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Molecular assessment of genetic identity and genetic stability in banana cultivars (*Musa* spp.) from China using ISSR markers

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Abstract

A study was carried out to identify of different cultivars and monitor somaclonal variations of banana during rapid mass micropropgation using Inter simple sequence repeats (ISSR) marker. DNA templates from 30 Banana cultivars were evaluated using 45 primers. Genetic diversity is analyzed by one statistical procedure: hierarchical classification. Total number of bands varied between the various cultivars from 5~9.The percentage of total polymorphism is about 85.1%.Four large group were obtained. Furthermore a homogenous amplification profile was observed for all the micropagated plants of 'Brazil' when compared to their mother plants; the developed profiles of different micropropagated clones were typical to that of the donor mother plants.

Keywords: cultivars identity, genetic stability, ISSR marker
Abbreviation: BAP ,Benzylaminopurine; CTAB, cetyl-trimethyl ammonium bromide; IAA, Indoleacetic acid; ISSR, Inter Simple Sequence Repeats; KIN, kinetin TDZ, N- phenyl – N´- 1,2,3 –thiadiazol 5-yl urea; UPGMA, Unweighted Pair-Group Method using arithmetic Average.

Introduction

Plantains and bananas *(Musa* spp.) are staple foods for rural and urban consumers in the humid tropics and an important source of rural income, particularly in some locations where smallholders produce them in compound or home gardens. World banana production is around 103 million tones annually, of which bananas cultivated for the export trade account for only 10% (Aurore et al. 2009). Hence, fruit harvested from bananas and plantains are important components of food security in the tropical world, and provide income to the farming community through local and international trade. Unfortunately many pests and diseases have significantly affected banana production. Largely due to poor quality cloned (Novak 1992). At the global level, the most serious constraint to the banana production is considered to be black sigaroka leaf spot disease caused by this fungus results in yield loses at 30~76% (Johanson et al., 2000). In the case of banana, improvement genetic improvement of bananas has been severely hampered by low female fertility levels the resulting from the triploid nature of cultivated bananas leading to irregular meiosis. Consequently, most cultivars exhibit low set. Banana improvement is also constrained by the long generation cycle and the large space (Ortiz et al., 1995). Tissue culture is propagation of banana has gained attention due to its potential to provide genetically uniform, pest and disease free planting materials. Micropropagation has a great commercial potential due to the speed of propagation, decreased production space requirement and the ability to multiply elite clones exhibiting superior growth and enhanced stress tolerance). But a phenomenon called somaclonal variation being a very serious problem in the banana tissue culture industry resulting in the production of undesirable plant off-type (Karp 1989; Phillips et al., 1994; Cullis 1992). Somaclonal variation at DNA level included gene methylation changes, DNA rearrangements and alterations in copy number (Brar et al., 1998). However, it is sometimes hard to differentiate such permanent somaclonal variability from transient epigenetic changes. In order to evaluate genetic variability, follow phylogenetic origin and extent of ecologically distinguished species or subspecies and to develop efficient crop breeding systems, plant breeders need to have a definitive identification both of cultivars and selections of crop plants. Reliable methods of identification are also required for the establishment of plant variety rights (Kjeldgaad et al., 1994). Unambiguous identification is especially important in a clonally-propagated crop such as banana (Kester 1983k). Commercial cultivators need to be sure that they are investing their time and money in propagating the specific cultivar that they have chosen on the basis of yield, harvest time, size and shape. Different methods are available to detect and monitor tissue culture-derived plants and cultivar identification the effect of mutagens on plants. Molecular markers allow a direct comparison of the effects on genotypes at the DNA level. Different molecular techniques have been developed and are widely used in many fields such as agriculture, biology medicine, etc for various purposes ,out of the available techniques, Inter Simple Sequence Repeats (ISSR)marker have proven to be simple, fast, cost effective, and versatile set of markers that repeatable amplification of DNA sequences using single primers. In the present study, the primary objectives were to use ISSR to examine the genetic integrity and uniformity of the important banana cultivars and tissue culture-derived plants and to generate useful DNA fingerprints to facilitate cultivar identification. In addition, the genetic relationships that exist between these cultivars were examined as well as genetic stability was monitored.

