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Full Length Article

Genetic Fidelity of *in vitro* Propagated Breadfruit (*Artocarpus altilis*) using Inter Simple Sequence Repeat Markers

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Abstract

A viable and simple method for the large scale production of breadfruit (*Artocarpus altilis*) using tissue culture is used at the Food and Agricultural Research Extension Institute in line with the Food Security policy of Mauritius. Shoot tip buds of *Artocarpus altilis* are initiated and multiplied on basal micropropagation medium supplemented with 4.4 µ*M* Benzyl adenine (BA). Direct systems of regeneration through the culture of organized meristems normally produce true-to-type plants. However, variations among *in vitro* raised plants have been widely reported. The aim of this study was to use ISSR markers to confirm the clonal fidelity of *in vitro* derived plantlets of breadfruit. DNA extraction of *Artocarpus altilis* was performed using a modified CTAB method and then purified using RNase and phenol treatment. The presence of DNA was verified on agarose gel electrophoresis and its yield and quality were determined by spectrophotometry. An average yield of 307.3 µL DNA was obtained but with some proteins, phenols and polysaccharides contamination was noted. The 63 markers generated by Inter Simple Sequence Repeat (ISSR) markers revealed a mean of 22.2% genetic variation and 77.7% genetic fidelity in the 15 week cultured plantlets and a mean of 1.9% genetic variation and 98.2% genetic fidelity in the 38 weeks cultured plantlets. These findings indicate that shoot meristems can be used as explants for ensuring clonal fidelity of micropropagated *A. altilis* and confirm the usefulness of ISSR markers as a certification tool for monitoring genetic stability during the regeneration process.

Keywords: *Artocarpus altilis*; Genetic fidelity; ISSR; Micropropagation

Introduction

Breadfruit (*Artocarpus altilis*, Moraceae) is a large evergreen woody species native to the Western pacific. *Artocarpus altilis* can be either seeded or seedless, but seedless ones occur mostly in the tropical regions (Ragone, 1997). It was introduced in Mauritius by the French in the year 1796 (Ragone, 1997) and it was an underutilized food crop, grown mainly in the backyard. Breadfruit is very rich in carbohydrates (Adebowale *et al*., 2005) and can be consumed at different stages of development (Ragone, 2011). *A. altilis* has medicinal properties that can be exploited for treating several diseases including high blood pressure and diabetes (Morton, 1987). It also provides materials such as wood for construction of buildings or boats and fibers for producing cloth (Ragone, 1997). In the context of local food security, breadfruit has been identified as an alternative staple crop for replacing wheat and rice and a profitable market for export by the Mauritian government.

Traditionally *A. altilis* is propagated by root cuttings, root shoot cuttings or by air layering (Ragone, 1991; Ragone, 2011). However, these methods are time consuming, labour intensive and expensive. Large-scale mass propagation of breadfruit is possible using plant tissue culture techniques with the capacity of producing a large number of disease-free and true to type plantlets in a short period of time (Rani and Raina, 2000). However studies have shown that after *in vitro* micropropagation, many of the regenerated plantlets are no longer exact copies of the parental plant and these variations are often heritable (Larkin and Scowcroft, 1981). These variations, termed somaclonal variations may be induced due to genotype, ploidy level, age of donor plant, explant source, tissue culture technique employed, culture conditions, duration of tissue culture and exposure to high level of exogenous hormones. Under tissue culture conditions the plant genome is under stress and therefore undergoes restructuration for adaptation to the *in vitro* conditions (McClintock, 1984). Due to its plasticity the plant genome can reorganize itself and alter its genetic expression so as to adapt to the new conditions. Many mechanisms can lead to somaclonal variations and these remain either unknown or theoretical in

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fruit crops (Skirvin *et al.*, 1994). These include point mutations, variation in chromosome number and arrangement, transposons activation, DNA methylation, changes in cytoplasmic DNA, chimaeral rearrangement and changes in gene expression.

