

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

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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, hyperpolymorphism 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

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

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 offtypes 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

| KU999099 GGAGCCTO, TCGCCCAAAGCCACCTOTAT KU999090 (TC)1 AAAGAGTAGCGAAAGCACCTCCTAT KX580069 (AAT)1,3 TGAAGACGCGGGTGAGGTTGGA KX580070 (TC)2,3 ACGGCTTCCTCTCAGCTCTTGGA KX580071 (TC)3,4 ACGGCTTCCTCTCAGCGAAAGCAAA KX580071 (TC)3,5 TGGCCTCAGCGAAAGCAAA KU999092 (GAGCG)4 TGGCCTCAGCGAAAGCAAA KU999093 (TA)3,4 ACCCCAATGCCCTCTTCTTTTTTTTTTTTTTTTTTTTTT | SI. | Primer Name | NCBI accession | Repeat type | Forward primer (5'-3') | Reverse primer (5'-3') | Functional annotation | Annealing temperature |
|---|-----|----------------|-------------------|---|-----------------------------|------------------------------|--|--------------------------|
| CIRGDESTR1 KU999089 (GCGACCT) ₀ TCGCCCAAAGCCACCTCCTAT CIRGDESTR2 KU999090 (TC) ₂ AAAGAGTAGCCAAAGCAAGTTTCAAGC CIRGDESTR3 KX580070 (TC) ₂ AAGAGTAGACCTCCTAA CIRGDESTR9 KX580070 (TC) ₂ ACGGCTTCCTCTCAGCCTCCAA CIRGDESTR9 KU999091 (TTC) ₂ TGGGCTCCTCTCAGCCTCCTCT CIRGDESTR10 KX580071 (TC) ₂ TGGCCTCAGCGAAAGGAGAA CIRGDESTR10 KX980071 (TC) ₂ ACGGCTTCCTCTCAGCCAAAGGAAA CIRGDESTR10 KV999093 (TA) ₂ ACGCCCAATGGCCACTTGTGAAC CIRGDESTR10 KU999094 (TC) ₂ ACGCCCAATGGCCCTTCTTTGTGAAC CIRGDESTR10 KU999098 (TC) ₂ ACGCCCAATGGCACCTTCTTTGGAAC CIRGDESTR19 KU999098 (TC) ₂ ACTTGTTGGAATGGACCACCACTAGGAAAA CIRGDESTR19 KU999100 (TC) ₂ ACTTGTGTGAACTAGCACACACACACACACACACACACAC | | | number | | , | | | (°C) |
| ChKGDEST82 KU999090 (TC) ₁₁ AAAGAGTAGCGAAAGCTACAGCC ChKGDEST84 KX580090 (TC) ₁₁ TGAAGACGGGGTGAGGTTGAGA ChKGDEST98 KX580070 (TC) ₂₂ ACGCCTTCCTCTCAGCCTCAGCTGAGGGAGA ChKGDEST100 KU999091 (TTC) ₁₂ TGGGATAGACCTTGTTGCTAT ChKGDEST101 KX580071 (TC) ₁₃ ACGCCACACCACCTCTTCTT ChKGDEST101 KX580071 (TC) ₁₃ ACGCCACACCACTCTTTTT ChKGDEST103 KU999092 (GAGGG) TGGCCTCGGCACCACCTCTTTTTTTTTTTTTTTTTTTTT | 1 | CnKGDEST81 | KU999089 | (GCGACCT), | TCGCCCAAAGCCACCCTCCTAT | TCGCCCGCAGGGAAAAATCCAC | DNA polymerase I | 58 |
