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Short communication

Potential of start codon targeted (SCoT) markers for assessment of genetic diversity of arecanut (*Areca catechu* L.)

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ABSTRACT

Gene-targeted markers constitute recent and novel marker systems, which are based on polymorphic sites existing within gene targeting regions. One among these marker systems is the Start Codon Targeted (SCoT), which is based on the short conserved region of plant genes, which neighbours the ATG start codon. SCoT markers, by virtue of both their higher length of the primers and annealing temperatures, are generally reproducible and have been reported to be highly polymorphic, compared to other dominant markers. In this study, analysis of genetic diversity among six arecanut accessions, *viz*., Mangala, Sumangala, Sreemangala, Mohitnagar, Swarnamangala and a natural dwarf mutant (Hirehalli Dwarf) was carried out using SCoT markers to evaluate the applicability of these markers in genetic diversity studies in arecanut. Using 10 SCoT primers, 82 band were produced among the accessions, of which 58 (70.73%) were found to be polymorphic. The highest genetic similarity value of 0.89 was found between the Swarnamangala and Mohitnagar and the lowest value of 0.63 was noticed between the Hirehalli Dwarf and Mohitnagar. The similarity coefficient values were then utilized to construct a dendrogram utilizing the unweighted pair group of arithmetic means (UPGMA). The cultivars were grouped depending on their geographical origins. The results obtained in this study indicate the suitability of SCoT marker system for genetic diversity analysis in arecanut.

Key words: Arecanut, genetic diversity, molecular markers, SCoT.

The arecanut palm (*Areca catechu* L.), from which the masticatory nut known commonly as 'betel nut' or 'supari' is obtained, belongs to the Arecaceae family under the tribe Areceae. *A. catechu* is the only cultivated species in the *Areca* genus. India is the major producer and consumer of arecanut, sharing 62% of the area and 60% of the production. Cultivation of arecanut palm is mainly concentrated in the states of Kerala, Karnataka, Assam, West Bengal, Meghalaya, Maharashtra and Tamil Nadu. Arecanut is a significant part of the religious, social and cultural celebrations and economic life of people in India; millions of people are depend on arecanut crop for their livelihood. It is also an ingredient in Ayurvedic and veterinary medicines (Lingappa *et al*., 6). Tender arecanuts are an excellent source of tannins, which is used for the production of natural dyes, tanning agents and adhesives (Sivaramakrishnan, 14). Based on the plant stature, arecanut palms are mainly classified into talls, semi-talls and dwarfs. Tall cultivars, which can attains a height of 18 to 20 m, have high yield potential, but are prone to wind damage and severe sun scorching. In addition, their tall stature makes farm operations cumbersome and expensive. Some of the improved tall cultivars with high yield potential, released by ICAR-CPCRI include Mohitnagar, Sumangala, Sreemangala and Swarnamangala, while Mangala is considered to be a semi-tall. Hirehalli Dwarf (HD) is the only dwarf identified till date and is considered to be a natural mutant (Naidu, 7). Compared to tall cultivars, the yield of HD is quite low (4-5 kg ripe nuts/ palm/ year); however, it has shown promise to be good source of crop improvement due to its short and compact nature (Ananda, 2). HD has been utilized to produce crop combination of HD with other high yielding tall cultivars. Till date, two such dwarf × tall hybrids have been released for commercial cultivars, *viz*., VTLAH-1 (HD × Sumangala) and VTLAH-2 (HD × Mohitnagar) (CPCRI, 5).

DNA-based molecular markers can surmount most of the limitations of morphological and biochemical markers. Different types of DNA markers being used in crop plants for the assessment of genetic diversity includes restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and microsatellites or simple sequence repeats (SSRs). In arecanut, RAPD markers have been utilized for genetic diversity assessment of accessions (Purushotham *et al*., 9; Bharath *et al*., 3). Recently, rapid advances made in the area of genomic research and enrichment of genomic resources have resulted in the designing/development of new marker systems, especially 'functional markers', so

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named as they are presumed to be linked to the gene of interest. The gene targeted marker, Start Codon Targeted Polymorphism (SCoT) was first illustrated by Collard and Mackill (4). SCoT markers are based on the short conserved region flanking the ATG translation start codon in plant genes that is conserved for all genes (Sawant *et al.*, 13). SCoT-PCR uses longer primers (18-mer length) and these markers display high polymorphism with added advantage of reproducibility. SCoT polymorphism marker technique has been successfully applied in many plants including date palm (Al-qurainy *et al*., 1) and coconut (Rajesh *et al*., 10). All these studies have revealed high polymorphism detection by SCoT markers. The objective of the present study was to assess the genetic diversity among the arecanut accessions using SCoT markers and determine the effectiveness of this marker system in arecanut.

