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Short Communication

Development of a SCoT-derived SCAR marker associated with tall-type palm trait in arecanut and its utilization in hybrid (dwarf x tall) authentication

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Abstract

Arecanut cultivars are generally tall, which is a major constraint in arecanut cultivation. High yielding tall cultivars have been crossed with natural dwarf mutant, Hirehalli Dwarf (HD) and hybrids have been developed and released for commercial cultivation. It is very difficult to distinguish selfed progenies of the female mother palm in the nursery stage using morphological traits. Start Codon Targeted (SCoT) primers were used to screen tall cultivars of arecanut and HD. One of the primers, SCoT11, produced an amplicon of around 1300 bp band, which was present in all the tall cultivars, but was absent in the dwarf accession. A SCAR marker, capable of distinguishing tall/dwarf trait in arecanut, was also developed and validated, which could ensure supply of genuine hybrid planting material to the farming community.

Key words: Arecanut, hybrids, SCoT, SCAR

Arecanut (*Areca catechu* L.) or betel nut is an extensively cultivated tropical palm, which plays an important role in social, cultural, religious, political and economic milieu of people in India, the Middle East and the Far East. The nuts form a popular masticatory in these regions (Gupta and Warnakulsuriya 2002). In India, arecanut is one of the important and remunerative plantation crops, occupying a prominent place in states of Karnataka, Kerala, Assam, Maharashtra, Goa, Tamil Nadu, Meghalaya and Andaman & Nicobar Islands. India dominates the world scenario in arecanut, being the largest producer and consumer of arecanut, holding 62 per cent of the area and 60 per cent of the production (Anon. 2014).

Arecanut palms are generally tall stemmed, erect palm reaching a height of above 30 m. Some of the improved tall cultivars with high yield potential developed at ICAR-CPCRI include *Mangala*, Sumangala, Sreemangala and Mohitnagar (Ananda 2002). Unavailability of skilled climbers for harvesting and carrying out plant protection measures and also increased wages for the climbers have posed great difficulty to arecanut farmers in recent years. Also, the tall cultivars are susceptible to severe sun scorching with farmers losing about 15-20% of the population due to sun scorching every year.

Hirehalli Dwarf (HD), a natural arecanut mutant identified for its short stature (Naidu 1963), is a good genetic source for arecanut improvement. However, the yield of HD is quite low. HD has been used in hybridization programmes with a view to develop dwarf and compact palms and to facilitate more palms per unit area and easy harvesting (Anon. 2007). Many inter-specific hybrids between HD and tall cultivars have been developed and evaluated for high yield and dwarfness as a part of breeding programmes at ICAR-CPCRI, which will beneficial by way of increased returns and reduced cost of various cultural operations. Combinations of HD crossed with Sumangala ('VTLAH-1') and HD crossed with Mohitnagar ('VTLAH-2') have exhibited their superiority for dry kernel yields per palm per year in the early phases of bearing. These hybrids have also shown reduced canopy area, desired

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dwarfness, early stabilization of yield, high recovery of dry kernel (26.45 %) and can be protected from sun scorch and heavy winds. It has been estimated that the dwarf hybrids could save nearly 40% of the cost of cultivation (Anon. 2007).

Arecanut palm is monoecious, with both male and female flowers occurring in the same spadix. It is essentially a cross-fertilized species (Bavappa and Ramachander 1967), but selfing of flowers, to a small extent, has also been reported (Murthi 1977). In HD, a mean of two days in intra-spadix overlapping and four days in inter-spadix overlapping have been reported (Ananda and Rajesh 2006). The possible impurity in hybrid nut production in arecanut could be the selfing of female parent. Arecanut, being a long duration perennial crop, one of the challenges in hybridization programmes is the rapid and accurate assessment of the purity of hybrid seeds before they are supplied to farmers. There is an urgent need for a rapid, accurate and cost effective method for assessing the genetic purity of hybrids. Molecular marker analyses offer substantial improvement for seed quality control and evaluation of genetic purity of hybrids because of their convenience, effectiveness, ease and reliability, compared to morphological traits and biochemical analysis.

