi SK, Zhang CL, Slater A, Madassery J. RAPD analysis of a variant of banana

(*Musa sp.*) cv. grande naine and its propagation via shoot tip culture. *In vitro Cell Dev Biol-Plant.* 2006;42(2):188-92.

19. Ray T, Dutta I, Saha P, Das S, Roy SC. Genetic stability of three economically important micropropagated banana (*Musa spp.*) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers. *Plant cell, tissue and organ cult*. 2006;85(1):11-21.
20. Sandoval, J. and Muller, L. Considerations la conservation in vitro de Musaceae, possibilities and limitations. *Association Bananera National (ASBANA) Costarico*. 1989. 31, 21-24.
21. Côte FX, Sandoval JA, Marie P, Auboiron E. Variations in micropropagated bananas and plantains: literature survey. *Fruits*. 1993;48(1):15-23.
22. Singh R, Nagappan J, Tan SG, Panandam JM, Cheah SC. Development of simple sequence repeat (SSR) markers for oil palm and their application in genetic mapping and fingerprinting of tissue culture clones. *Asia-Pac J Mol Biol Biote*. 2007;15(3):121-31.
23. CIRAD. Centre de co-operation internationale recherché agronomique pour i.e., development. http://www.cirad.fr/en/actualite/communique. php. 2003.
24. Godwin ID, Aitken EA, Smith LW. Application of inter simple sequence repeat (ISSR) markers to plant genetics. *Electrophoresis.* 1997;18(9):1524-8.
25. Osipova ES, Koveza OV, Troitskij AV, Dolgikh YI, Shamina ZB, Gostimskij SA. Analysis of specific RAPD and ISSR fragments in maize (Zea mays L.) somaclones and development of SCAR markers on their basis. *Russ J Genet*. 2003;39(12):1412-9.
26. Devarumath R, Nandy S, Rani V, Marimuthu S, Muraleedharan N, Raina S. RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and *C. assamica* ssp. assamica (Assam-India type). *Plant cell reports*. 2002;21(2):166-73.
27. Venkatachalam L, Sreedhar RV, Bhagyalakshmi N. Genetic analyses of micropropagated and regenerated plantlets of banana as assessed by RAPD and ISSR markers. *In Vitro Cell Dev Biol -Plant*. 2007;43(3):267-74.
28. Podwyszynska M, Niedoba K, Korbin M, Marasek A. Somaclonal variation in micropropagated tulips determined by phenotype and DNA markers. InXXII International Eucarpia Symposium, Section Ornamentals, Breeding for Beauty 714 2006:211-220.
29. Martins M, Sarmento D, Oliveira MM. Genetic stability of micropropagated almond plantlets, as assessed by RAPD and ISSR markers. *Plant Cell Rep*. 2004;23(7):492-6.