Identification and utilization of informative EST-SSR markers for genetic purity testing of coconut hybrids

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Abstract
Coconut palms are categorized into two forms, viz., ‘talls’ and ‘dwarfs’ which are being utilized to produce hybrids through the process of inter-varietal or intra-varietal crosses. Hybrid coconut seedlings are generally identified and selected based on morphological traits by plant breeders, which is quite difficult and requires expertise. Even minor errors in identification may adversely affect breeding programs in coconut, which is spread over many decades. In this study, we have utilized thirty EST-SSR markers, derived from existing coconut leaf transcriptome data, for screening polymorphism between eighteen coconut parental lines. The polymorphic primers capable of differentiating the parental palms were then utilized successfully for assessment of purity of hybrids derived from these parents. Thus, the current study demonstrates the utility of EST-SSR markers in determining the genetic purity of hybrids in coconut.

Keywords: Coconut, hybrids, genetic purity, EST-SSR

Introduction
Generating and testing hybrid varieties of coconut are currently a major field of research in many countries with the objectives of increasing yield of nuts, oil content and also tolerance to abiotic and biotic stresses. There are many hybrids being developed and researched upon to cater to the climate, soil conditions and needs of each individual location. The two major varieties of coconut palms are ‘talls’ and ‘dwarfs’ (Narayana and John, 1949) with dwarfs (even though fewer than 5 per cent of the world coconut population) being in higher demand for genetic studies due to their quick emission of inflorescence and early germination (Bourdeix et al., 2008). Talls take a longer time to flower (~6 years) but live much longer (~100 years) when compared to dwarfs (~60 years). Talls (var. typica) and dwarfs (var. nana) also differ in their breeding behaviour with the talls being allogamous (cross-fertilizing) and dwarfs being autogamous (self-fertilizing) (Arunachalam and Rajesh, 2008). Inter-varietal crosses between a dwarf male parent with a tall female parent (T x D) as well as tall male parent with a dwarf female parent (D x T) and intra-varietal crosses (T x T and D x D) are methodologies followed for the development of hybrids (Arunachalam and Rajesh, 2008).

Hybrid varieties that provide better resistance to various diseases and enhanced yield have been successfully developed in coconut. Kalpa Sankara, a hybrid resistant to root (wilt) disease has been derived by crossing Chowghat Green Dwarf (CGD) and West Coast Tall (WCT) (Nair et al., 1996). Hybrids developed between Vanuatu Tall (VTT) and Rennell Island Tall (RIT) have been reported to possess better resistance towards coconut foliar decay disease, which is endemic to Vanuatu in the South Pacific (Labouisse et al., 2011). Recently, Kalpa Samrudhi, a cross between Malayan Yellow Dwarf (MYD) and WCT, has been developed which...
provides a much higher nut yield, copra content as well as oil output when compared to its parents (Jerard et al., 2015).

Even though the development of hybrids has contributed significantly for the increased productivity of coconut, the timely production and ample supply of hybrid seedlings, which are genetically pure, to the farmers is the key factor determining the success of hybrid technology in this crop. Morphological descriptors currently used for seed purity assessment in coconut include petiole colour, days taken for germination, seedling vigour and higher collar girth (Rajesh et al., 2014). Although hybrid purity assessments based on morphology are extensively taken up, they are often affected by environment; in addition, requirement for time and resources for such an assessment is tremendous. Selection by petiole colour, which is generally utilized marker to select hybrid seedlings in nurseries, is authentic only if parents used are homozygous for red, yellow or green petiole (Rajesh et al., 2014). Some of the drawbacks of utilizing morphological traits for genetic purity testing of coconut hybrids are that they are cumbersome and subjected to environmental influences. Furthermore, many of the varieties and hybrids are phenotypically less distinct resulting in difficulty in accurate morphological evaluation.

