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Chapter 14

Arecanut

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1. Introduction

The genus *Areca* L. belongs to the sub-tribe Arecinae and tribe Arecae in the family Arecaceae and comprises 45 species (Plant List, 2013), growing in hot and humid tropical regions of the world. Among those, *Areca catechu* L. is the only cultivated species, cultivated mainly in Bangladesh, China, India, Indonesia, Malaysia, Myanmar, the Philippines, Sri Lanka, Thailand and Vietnam. It is believed that the South East Asian region is the centre of origin for *A. catechu* L. (Bavappa *et al.*, 1982). It is a monoecious, unbranched palm, widely used for masticatory purposes, either alone or along with slaked lime, betel leaf (*Piper betle* L.) and tobacco. The nuts also play a major role in many of the social, religious functions, known for its medicinal importance. The nuts are being sold as ripe, dried, cured and processed forms.

In India, the *Areca* palm is commonly cultivated in the plains and foothills of the Western Ghats region of states such as Kerala, Karnataka, Goa and some parts of Maharashtra and Gujarat and in the North Eastern states of Assam, Meghalaya and West Bengal. India stands top in both area and production of arecanut in the world, where it is grown in an area of 446 thousand hectares with production of 609 thousand tones (FAOSTAT, 2013). The country accounts for 57 per cent in area and 53 per cent of the total world production, of which the three states *viz.*, Kerala, Karnataka and Assam contributing 90 per cent of area under cultivation and 95 per cent of the production (Rajagopal and Balasimha, 2004). The crop provides economic security for millions of people and for many sole means of livelihood in the Indian sub-continent, South East Asian countries and also in some of the Pacific islands (Sankaran *et al.*, 2013).

2. Genetic Diversity in Arecanut

A number of cultivars (ecotypes) have been identified from various arecanut growing regions within the country as well as from other parts of the world (Bavappa, 1963). A field gene bank is being maintained at ICAR-Central Plantation Crops Research Institute (CPCRI), Regional Station, Vittal, Karnataka, which is considered to be the largest assemblage of the *Areca* germplasm in the world. A total of 173 accessions have been collected so far (ICAR-CPCRI, 2016), that includes 23 exotic accessions from various South East Asian countries representing three species *viz.*, *Areca catechu* L., *A. concinna* Thw. and *A. triandra* Roxb. The indigenous collections, numbering 150, comprise of collections from Assam, Goa, Gujarat, Karnataka, Kerala, Maharashtra, Meghalaya, Tamil Nadu, West Bengal, and Andaman and Nicobar group of Islands (Ananda, 2006).

Earlier work in arecanut were mainly focused on studies on floral morphology, cytogenetics, biochemistry, plant pathology and plant physiology (Bhat, 1982; Joshi and Reddy, 1982; Bhat, 1985). Evaluation of arecanut germplasm has been attempted using morphological traits and yield criteria (Ananda *et al.*, 2000; Rajesh, 2007). The information gathered regarding the diversity, relationship, phylogeny among arecanut accessions using morphological and biochemical approaches are not reliable as environmental factors greatly influence yield, growth and development of perennial crops. Hence, DNA-based marker systems have been utilized for assessment of genetic diversity in arecanut in recent years

3. Using DNA-based Markers in Arecanut

In arecanut, the most widely used molecular marker has been the Random Amplified Polymorphic DNA (RAPD), since it is cost effective, easy to handle, versatile, and can distinguish slight changes in the polymorphic DNA among the populations. Rajesh *et al.* (2007) carried out optimization of RAPD protocol for arecanut. Purushotham *et al.* (2008) carried out assessment of extent of genetic diversity in 11 arecanut cultivars collected from the Western Ghats regions of India using RAPD technique. Two major clusters were formed of which ten cultivars formed the major cluster ('A') while Mohitnagar alone formed a separate cluster ('B') (Figure 14.1). Two unique amplicons produced by primers OPA-13 and OPA-15 were specific to all the cultivars in cluster 'A'. The cluster 'A' was subdivided into two minor clusters, 'A_1' and 'A_2' comprising of nine cultivars and one cultivar respectively. The sub-cluster 'A_1' consisted of two minor clusters 'A_{1a}' and 'A_{1b}', with Mohitnagar *inter se* forming one group while eight cultivars grouped in to another, segregated into three groups. The Maidhan varieties were closely linked with each other while the exotic Saigon-12 formed a distinct clade in this group. An unique band produced by the primer OPD-05 was specific Cluster 'A_2'. The results of the study revealed that despite their narrow distribution in the Western Ghats, the accessions showed moderate polymorphism.