Materials and methods

Plant material

Thirty commercially grown banana cultivars were in involved in this study collected from the *Musa* germplansm Center of Hainan, and various parts of plantation and maintained in the experiment garden. They are shown in Table 1.

In vitro propagation

Meristem-tip cultures of banana were derived from shoot apexes. Explants (ca. 10 x 10 x 6 mm) obtained from decapitated shoot apexes of suckers were surface sterilized by 70% ethanol for 20 seconds, then incubated in a 5% solution of sodium hypo chloride for 20 min followed by three rinses in sterile distilled water. The effects of cytokinins [Benzylaminopurine (BAP), kinetin (KIN) and N- phenyl – N´- 1,2,3 –thiadiazol 5-yl urea (TDZ)] combined with auxin [Indoleacetic acid (IAA)] were evaluated on basal Murashige and Skoog (MS) medium. The pH was adjusted to 5.7 with 1M NaoH before agar and charcoal was added. The cultures were maintained at 25 ºC with 16 h photoperiod at a photosynthetic photon flux density of 120µmol /m ²/ s. Sub-culturing was carried out at 45-day intervals. All treatments were performed on three replications of 10 explants in experiments employing a completely randomized design.

Genomic DNA isolation

For variety identification, DNA of the 30 China banana cultivars was extracted from the leaves. In the separate experiment DNA was extracted from the leaves of the parental genotype and regenerated plants of the first, third, fifth, seventh, ninth and eleventh sub-cultures for genetic fidelity study. Total DNA was isolated by a modified cetyl-trimethyl ammonium bromide (CTAB) extraction technique. Approximately 0.2 g of fresh leaf tissue was ground in liquid nitrogen and added to 700 µl of extraction buffer (2% CTAB, 100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 0.2% β- mercaptoethanol, pH=8) and incubated at 60℃ for 30 min. The aqueous solution was extracted with 600 µl of chloroform: isoamyl alcohol (24:1) and centrifuged for 20 min at 12000 rpm. The extraction was repeated and 1 ml 96% cold ethanol was added to aqueous phase and left at -20℃ for 20 min to precipitate the nucleic acid. The samples were then centrifuged for 10 min at 12000 rpm. The precipitated nucleic acid was washed with 1 ml 70% ethanol, left to air dry, rehydrated in 100µl TE buffer and digested with 1µl RNase (10 mg/ml, Fermentas, Germany) for 45 min at 37℃. The quality of genomic DNA was examined by agarose (1%) gel electrophoresis and quantified spectrophotometically at A260 and A280 nm. Each sample was diluted to 50 ng/µl in TE buffer and stored at -20℃

Selection of ISSR Primers

To select suitable primers for the study of cultivars of Banana, 45 ISSR primers (UBC primer set No. 9, Biotechnology Laboratory, University of British Columbia, Vancouver, Canada, http://www.ubc.ca/) were screened using two DNA samples. From the preliminary screening, 12 primes that could amplify visible bands were selected for further examination. Eventually, 8 ISSR primers gave the strongest, clearest, and most reproducible bands were then chosen for amplification of all DNA samples (Table 2).

