Research Article

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Assessment of genetic fidelity of arecanut plantlets derived through direct somatic embryogenesis by RAPD markers

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Abstract

Random amplified polymorphic DNA (RAPD) markers were used to evaluate clonal fidelity of plantlets derived through direct somatic embryogenesis from Yellow Leaf Disease (YLD) resistant arecanut palms. Pair wise genetic similarities were generated by Jaccard's coefficient using the RAPD banding pattern between each mother palm and its progenies (eight plantlets/palm). Mother palms and its progenies were showing high similarity (99 % in one case and 98% in one palm). The low level of variability shown by plantlets of direct somatic embryogenesis can be exploited for large-scale multiplication of elite arecanut palms.

Key words: *Areaca catechu* L., clonal fidelity, *in vitro* multiplication, RAPD, YLD resistant

Introduction

In vitro propagation is a promising technique for rapid and large-scale production of disease free planting material in a number of crops. However, scaling up of any tissue culture protocol is severely hindered due to incidence of somaclonal variations as a result of various factors in culture conditions. Hence a strict quality check in terms of genetic similarity of the progeny becomes compulsory.

Molecular markers are invaluable tools for establishing the genetic uniformity of tissue culture derived plantlets. Among the different molecular markers available, RAPD markers are preferred due to their cost effectiveness, technical simplicity and non-requirement of sequence information of template DNA. RAPD technique has been successfully used for assessment of clonal fidelity in turmeric (Neeta *et al.,* 2001), piper (Chaveerach *et al.,* 2002) and in monocots such as banana (Smith, 1988; Vuylsteke *et al.*, 1991), sugarcane (Saini *et al.*, 2004) etc.

The plantlets derived from direct somatic embryogenesis usually are unicellular in origin and hence are genetically uniform. Direct somatic embryogenesis was reported for the first time in arecanut tissue culture by Karun *et al.* (2004) and plantlet regeneration via direct and indirect somatic embryogenesis by Radha *et al.,* 2006. Assessing genetic fidelity of perennial crops is very much important, as these crops will remain in the field for a long time. In the present study, RAPD technique was employed to study the genetic fidelity of plantlets derived via direct somatic embryogenesis from YLD resistant arecanut palms.

Materials and Methods

In vitro multiplication

The protocol for arecanut somatic embryogenesis and plantlet regeneration from leaf and inflorescence explants developed by Karun *et al.* (2004) was employed. Inflorescence explants from two mother palms coded for convenience as H and G., resistant to YLD at hot spot garden (Sullya Taluk of South Kanara District) were chosen for the study.

DNA isolation and RAPD marker analysis

Eight plantlets derived through direct somatic embryogenesis (DSE) of the aforesaid mother palms were used for the study. Immature leaf samples collected from both mother palms and its progenies were used as plant material for DNA extraction. The genomic DNA was extracted by using DNeasy Plant Mini Kit (Qiagen).

Forty primers of the series OPM, OPAF and OPC (Operon Biotechnologies, Germany) were screened. Amplification reactions (15 il final volume) contained 10 ng genomic DNA, 200 iM of dNTPs, 10 pmol/il of each primer, 1X *Taq* DNA buffer and 0.5 units of *Taq* DNA polymerase and overlaid with one drop of mineral oil. PCR was performed in Thermal Cycler (BioRad) with

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initial denaturation at 94°C for 5 min. followed by 40 cycles of denaturation at 94°C for 1 min., annealing at 42°C for 1 min. and extension at 72°C for 1 min., finally ending with one cycle of 72°C for 7 min. After amplification, the PCR product was stored at –20°C till electrophoresis. Amplified products were mixed with 3ì l of 6X gel loading dye [0.25% bromophenol blue and 40% (w/v) sucrose in H_2O] before loading. The PCR products were separated by agarose gel (1.5%) electrophoresis in 1X TBE buffer. Ethidium bromide-stained gels were documented using Syngene UK Gel Documentation and Analysis System.

