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Studies on genetic identification and genetic fidelity of cultivated banana using ISSR markers

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Abstract

Molecular identification of different varieties is an important element for efficient and effective management of banana genetic resources. Genetic uniformity of *in vitro* raised plants also a prerequisite for production of quality planting material of banana. Present study showed the use of ISSR marker for identification of different varieties of banana and detection of genetic uniformity of micropropagated plantlets. Fifteen ISSR primers used to differentiate the four cultivated banana. Based on those identity markers, the genetic distance between varieties was estimated and their genetic relationship was found out. The banana varieties having genome constitution AAA were grouped together whereas 'Bantala' variety is out grouped with genome constitution BBB. A homogenous amplification profile was observed for all the micropropagated plants of 'Bantala' when compared to mother plant. In case of 'Grand Naine', few plants showed variation at the DNA level in primer IG-13. But, morphologically they are identical as compared with original mother plant. The results confirmed that the clonal fidelity of *in vitr*o raised plantlets and corroborate the fact that *in vitro* multiplication is the safest mode for multiplying of true to type plants.

Keywords: Banana; genetic stability; ISSR marker; *In vitro*; Shoot multiplication

Abbreviations:

Ads_Adenine sulfate; BA_6benzylaminopurine; 2,4-D_2,4-dichlorophenoxyacetic acid; ISSR_ Inter Simple Sequence Repeats; IAA_indole-3-acetic acid; Kn_Kinetin; NAA_1-napthalene acetic acid

Introduction

Banana and plantains (clones of the genus *Musa,* family Musaceae) are among the oldest and the most valued crop plants of the tropics and sub-tropics all over the world and the trade generates considerable income in almost all part of the globe. With respect to the total volume, banana ranks first among fruit crops with an annual production of about 103 million tones annually (FAO, 2004, Aurore *et al*., 2009). Total world production is approximately 81 million tones per year and 90% of fruits are used for domestic consumption (FAO, 2004). They are considered to be one of the most important sources of energy in the diet of people living in tropical humid regions. Banana plants also provide useful by-products, such as fiber, vegetables, beer, wine and vinegar (Hammond *et al*., 1996; Aurore *et al*., 2009). Unfortunately, the fruit production is hampered by diseases and pests (Stover, 1972; 1977; 1980) largely due to poor quality clones (Novak, 1992). Yellow sigatoka is one of the serious diseases affecting the banana crop. In fact, improvement by traditional breeding procedure is difficult and time consuming endeavors because of the high sterility, polypiody and long generation times of most edible varieties. The modern tissue culture technology provides an opportunity to develop new germplasm better adapted to changing demands. 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 through out the year (Dhawan and Bhojwani, 1986).

Table 1. List of fifteen primers that produced polymorphic markers among the *Musa* varieties studied

Primer	Primer sequence	Total no. of bands	No. of Polymorphic bands	% of polymorphic
IG-01	AGGGCTGGAGGAGGGC	07	3	42.8
IG-02	AGAGGTGGGCAGGTGG	09	4	44.4
IG-03	GAGGGTGGAGGATCT	05	2	40.0
IG-05	GACAGACAGACAGACA	06	3	50.0
IG-06	GACAGATAGACAGATA	12	6	50.0
IG-09	$(AG)_8T$	12	5	41.6
IG-10	$(AG)_8C$	09	4	44.4
IG-11	$(AC)_8T$	16	8	50.0
IG-12	$(AC)_8G$	09	4	44.4
IG-13	$(GA)_8A$	05	2	40.0
IG-14	$(GA)_8T$	14	5	35.7
IG-15	$(CT)_8G$	13	6	46.1
IG-18	$(GA)_8C$	05	2	40.0
IG-19	$TGG(AC)_7$	09	4	44.4
IG-23	$ACG(GT)_7$	10	4	40.0
Total	----------	135	62	45.9

In the last few years, *in vitro* propagation has revolutionized commercial nursery business (Pierik, 1991). Recent progress of banana tissue culture and its biotechnological application has been reviewed (Rout *et al*., 2000a).