Genetic fidelity of arecanut plantlets that were derived through direct somatic embryogenesis from the Yellow Leaf Disease (YLD) resistant arecanut palms was carried out by Karun *et al.* (2008) utilizing RAPD markers (Figure 14.2). Eight plantlets derived through direct somatic embryogenesis from the YLD mother

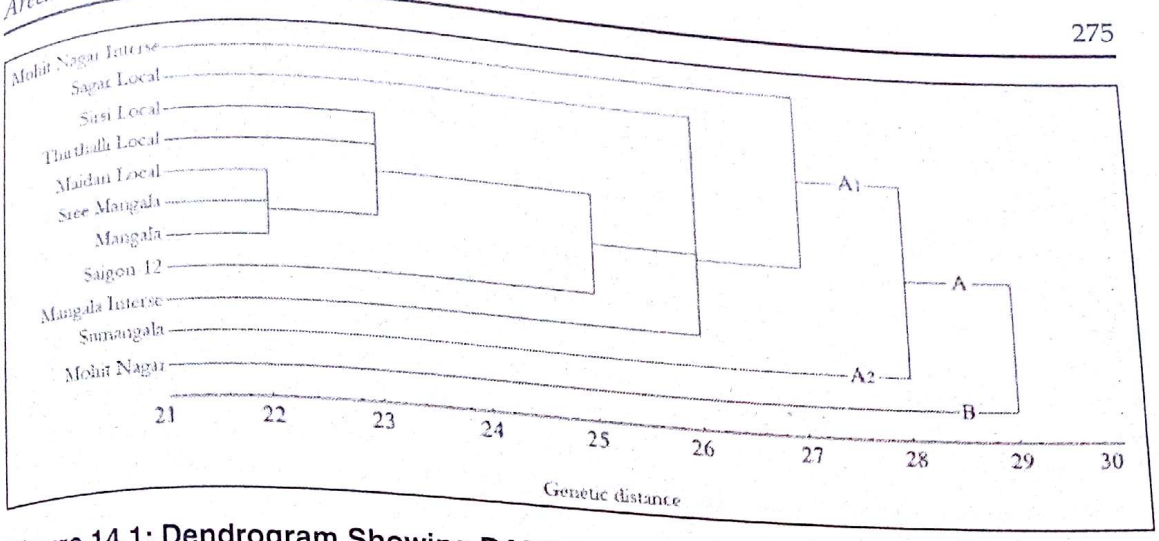

Figure 14.1: Dendrogram Showing RAPD Marker Based Genetic Distance among the 11 Arecanut Cultivars from Western Ghats (Purushotham *et al.*, 2008).

palms were studied. It was concluded that the plantlets derived from direct somatic embryogenesis showed less variation under *in vitro* conditions and hence the inflorescence culture derived plantlets could be employed for mass multiplication for the desirable qualities in the elite palms.

Sankaran *et al.* (2013) assessed the genetic diversity among 10 arecanut accessions from the Andaman and Nicobar Islands using RAPD markers. Among the 30 RAPD primers, 11 were selected to detect polymorphism based on their reliability in pooled DNA. They considered the primers OPF-16, which produced maximum number of bands (49), OPF-8 and OPH-35 (producing 48 bands each), OPF-41 and OPF-9 each produced a minimum number of 39 bands. Their studies showed that the percentage of polymorphism ranged from 50 to 100, with primers OPH-8, OPH-35, OPP-46 and OPF-8 showing 100 per cent polymorphism while OPF-1 showing only 50 per cent. The diversity analysis revealed that the accessions were grouped in two clusters as wild ones segregated from the cultivated nine accessions. The cluster of cultivated ecotypes was further split into two clades with four and five accessions respectively. These results were in conformity with those obtained