The maintenance of a particular cultivar is vital for the production of true-to-type and genetically uniform plants, so as to meet consumer requirements in terms of yield and quality. Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Random Fragment Length Polymorphism (RFLP) and IRAP are powerful and valuable tools for assessing genetic integrity in tissue culture-derived plants because of their reliability, high accessibility and quickness to assay. Inter Simple Sequence Repeat (ISSR) markers have been greatly used for detecting polymorphisms in various tissue culture derived plants. ISSR is a genotyping technique based on variation in the regions between microsatellites. ISSR is very informative, highly reproducible, and shows high polymorphism level when compared with RAPD (Pathak and Dhawan, 2012). Alizadeh and Singh (2009) have employed ISSR markers to detect variations in micropropagated grape (*Vitis* spp.*)* rootstock genotypes. Palombi *et al*. (2007) successfully employed ISSR for the assessment of genetic fidelity in *Actinidia arguta*. In this study, we used ISSR markers to assess the effectiveness of the tissue culture protocol in producing identical clones. Murch *et al*. (2007) have successfully micropropagated three cultivars of breadfruit using mature axillary shoot buds and have used flow cytometry to confirm the ploidy status of the plantlets but to date no study has been reported the use of molecular markers to confirm the genetic fidelity of micropropagated breadfruit plants. Hence, the aim of this study is to validate the use of shoot meristems as explants for ensuring clonal fidelity of micropropagated *A. altilis* and to confirm the usefulness of the ISSR technique to endorse same.

Materials and Methods

Plant Material and Culture Conditions

Shoot tips of *Artocarpus altilis* obtained from grown suckers cultured and multiplied on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) supplemented with and 4 µ*M* Benzyl adenine were provided from the Food and Agricultural Research Extension Institute (Mauritius). The cultures were kept in growth rooms at 25±2ºC and 16 h photoperiod with a light intensity of 2000–2500 lux. Subculture was performed every 5–7 weeks. Apical meristem (1–2 mm long) buds were excised from shoot tips and inoculated on fresh MS and Benzyl adenine for regeneration. The plantlets aged 15 weeks (subculture level 3) and 38 weeks (subculture level 8) were used for investigating genetic stability.

DNA Extraction

Genomic DNA was isolated using a modified Doyle and Doyle (1990) protocol. One gram of fresh leaf tissue was ground to a fine powder in a pre-chilled mortar using liquid nitrogen. The grindate was transferred to a 15 mL corning tube containing 10 mL pre heated (60–65ºC) 2 x CTAB buffer to which 0.2 g PVP and 20 µL *β*-mercaptoethanol were added. The corning tube was then placed in a 60ºC water bath (Clifton) for 30–45 min with frequent swirling (approximately every 10 min). After incubation, 750 µL of mixture was dispensed into 1.5 mL eppendorf tubes and 500 µL of chloroform: isoamylalcohol (24:1) was added to each tube. The eppendorf tubes were then inverted several times for proper mixing of contents and then micro-centrifuged (Heraeus Sepatech Biofuge 13) at 9500 g for 15 min. The aqueous layer formed was then removed using a wide bore pipette and placed in clean 1.5 mL eppendorf tubes. Then 150 µL of 5 *M* NaCl was added for reducing polysaccharides contaminants followed by 2/3 volume of ice-cold isopropanol and the tubes were left overnight at -20ºC for precipitation to take place. After that, the tubes were spun at maximum speed for 15 min at 4ºC. The supernatant was poured off and the pellet washed with 70% alcohol by spinning at 16000 *g* for 5 min. The ethanol was then aspirated off and the pellet was air dried for 15 min in an evaporator (Jouan RC 1009) at 45ºC. The pellet was re-suspended in 50 µL TE buffer and kept at 4ºC to dissolve.

DNA purification was then performed by incubating extracted DNA with RNase at 37°C for 1.5 h (1 µL RNase (10 mg/mL) added for each 50 µL DNA). Then 50 µL of 3 *M* sodium acetate was added, followed by an equal volume of phenol: Chloroform: isoamyl alcohol (25:24:1). The contents of the tube were mixed by inverting the eppendorf several times. The mixture was spun at 10,000g for 5 min and the supernatant was collected and washed with an equal volume of chloroform: isoamylalcohol (24:1) by spinning at 9500g for 5 min. The supernatant was then transferred into a clean eppendorf tube and an equal volume of ice cold isopropanol was added. The tube was left overnight at -20°C for DNA precipitation and micro centrifuged at maximum speed for 10 min. The supernatant was discarded and washed with 70% alcohol. The pellet was air dried and re-dissolved in 100 µL sterile TE buffer and stored at -20°C for later use.