Data analysis

Bands were scored as either present (1) or absent (0). The SIMQUAL program (NTSYS v. 2) was used to calculate Jaccard's similarity coefficient and a dendrogram of genetic relatedness was drawn by means of the unweighted pair group method with arithmetic average (UPGMA) analysis.

Results and Discussion

***In vitro* multiplication**

Direct somatic embryos were developed from rachillae of the inflorescence. A total of 120 cultures each were initiated from two palms. In the palms H and G, a total of 36 and 31 direct somatic embryos were formed, respectively. Fully developed plantlets were selected for hardening and these plantlets were utilized for the clonal fidelity studies.

RAPD marker analysis

RAPD analysis of two mother palms and their progenies were carried out in order to confirm the genetic fidelity. The results were obtained as patterns of band from *in vitro* propagated progenies and their respective mother palm. The 15 RAPD primers gave rise to a total of 134 scorable bands across mother palm H and its progenies and its details are shown in Table 1a. The same primers gave rise to a total of 143 scorable bands across mother palm G and its eight progenies, the details of which are given in Table 1b. Out of 40 primers screened, 15 showed good amplification, of which 13 of them produced monomorphic bands across all *in vitro* raised plantlets and its corresponding mother palm (Fig. 1). However, primer OPAF 2 showed polymorphism between mother palm H and its progenies where as primer OPM 7 and OPAF 2 showed polymorphism between mother palm G and its progenies. Number of bands for each primer varied from 5 to 13, with an average of 8.9 bands per primer for mother palm H and its progenies where as it ranged from three to 15, with an average of 9.5 bands per primer for mother palm G and its progenies.

Genetic similarity and Cluster analysis

Pair wise genetic similarities were generated by Jaccard's coefficient using the banding pattern between each mother palm and its progenies. Mother palm H and its clones showed maximum similarity value of 0.99. The mother palm G and its seven progenies were showing a similarity value of 0.98 but one progeny (G8) was showing only 0.93 similarity value. This might be due to point mutation by the influence of growth regulators used in culture medium.

The binary matrix of RAPD data was also used to construct UPGMA dendrogram of each mother palm and its progenies. The dendrogram obtained from UPGMA

Table 1a. Primer details and size of the amplicons generated by 15 RAPD primers used for the clonal fidelity test in mother palm H and its progenies (H1-H8)

Primer	Nucleotide Sequence	Fragment Range	Total no.of monomorphic bands	Total no.of polymorphic bands	Total no.of bands	G+C (%)
OPM 2	ACAACGCCTC	300-1450	10	0	10	60
OPM 7	CCGTGACTCA	300-2000	9	1	10	60
OPC1	TTCGAGCCAG	300-1500	8	0	8	60
OPC 7	GTCCCGACGA	300-1500	9	0	9	70
OPM 5	GGGAACGTGT	300-1400	7	0	7	60
OPM 12	GGGACGTTGG	350-1400	7	0	7	70
OPM 13	GGTGGTCAAG	400-1500	11	0	11	60
OPM 14	AGGGTCGTTC	300-1000	5	0	5	60
OPM 18	CACCATCCGT	500-2000	10	0	10	60
OPAF 2	CAGCCGAGAA	300-2500	12	1	13	60
OPAF 6	CCGCAGTCTG	250-2000	13	0	13	70
OPAF 12	GACGCAGCTT	400-1500	8	0	8	60
OPAF 16	TCCCGGTGAG	100-1500	8	0	8	70
OPAF 10	GGTTGGAGAC	400-1500	9	0	9	60
OPAF 19	GGACAAGCAG	500-1500	6	0	6	60