Table 1. List of Musa cultivar and ISSR clusters in the present study

Code	Cultivar	Genome	ISSR
H1	Brazil	AAA	01
R2	8818	AAA	02
H2	Thailand	AAA	03
H3	Brazil Baizhong	AAA	04
H4	Brazil Huangzhong	AAA	05
H5	Williams	AAA	06
R7	Vallery	AAA	07
R8	Tumoc	AAA	08
R9	Dongguan Dajiao	AABB	09
G1	Hongjiao	AAA	10
H11	Fenjiao	ABB	11
Y12	Hekouyebajiao	ABB	12
Y13	Suanbajiao	ABB	13
Y14	Huanongdajiao	ABB	14
Y15	Honghe'aijiao	AAA	15
G2	Huanong7	AAA	16
H6	Huanongtianjiao	AAA	17
H7	Dongguandajiao1	AABB	18
T2	Beida'aijiao	AAA	19
H8	Sanvafenjiao	ABB	20
H9	Mijiao	AAB	21
H10	Gaojiaoyajiao	AAA	22
G3	Zhongjiaoyajiao	AAA	23
G4	Mumianjiao	ABB	24
G5	Xiaomijiao	AAB	25
G6	Huangdijiao	AA	26
G7	Beijao	AAA	27
T3	Taiwanjiao	AAA	28
G8	Guangjiaodundilei	AA	29
G9	Jinshouzhi	AAAB	30

DNA amplification

All reaction were carried out in a final volume of 25µl containing 5 µl 10xPCR buffer, 0.2 mM dNTP, 1.5 mM MgCl2 ,0.4 mM each of primers, 1.0 U Taq polymerase, and 1.0 µl genomic DNA from banana per reaction. The final volume was adjusted to 25µl with sterile Milli-Q water. Amplification was performed in thin-walled PCR tubes. The ISSR-PCR amplification was performed on PTC200 DNA thermal cycle (TM Research) with modification of the annealing temperature to optimize the reaction mixtures were denatured at 94℃ for 5 min and subjected to 44 cycles of 30s at 94℃, 45s at 48-55℃, 1.5min at 72℃, and final extension step of 10 min at 72℃ and eventually stored at 4℃. Ampicons were electrophoresed in 1.5% agarose gels with 1xTAE buffer at 5V/cm. Band patterns were photographed

Table 2. Features of ISSR prime used for ISSR-PCR of 30 banana cultivars and the amplified products

Sequence	Annealing Temperature(℃)	Amplified bands		
		Total	Polymorph bands	%Polymorphism
$(CT)_8T$	55	5	4	80.0
$(CA)_8RC$	52	6	5	83.3
$(AC)_8YT$	55	4	3	75.0
$(CA)_8RG$	55	6	6	100.0
$(GA)_8YG$	53	7	6	85.7
$(GA)_8YC$	55	8	7	87.5
$(CA)_8A$	57	9	8	88.9
$(GA)_8YT$	57	9	7	77.8

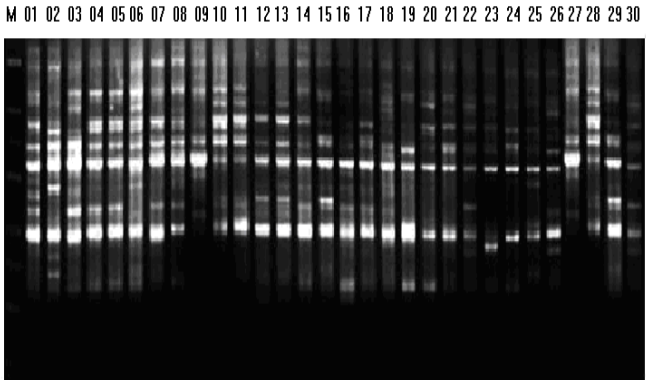

Fig1. ISSR marker profiles of 30 cultivars of Banana generated by primer $(GA)_8YC$ in 1.2 percent agarose gel

under UV light (302 nm) after staining with ethidium bromide (EB). The size of amplified fragment was estimated by comparison to a DNA marker DL2000 (Takara, Biotechnology Co. Ltd, Danlian, China)

Data analysis

Amplification product profiles were scored for the presence (1) or absence (0) of bands. Molecular size of the amplified fragments was estimated using 100 bp DNA ladder (Fermentas). The experiments were repeated twice for each cultivar to confirm the repeatability and only reliable and repeatable bands were considered. Genetic similarity between pairs was calculated according to Jaccard's similarity coefficient（Jaccrard,1980）, followed by cluster analysis by the UPGMA in the SAHN program of NTSYS-pc version 2.1 (Rohlf, 1998).