DNA-based markers, because of their rapidity in estimation, ease of use and cost-effectiveness, have become indispensable for use in variety identification, diversity and linkage-mapping studies. Among the common molecular markers, SSR (simple sequence repeat) are generally preferred due to their abundance, co-dominant inheritance, presence over the whole genome, higher reproducibility, multi-allelic nature, hypervariability and high transferability across species/genera (Varshney et al., 2005). SSRs have been developed and utilized in coconut for genetic diversity studies (Rivera et al., 1999; Perera et al., 2000; Meerow et al., 2003; Rajesh et al., 2008 a,b).

The use of SSRs for the authentication/differentiation of hybrids is a widely accepted procedure in many crops (Antonova et al., 2006; Sundaram et al., 2008; Naresh et al., 2009) and has been previously used in coconut too. SSR-based identification of Kalpa Sankara hybrids has been reported by Rajesh et al. (2012). In a cross between Sri Lanka Yellow Dwarf (SLYD) and Sri Lanka Tall (SLT), progenies with yellow colour were removed as selfed progenies based on visual observations (since SLYD petioles are yellow in colour), but SSR analysis later on proved that at least 11 per cent of the discarded yellow seedlings were actually hybrids (Perera, 2010).

Although genomic SSR markers have been utilized for genetic purity studies in plants traditionally, their high cost and time involved in this process have restricted their utilization. The number of SSR markers available in coconut is limited. With the exponential accumulation of data in EST databases, EST-derived SSRs (EST-SSRs) are being utilized these days for various molecular studies. EST-SSRs are also advantageous in that these SSRs might be from gene sequences that are functional, ESTs being located in the coding region of a gene. EST-SSR markers have been utilized earlier in genetic purity assessment of annual crops like safflower (Naresh et al., 2009) and castor (Pranavi et al., 2011; Gouri Shankar et al., 2013), but there are no such reports in perennial tree crops. In this study, we aim to identify novel markers that could decisively validate different coconut hybrids through the use of EST-SSRs.

Materials and methods

Plant materials

The plant materials used for hybrid authentication using molecular markers consisted of tall and dwarf parents and their offsprings collected from the ICAR-CPCRI Farm, Kasaragod, Kerala, India. A total of 18 parental lines and 103 progenies were used for the study (Table 1).

DNA isolation

DNA was extracted from spindle leaves of parental palms and their progenies following the modified method of Rajesh et al. (2013). To check the DNA purity, it was run in 0.8 per cent agarose gel, stained with ethidium bromide and visualized in a gel documentation system.

Assessment of parental polymorphism using EST-SSR markers

Initially, all the parental palms used in hybrid seed production were screened using the 30 novel
Table 1. Details of parental palms used for hybrid authentication studies and EST-SSR primers showing parental polymorphism

| Cross no. | Parents                          | EST-SSR primer showing polymorphism |
|-----------|----------------------------------|-------------------------------------|
| 1         | CGD Chowghat Green Dwarf         | CnKGDEST126                         |
|           | WCT West Coast Tall              | CnKGDEST117                         |
| 2         | MYD Malayan Yellow Dwarf         | CnKGDEST126                         |
|           | TPT Tiptur Tall                  |                                     |
| 3         | COD Chowghat Orange Dwarf        | CnKGDEST126                         |
|           | WCT West Coast Tall              |                                     |
| 4         | GBGD Gangabondam Green Dwarf     | CnKGDEST130                         |
|           | PHOT Philippines Ordinary Tall   |                                     |
| 5         | GBGD Ganga Bondam Green Dwarf    | CnKGDEST130                         |
|           | LCT Laccadive Ordinary Tall      |                                     |
| 6         | LCT Laccadive Ordinary Tall      | CnKGDEST130                         |
|           | CCNT Cochin China Tall           |                                     |
| 7         | GBGD Gangabondam Green Dwarf     | CnKGDEST130                         |
|           | FJT Fiji Tall                    |                                     |
| 8         | WCT West Coast Tall              | CnKGDEST126, CnKGDEST117            |
|           | COD Chowghat Orange Dwarf        | CnKGDEST117                         |
| 9         | LCT Laccadive Ordinary Tall      | CnKGDEST117                         |
| 10        | COD Chowghat Orange Dwarf        | CnKGDEST117                         |
|           | CCNT Cochin China Tall           |                                     |
| 11        | CGD Chowghat Green Dwarf         | CnKGDEST117                         |
|           | CCNT Cochin China Tall           |                                     |
| 12        | MYD Malayan Yellow Dwarf         | CnKGDEST117                         |
|           | SNRT San Ramon Tall              |                                     |
| 13        | MOD Malayan Orange Dwarf         | CnKGDEST117                         |
|           | SNRT San Ramon Tall              |                                     |
| 14        | MGD Malayan Green Dwarf          | CnKGDEST117                         |
|           | CCNT Cochin China Tall           |                                     |
| 15        | CRD Cameroon Red Dwarf           | CnKGDEST117                         |
|           | CCNT Cochin China Tall           |                                     |
| 16        | COD Chowghat Orange Dwarf        | CnKGDEST117                         |
|           | SNRT San Ramon Tall              |                                     |
| 17        | GBGD Gangabondam Green Dwarf     | CnKGDEST117                         |
|           | SNRT San Ramon Tall              |                                     |
| 18        | MYD Malayan Yellow Dwarf         | CnKGDEST117                         |
|           | CCNT Cochin China Tall           |                                     |