In vitro production of plants involves the application of plant growth regulator, such as auxin, for initiation. Nevertheless, these auxins are known to be associated with genetic instability in plants, a phenomenon called somaclonal variation (Karp, 1989; Cullis, 1992). Mostly somaclonal variation also occurs as responses to the stress imposed on the plant in culture conditions and are manifested in the form of DNA methylations, chromosome rearrangements and point mutations (Phillips *et al*., 1994). Although somaclonal variations may be used as a source for variation to get superior clones, it could be a serious problem in plant tissue culture industry where the aim is to develop identical propagules of a desired variety resulting in the production of undesirable traits or plant off-types (Karp, 1993; Cassells *et al*., 1999). Scaling up of any micropropagation protocol is severly hindered due to incidence of somaclonal variations, so a stringent quality check in terms of genetic similarity of progeny becomes mandatory. Any system, which can significantly reduce or eliminate variation generated during tissue culture, can be of much practical utility. Traditionally, morphological description, physiological supervision, karyological analysis, biochemical estimations and field assessment were used to detect any types of genetic variations, but presently molecular markers have complemented over traditional methods to detect and monitor the genetic fidelity of tissue culture derived plantlets and variety identification. This is apparent in studies conducted to screen somaclonal variations produced in tissue cultured derived plants such as in turmeric (Salvi et al., 2001), *Lillium* (Varshney et al., 2001), Strawberry (Gaafer and Saker, 2006) and *Swertia chirayita* (Jhosi and Dhawan, 2007). Out of the available techniques, ISSR markers have proven to be a reliable, reproducible, easy to generate, inexpensive and versatile set of markers that relies on repeatable amplification of DNA sequences using single primers. This is apparent in studies conducted to screen somaclonal variations produced in tissue cultured derived plants such as in *Swertia chirayita* (Jhosi and Dhawan, 2007).The present communication is to document the identification of Indian cultivated banana varieties and also to establish the genetic integrity and uniformity of the tissue culture-derived plantlets through ISSR markers.

Materials and methods

Plant material

Four commercially grown banana varieties namely ‘Bantala’, ‘Grande Naine’, ‘Patakapura’ and ‘Robusta’ were collected from the various parts of Orissa, India and maintained in the experimental garden. The genomic size of the variety ‘Bantala’ was BBB and other three varieties (‘Grande Naine’, ‘Patkapura’ and ‘Robusta’) were AAA.

Table 2. Similarity coefficients among four *Musa* genome based on ISSR fingerprints

Cultivars	Grand Naine	Bantala	Patkapura	Robusta
Grand Naine	1.00			
Bantala	0.53	1.00		
Patkapura	0.63	0.59	1.00	
Robusta	0.67	0.55	0.62	1.00

In vitro propagation

Rhizomes of *Musa acuminata* varieties were collected from experimental garden and used for *in vitro* study. The rhizomes were washed with 5% (v/v) teepol' detergents (Qualigen, Mumbai, India) for 30 min, then surface sterilized in 0.5% (w/v) mercuric chloride for 40min and kept overnight in 1% (w/v) polyvinylpolypyrrolidone (MW-6000). Each rhizome were then sliced into 30-35 pieces (5-6 mm diameter) and used as initial explant. The rhizome segments were then transferred to agar basal culture medium containing macro and micro nutrients as recommended by MS (Murashige and Skoog, 1962), supplemented with 100 mg l^{-1} myo-inositol, 30 gm l^{-1} sucrose, 0.5 mg l^{-1} nicotinic acid, 0.5 mg l^{-1} pyridoxine-HCl, 0.5 mg l^{-1} thiamine-HCl, 2 mg l^{-1} glycine and also fortified with different concentrations (0, 1.0,2.0, 3.0, 4.0, 6.0 mg l^{-1}) of BA or Kn, Ads (50, 100, 150, 200 mg l^{-1}) in combination with IAA, NAA and 2,4-D (0, 1.0, 1.5 and 2.0 mg l^{-1}) for shoot proliferation and multiplication. All the growth regulators were added prior to autoclaving. The media was solidified with 0.7 % (W/V) agar. Media was sterilized for 15 min at 121°C (15 lb psi pressure). Cultures were incubated at 25±1°C under 16 h/8 h photoperiod with light intensity of 3000 lux and 50 – 60% relative humidity (RH). After 3 - 4 weeks of culture, the domes with 2-3 pairs of leaf primordias were transferred to MS medium with 4.0 - 6.0 mg l^{-1} BA, 100-200 mg l^{-1} Ads and 1.0-2.0 mg l^{-1} IAA for further shoot multiplication and growth. The cultures were subcultured every 4 weeks on fresh medium of same composition. For root induction, excised microshoots (1-2 cm length) were transferred to half strength basal MS medium supplemented with different concentrations of activated charcoal (100, 200, 300, 400, 500 and 600 mg l^{-1}) and 2% sucrose (w/v) with or without 0.25, 0.5 and 1.0 mg l^{-1} IAA or IBA. Rooted micropropagules were thoroughly washed and planted in earthen pot containing mixture of sand, soil and cow dung manure in the ratio of 1: 1: 1 (w/v) and were kept in green house with 25 - 28 °C and >85% relative humidity for acclimatization. The data pertaining to mean percentage of culture showing response, number of shoots/culture, mean percentage of rooting and no of roots/shoot were statistically analyzed by the Post-Hoc Multiple Comparison test (Marascuilo and McSweeney, 1977).