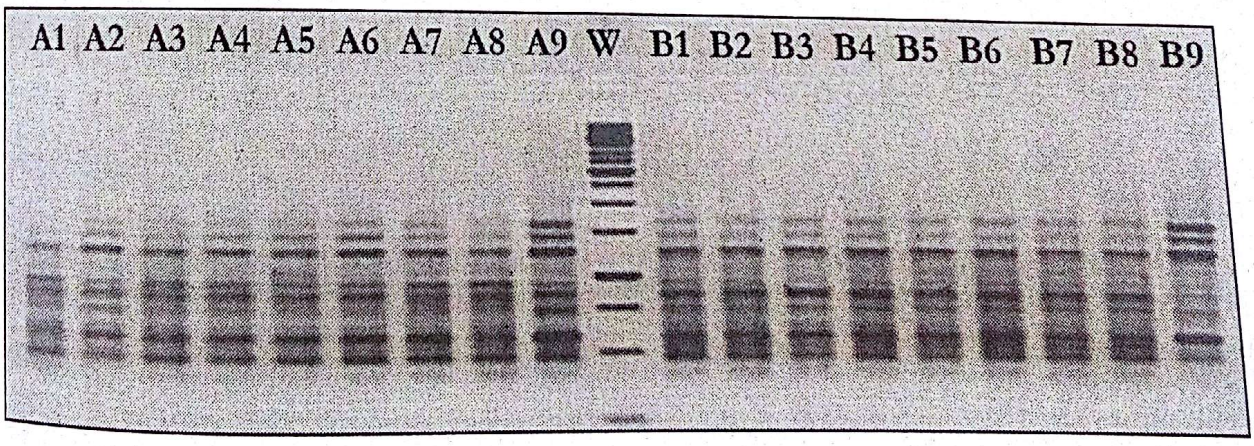

Figure 14.2: RAPD Banding Profile of *in vitro* Propagated Plantlets and Field Grown Mother Palms [A1-A8: Plantlets derived from the mother palm A9; B1-B8: Plantlets derived from the mother palm B9] (Karun *et al.*, 2008).

by Purushotham *et al.* (2008) as huge variability among the wild and cultivated genotypes was observed. It implied that selection of appropriate accessions of arecanut for hybridization or breeding programs is imperative.

Hu *et al.* (2009) reported isolation of nine novel microsatellite loci from *A. catechu* germplasm conserved in Taiwan. When these SSRs were utilized for germplasm evaluation, 5-15 alleles were detected; null alleles were also recorded in five loci. The first large scale studies of estimation of genetic diversity in arecanut were conducted by Bharath *et al.* (2012), who studied the genetic diversity among 60 arecanut accessions utilizing microsatellite markers. Nine microsatellite markers specific to arecanut, isolated earlier by Hu *et al.* (2009), were employed for analysis. The accessions studied were the collections from Konkan, North East region and Andaman and Nicobar Islands of Indian sub-continent, and exotic collections from different arecanut growing regions of South East Asia and Pacific Islands. The results showed that all of the microsatellites showed polymorphism except one. The cluster analysis revealed that they formed two major clusters: Cluster I comprised of exclusively the Konkan collections and Cluster II was formed due to collections from North East and exotic collections. Among the collections from India, Konkan collections formed two distinct clusters.

Bharath *et al.* (2015) carried out RAPD analysis using 14 polymorphic in 60 accessions [43 indigenous (Konkan I and II, Maidhan, North East and Andaman and Nicobar Islands) and 17 exotic germplasm] collected from various parts of South East Asia and Pacific region (British Solomon Islands, China, Fiji, Indonesia, Singapore, Sri Lanka and Vietnam), and conserved in the field gene bank of ICAR-CPCRI, Regional Station, Vittal, Karnataka, India. While analyzing the results obtained from RAPD analysis (Figure 14.3), a maximum of 13 bands were obtained using the primers OPF-6 while only five amplified fragments were obtained for OPAF-19. Shannon's indices showed a great variation with minimum values for OPAF-19 and maximum for OPAF-6 and the minimum gene diversity was recorded for OPAF-6 and maximum for OPM-13. The cluster analysis revealed that the genotypes were segregated into two clusters with Cluster I comprised of all exotic accessions, North East accessions and those from Andaman and Nicobar Islands,