Results

Production of ISSR Amplification

A total of 54 strong, clear, and reproducible bands were amplified from 30 cultivars using the 8 selected ISSR primes, of which 46 were polymorphic (85.1%) (Table2). The number of DNA amplified bands varied between three and eight with a range of 100~2500bp (Fig. 1& Fig. 2). The maximum number of fragments was found to be eight and was obtained using primer $(CA)_8$ A and $(GA)_8$ YT, whereas the least number of fragments was three that was obtained with $(AC)_8$ YT. The highest level of polymorphism (100%) was obtained with primer $(CA)_8$ RG, whereas the lowest was 77.8% and was obtained with primer $(GA)_8$ YT (Table 2).

Cluster Analysis

Genetic similarity among the cultivars was estimated using similarity coefficient matrix based on ISSR bands scored. Pair wise values of similarity coefficient (Fig. 3) The dendrogram was constructed based on the similarity matrix using UPGMA method indicates that the 30 cultivars divided them into four clusters. One cluster having variety '8818', 'Tumoc', 'Vallery', 'Thailand', 'Brazil', 'Xiaomijiao', 'Honghe'aijiao', 'Beida'aijiao', 'Gaojiaoyajiao', variety of 'Williams'; 'Brazil Huangzhong,' 'Huanongtianjiao' 'Gaojiaodundilei', T'aiwanjiao', 'Mumianjiao,' 'Jingshouzhi', 'Huanong 7', 'Zhongjiaoyajiao', 'Brazil Baizhong', 'Hongjiao' has been grouped together in the second cluster. The third cluster including variety 'Hekouyebajiao', 'Dongguan Dajiao', 'Huangdijiao', 'Beijiao', 'Suanbajiao', 'Huanongdajiao', 'Sanyafenjiao', 'Gaojiaoyajiao'.

Analysis of genetic fidelity of in vitro raised plantlets

The quality of *in vitro* derived regenerated was screened with five ISSR primers that have showed monomorphic among the plantlets. The banding pattern of PCR amplified product from micropropagated plantlets was found to be monomorphic for

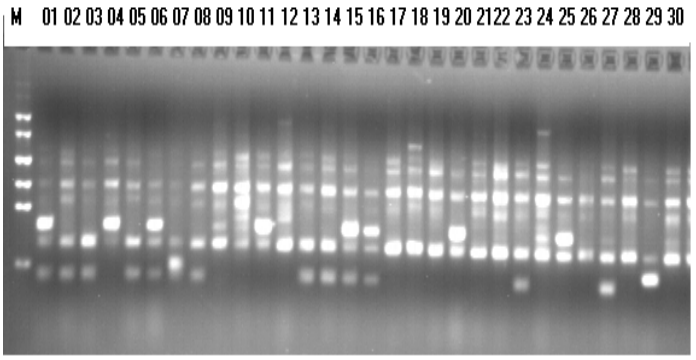

Fig 2. ISSR marker profiles of 30 cultivars of Banana generated by primer $(CA)_8$ A in 1.2 percent agarose gel