Genetic characterization

For variety identification study, genomic DNA was extracted from young unfurled leaves of four varieties of banana i.e. 'Bantala', 'Grande Naine (G-9)', 'Patkapura' and 'Robusta'. Five samples from each variety were used for this experiment. In a separate experiment, the young leaves were collected from *in vitro* raised plantlets (15 plantlets from each variety) grown under greenhouse for genetic fidelity study. The genomic DNA was isolated by using modified method of Doyle and Doyle (1990). An aliquot of 2 µl of the preparation was checked on 0.8 % (w/v) agarose gel for estimating the amount and purity of DNA isolated comparing it against a known weight of uncut lambda DNA. The yield of DNA was sufficient per gram of leaf tissue (200–800 ng). Thirty-five customs synthesized (M/s Bangalore Genei, Bangalore) ISSR primers were chosen for the study. Polymerase chain reactions (PCR) with single primer was carried out in a final volume of 25 µl containing 20 ng template DNA, 100 µM of each deoxyribonucleotide triphosphate, 20 ng of primer 1.5 mM $MgCl_2$, 1x *Taq* buffer (10 mM Tris-HCl [pH-9.0], 50 mM KCl, 0.01% gelatin), and 0.5 U *Taq DNA polymerase* (M/S Bangalore Genie, Bangalore, India). Amplification was performed in a PTC-100 thermal cycler (M J Research, Watertown, MA, USA) programmed for a preliminary 2 min denaturation step at 94 ^{0}C, followed by 40 cycles of denaturation at 94 ^{0}C for 20s, annealing at required temperature (depending on nucleotide sequence of ISSR primer) for 30s and extension at 72 ^{0}C for 1 min, final extension at 72 ^{0}C for 10 min. The separation of amplified DNA fragments was performed by electrophoresis in 1.5% (w/v) agarose gel along with a molecular weight marker (1.0 Kb plus ladder, M/S Bangalore Genie, Bangalore, India), pre stained with ethidium bromide and visualized under UV light. Gel photographs were scanned through a Gel Documentation System (BioRad, California, USA) and the amplification product sizes were evaluated using the software Quantity One (BioRad, California, USA). All the PCR reactions were repeated twice. The PCR amplification products were scored visually for presence or absence between samples and transcribed into binary format. The data was used to calculate Dice coefficient of similarity. The similarity measures