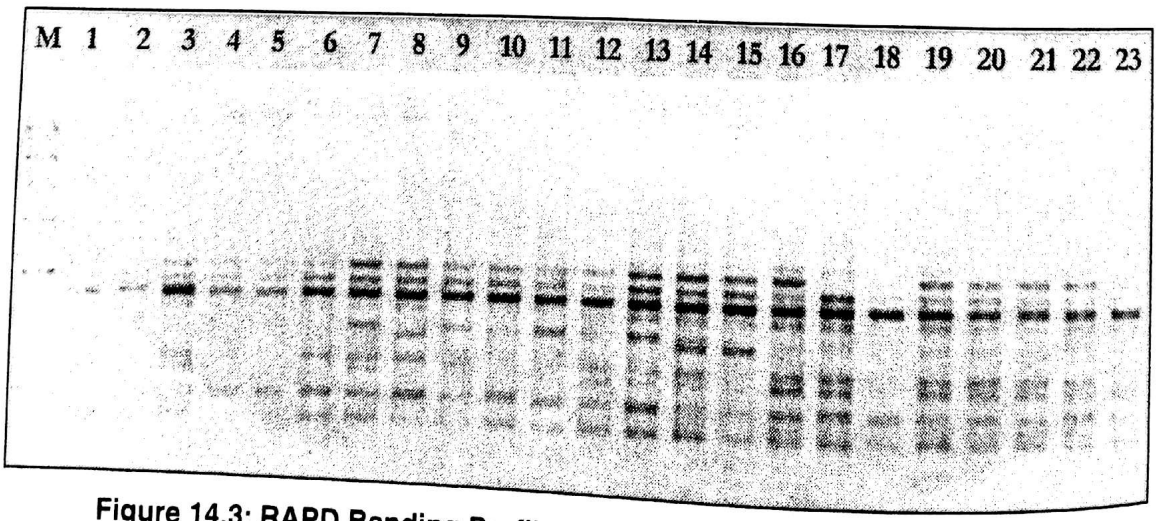

Figure 14.3: RAPD Banding Profiles Generated using Primer OPAF-2 in Arecanut Accessions. M: Standard 1 Kb ladder (M) (Bharath *et al.*, 2015).