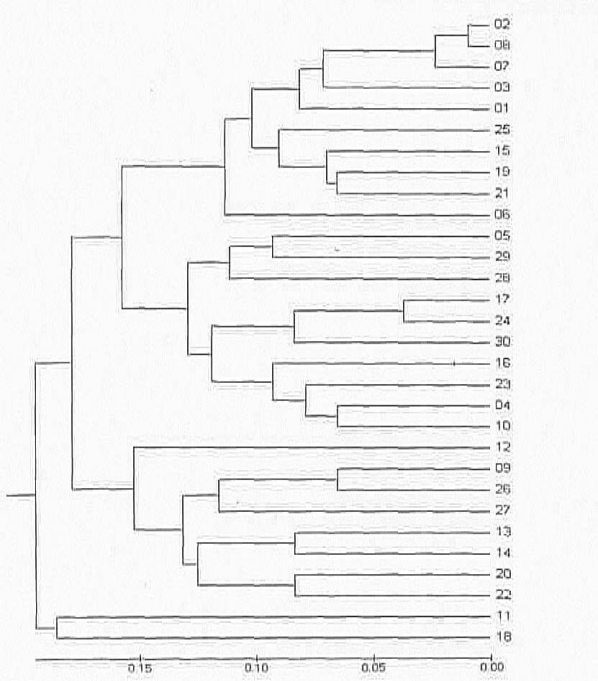

Fig 3. Dendrogam generated using UPGMA analysis showing relationships between banana cultivars

most of the primer tested. The identical ISSR banding pattern of eighteen in vitro raised plantlets and theirs mother control plant have showed in Fig.4. ISSR primer UBC487 showed identical DNA profiles as compared with mother plants. Absence of genetic variation using the RAPD and ISSR marker system has been reported in several cases such as micropropagated shoots of three banana cultivars, i.e. Robusta, Giant Govenor and Martaman (Ray et al., 2006). Somatic embryogenesis-derived regenerants of oil palm (Rival et al., 1998), micropropagated teak (Gangopadhyay et al., 2003), somatic embryo derived sweet potato plants (Sharma et al. 2004) and axillary bud proliferation in chestnut rootstock hybrids (Carvalho et al., 2004). Raimondi et al. (2001) observed no intraclonal variation using RAPD analysis in asparagus plantlets.

Discussion

Molecular marker have been employed in the characterization and evaluation of genetic diversity in Musa cultivars and monitoring somaclonal variations of during rapid mass micropropgation, including restriction fragment length polymorphism (RFLP) (Gawel et al., 1992; Fauré et al., 1994; Carreel et al., 2002); random amplified polymorphic DNA (RAPD) (Bhat et al., 1995; Pillay et al., 2000, 2001; Masoud et al., 2008; Brown et al., 2009); amplified fragment length polymorphism (AFLP) (Loth et al., 2000; Ude et al., 2002a; 2002b); microsatellites or simple sequence repeats (SSRs) (Lagoda et al., 1998a; Crouch et al., 1998; Kaemmer et al., 1997; Grapin et al.,1998; Silvana et al., 2003) and Inter simple sequence repeats (ISSR) (Rout et al., 2009). Some molecular methods including RAPD, AFLP, RFLP, SSR and

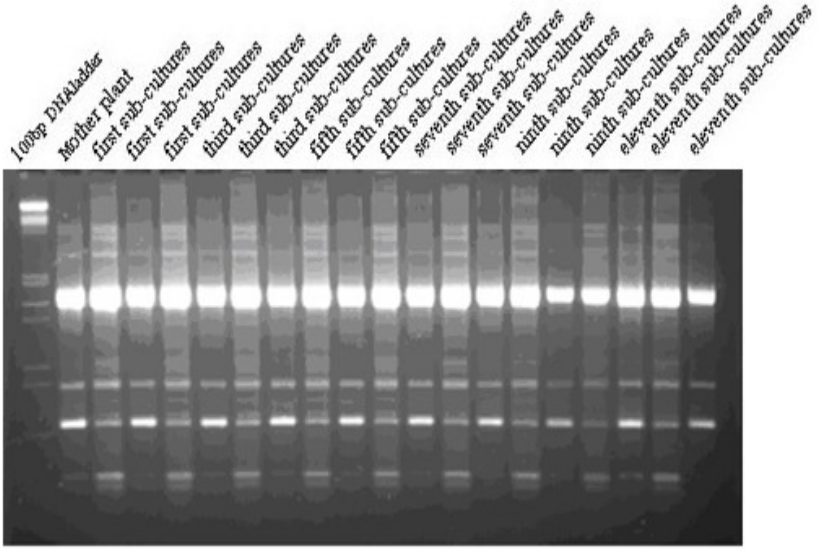

Fig 4. ISSR marker profiles of Banana mother plant (lane1) and long-term micropropagated shoots