PCR Amplification

PCR reactions were performed in a volume of 25 µL containing 1X Taq PCR Buffer, 2.5 m*M* $MgCl_2$, 0.20 m*M* dNTP Mix, 0.5 µ*M* primer, 1 U Taq polymerase, 20–50 ng DNA template and made up to final volume with sterile Millipore water using the Applied Biosystems 2720 Thermal Cycler. ISSR PCR reaction program consisted of initial denaturation of 94ºC for 7 min; 35 cycles of 94ºC for 30 s; 53ºC for 1 min (depending on optimized annealing temperature); 72ºC for 2 min and a final extension of 72ºC

for 10 min. 50 ISSRs (ISSR-1 to ISSR-25) were screened for polymorphic bands. Amplicons were separated on 2.0% agarose gel in 0.5 X TBE buffer and visualized by ethidium bromide staining under UV illumination (UVP model M20); their sizes were estimated by comparison with O Gene Ruler 1kb Plus DNA ladder (Fermentas).

Data Analysis

Data analysis was conducted on the products which were consistent, well resolved and reproducible over two amplifications. The markers generated by each primer were scored visually from the gel. A matrix of binary data was built, whereby the presence of the band is indicated by '1' and the absence by '0'. A UPGMA dendrogram using hierarchical clustering was built using NTSYS-PC (Rohlf, 2005) and DAR win 5 software (Perrier and Jacquemoud-Collet, 2006).

Results

Assessment of Genetic Stability

Out of 50 ISSR primers tested, only 10 primers (ISSR-1, 2, 5, 8, 10, 14, 15, 18, 19 and 20) produced clear, reproducible and scorable bands that were further used for assessing genetic fidelity (Table1). ISSR 1, 5, 8, 15, 18 and 19 revealed 100% monomorphism for both *in vitro* plantlets of 3 and 8 subculture levels. Polymorphic bands were observed with primers ISSR 2, 10, 14 and 20 in some of the *in vitro* plantlets. These 10 ISSR primers generated 63 markers that ranged in size from 170 bp (ISSR-1) to 5000 bp (ISSR 10). The number of scorable bands for each primer varied from 3 (ISSR-14 and ISSR-15) to 15 (ISSR-1), with an average of 6.3 bands per primer. A total number of 663 bands were generated with the 10 ISSR primers.

The banding profiles generated by the 10 ISSR primers were compared for the micropropagated plantlets and the mother plant. Primer ISSR-1 produced 15 DNA fragments of 170 to 2000 bp common to all 10 micropropagated plants. For ISSR-2, DNA fragments of 600, 850, 1250 and 1800 bp were amplified in all clones. However, a DNA fragment of 1800 bp was absent in sample 9. The primer ISSR-5 gave rise to 4 DNA fragments of 2500 to 4500 bp similar in all samples. Furthermore ISSR-8 yielded 5 DNA segments of 900 to 1800 bp, which were similar in all clones. As for ISSR-10, 5 bands were produced common to all samples except 5000 bp DNA fragment missing in samples 2, 3, 4 and 5. Concerning ISSR-14 generated 3 markers common to most samples however a DNA fragment of 1200 bp was missing in samples 2, 3, 4, 5, 7 and 10 while in samples 2, 3 and 4 a DNA fragment 1300 bp was absent. For ISSR-15, 3 markers of 200 to 800 bp were common to all *in vitro* plantlets. Moreover ISSR-18 produced 10 bands of 300 to 3500 bp common in all the samples. In addition, ISSR-19 generated 6 markers of sizes 280 to 1300 bp identical in all clones. Finally, ISSR-20 produced 8 markers of sizes 350 to 1300 bp common in most samples except in samples 4 and 5 bands of 900, 1200 and 1300 bp were missing (Fig. 1).