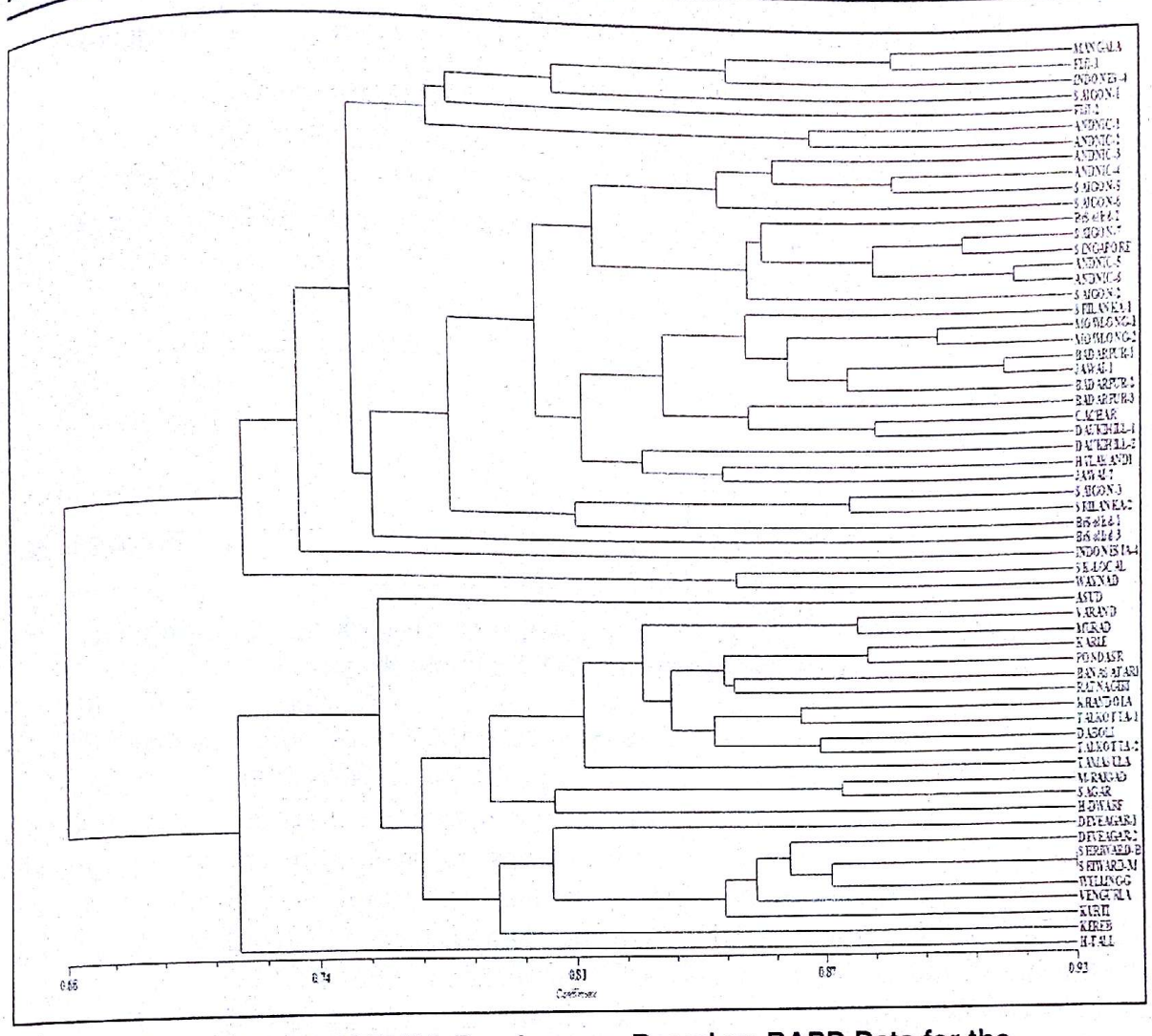

Figure 14.4: A UPGMA Dendrogram Based on RAPD Data for the 60 Arecanut Accessions (Bharath *et al.*, 2015).

while Cluster II comprised of purely the indigenous ones, *viz.*, Konkan I, Konkan II and Maidhan collections. It was observed that collections from North East and Andaman and Nicobar Islands shared similarity with the local South Kanara and Wayanad accessions in the Cluster I, despite their geographical distances. Hirehalli Tall, a Maidhan collection, formed a distinct accession in Cluster II. It was concluded that there existed a high level of genetic diversity among those sixty accessions that were analyzed.

Rajesh *et al.* (2016a) undertook analysis of genetic diversity among six arecanut accessions *viz.*, Mangala, Sumangala, Sreemangala, Mohitnagar, Swarnamangala and a natural dwarf mutant (Hirehalli Dwarf), using SCoT markers to evaluate the applicability of these markers in genetic diversity studies in arecanut. Using 10 SCoT primers, described by Collard and Mackill (2009), 82 band were produced among the accessions, of which 58 (70.73 per cent) 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 dendrogram constructed utilizing the UPGMA grouped the cultivars based on their geographical origins, with Hirehalli Dwarf forming a distinct accession (Figure 14.5).

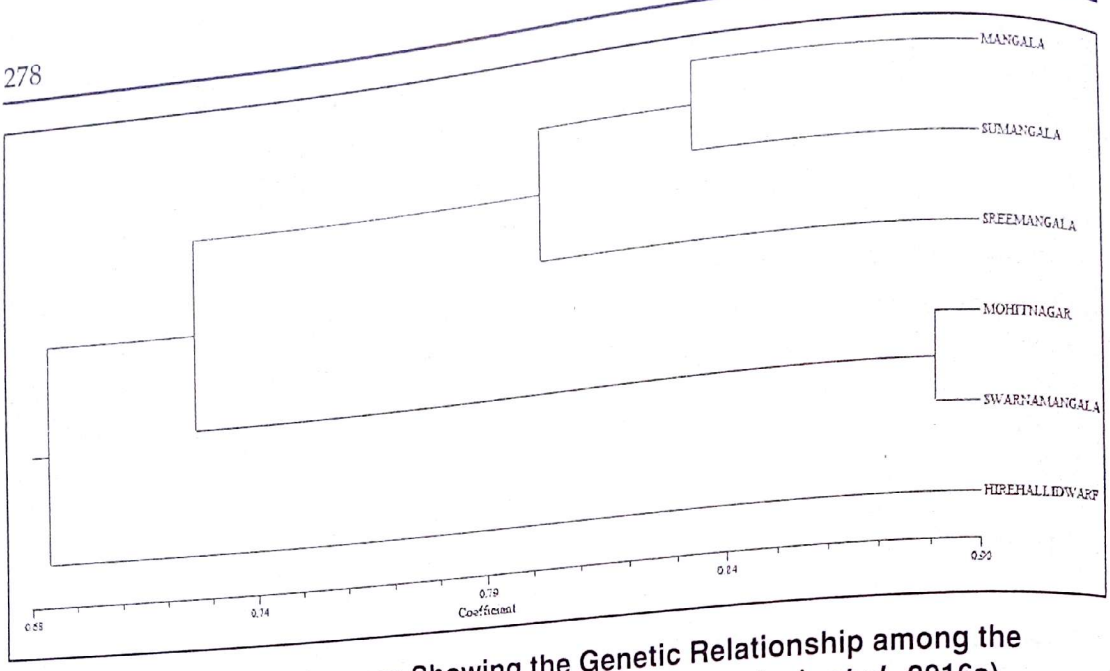

Figure 14.5: Dendrogram Showing the Genetic Relationship among the Six Arecanut Accessions using SCoT Analysis (Rajesh *et al.*, 2016a).