EST-SSR primers (Table 2), which were mined from leaf transcriptome data of Chowghat Green Dwarf cultivar (Rajesh et al., 2015) as per the procedure reported in Preethi et al. (2014). PCR reactions were performed in volumes of 20 µL and contained genomic DNA (35 ng), 10 mM of each dNTPs (MBI Fermentas), 0.2 µM primer (Sigma), 3 Units of Taq DNA polymerase (MBI Fermentas) and 10X buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂]. The amplification conditions followed were: initial denaturation step at 94 °C for 2 minutes, 39 cycles at 94 °C for 1 minute, 55 °C for 1 minute and 72 °C for 1 minute 30 seconds and concluding with a final extension at 72 °C for 10 minutes.

The amplicons were separated on 3 per cent agarose gel and photographed on a digital gel documentation and image analysis system after staining with ethidium bromide. Polymorphic primers capable of differentiating the parental palms were then utilized for hybrid purity assessment studies.

Results and discussion

Thirty novel EST-SSR primers were used to screen polymorphism among eighteen parental lines. Those primers capable of detecting polymorphism among the parental palms in a particular cross were selected (Table 1). Confirmation of the results was achieved through repeated testing. For all these markers, the alleles present in the parents were of different sizes and both parental alleles were detected in the hybrids, EST-SSRs being co-dominant markers.