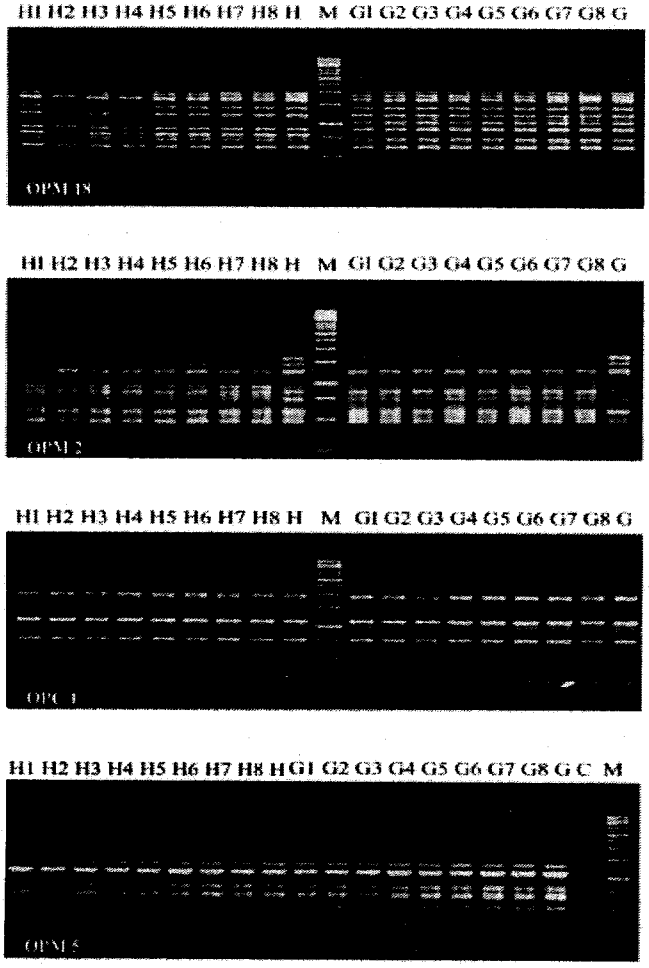

Fig. 1. **RAPD banding profile of *in vitro* propagated plantlets and field grown mother palms**
[H : Mother palm of H1-H8; G: Mother palm of G1-G8; M: 1 kb ladder; C : Control]

grouped the mother palm H and its eight progenies into two clusters (Fig. 2). All the six progenies and mother palm H grouped in to cluster I with a similarity value of 0.992. Cluster II had only two progenies H5 and H7 with a similarity value of 0.99.

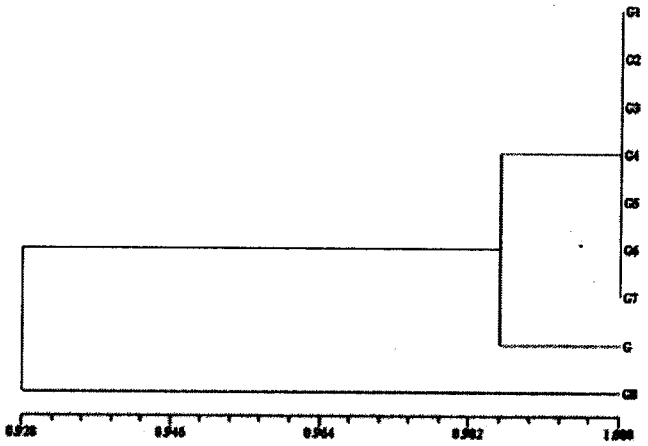

Fig. 2. **Dendrogram showing the genetic relationship between mother palm (H) and its progenies (H1-H8)**

The UPGMA dendrogram of mother palm G and its eight clones (Fig. 3) showed two clusters. The cluster I contained seven clones and mother palm G with a similarity value of 0.984 where as only one progeny separated into a separate cluster with a similarity value of 0.93.

Sugarcane clones derived through meristem culture were found genetically identical and more than 97 % fidelity was maintained (Devarumath *et al.*, 2007).