The 63 markers generated by ISSRs revealed a mean of 22.2% genetic variation and 77.7% genetic fidelity in the *in vitro* plantlets at 3 subculture levels and a mean of 1.9% genetic variation and 98.2% genetic fidelity in the *in vitro* plantlets at 8 subculture levels (Table 2 and 3). A dendogram (Fig. 2) was generated using the markers generated and it shows the genetic uniformity of the *in vitro* plantlets.

Discussion

The present study was carried out to assess the clonal fidelity of the *in vitro* regenerated *A. altilis* using ISSR markers. ISSR is a PCR based technique that amplifies DNA segments present between two identical microsatellites oriented in opposite direction and at relatively amplifiable distance along the DNA strand (Reddy *et al.*, 2002). These primers bind to simple sequence repeats and amplify the inter-SSR regions of different sizes across the eukaryotic genome. The use of ISSR to ascertain clonal fidelity has been well documented in various studies because it is simple, highly informative, cost effective and well known for its high reproducibility and polymorphisms. ISSR generates a larger number of polymorphic markers per primer because of the large amount of SSR regions in the plant genome (Pathak and Dhawan, 2012).

Based on 63 markers generated following amplification with the 10 ISSR primers, a mean of 77.7% genetic fidelity in clones at 3 subculture levels and a mean of 98.2% genetic fidelity in clones at 8 subculture levels with respect to the mother plant were revealed (Table 2 and 3). The high percentage of monomorphism (77.7% and 98.2%) in regenerated plantlets confirmed the effectiveness of shoot tip meristems as ideal sources of explants for guaranteeing true to type plantlets.

The use of direct regeneration systems via organized meristem cultures such as enhanced axillary branches are preferred (Vasil, 1994), because they can resist genetic changes occurring during cell division and differentiation and produce true to type plants (Shenoy and Vasil, 1992). The use of shoot tip explants from grown suckers of breadfruit yielded true to types with an overall 88% monomorphisms but with some minor variations. The slight variations can be accounted by the loss of the stabilizing influence of the plants during *in vitro* culture and therefore leading to somaclonal variations (Karp *et al*., 1994). Polymorphisms revealed by the ISSR patterns may be explained first by experimental errors. However, it is possible that variations have arisen as a result of the loss or gain of primer annealing as a result of point mutation, insertion, deletion or transposable elements (Peschke *et al*., 1991). Under *in vitro* stresses, plant tissues usually reorganize their genome to adapt to the exposed conditions.

Table 1: List of reproducible and scorable ISSR primers used for assessing genetic fidelity of *in vitro* raised *A. altilis*

Primers	Primer sequences (5' to 3')	Optimized annealing temperature (^{0}C)	Number of markers	Product Sizes (bp)
ISSR-1	$(CA)_6GG$	46	15	170-3000
ISSR-2	$(CT)_8AC$	53	4	600-1800
ISSR-5	$(CT)_8GC$	53	4	2500-4500
ISSR-8	$(GA)_6GG$	53	5	900-1800
ISSR-10	$(GACA)_4$	46	5	450-5000
ISSR-14	$(CTC)_3GC$	53	3	600-1300
ISSR-15	$(CA)_8T$	53	3	200-800
ISSR-18	$(GA)_8A$	53	10	300-3500
ISSR-19	$CTC(GA)_7$	46	6	280-1300
ISSR-20	$ACA(GT)_7$	46	8	350-1300

Table 2: Percentage of monomorphism and polymorphism of *in vitro* regenerated *A. altilis* plantlets at 3 subculture level revealed by ISSR markers

Primers	% Monomorphism	% Polymorphism
ISSR-1	100	0
ISSR-2	100	0
ISSR-5	100	0
ISSR-8	100	0
ISSR-10	84	16
ISSR-14	53.3	46.7
ISSR-15	100	0
ISSR-18	100	0
ISSR-19	100	0
ISSR-20	85	15
Mean	92.3%	7.8%