The hybridity of 14 F₁ plants derived from CGD x WCT were tested through the use of CnKGDEST126 and CnKGDEST117 primers, which displayed polymorphism between the parental lines. Out of 14 F₁ progenies, a total of 11 were confirmed to be true hybrids while three were deduced to be selfed or off types using CnKGDEST117 primers (Fig. A). Out of a total of six F₁ progenies tested from a cross between MYD and TPT, two offsprings were deduced to be off-types and the other four as true hybrids using the primer CnKGDEST126 (Fig. 1B). In a cross between COD x WCT, two pure hybrids and two selfed F₁ progenies were detected using the primer CnKGDEST126 (Fig. 1C). F₁ progenies of the
| Sl. No. | Primer Name | Accession | Forward Primer | Reverse Primer | Functional Annotation | Annealing Temperature (°C) |
|--------|-------------|-----------|----------------|----------------|-----------------------|----------------------------|
| 1      | CnKGDEST81  | KU999089  | TGGCCGGGAAGAAAAGCATT | TCGCCCAAAGCCACCCTCCTA | DNA polymerase I | 58 |
| 2      | CnKGDEST82  | KX580069  | (GCGACCT) | TCGCCCGCAGGGAAAAATCCAC | Keratin-associated protein 10-12-like receptor-like protein kinase | 60 |
| 3      | CnKGDEST84  | KX580070  | (TC) | AAAGAGTAGCGAAAGCAAGTTTCAAGC | Leucine-rich repeat transcription factor-like protein | 59 |
| 4      | CnKGDEST87  | KU999090  | (AAT) | TGAAGACGCGGGTGAGGTTGGA | Leucine-rich repeat receptor-like protein kinase | 59 |
| 5      | CnKGDEST96  | KU999091  | (TTC) | TGGGATAGACCTTGGTCTGTTGCTAT | Serine threonine protein kinase | 57 |
| 6      | CnKGDEST100 | KU999092  | (GAGGCG) | TGGCCCTCAGCGAAAGGGAGAA | 50s ribosomal protein l3 | 59 |
| 7      | CnKGDEST101 | KX580071  | (CT) | TGGATATCACAGCCCTTCCATGCT | Squalene synthase | 57 |
| 8      | CnKGDEST103 | KU999093  | (TA) | ACCCCAATGCCCGTGTGTGAAC | Midasin | 59 |
| 9      | CnKGDEST95  | KU999094  | (TC) | ACGGCACCAATTGGGTCAGACG | Phosphomethyl pyrimidine synthase, chloroplastic | 58 |
| 10     | CnKGDEST106 | KU999095  | (AT) | TCTGATGGCACCCGCATTGGAG | ACT domain containing protein | 59 |
| 11     | CnKGDEST85  | KU999096  | (CT) | TGGATATCACAGCCCTTCCATGCT | Squalene synthase | 57 |
| 12     | CnKGDEST90  | KU999097  | (TA) | GGCACAACCAGTGTCTCTTTGGCA | F-box protein | 59 |
| 13     | CnKGDEST98  | KU999098  | (GT) | AGACCCTCATGCACTAGGCCAC | Isovaleryl-CoA dehydrogenase, mitochondrial | 58 |
| 14     | CnKGDEST199 | KU999099  | (GA) | ACTTGTTGGGATAGGGTGCGGC | Glutamate receptor 3.5 isoform X1 | 59 |
| 15     | CnKGDEST107 | KU999100  | (TC) | TGTCAAGCAGCCAACTCCGAT | Serine/arginine-rich splicing factor SR30 | 59 |
| 16     | CnKGDEST108 | KU999101  | (GA) | AGCTGGGA TGGAAAGCAAAGGGC | Early flowering 2-like isoform X1 | 58 |
| 17     | CnKGDEST109 | KU999102  | (TG) | ACTCTGCTGCTTTTCCAGACAGGT | Myeloid transcription factor-like protein | 59 |
| 18     | CnKGDEST110 | KU999103  | (AG) | TGGTTGTCCTTGGA | UV-damaged DNA-binding protein | 58 |
| 19     | CnKGDEST111 | KU999104  | (AG) | AGCTGGGA TGGAAAGCAAAGGGC | Early flowering 2-like isoform X1 | 59 |
| 20     | CnKGDEST112 | KU999105  | (AT) | AGCGTTGAGGAGGAGGGAGAC | Myeloid transcription factor-like protein | 59 |
| 21     | CnKGDEST113 | KU999106  | (AG) | AGCTGGGA TGGAAAGCAAAGGGC | Early flowering 2-like isoform X1 | 59 |
| 22     | CnKGDEST114 | KU999107  | (AG) | AGCTGGGA TGGAAAGCAAAGGGC | Myeloid transcription factor-like protein | 59 |
| 23     | CnKGDEST115 | KU999108  | (AG) | AGCTGGGA TGGAAAGCAAAGGGC | Early flowering 2-like isoform X1 | 59 |
| 24     | CnKGDEST116 | KU999109  | (AG) | AGCTGGGA TGGAAAGCAAAGGGC | Myeloid transcription factor-like protein | 59 |
| 25     | CnKGDEST117 | KX580073  | (TC) | CGCATGGGAGGCT GAGGCAAAA AAGGGGCCTCTTCCCATGCCTT | Ethylene-responsive transcription factor | 59 |
| 26     | CnKGDEST118 | KX580074  | (TA) | CGCATGGGAGGCT GAGGCAAAA AAGGGGCCTCTTCCCATGCCTT | Ethylene-responsive transcription factor | 59 |
| 27     | CnKGDEST119 | KX580075  | (TC) | CGCATGGGAGGCT GAGGCAAAA AAGGGGCCTCTTCCCATGCCTT | Ethylene-responsive transcription factor | 59 |
| 28     | CnKGDEST120 | KX580076  | (AG) | AGCTGGGA TGGAAAGCAAAGGGC | Early flowering 2-like isoform X1 | 59 |
| 29     | CnKGDEST121 | KX580077  | (AG) | AGCTGGGA TGGAAAGCAAAGGGC | Early flowering 2-like isoform X1 | 59 |
| 30     | CnKGDEST122 | KX580078  | (CT) | AGCTGGGA TGGAAAGCAAAGGGC | Early flowering 2-like isoform X1 | 59 |
crosses GBGD x PHOT (Fig. 1D), GBGD x LCOT (Fig. 1E) and LCOT x CCNT (Fig. 1F) were all confirmed to be true hybrids when checked with primer CnKGDEST130. Two selfed F₁ progenies were detected out of a total of eight probable hybrids in GBGD x FJT cross using the primer CnKGDEST130 (Fig. 1G). The primer CnKGDEST117 could aid in identifying one offtype from among ten F₁ progenies with the others confirmed as true hybrids in WCT x COD (Fig. 1H). LCT x COD cross revealed three offtypes and seven pure hybrids using the primer CnKGDEST117 (Fig. 1I).