ISSR indicated that there were different among cultivars (Bhat et al., 1995; Onguso et al., 2004).Tough some other RPAD and SSR groups have found no correlation (Silvana et al., 2003; Carreel et al., 2002). Furthermore Rout (Rout et al., 2009) have found a good correlation between ISSR clusters and Musa cultivars. ISSR are present to detect difference between SSRs (Ziekiewicz et al., 1994). Compared with other molecular markers, ISSR can reveal high polymorphism, which is helpful in distinguishing individual at inter-and/or intra-species level. Moreover, ISSR has its specific advantage over other markers as follows: no prior sequence information, simple operation, high stability and low cost. Therefore ISSR has been proposed as a more economical and reliable DNA marker system (Bornet et al., 2001,). In this study, we have used the ISSR markers as tools, in survey of the genomes of 30 *Musa* cultivars obtained from Hainan province of South China. In total 85.1% of the bands generated using ISSR were polymorphic, which provided evidence of a large genetic diversity among the tested cultivars. The most cultivars bananas in China are from Brazil subgroup, and there are innumerous unclassified landraces with localized importance, recognized by a local name associated with the Brazil subgroup. Here, we have shown that most of the landraces evaluated are highly similar or identical to the cultivar 'Brazil'. However, the landrace 'Dongguandajiao1', with doubtful classification, did not in e cluster with 'Dongguandajiao', despite the clear name relationship with each other. It is possible this two landraces with similar morphological and /or fruit quantity attributes. The division of *Musa* cultivars into subgroups is based on common origin or mutations in a cultivars, although these mutations may have important effects for commercialization (Dantas et al., 1997). Such mutations are mainly reflected in changes in the color of leaves as well as changes in the size of the plants, while maintaining fruits quality (Jenny et al., 1999). Large variations in morphological characteristics do not necessarily reflect the same degree of genetic variation (Gawel et al., 1992). According to Jenny et al. (1999), the variability within each subgroup is mainly dependence on the genotype and the frequency with which each clone is multiplied and planted .High levels of genetic similarity are expected between cultivars from the same subgroup because they share a common origin .The observation of monomorphic bands profiles among cultivars of the same subgroup reflects the maintenance of allele composition during successive cycles of vegetative propagation. The used of ISSR to discriminate between somatic mutants and the clone from which the mutants originated have been studied in *Musa* (Venkatachalam et al., 2007). The China banana has numerous clones in order to suit the local environment. The present investigation is to identify the clone and study the genetic uniformity of the in vitro plants. The results obtained from the investigation will be helpful for proper identification and of new cultivars and selection of parents suitable for creating of mapping population. It will also help in the identification of duplications among the accession in tissue culture germplasm banks and in the fields

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References

Aurore G, Parfait B, Fahrasmane L (2009) Bananas raw materials for making processed food products. Trends in Food Science& Technology 20:78-91

Bhat KV, Jarret RL (1995) Random amplified polymorphic DNA and genetic diversity in Indian germplasm. Genet Res Crop Evol 42:107-118

Bornet B, Branchard M (2001) Nonanchored inter simple sequence repeat (ISSR) marker: Reproducible and specific tools for genome fingerprinting. Plant Molecular Biology Reporter 19:209-215

Brar DS, Jain SM (1998) Somaclonal variation: mechanisms and applications in crop improvement In: Jain S M ,Brar D S, Ahloowalia B S (eds).Somaclonal variation and induced mutations in crop improvemen. pp: 17-37. Boston, USA: Kluwer Academic Publishers.