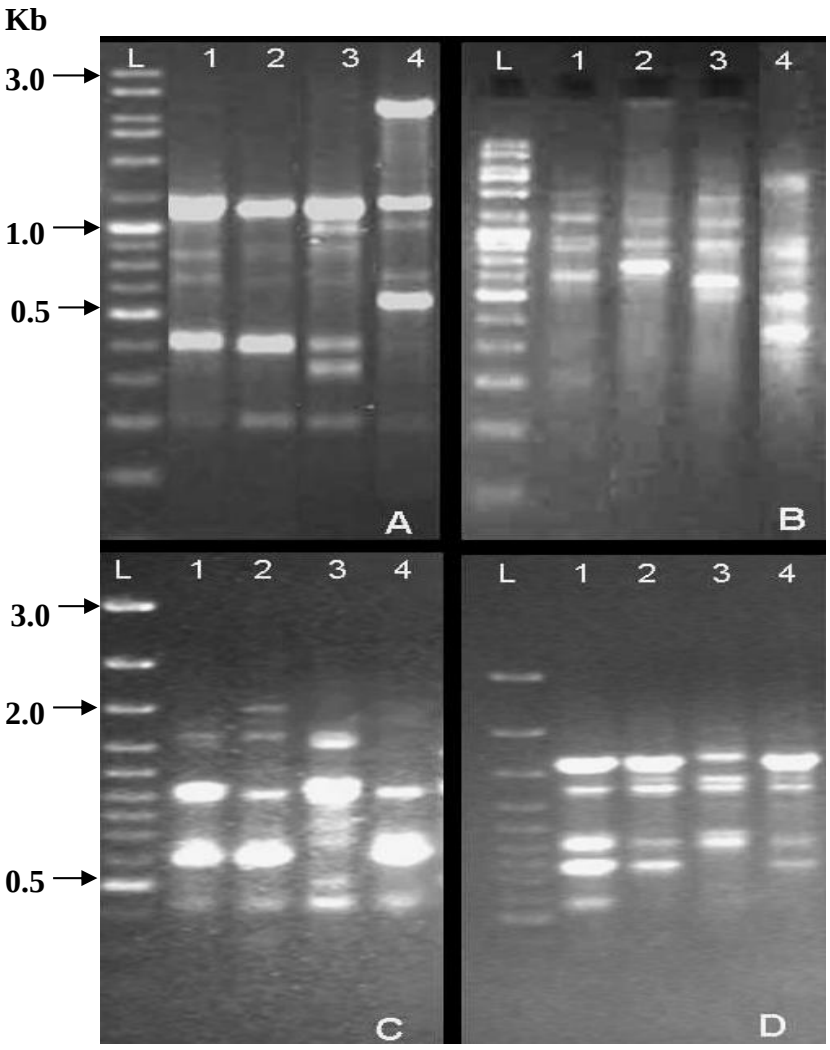

Fig 1. Amplification pattern in four varieties of *Musa accuminata* produced by using ISSR primers IG-03 (A), IG-11(B), IG-15 (C) and IG-23 (D). L represents low range DNA ladder and lane 1-4 represents the *Musa* varieties 'Grand Naine', 'Robusta', 'Patkapura' and 'Bantala' respectively

were subjected to unweighted pair group method for arithmetic averages (UPGMA) and ploted in a phenogram using NTSYS-pc Ver. 2.1 (Rolhf, 2001). Bootstrap analysis was made to know the significance of the cluster.

Results and Discussion

Genetic characterization of cultivated banana

Molecular markers have been used successfully to determine the degree of relatedness among individuals or group of accessions to clarify the genetic structure or variation among accessions, population, varieties and species. On the basis of number, intensity and reproducibility of ISSR bands, fifteen primers were selected out of the thirty-five primers tested in four cultivated banana varieties (Table 1). Bands with same mobility were considered as identical irrespective of their band intensity. Very weak band with negligible intensity and smear bands were both excluded from the final analysis. ISSR banding profile of four cultivated varieties of banana are shown in Fig. 1 A & B. A total of 135 fragments were scored that varied from 05 (IG-03, IG-13 and IG-18) to 16 (IG-11) with an average of 9 bands per primer. The polymorphic bands ranged from 02 to 08 with an average of 04 bands per primer approximately. The size of scored bands ranged from 0.22 to 2.7 Kbp. Twenty-two bands in total were variety's specific (present in single variety while absent in rest), highest being eight unique fragments in 'Bantala'. Genetic similarity among the varieties was estimated using similarity coefficient matrix based on ISSR bands scored. Pair wise values of similarity coefficient (Table 2) ranged from a mini-