Progenies of crosses between COD x CCNT (Fig. 2A), CRD x CCNT (Fig. 2B) and MYD x CCNT (Fig. 2C) showed true hybrids in all the lanes of the F₁ progenies used for testing with the primer CnKGDEST117. In CGD x CCNT (Fig. 2D) and MYD x SNRT (Fig. 2E), out of four progenies, two pure hybrids and two offtypes were identified using the primer CnKGDEST117. The same primer, CnKGDEST117, was used for the assessment of hybrid purity in MOD x SNRT (Fig. 2F) and MGD x CCNT (Fig. 2G) which showed that out of four F₁ progenies, only one was a true hybrid with the others being offtypes. Assessment of progenies of COD x SNRT with the primer CnKGDEST117 revealed that there was an offtype among the four F₁ progenies (Fig. 2H). In the cross between GBGD
and SNRT, when tested with the primer CnKGDEST117, a total of three selfed progenies were detected among the four F$_1$ progenies tested (Fig. 2I).

Identifying hybrids in an early stage is of prime importance for breeders; using morphological markers for this purpose is an unreliable method to identify a hybrid mainly due to the fact that the morphological traits are limited, display dominant expression thus reducing statistical capability, are influenced by the environment and they might change according to the development phase of the plant (Kumar et al., 2009). Despite these disadvantages, morphological traits like petiole colour, days taken for germination, seedling vigour in terms of leaf production and higher collar girth over a specific duration are still utilized for identification of hybrids in coconut (Rajesh et al., 2014). With reference to a perennial crop like coconut, it is also of utmost importance that proper hybrid identification be done at an early stage due to the long time that it takes to grow, flower and
bear fruit. Commercial hybrids are hugely popular in coconut with both public and private sectors being actively involved in the development of hybrids. This necessitates strict quality control with respect to monitoring seed genetic purity at various production stages for the success of hybrid technology among stakeholders.

Presently, EST-SSRs have emerged as an important category of molecular markers due to their ease of availability, their hyper variability nature, their aptness for high throughput analysis, their high rate of polymorphism and cross-transferability in comparison to other available markers (Poczai et al., 2013). EST-derived SSR markers possess great potential for use in marker assisted selection (MAS), for developing high yielding varieties, molecular mapping and quantitative trait loci (QTL) analysis (Varshney et al., 2005). In coconut, they are few reports on identification of EST-SSR markers in coconut (Xiao et al., 2013; Xia et al., 2014). However, the present study is the first report of hybrid authentication studies in coconut utilizing EST-SSR markers. Furthermore, the markers identified through this study could be utilized in assessments of purity of hybrid seedlings and identification and subsequent elimination of selfed progenies from seedling nurseries, resulting in considerable economy with respect to time and resources.