Brown N, Venkatasamy S, Khittoo G, Bahorun T, Jawaheer S (2009) Evalution of genetic diversity between 27 banana cultivars (*Musa* spp.) in Mauritius using RAPD markers. African Journal of Biotechology 8(9):1834－1840

Carreel F, Leon D G, Lagoda PJL, Lanaud C, Jenny C, Horry JP, Du Montcel HT (2002) Ascertaining maternal and paternal lineage within *Musa* by chloroplast and mitochondrial DNA RFLP analyses. Genome 45:679-692

Cravalho LC, Goulao L, Oliveita C, Goncalves JC, Amancio S (2004). RAPD assessment for identification of clonal fidelity and genetic stability of in vitro propagated chestnut hybrids. Plant Cell Tiss Org Cult 77:23-27

Crouch HK, Crouch RL, Jarret RL, Cregan PB, Ortiz R (1998) Segregation at microsatellite loci in haploid and diploid gametes of *Musa*. Crop Sci 38:211-217

Cullis CA (1992) The molecular biology of plant cells and cultures. In: Fowler MW, Warren GS (eds.) Plant Biotechnology pp: 19-32. Oxford: Pergamon Press.

Dantas JLL, Shepherd K, Silva SO, Soares-Filho WS (1997) Classifieacáo botanica origem evolucao e distritubicáo geographic. In: Alves EJ (ed.) A Cultura da Banana: aspectos técnicos, socioeconn Ônics e agroindustriais pp.27-34. Brasilia, Embrapa.

Fauré S, Noyer JL, Carrel F, Horry JP, Lanaud C (1994) Maternal inheritance of chloroplast genome and paternal inheritance of mitochondrial genome in banana (*Musa* acuminata). Curr Genet 25:13-15

Gawel NL, Jarret RL, Whittemore AP (1992) Restriction fragment length polymorphism (RFLP)-base phylogenetic analysis of *Musa*. Theor appl. Genet 84:265-290

Geangopadhyay G, Geangopadhyay SB, Poddar R, Fupta S (2003) Micropropagation of Tectona grandis:assessment of genetic fidelity. Biol Plant.46:459-461

Grapin A, Noyer J, Carreel F, Dambier D, Baurens FC, Lanaud C, Lagoda PJL (1998) Diploid *Musa* acuminate genetic diversity assays with sequence-tagged microsatellite sites. Electrophoresis 19:1374-1380

Jaccard P (1980) Nouvelles researchers sur la distribution florale.Société Vaudoise des Sciences Naturelles 44, 22_270

Jenny C, Carreel F, Tomekpe K, Perrier X, Dubois C, Horry J P, Monteel HT (1999) Les bananiers. In: P. Hamon, M seguin, X Perrier, J C Glastzman(eds) .Centre de Coopération Internationale en Recherche Agronomique pour le Développement.Diversité des Plantes Tropicales pp.113-139.Montpellier,CIRAD

Johanson A, Tushemereirwe W, Kramura E B (2000) Distribution of sigatoka leaf spots in Uganda as determined by species-specific polymerase chain reaction (PCR.) In: Craenen K, Ortiz R., Karamura EB, Vuylsteke (eds.) Proceedings of the International Conference on Banana and Plantain for Africa Acta Horticurae.International Society for Horticultural Science (ISHS) pp.319-334 Leuven,Belgium.

Kaemmer D, Fisher D, Jarret RL, Baurens FC, Grapin A, Dambier D, Noyer JL, Lanaud C, Khal G, Lagoda PJ L (1997) Molecular breeding in the genus *Musa*: a strong case for STMS marker technology. Euphytica 96:49-63

Karp A (1989) Can genetic instability be controlled in plant tissue cultures? Plant Tissue Culture 58: 2-11.