Table 3: Percentage of monomorphism and polymorphism of *in vitro* regenerated *A. altilis* plantlets at 8 subculture level revealed by ISSR markers

Primers	% Monomorphism	% Polymorphism
ISSR-1	100	0
ISSR-2	95	5
ISSR-5	100	0
ISSR-8	100	0
ISSR-10	100	0
ISSR-14	86.7	13.3
ISSR-15	100	0
ISSR-18	100	0
ISSR-19	100	0
ISSR-20	100	0
Mean	98.2%	1.9%

This occurs due to the high plasticity of plants. According to Larkin and Scowcroft (1981), *in vitro* stresses during the micropropagation stages may result in various phenotypic and genetic changes. Studies suggest that somaclonal variations arise as a result of three way interactions of initial explants, the *in vitro* culture conditions and the genotype (Venkatachalam *et al*., 2007) and other reports support that variations are directed by the plant genotypes rather than the culture conditions (Pathak and Dhawan, 2012). The presence or absence of markers may be explained by the single base change at the primer annealing (Rani *et al.*, 1995), thus suggesting that the *in vitro* conditions have induced variable changes in the genome of the different *in vitro* plantlets (Sheida *et al*., 2008).

Various types of mutations have been described in somaclonal variants, including point mutations, gene duplication, chromosomal rearrangements, and chromosome number changes (Kaeppler *et al.*, 2000). The movement of transposable elements and changes in DNA methylation, possibly through the function of small interfering RNA (Lippman *et al*., 2003), have also been implicated as potential mechanisms behind some somaclonal variations. The donor genotype may also lead to variations if components of the plant genome such as repetitive DNA sequences become unstable during the culture process (Lee and Phillips, 1988) and this accounts why some plant genomes are more resistant to somaclonal variations than others.

Variations may also arise as a result of long exposure to plant growth hormones (El-Dougdoug *et al*., 2007) and longer cultures (Modgil *et al*., 2005; Huang *et al*., 2009).

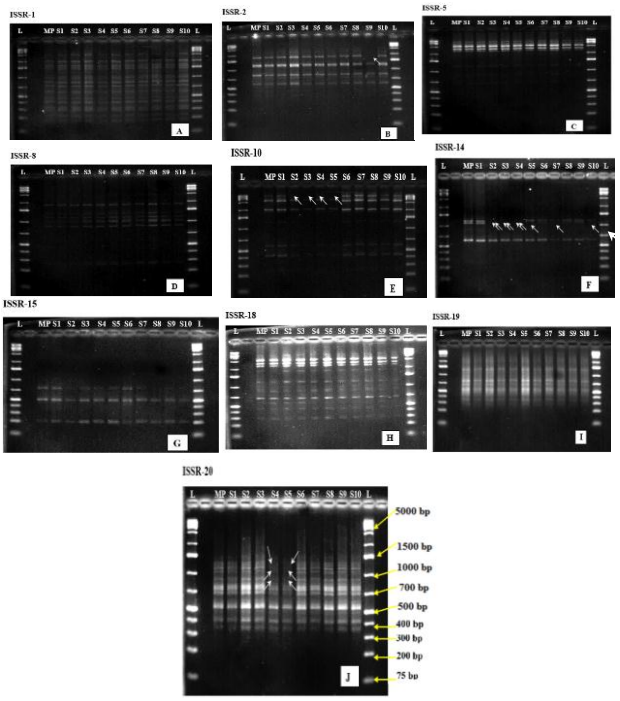

Fig. 1A-J: ISSR profiles generated by primer ISSR-1 (A), ISSR-2 (B), ISSR-5(C), ISSR-8(D), ISSR-10(E), ISSR-14(F), ISSR-15(G), ISSR-18(H), ISSR-19(I) and ISSR-20(J). ISSR bands of mother plant are indicated in lane MP. Lanes S1 to S5 and lanes S6 to S10 are ISSR profiles of clones at 3 and 8 subculture levels respectively. Band size of fragments as compared with markers (O Gene Ruler 1kb Plus DNA ladder (Fermentas) is indicated by L. White arrows show variations