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References
Antonova, T.S., Guichet, S.Z., Tchelustnikova, T.A. and Ramasanna, S.A. 2006. Development of marker system for identification and certification of sunflower lines and hybrids on the basis of SSR analysis. Helia 29(45): 63-72.

Arunachalam, V. and Rajesh, M.K. 2008. Breeding of coconut palm (Cocos nucifera L.). CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources no. 053. p. 1-12.

Bourdeix, R., Leclerc, C., Thampan, P.K., Baudouin, L. and Joly, H. 2008. Modern and natural coconut hybrids in southern India: Natural, technical and social facts. Journal of Ethnobiology 28(1): 39-54.

Gouri Shankar, V., Venkata Ramana Rao, P., Bindu Priya, P., Nagesh Kumar, M.V., Ramanjaneyulu, A.V. and Vishnuvardhan Reddy, A. 2013. Genetic purity assessment of castor hybrids using EST-SSR markers. SABRAO Journal of Breeding and Genetics 45(3): 504-509.

Jerard, B.A., Niral, V., Samsudeen, K., Nair, R.V., Jayabose, C. and Thomas, G.V. 2015. Development of a Dwarf x Tall coconut hybrid ‘Kalpa Samrudhi’. Journal of Plantation Crops 43(1): 46-52.

Kumar, P., Gupta, V.K., Misra, A.K., Modi, D.R. and Pandey, B.K. 2009. Potential of molecular markers in plant biotechnology. Plant Omics Journal 2(4): 141-162.

Labouisse, J., Sileye, T., Bonnot, F. and Baudouin, L. 2011. Achievements in breeding coconut hybrids for tolerance to coconut foliar decay disease in Vanuatu, South Pacific. Euphytica 177(1): 1-13.

Meerow, A.W., Wisser, R.J., Brown, J.S., Kuhn, D.N., Schnell, R.J. and Broschat T.K. 2003. Analysis of genetic diversity and population structure within Florida coconut (Cocos nucifera L.) germplasm using microsatellite DNA, with special emphasis on the Fiji Dwarf cultivar. Theoretical and Applied Genetics 106(4): 715-726.

Nair, M.K., Koshy, P.K., Jacob, P.M., Nair, R.V., Bhaskara Rao, E.V.V., Namboothiri, K.U.K. and Iyer, R.D. 1996. A root (will) disease resistant coconut hybrid and strategy for resistance breeding. Indian Coconut Journal 27: 2-5.

Narayana, G.V. and John, C.M. 1949. Varieties and forms of the coconut. Madras Agricultural Journal 36: 349-366.

Naresh, V., Yamini, K.N., Rajendrakumar, P. and Dineshkumar, V. 2009. EST-SSR marker based for the genetic purity assessment of safflower hybrids. Euphytica 170(3): 347-353.

Perera, L. 2010. Hybrid testing and variety identification of coconut (Cocos nucifera L.) in Sri Lanka using microsatellite markers. CORD 26(1): 39-46.

Perera, L., Russell, J. R., Provan, J. and Powell, W. 2000. Use of microsatellite DNA markers to investigate the level of genetic diversity and population genetic structure of coconut (Cocos nucifera L.). Genome 43(1): 15-21.

Poczai, P., Varga, I., Hyvonen, J. and Bell, N.E. 2012. Genomics meets biodiversity: Advances in molecular marker development and their applications in plant genetic diversity assessment. In: The Molecular Basis of Plant Genetic Diversity. (Ed.) Caliskan, M. Intech Publishers. DOI: 10.5772/33614.

Pranavi, B., Sitaram, G., Yamini, K.N. and Dineshkumar, V. 2011. Development of EST-SSR markers in castor bean (Ricinus communis L.) and their utilization for genetic purity testing of hybrids. Genome 54(8): 684-691.