One of the outcomes of the recent progress made in genomic research has been a shift from the use of random DNA markers to novel, gene-targeted, functional markers (Poczai et al. 2013). Start codon targeted polymorphism (SCoT) is a simple and novel marker system based on the short conserved region flanking the ATG translation start codon in plant genes (Collard and Mackill 2009). Compared to RAPD and ISSR markers, SCoT markers are more reproducible, primer length and annealing temperature not being the sole factors determining reproducibility (Gorji et al. 2011). The utility of this primer in genetic diversity analysis has been reported in a number of plant species (Gorji et al. 2011; Guo et al. 2012; Luo et al. 2012), but there is no report on the application of SCoT markers in arecanut.

The plant materials used consisted of five palms each from six tall arecanut accessions (Mangala, Sumangala, Sreemangala, Mohitnagar, Swarnamangala and Hirehalli Tall) and 12 palms of HD accession. DNA was extracted from spindle leaves of the accessions by a modified SDS method (Rajesh et al. 2015). Two bulk DNA samples (Tall and Dwarf) were prepared by pooling an equal amount of DNA sample for screening. The first pool consisted of DNA of 30 tall palms (five palms of each tall accession; six accessions) and the second pool consisted of DNA of 12 HD palms. High molecular weight genomic DNA was isolated from the spindle leaves of the entire arecanut palms sampled. DNA yield of 500-700 ng of DNA per 100 mg of tissue was obtained using the extraction procedure. An absorbance ratio (A260/A280) of 1.6-1.8 indicated that there were no significant levels of protein/polysaccharide/RNA contamination.

A total of 24 SCoT primers (SCoT 1-SCoT 24), described by Collard and Mackill [10] were used for screening. The PCR reactions were performed in a volume of 20 µl which including 30 ng of genomic DNA, 10 µM primer, 10 mM of each dNTPs (Fermentas), 10×buffer (10 mM Tris-HCl, pH 8.3) and 3 Units of Taq DNA polymerase (Fermentas). The PCR cycling condition consisting of an initial denaturation (3 min at 94^{o}C) followed by 35 cycles at denaturation (1 min at 94^{o}C), annealing (1 min at 52^{o}C) and extension (2 min at 72^{o}C), with a final extension (5 min at 72^{o}C). After amplification, the PCR products were separated on 1.2% agarose gel in 1X TBE buffer by electrophoresis stained with ethidium bromide. The gel visualized in a gel documentation system. Each reaction was repeated thrice under the same condition for consistency of polymorphic bands.

All the 24 SCoT primers screened (SCoT primers 1-24; [10]) amplified arecanut genomic DNA. Only one of the primers, SCoT 11 (5'- AAGCAATGGCTA CCACCA-3'), showed a highly reproducible polymorphic band of around 1300 bp in tall DNA pool, which was absent in the dwarf DNA pool. The polymorphic band, between tall and dwarf DNA pools, were then validated in individual tall and dwarf accessions [30 tall palms (five palms of each of six tall accessions) and 12 dwarf palms of HD]. The analysis confirmed that the primer SCoT 11 produced a unique band of around 1300 bp in all the tall accessions, whereas similar amplicon was missing in all the HD palms tested (Fig. 1). This polymorphic band was excised directly from agarose gel, purified, cloned and sequenced.

BLAST analysis revealed that the cloned sequence (Genbank accession No. KP221658) had 94% homology to date palm hexose carrier protein. Based on this sequence, a pair of SCAR primers, specific to tall accessions, were designed (Fwd: 5'-CACCACCACCACCTTCCTATG-3''; Rev: 5'-GGCTACCACCATAAGGCAGAG-3'). These SCAR

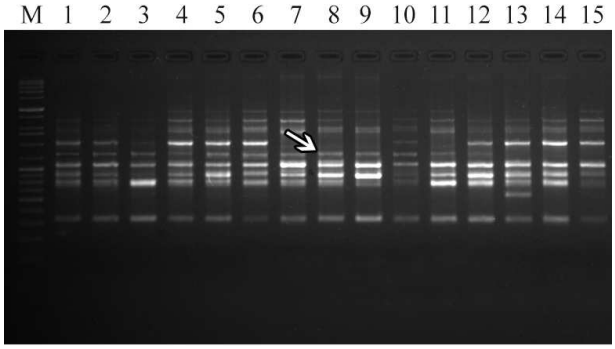

Fig. 1. SCoT banding pattern of individual tall and dwarf palms (HD) with the primer SCoT11. M: High range ladder. Lanes 1-2: Mangala; 3-4: Sumangala; 5-6: Sreemangala; 7-8: Mohitnagar; 9-10: Swarnamangala; 11-12: Hirehalli Tall; 13-15: HD palms. Arrowhead indicates polymorphic band around 1300 bp specific to tall palms