Rajesh *et al.* (2016b) carried out studies utilizing Start Codon Targeted (SCoT) markers to identify molecular marker(s) capable to differentiate tall/dwarf trait in arecanut. Tall cultivars (Mangala, Sumangala, Sreemangala, Mohitnagar, Swarnamangala and Hirehalli Tall) and the natural mutant of arecanut (Hirehalli Dwarf) were screened utilizing 25 SCoT primers described by Collard and Mackill (2009). One of the primers, SCoT11, produced an amplicon of around 1300 bp band specific to all the tall cultivars, which was absent in the dwarf cultivars (Figure 14.6). The DNA fragment was purified, cloned and sequenced. 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.

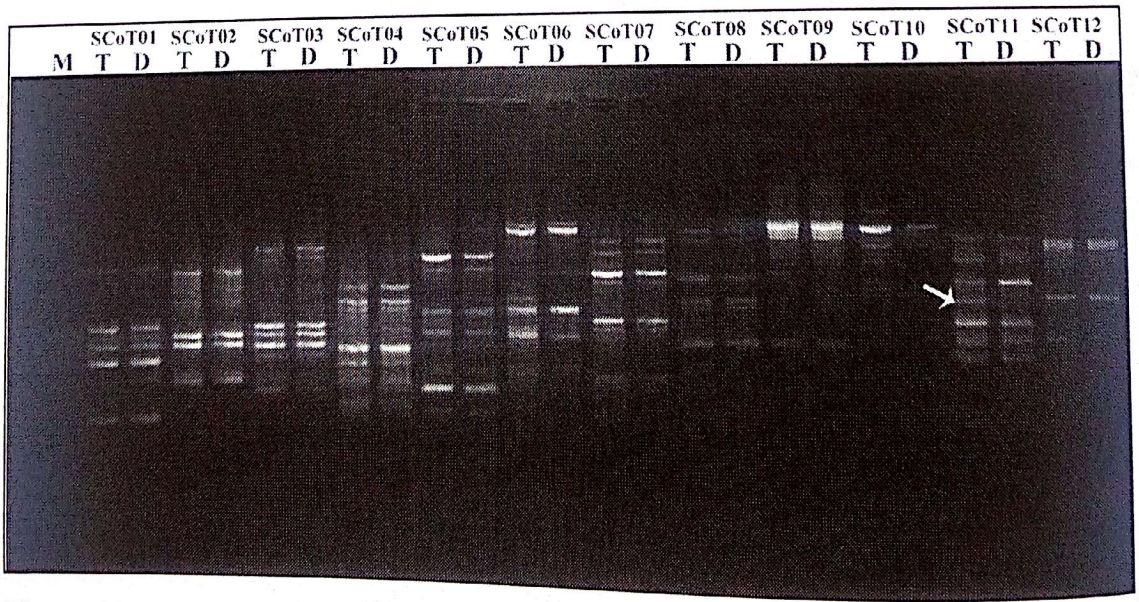

Figure 14.6: Banding pattern of pooled DNA of tall and dwarf palms with the primers SCoT01- SCoT12. Arrowhead represent polymorphic band of SCoT11 primer specific to tall accession. M: High range ladder. T: Tall bulk. D: Dwarf bulk.

4. Conclusion

In perennial crops such as arecanut, the morphological and biochemical methods for characterization of germplasm/evaluation of genetic relationships, yield parameters *etc.* have their own limitations. Hence, advanced biotechnological approaches, such as utilization of DNA-based molecular markers, are to be explored. Despite its demonstrated utility in other crops, only few studies have been carried out in arecanut using molecular markers. As arecanut is an economically important plantation crop, there is a need to characterize the arecanut germplasm using molecular markers. Such studies would aid in selection of desirable parents and clones with desirable traits among arecanut germplasm grown in various agro climatic zones and geographical regions of the world.

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