Preethi, P., Rajesh, M.K., Naganeeswaran, S., Shafeeq Rahman, Karun, A. 2014. Identification of EST-SSRs
in coconut (Cocos nucifera L.) by deep transcriptome sequencing. In: Book of Abstracts of National Seminar on New Horizons and Challenges in Biotechnology and Bioinformatics. (Eds.) Rajesh, M.K., Muralikrishna, K.S., Karun, A. and Chowdappa, P. CPCRI, Kasaragod, India.

Rajesh, M.K., Arunachalam, V., Nagarajan, P., Lebrun, P., Samsudeen, K. and Thamban C. 2008a. Genetic survey of 10 Indian coconut landraces by simple sequence repeats (SSRs), *Scientia Horticulturae* **118**(4): 282-287.

Rajesh, M.K., Jerard, B.A., Preethi, P., Thomas, R.J. and Karun A. 2014. Application of RAPD markers in hybrid verification in coconut. *Crop Breeding and Applied Biotechnology* **14**(1): 36-41.

Rajesh, M.K., Jerard, B.A., Preethi, P., Thomas, R.J., Fayas, T.P., Rachana, K.E. and Karun, A. 2013. Development of a RAPD-derived SCAR marker associated with tall-type palm trait in coconut. *Scientia Horticulturae* **150**: 312-316.

Rajesh, M.K., Nagarajan, P., Jerard, B.A., Arunachalam, V. and Dhanapal R. 2008b. Microsatellite variability of coconut accessions from Andaman and Nicobar Islands. *Current Science* **94**(12): 1627-1631.

Rajesh, M.K., Thomas, R.J., Rijith, J., Shareefa, M. and Jacob P.M. 2012. Genetic purity assessment of D x T hybrids in coconut with SSR markers. *Indian Journal of Genetics and Plant Breeding* **72**(4): 472-474.

Rajesh, M.K., Rachana, K.E., Naganeeswaran, S., Shafeeq, R., Thomas, R. J., Shareefa, M., Merin, B. and Anitha Karun (2015) Identification of expressed resistance gene analog sequences in coconut leaf transcriptome and their evolutionary analysis. *Turkish Journal of Agriculture and Forestry* **39**: 489-502.

Rivera, R., Edwards, K.J., Barker, J.H.A., Arnold, G.M., Ayad, G., Hodgkin, T. and Karp A. 1999. Isolation and characterization of polymorphic microsatellites in *Cocos nucifera* L. *Genome* **42**(4): 668-675.

Sundaram, R.M., Naveen Kumar, B., Biradan, S.K., Balachandran, S.M., Mishra, B., Ilyas Ahamed, M., Viraktamath, B.C., Ramesha, M.S. and Sharma, N.P. 2008. Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. *Euphytica* **163**(2): 215-224.

Thomas, R.J., Jacob P.M. and Nair R.V. 2010. Kalpa Sankara, a coconut hybrid (CGD X WCT) suitable for the root (wilt) disease prevalent tract of Kerala. *Plant Horti Tech* **10**: 28-31.

Varshney, R.K., Sigmund, R., Börner, A., Korzun, V., Stein, N., Sorrells M.E., Langridge P. and Graner, A. 2005. Interspecific transferability and comparative mapping of barley EST-SSR markers in wheat, rye and rice. *Plant Science* **168**(1): 195-202.

Xia, W., Zheng, L., Yi, L., Annali, S.M., Haikuo, F., Yaodong, Y., Songlin, Z. and Ming, P. 2014. Development of gene-based simple sequence repeat markers for association analysis in *Cocos nucifera*. *Molecular Breeding* **34**(2): 525-535.

Xiao, Y., Luo Y., Yang, Y., Fan, H., Xia, W., Mason, A.S., Zhao, S., Sager, R. and Fei Q. 2013. Development of microsatellite markers in *Cocos nucifera* and their application in evaluating the level of genetic diversity of *Cocos nucifera*. *Plant Omics Journal* **6**(3): 193-200.