primers were used to amplify individual tall and dwarf arecanut accessions. The reaction mixture consisted of 20 µl which including 30 ng of genomic DNA, 2 µM of each primer, 10mM of each dNTPs (MBI Fermentas), 10×buffer (10 mM Tris-HCl, pH 8.3) and 3 Units of Taq DNA polymerase (Fermentas). PCR amplifications were carried out with an initial denaturation at 94^{o}C for 3 min followed by 35 cycles at 94^{o}C for 1 min, annealing at 65^{o}C for 1 min and 72^{o}C for 5 min with a final extension at 72^{o}C for 5 min. The amplified products were run on 1.2% agarose gel stained with ethidium bromide and visualized under gel documentation system. This primer gave amplification in all the individual tall accessions, but not in the HD palms (Fig. 2).

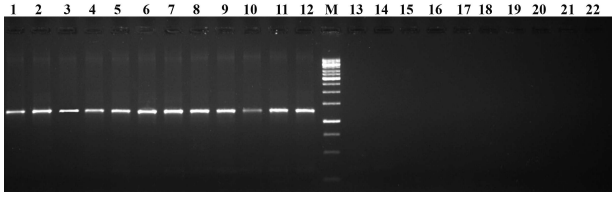

Fig. 2. Validation of tall palm specific SCAR marker. Lanes 1-2: Mangala; 3-4: Sumangala; 5-6: Sreemangala; 7-8: Mohitnagar; 9-10: Swarnamangala; 11-12: Hirehalli Tall; M: 1 Kb ladder; 13-22: HD palms

The SCAR marker was validated in the progenies of two D x T crosses [VTLAH-1: HD x Sumangala; VTLAH-2: HD x Mohitnagar]. DNA was extracted from eight progenies and parents of a HD x Sumangala cross and 18 progenies of a HD x Mohitnagar cross and these were analyzed using the designed SCAR marker. Selfed progenies of the particular HD mother palm were also included in this analysis. While a single, bright band of around 1300 bp was obtained in tall parents (Sumangala and Mohitnagar) and hybrids of two D x T crosses (VTLAH-1, VTLAH-2), an amplicon was not visible in HD sample and the selfed progenies of HD (Fig. 3A, B).

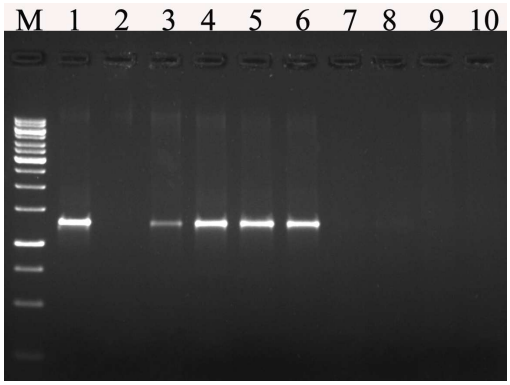

Fig. 3. A. PCR amplification of VTLAH-1 hybrids and parental palms with SCAR marker. M: 1 Kb ladder. Lane 1: Tall parent (Sumangala); 2: Dwarf parent (Hirehalli Dwarf); 3-6: VTLAH-1 (HD x Sumangala) hybrids; 7-10: Selfed progenies of HD

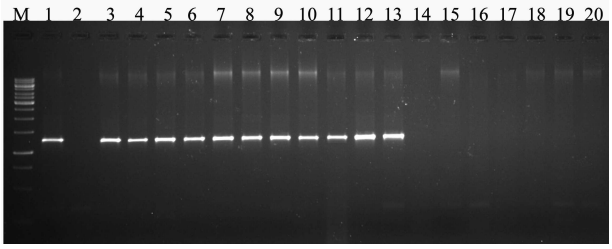

Fig. 3. B. PCR amplification of VTLAH-2 hybrids and parental palms with SCAR marker. M: 1 Kb ladder. Lane 1: Tall parent (Mohitnagar); 2: Dwarf parent (Hirehalli Dwarf); 3-13: VTLAH-2 (HD x *Mohitnagar*) hybrids; 14-20: Selfed progenies of HD

To conclude, the SCAR marker developed would be an invaluable tool which can facilitate hybrid purity assessment in a perennial crop like arecanut and ensure supply of true hybrids to farmers.

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