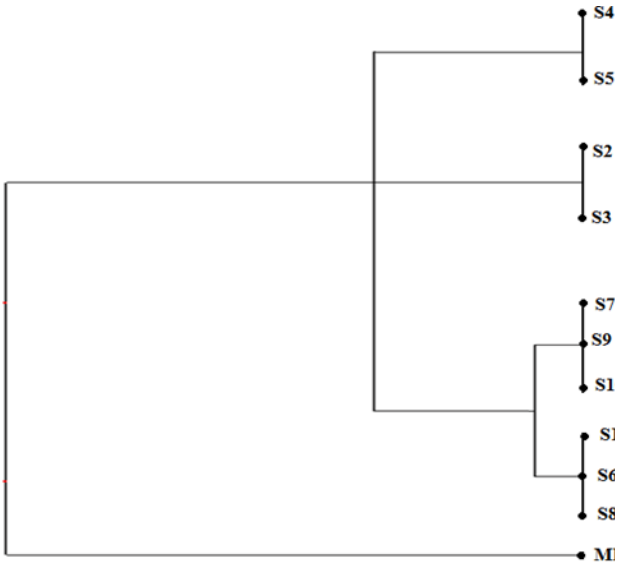

Fig. 2: Dendogram illustrating the relationship among the *in vitro* and the mother plants using ISSR markers. MP (Mother Plant); S1-S5 (*in vitro* plantlets at 3 subculture levels) and S6-S10 (*in vitro* plantlets at 8 subculture levels)

Conclusion

This is a novel report on the use of ISSR markers to ascertain genetic fidelity in micropropagated breadfruit. These findings validate the use of meristem-based *in vitro* regeneration systems to warrant genetic stability of regenerated plantlets to produce clones for commercial exploitation. The results obtained from this study are promising and showed that ISSR markers can be employed as molecular tools for assessing the genetic integrity of micro-propagated *A. altilis* on a commercial scale. Therefore, plantlets at 38 weeks of culture time or less would be recommended for acclimatization with probably the minimum amount of genetic variants.

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References

Adebowale, K.O., B.I. Olu-Oqolabi, E.K. Olawumi and O.S. Lawal, 2005. Functional properties of native, physically and chemically modified breadfruit (*Artocarpus artilis*) starch. *Indus. Crops Prod.*, 21: 343–351

Alizadeh, M. and S.K. Singh, 2009. Molecular assessment of clonal fidelity in micropropagated grape (*Vitis* spp.) rootstock genotypes using RAPD and ISSR markers. *Iran. J. Biotechnol*., 7: 37–44

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. *Focus*, 12: 13–15

El-Dougdoug, K.H.A., H.M.S. El-Harthi, H.M. Korkar and R.M. Taha, 2007. Detection of somaclonal variations in Banana tissue Culture using isozymes and DNA fingerprint analysis. *J. Appl. Sci. Res*., 3: 622–627

Huang, W.J., G.G. Ning, G.F. Liu and M.Z. Bao, 2009. Determination of genetic stability of long-term micropropagated plantlets of *Platanus acerifolia* using ISSR markers. *Biol. Plant.*, 53: 159–163

Kaeppler, S.M., H.F. Kaeppler and S. Rhee, 2000. Epigenetic aspects of somaclonal variation in plants. *Plant Mol. Biol*., 43: 179–188

Karp, A., 1994. Origins, causes and uses of variation in plant tissue cultures. *In*: *Plant Cell and Tissue Culture*, pp: 139–152. Vasil, I.K. and T.A. Thorpe (eds.). Kluwer Academic Publishers, Germany

Larkin, P. and Scowcroft, 1981. Somaclonal variation a novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet*., 60: 197–214

Lee, M. and R.L. Phillips, 1988. The Chromosomal basis of somaclonal variation. *Ann. Rev. Plant Physiol. Plant Mol. Biol*., 39: 413–437

Lippman, Z., B. May, C. Yordan, T. Singer and R. Martienssen, 2003. Distinct mechanisms determine transposon inheritance and methylation via small interfering RNA and histone modification. *PLoS Biol*., 1: 420–428