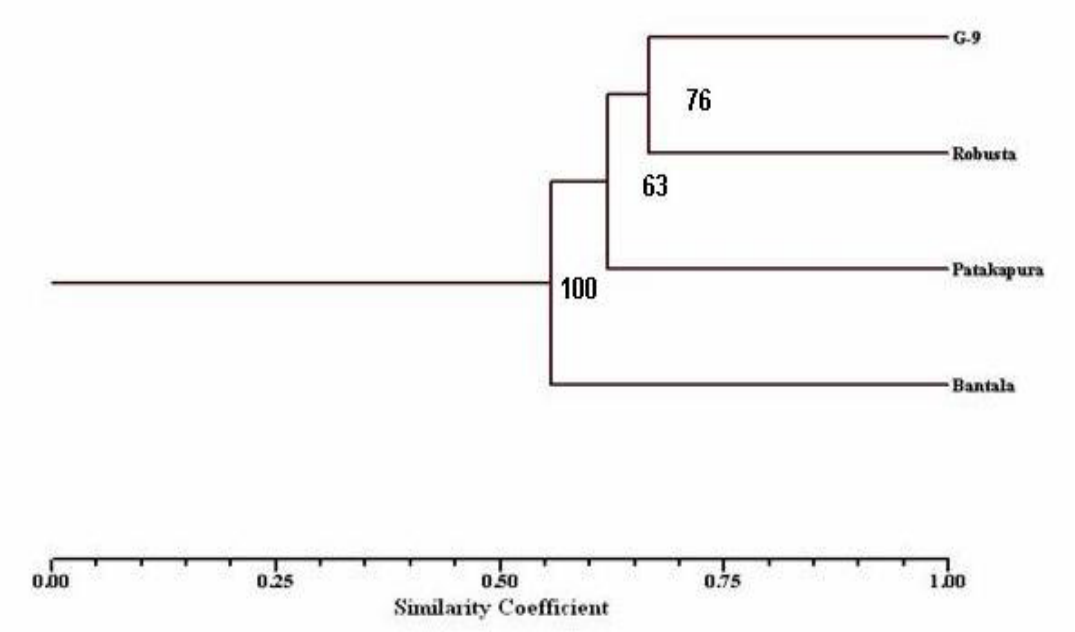

Fig 2. A dendrogram showing the phylogeny of 4 *Musa acuminata* varieties using 15 ISSR primers. The number present in the nodal points are indicating the significance (bootstrap analysis) to the cluster.

mum of 0.53 between 'Bantala' and 'Grand Naine' to a maximum of 0.67 between 'Robusta' and 'Grand Naine'. The dendrogram was constructed based on the similarity matrix using UPGMA method indicates that the four varieties divided them into two clusters at 54% similarity. One group having only variety 'Bantala' and the other having the rest three varieties i.e. 'Grand Naine', 'Robusta' and 'Patkapura'. In the second cluster, 'Grand Naine' and 'Robusta' has been grouped together sharing a similarity of 66%. Bootstrap analysis showed that 'G-9' and 'Robusta' have 76 times significance of the clustering and 'G-9' and 'Robusta' have 64 times significance with 'Patkapura' (Fig. 2). Bhat and Jarret (1995) used 49 RAPD primers to characterize of Indian *Musa* germplasm, whereas only 15 ISSR primers were enough to distinguish four varieties included in the present study. The genetic analysis of the different varieties/species of *Musa* by using various molecular markers have been reported (Jin et al., 2000; Ude et al., 2002 a,b; Creste et al., 2003). Onguso *et al*. (2004) were able to distinguish 20 selected banana cultivars from different regions of Kenya using 19 RAPD primers. The present result indicates that the clustering pattern formed within the four varieties was varied with morphological characters as well as genome constitute. The varieties 'Grand Naine', 'Robusta' and 'Patkapura' having the genome AAA are in the same group whereas; 'Bantala' having genome BBB is separated from them.

In vitro culture

The present investigation showed that the high-frequency shoot regeneration from apical meristematic domes derived from rhizome explants of four *Musa* varities by manipulating the growth regulators. The morphogenetic response was varied with different combinations and concentrations of cytokinins and auxins (data not shown). The mean percentage frequency of shoot buds proliferating from rhizome explants ranged from 10.2 to 92.8 after eight weeks of culture. The media with BA (6.0 mg l^{-1}) and Ads (150 mg l^{-1}) induced rapid development of green meristematic domes with 2 - 3 leaf primordia (Fig. 3A). The meristematic domes were cut longitudinally and transferred to different media for shoot multiplications. Among the various media tested, the media containing 4.0 mg l^{-1} BA along with 150 mg l^{-1} Ads and 1.5 mg l^{-1} IAA showed a significant effect on shoot bud multiplication (92.8%) within 8 weeks of culture (Table 3). Increasing of BA concentration up to 6.0 mg l^{-1} resulted higher rate of shoot multiplication (Fig 3). The regulatory action of cytokinins, auxins and apical dominance on *in vitro* shoot proliferation is well documented (Wickson and Thimann, 1958). Though BA alone helped in shoot regeneration and growth, the presence of adenine sulphate and IAA promoted high frequency of shoot multiplication (Rout *et al*., 2000b). These results are consistence with earlier reports indicating the influ-