A total of 24 arecanut samples were used in the present study comprising of four palms each from five tall arecanut cultivars (Mangala, Sumangala, Sreemangala, Mohitnagar and Swarnamangala) and Hirehalli Dwarf. Extraction of DNA was carried out from arecanut spindle leaves by a modified SDS method (Rajesh *et al*., 11). The quality and quantity of extracted DNA was established using spectrophotometery and agarose gel electrophoresis. The extracted DNA, after diluting to 20 ng/µl, was stored at -20°C till further use. Ten SCoT primers (Collard and Mackill, 4) were used for amplification of 24 arecanut DNA samples. Amplification reactions were undertaken in volumes of 20 µl which included 30 ng of genomic DNA, 10 µM of the primer (Sigma), 10 mM of each dNTPs (Bangalore Genei), 10X buffer (10 mM Tris-HCl, pH 8.3) and 3 units of *Taq* DNA polymerase (Bangalore Genei). The PCR cycling conditions were as follows: initial denaturation at 94ºC for 3 min., followed by 35 cycles of denaturation at 94ºC for 1 min., annealing at 52ºC for 1 min. and extension at 72ºC for 2 min. A final extension was provided at 72ºC for 5 min. All primers were amplified using the same condition. After amplification, the PCR products were separated on 1.2% agarose gel in 1X TBE buffer by electrophoresis and stained with ethidium bromide. The gels were visualized in a gel documentation system. Each reaction was repeated twice to ensure consistency of results obtained. The PCR amplified SCoT primer bands were scored as absent (0) and present (1) and only clear, reproducible and unambiguous bands were considered for the scoring. Software package NTSYS-PC version 2.0 (Rohlf, 12) was used for the further analysis. Genetic similarity between the arecanut accessions were calculated using similarity matrix generated by estimating Jaccard's similarity coefficient. These similarity coefficients were then utilized for cluster analysis. Dendrogram, depicting the phylogenetic relationships among the arecanut accessions, was constructed using Unweighted Pair-Group Method (UPGMA). The formulae provided by Powell *et al*. (8) and Smith *et al*. (15) were utilized to estimate the average Polymorphism Information Content (PIC) and Marker Index (MI).

Estimation of genetic diversity is a prerequisite for efficiently managing genetic resource of a crop, which can enable identification of suitable parental combinations and thus contribute to genetic improvement of the crop. SCoT markers are expected to be linked to functional genes and corresponding traits, thus the amplicons can be converted to gene targeted marker systems (Xiong *et al*., 16). Besides these markers are multilocus, which are helpful in obtaining high genetic polymorphism. This is one of the first studies, which make use of functional markers for evaluation of extent of genetic diversity amongst arecanut accessions.

All the 10 SCoT primers utilized in the present study showed polymorphism among the 24 arecanut palms and a total of 82 bands were detected. The number of bands ranged from five (SCoT05) to 11 (SCoT20) with an average of 8.2 bands per primer. Among these, 58 (70.73%) bands were polymorphic, the number of polymorphic bands varying from three (SCoT02 and SCoT08) to nine (SCoT05) with an average of 5.7 per primer. The highest polymorphism was detected with the primer SCoT06 (100%) and lowest for the primer SCoT02 with 50% polymorphism (Table 1). In an earlier study in arecanut, only 57.5% polymorphism could be detected using RAPD markers (Purushotham *et al*., 9).

The PIC value ranged from 0.21 for the primer SCoT12 and SCoT13 to 0.41 for the primer SCoT20. The informativeness of each primer was calculated according to MI, the value of which varied from 0.78 to 3.30. Based on the high MI, four informative primers SCoT 20 (MI = 3.30), SCoT 05 (MI = 2.63), SCoT 23 (MI = 2.48) and SCoT 06 (MI = 2.22) were identified. The number of amplicons and PIC of the SCoT markers observed in arecanut from the present study is comparable to the results obtained in other studies in coconut (Rajesh *et al*., 10).

The similarity coefficient's between pairs of accessions were arrived at by calculating the Jaccard's similarity coefficient (Table 2), which ranged from 0.63 (between the accession HD and Mohitnagar) to 0.89 (between the accessions Swarnamangala and Mohitnagar). Earlier, Purushotham *et al*. (9) reported moderate genetic diversity among 11 arecanut cultivars of Western Ghats regions of India using RAPD markers and reported that a maximum genetic distance of 47% between cultivars.

Table 1. Percent polymorphism, polymorphism information content (PIC) and marker index (MI) obtained in arecanut accessions utilizing 10 SCoT primers.