McClintock, B., 1984. The significance of responses of the genome to challenge. *Science*, 226: 792–801

Modgil, M., K. Mahajan, S.K. Chakrabarti, D.R. Sharma and R.C. Sobti, 2005. Molecular analysis of genetic stability in micropropagated apple rootstock MM106. *Sci. Hortic.*, 104: 151–160

Morton, J., 1987. Breadfruit. *In*: *Fruits of Warm Climates*, pp: 50–58. Morton Collectanea. University of Miami, Coral Gables, Florida, USA

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Plant Physiol*., 15: 473–497

Murch, S.J., D. Ragone, W.L. Shi, A.R. Alan and P.K. Saxena, 2007. *In vitro* conservation and sustained production of breadfruit (*Artocopus altilis*, Moraceae): Modern technologies to improve distribution of a traditional tropical crop. *PLoS Biol.*, 1: 420–428

Palombi, M.A., B. Lombardo and E. Caboni, 2007. *In vitro* regeneration of wild pear (*Pyrus pyraster* Burgsd) clones tolerant to Fe-chlorosis and somaclonal variation analysis by RAPD markers. *Plant Cell Rep*., 26: 489–496

Pathak, H. and V. Dhawan, 2012. ISSR assay for ascertaining genetic fidelity of micropropagated plants of apple rootstock Merton 793. *In Vitro Cell. Dev. Biol*., 48: 137–143

Perrier, X. and J.P. Jacquemoud-Collet, 2006. *DAR win Software*. Available at: http://darwin.cirad.fr/darwin

Peschke, V.M., R.L. Philip and B.G. Gengenbach, 1991. Genetic and molecular analysis of tissue-culture- derived Ac elements. *Theor. Appl. Genet*., 82: 121–129

Ragone, D., 1991. *Collection, Establishment and Evaluation of a Germplasm Collection of Pacific Island Breadfruit*. A Thesis submitted for Ph.D. Dissertation. University of Hawaii, Honolulu, USA

Ragone, D., 1997. *Breadfruit*. *Artocarpus altilis* (Parkinson) Fosberg. Promoting the conservation and use of underutilized and neglected crops 10. International Plant Genetic Resources Institute, Rome, Italy

Ragone, D., 2011. *Farm and Forestry Production and Marketing Profile for Breadfruit* (*Artocarpus altilis*). Permanent Agriculture Resources (PAR), Holualoa, Hawaii, USA

Rani, V. and S.N. Raina, 2000. Genetic fidelity of organized meristem-derived micropropagated plants: a critical reappraisal. *In Vitro Cell. Dev. Biol*., 36: 319–330

Rani, V., A. Parida and S.N. Raina, 1995. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in micropropagated plants of *Populus deltoides* Marsh. *Plant Cell Rep*., 14: 459–462

Reddy, M.P., N. Sarla and E.A. Siddiq, 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, 128: 9–17

Rohlf, F.J., 2005. *NTSYSpc (Numerical Taxonomy and Multivariate Analysis System)*. Version 2.2, 2005, Exeter Software, Applied Biostatistics Inc., New York, USA

Sheida, M., H. Aminpoor, Z. Noormohammadi and F. Farahani, 2008. RAPD analysis of somaclonal variation in banana (*Musa acuminata* L.) cultivar Valery. *Acta Biol. Szegediensis*, 52: 307–311

Shenoy, V.B. and I.K. Vasil, 1992. Biochemical and molecular analysis of plants derived from embryogenic cultures of napier grass (*Pennisetum purpureum* K. Schum.). *Theor. Appl. Genet*., 83: 947–955

Skirvin, R.M., K.D. Mcpheeters and M. Norton, 1994. Sources and frequency of somaclonal variation. *Hortic. Sci*., 29: 1232–1237

Vasil, I.K., 1994. Automation of plant propagation. *Plant Cell Tiss. Org*., 9: 105–108

Venkatachalam, L., R.V. Sreedhar and Bhagyalakshmi, 2007. Micro propagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR markers. *Plant Growth Regul*., 51: 193–205

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