Table 3. Effect of growth regulators on shoot proliferation and multiplication of *Musa accuminata* cv. Grand Naine after 8 weeks of culture

MS + Growth regulators (mg l^{-1})			% of explants showing shoot multiplication (Mean± SE)*	Av. Number of shoot-buds/culture (Mean± SE)*
BA	Ads	IAA		
0	0	0	0	0
1.0	0	0	10.2 ± 0.8 a	2.6 ± 0.8 a
1.0	50		35.2 ± 0.6 b	4.2 ± 0.4 b
2.0	50	0	58.4 ± 1.2 c	5.4 ± 0.5 b
3.0	100	0	66.6 ± 0.8 d	8.3 ± 0.6 c
4.0	150	0	74.2 ± 1.0 e	12.5 ± 0.7 d
4.0	150	1.0	81.4 ± 0.7 g	18.2 ± 0.8 e
4.0	150	1.5	92.8 ± 1.2 j	22.4 ± 1.0 g
6.0	150	0	88.2 ± 1.1 h	18.8 ± 0.7 e
6.0	150	1.5	90.5 ± 0.6 i	20.4 ± 0.7 f
4.0	200	0	77.5 ± 1.0 f	18.7 ± 0.5 e
6.0	200	1.0	74.6 ± 1.0 e	17.4 ± 0.6 e
6.0	200	1.5	75.2 ± 0.8 e	18.2 ± 0.8 e

*Mean of 15 cultures per treatment; repeated three times.
Shoot length less than 0.2 cm is not counted.
Mean having same letter in a column were not significantly different by Post-Hoc Multiple Comparison test $P< 0.05$ level.

ence of cytokinins and auxins on shoot multiplication of different explant of *Musa species* (Krikorian and Cronauer, 1984). The rate of multiplication was high up to 7th subculture and decline in subsequent subcultures. This might be due to imbalance between the exogenous and endogenous growth regulators and ionic concentration of nutrient salts. The isolated multiple shoots were transferred to rooting medium having ½ strength MS medium supplemented with 500 mg l^{-1} activated charcoal, 2 % (w/v) sucrose and without any growth hormone. The percentage of rooting was 95.8 % within 2 weeks of transfer (Fig.3C). The rooted propagules were then transferred to greenhouse for 3 weeks and subsequently to open field with 94% survivality. The plants grew well and attained 10-12 cm within 6 weeks (Fig. 3D). The acclimatized plants exhibited normal development and no morphological variation was noticed under field condition (Fig. 3E).

Analysis of genetic fidelity of in vitro raised plantlets

The quality of *in vitro* derived regenerates was screened with ten 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 most of the primer tested. In 'Bantala', no polymorphism was detected in the profile, however some weak bands were absent in some of the regenerates though their frequency was quite low and were not reproducible when repeated. The identical ISSR banding pattern of fifteen in vitro raised plantlets and their mother control plant have shown in Fig. 4A. Most of the primers showed identical DNA profiles as compared with mother plant. In case of 'Grand Naine' few plants showed variation in the PCR profile generated through the primer IG-13 (Fig. 4B).Very few plants showed variation at the DNA level but morphologically they are identical with each other. Shenoy and Vasil (1992) reported that micropropagation through meristem culture are generally less subjected to genetic changes that might occur during cell differentiation under *in vitro* conditions. Ray *et al* (2006) highlighted the genetic stability of the micropropagated plants of three banana cultivars i.e. Robusta (AAA), Giant Govenor (AAA) and Martaman (AAB) by using 21 RAPD and 12 ISSR primers. They found three somaclonal variants from 'Robusta' and three from 'Giant Govenor". Harirah and Khalid (2006) used eighteen arbitrary decamer primers to study the clonal fedility of Musa acuminate Cv. Berangan. They found that all the regenerated plants were monomorphic. No somaclonal variation was detected. In some cases, regeneration process is prone to somatic variation resulting in off-types as in case of *Populus termuloides* (Rahman and Rajora 2001) and tea clones (Devarumath *et al*., 2002). Variation is induced by different genetic and epigenetic mechanisms that are likely to be reflected in the banding pattern developed by employing different

Fig 3. Micropropagation protocol of *Musa acuminate* Var. Grand Naine (A- Proliferation of meristems, B- Shoot multiplication, C- Induction of rooting, D- Plantlets acclimatized in greenhouse, E- *In vitro* raised plants grown in the field).