Kester DE (1983) The clone in horticulture. Hort Sci 75:831-837

Kjeldgaad RH, Marsh DR (1994). Intellectual property rights for plants. Plant Cell 6:1524-1528

Lagoda PJL, Dambier D, Grapin A, Baurens FC, Lanaud C, Noyer JL (1998) Nonradioactive sequence-tagged microsatellite site analyses: A method transferable to the tropics. Electrophoresis 19:152-157

Loth JP, Kiew R, Set O, Gan LH, Gan YY (2000) Amplified fragment length polymorphism fingerprinting of 16 bananas cultivars (*Musa* cvs.). Mol Phylogenet Evol 17:360-366

Masoud S, Heywa A, Zahra N, Farah F (2008) RAPD analysis of somaclonal variation in banana(*Musa* acuminate L.) cultivar valery. Acta Biologica Szegediensi 52(2):307-311

Novak FJ (1992) *Musa* (Banana, Plantain). In: Hammerschlag FA, Litz R (eds.)

Onguso JM, Kahangi EM, Ndiritu DW, Mizutani F (2004) Genetic characterization of cultivated bananas and plantains in Kenya by RAPD markers. Scientia Hortic 99:9-20

Ortiz R, Ferris RSB, Vuylsteke DR (1995) Banana and plantain breeding. In: Gowen SR (eds.) Banana and Plantains, pp.110-146.Chapman and Hall, London perennial fruits crops, Wallingford, pp. 449-488,CAB International, UK

Phillips RL, Kaeppler SM, Othoft P (1994) Genetic instability of plant tissue cultures. Break down of normal controls. Proc. Nat Acad. Sci. USA 91:5222-5226

Pillay M, Ogundiwin E, Nwakanma DC, Ude G, Tenkouano A (2001) Analysis of genetic diversity and relationships in East African banana germplasm. Theor Appl Genet 102:965-970

Pillay M, Nwakanma DC, Tenkoano A (2000) Identification of RAPD markers linked to A and B genome sequences in *Musa* L. Genome 43:763-767

Raimondi JP, Masuelli RW, Camadro EL (2001) Assessment of somaclonal variations in asparagus by RAPD fingerprinting and cytogenetic analysis. Scientia Hort 90:19-29

Ray T, Dutta I, Saha P, Das S, Roy SC (2006) 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 Tiss Organ Cult 85:11-21

Rival A, Bertrand L, Beule T, Combes MC, Trouslot P (1998) Suitability of RAPD analysis for the detection of somaclonal variants in oil palm (Elaeis guineensis Jacq). Plant Breed117:73-76

Rohif FJ (1998). NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.1.Applied Biostatics, New York

Rout GR, Senapati SK, Aparajita S, Palai SK (2009) Studies on genetic identification and genetic fidelity of cultivars banana using ISSR marker. Plant Omics Journal 2(6):250-258

Sharman SD, Ghosh SA, Mandal BB, Srivastava PS (2004) Somatic embryogenesis in a range of genotypes and genetic stability of the plants derived from somatic embryos using morphological and RAPD markers in sweet potato. J Plant Biotech 6:119-124

Silvana C, Augusto TN, Sebastáo de OS, Antonio F (2003) Genetic characterization of banana cultivars(*Musa* spp.) from Brazil using microsatellite markers. Euphytica 132:259-268

Ude G, Pillay M, Nwakanma D, Tenkouano A (2002a) Analysis of genetic diversity and sectional relationship in *Musa* using AFLP marker. Theor Appl Genet.104:1239-1252

Ude G, Pillay M, Nwakanma D, Tenkouano A (2002b) Genetic diversity in *Musa* acuminate Colla and *Musa* balbisiana Colla and some of their natural hybrids using AFLP marker. Theor. Appl. Genet 104:1246-1252

Venkatachalam L, Sreedhar RV, Bhagyalakshmi N (2007) Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR markers. Electron J of Biotechnol.15:106-113

Ziekiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-Anchored polymerase chain reaction amplification. Genomics 20:176-183