Primer name	Primer sequence (5'-3')	No. of amplified bands	No. of polymorphic bands	Polymorphic ratio (%)	PIC value	Marker index (MI)
SCoT 02	CAACAATGGCTACCACCC	6	3	50%	0.38	1.14
SCoT 05	CAACAATGGCTACCACGA	10	9	90%	0.29	2.63
SCoT 06	CAACAATGGCTACCACGC	7	7	100%	0.32	2.22
SCoT 08	CAACAATGGCTACCACGT	5	3	60%	0.26	0.78
SCoT 11	AAGCAATGGCTACCACCA	8	4	50%	0.32	1.26
SCoT 12	ACGACATGGCGACCAACG	7	5	71.4%	0.21	1.04
SCoT 13	ACGACATGGCGACCATCG	8	5	62.5%	0.21	1.07
SCoT 20	ACCATGGCTACCACCGCG	11	8	72.7%	0.41	3.30
SCoT 23	CACCATGGCTACCACCAG	10	8	80%	0.31	2.48
SCoT 24	CACCATGGCTACCACCAT	10	6	60%	0.25	1.50

Table 2. Similarity matrix of six arecanut accessions based on SCoT data.

Accession	Mangala	Sumangala	Sreemangala	Mohitnagar	Swarnamangala	Hirehalli Dwarf
Mangala	1.00					
Sumangala	0.84	1.00				
Sreemangala	0.77	0.84	1.00			
Mohitnagar	0.68	0.70	0.77	1.00		
Swarnamangala	0.70	0.72	0.76	0.90	1.00	
Hirehalli Dwarf	0.73	0.69	0.70	0.63	0.67	1.00

The similarity coefficients generated from the SCoT data were used to construct a dendrogram using UPGMA analysis (Fig. 1). Dendrogram separated the six accessions into two major clusters diverging at the simlarity coeficient of 0.68. The first cluster was again sub-divided into two. The first sub-cluster included the three exotic accessions, *viz.*, Mangala (an introduction from China), Sumangala (an introduction from Indonesia) and Sreemangala (an introduction from Singapore). The second sub-

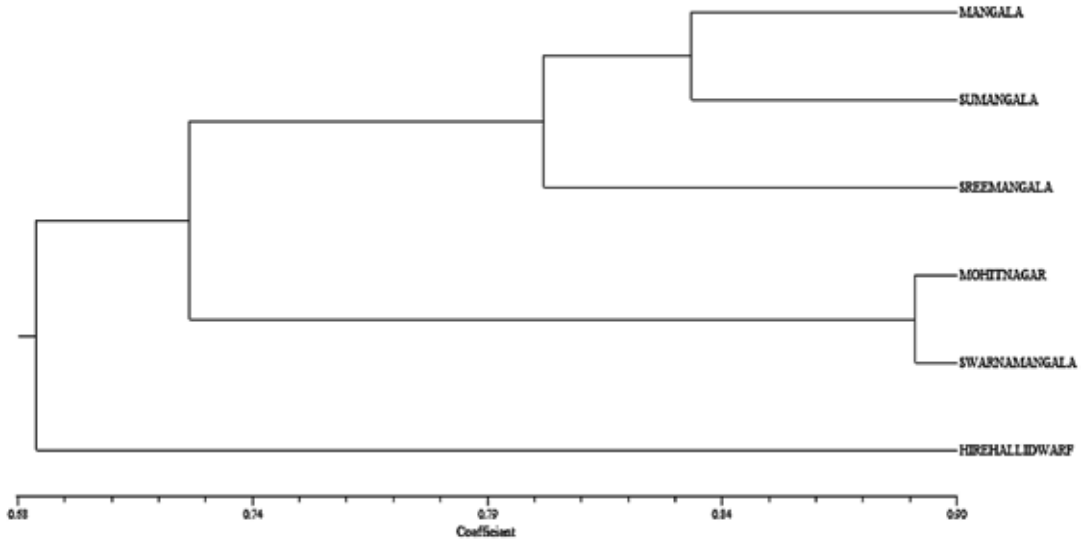

Fig. 1. Dendrogram showing the genetic relationship among the six arecanut accessions.

cluster comprised of the indigeneous accession Mohitnagar and Swarnamangala (a selection from Vietnam). Hirehalli dwarf formed a separate cluster, which shows its diverse relationship to exotic and indigeneous tall accessions. It was observed from the results that the dendrogram was in agreement with goegraphic origin of arecanut, eventhough the trend was not absolute.

It can be concluded from the present study that SCoT markers provided information on the existence of high genetic diversity among the exotic and indigenous arecanut accessions. The genetic polymorphism generated by SCoT markers could be utilized for deciphering the breeding history of the domesticated genotypes.

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