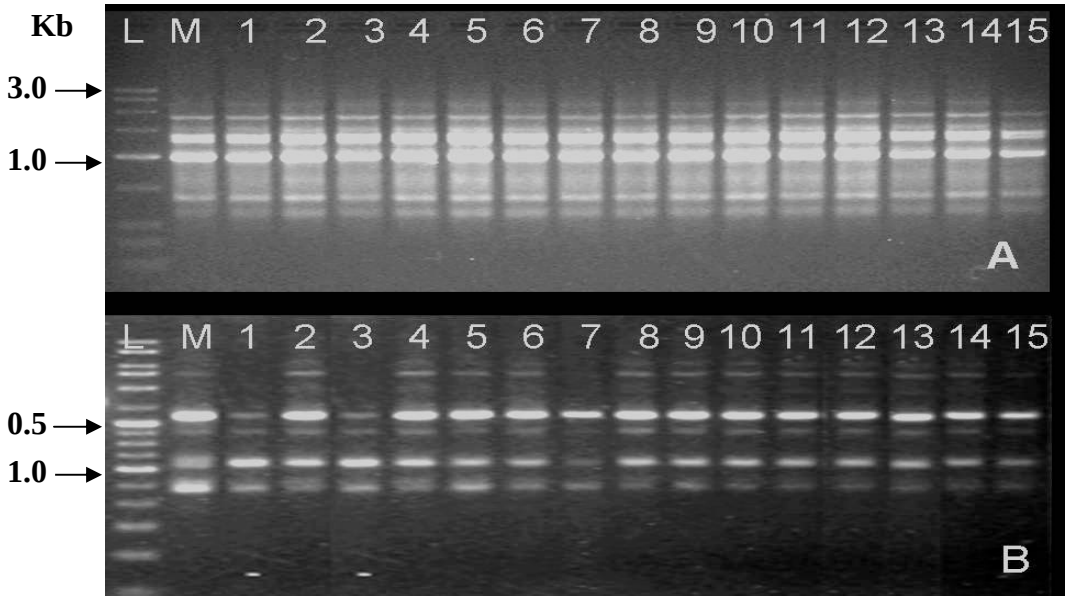

Fig 4. Gel showing the homogenous amplification pattern in 15 randomly chosen tissue culture raised plantlets of 'Bantala' (A) and 'Grand Naine' (B) by ISSR primer IG-13. L- represents low range DNA ladder, M- represents the mother explants source and lane 1-15 represents the tissue-cultured raised plantlets

marker system. However, the reliability and efficiency of molecular markers in detecting large scale genome arrangements have been frequently questioned. Since simple sequence repeat based primer target the fast evolving and hyper variable DNA sequence, ISSR markers are considered suitable to detect variation among tissue cultured produced plants (Rahman and Rajora, 2001; Ray *et al*., 2006; Joshi and Dhawan, 2007).

The Indian banana has evolved to suit the local environment giving rise to a numerous clones. The present investigation is to identify the clone and also to study the genetic uniformity of the *in vitro* raised plants, which is pre-requisite for mass propagation. The results obtained from the investigation will be helpful for proper identification and author's property rights protection 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 also in the field.

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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 *Musa* germplasm. Genet Resource Crop Evol 42:107-118

Cassells AC, Joyce SM, Curry RF, McCarthy TF (1999) Detection of economic variability in micropropagation. In: Altman A., Ziv M, Izhar, S (Eds), Plant Biotechnology and *In vitro* Biology in the 21st century, Kluwer Academic Publishers, The Netherlands

Creste S., Neto AT, Silva SO, Figueira A (2003) Genetic characterization of banana cultivars (*Musa* spp.) from Brazil using microsatellite markers. Euphytica, 132:259-268

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

Devarumath R, Nandy S, Rani V, Marimuthu S, Muraleedharan N, Raina S (2002) RAPD, ISSR and AFLP fingerprint 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 Rep. 21: 166-173.

Dhawan V, Bhojwani SS. (1986) Micropropagation in crop plants. Glimpses Plant Res., 7: 1– 75.