Table 1b. Primer details and size of the amplicons generated by 15 RAPD primers used for the clonal fidelity test in mother palm G and its progenies (G1-G8)

Primer	Nucleotide Sequence	Fragment Range	Total no.of monomorphic bands	Total no.of polymorphic bands	Total no.of bands	G+C (%)
OPAF 19	GGACAAGCAG	500-1500	6	0	6	60
OPM 2	ACAACGCCTC	300-1450	11	0	11	60
OPM 7	CCGTGACTCA	300-2500	11	0	11	60
OPC1	TTCGAGCCAG	300-1500	8	0	8	60
OPC 7	GTCCCGACGA	300-1500	9	0	9	70
OPM 5	GGGAACGTGT	300-1400	7	0	7	60
OPM 12	GGGACGTTGG	350-1400	8	0	8	70
OPM 13	GGTGGTCAAG	400-1500	12	0	12	60
OPM 14	AGGGTCGTTC	300-1000	7	0	7	60
OPM 18	CACCATCCGT	500-2000	10	0	10	60
OPAF 2	CAGCCGAGAA	300-2500	3	12	15	60
OPAF 6	CCGCAGTCTG	250-2000	14	0	14	70
OPAF 12	GACGCAGCTT	400-1500	7	0	7	60
OPAF 16	TCCCGGTGAG	100-1500	9	0	9	70
OPAF 10	GGTTGGAGAC	400-1500	9	0	9	60
OPAF 19	GGACAAGCAG	500-1500	6	0	6	60

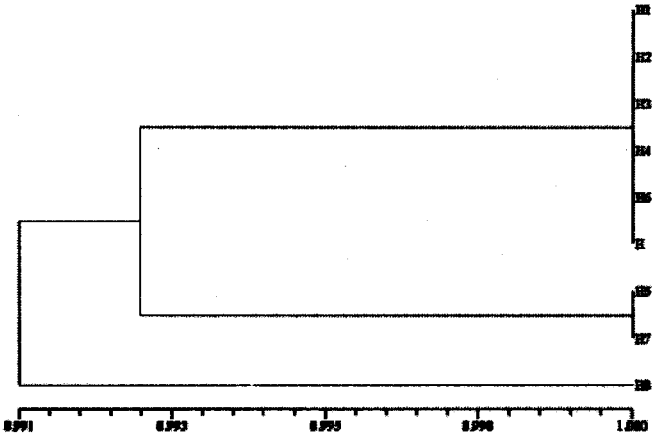

Fig. 3. Dendrogram showing the genetic relationship between mother palm (G) and its progenies (G1-G8)

In *Robinia pseudoacacia*, cluster analysis generated using RAPD markers, revealed genetic variation among the 18 micropropated plants derived from a single mother plant (Bindiya *et al.*, 2003). The rates of somaclonal variation in banana plants derived from meristem culture varied from 0 to 70 % according to genotype (Smith, 1988; Vuylsteke *et al.*, 1991).

Genetic stability of plantlets derived through indirect somatic embryogenesis in arecanut revealed 15-20 % variability in a study conducted earlier. Damasco *et al.* (1996) reported that the source of explants and mode of regeneration play an important role in determining the presence or absence of variation. Somaclonal variation arises mainly due to alteration in auxin-cytokinin concentrations, duration of *in vitro* culture, *in vitro* stress due to unnatural conditions altered diurnal rhythm and nutritional conditions (Modgil *et al.*, 2005). Moreover, cultured plant tissues have also been reported to undergo high levels of oxidative stress due to a reactive oxygen species formed within the cells and the latter is known to cause DNA damage (Aimee et al., 1988). Present study revealed only one clone G8 with greater variation (7%) when compared to the mother palm. This might be due to point mutations or insertions, deletion or inversions. The remaining clones were found to be 98 to 99% similar to the mother palm. More significantly, the results show the importance of confirming the genetic integrity of tissue cultured plantlets before transferring to the field.

Conclusion

In this study, the assessment of genetic variability using RAPD markers in plantlet derived from direct somatic embryogenesis of inflorescence culture showed less variation in *in vitro* regenerated plantlets which indicates that direct somatic embryogenesis from inflorescence culture can be employed for the mass multiplication of elite palms with desirable qualities.

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