Doyle JJ, Doyle JL (1990) Isolation of Plant DNA from fresh tissue. *Focus* 12: 13-15

FAO, Agricultural Production Yearbook. FAO, Rome. 2004

Gaafer RM, Saker MM (2006) Monitoring of Cultivars identity and genetic stability in strawberry Varieties grown in Egypt. World Jour. Agril Sci. 2: 29-36

Hammond JB., Egg R., Diggins D, Coble GC (1996) Alcohol from bananas. Bioresources Technology, 56:125-130

Harirah AA, Khalid N (2006) Direct regeneration and RAPD assessment of male inflorescence derived plants of *Musa acuminata* Cv. Berangan. Asia Pacific Jour Mol Biol Biotech. 14:11-17

Joshi P, Dhawan V (2007) Assessment of genetic fredility of micropropagated *Swetia chirayita* plantlets by ISSR marker assay. Biol. Planta. 51(1): 22-26

Jin PL, Kiew R, Set O, Gan LH, Gan YY (2000) Amplified fragment length polymorphism fingerprinting of 16 banana cultivars (*Musa* cvs) Mol Phylogenetics and Evol. 17:360-366

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

Karp A (1993). Are your plants normal? Genetic instability in regenerated and transgenic plants. Agro-Food-Industry Hi-Tech May/ June 7-12

Krikorian AD, Cronauer SS (1984) Banana. In: Sharp WR, Evan DA, Ammirato PV, Yamada Y (eds) Handbook of Plant Cell Culture, Vol. II, MacMillan, pp. 327-348, New York

Marascuilo LA, McSweeney M (1977) Post-Hoc Multiple Comparison in sample preparations for test of homogeneity. In: McSweeney M, Marascuilo, LA (eds), Non-parametric and distribution free methods the social sciences. CA, Books/Cole Publ., pp. 141-147, USA

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plantarum 15: 473–497

Novak FJ (1992) *Musa* (Banana, Plantain). In: Hammerschlag FA, Litz R (eds.) Biotechnology of perennial fruits crops, Wallingford, pp. 449-488, CAB International, UK

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

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

Pierik RLM (1991) Horticulture new technologies and applications proceeding of the international seminar on new frontiers in horticulture. Curr Plant Sci Biotechnol Agric. 12:141–145

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

Rahman M, Rajora O (2001) Microsatellite DNA somaclonal variations in micropropagated trembling aspen (*Populus tremuloides*). Plant Cell Rep. 20: 531- 536

Rolhf FJ (2001) NTSYS-pc numerical taxonomy and multivariate analysis system. Version 2.1, Exeter Publising Ltd., Setauket, New York. USA

Rout GR, Samantaray S, Das P (2000a) Biotechnology of the banana: a review of recent progress. Plant Biol. 2:512-524

Rout GR, Samantaray S, Das P (2000b) Augmenting *in vitro* shoot multiplication with growth regulators and light conditions in *Musa accuminata* Cv. Drawf Cavendish. Acta Biologica Cracoviensia Series Botanica 43:15-21

Salvi ND, Geoge L, Eapen S (2001) Plant regeneration from the leaf base callus of turmeric and random amplified polymorphic DNA analysis of regenerate plants. Plant Cell Tiss Org Cult. 66:113-119

Shenoy VB, Vasil IK (1992) Biochemical and molecular analysis of plants derived from embryonic tissue cultures of Napier grass (*Pannisetum purpurem* K. Schum). Theor Appl Genet. 83: 947-955

Stover RH (1972) Banana, plantain and abaca diseases. Surrey, U.K.: Commonwealth Mycol Inst, Kew.

Stover RH (1977). Banana (*Musa spp*.). In: Hewitt WR, Chirappa S (eds.), Plant Health and Quarantine in International Transfer of Genetic resources, Boca Raton, CRC press, UK

Stover RH (1980) Sigatoka leaf spots of bananas and plantains. Plant Diseases, 64, 750-755

Ude G, Pillay M, Nwakanma D, Tenkouano A (2002a) Analysis of genetic diversity and sectional relationships in *Musa* using AFLP markers. Theor. Appl. Genet., 104:1239-1245

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

Varshney A, Laxmikumaram M, Srivastava PS, Dhawan V (2001) Establishment of genetic fredility of *in vitro* raised *Lillium* bulblets through RAPD markers. In Vitro Cell. Dev. Biol. Plant 37: 227-231

Wickson M, Thimann KV (1958) The antagonism of auxin and kinetin in apical dominance. Plant Physiol. 11: 63-74