| ChKGDEST84 KX580069 (AAT) ₁₃ TGAAGACGGGGTGAGGTTGGA ChKGDEST87 KX580070 (TC) ₂₀ ACGCTTCCTCTCAGCCTCATTGCTAT ChKGDEST10 KU999091 (TTC) ₁₃ ACGCCTTCCTCAGCGAAAGGGAAA ChKGDEST101 KX580071 (TC) ₁₃ AGGCCTGCACCTCTTCTT ChKGDEST101 KX580071 (TC) ₁₃ AGGCCTGCACCTCTTCTT ChKGDEST101 KU999093 (TD) ₂₁ ACGCCATCGCACTCTTCTT ChKGDEST95 KU999094 (TD) ₂₁ ACGCCACCCCTTCTCTTCTT ChKGDEST96 KU999099 (TD) ₂₁ ACGCCACCATTGGACCCCCTTTCTT ChKGDEST97 KU999099 (TD) ₂₁ TCTGATGGACCCCCATTGGCAC ChKGDEST19 KU99909 (TO) ₂₁ AGACCTCATGCACTTTGGAC ChKGDEST19 KU999100 (TO) ₂₁ TGTCCATGCTTTTGGACACTTTGCCACCACACACACACAC | 2 | CnKGDEST82 | KU999090 | $(TC)_{21}$ | AAAGAGTAGCGAAAGCAAGTTTCAAGC | TGGACAAAGAGACAGACAGAC | Keratin-associated protein 10-12-like | 09 |
| ChKGDEST87 KX380070 (TO) ₂₀ ACGGCTTCCTCTCAGCCTCAA ChKGDEST96 KU999901 (TTO) ₂₀ TGGGATAGACCTTGGTCTGTTGTA ChKGDEST101 KU999092 (GAGCG) TGGCCTCAGCACCACTTCTTCTT ChKGDEST101 KX380071 (TO) ₂₀ AGGCCTGGCACCACTTCTTCTT ChKGDEST103 KU999094 (TO) ₂₀ ACGCCAATGCCCGTGTGTGAAC ChKGDEST104 KU999094 (TO) ₂₀ ACGCCAATGCCCGTGTGTGAAC ChKGDEST105 KU999096 (TO) ₂₁ TCTGATGGCACCACTTGGAC ChKGDEST106 KU999099 (TO) ₂₀ TGTGATGCCCTTTTGGAC ChKGDEST107 KU999099 (GA) ₂₀ ACTTGTTGGGATGGGCAC ChKGDEST119 KU999100 (TO) ₂₀ TGTCCATGCTTTTGGACTGCAC ChKGDEST119 KU999100 (TO) ₂₀ TGTCCATGCTTTTGGACTGCAC ChKGDEST119 KU999100 (TO) ₂₀ TGTCCATGCTCCACCCCAGGAAA ChKGDEST119 KU999100 (TO) ₂₀ TCTCTGCTCCACCCCATGGTCACCACCCACGGAAAA ChKGDEST113 KU999100 (TO) ₂₀ TCTCTGCTCCACCCCCATGGTCACCCCCACGGAAAA ChKGDEST113 KU999100 | 3 | CnKGDEST84 | KX580069 | | TGAAGACGCGGGTGAGGTTGGA | GGGAGCCAAAGGTGTCAAGGCA | Leucine-rich repeat | 65 |
| ChKGDEST96 KU999091 (TC)30 TGGGATGGACCTGGTGTGCTAT ChKGDEST100 KU999092 (TC)30 TGGGATGGACCTGGTGTGTAGAC ChKGDEST101 KX380071 (TC)31 TGGCCTGGCACCTGTTGTAGAC ChKGDEST103 KU999092 (TC)32 ACCCCAATGCCCGTGTTGAAC ChKGDEST104 KU999093 (TA)32 ACGCACCCAATTGGGTCAGACG ChKGDEST105 KU999094 (TO)32 TCTGATGGCACCCCGTTTGGAG ChKGDEST106 KU999099 (TA)32 TCTGATGGCACCCGCATTGGAG ChKGDEST106 KU999099 (GA)32 ACTTGTTGGGATGGGTCACCCACGCACCACGCACACGCACACGCACACACA | 4 | CnKGDEST87 | KX580070 | (TC) | ACGGCTTCCTCAGCCTCCAA | TGCCACTTGCCGGTGAAAAAGGT | Glycosyl transferase | 59 |
| ChKGDESTI00 KU999092 (GAGGCG), TGGCCCTCAGCGAAAGGGAGAA ChKGDESTI01 KX580071 (TC)3,3 AGGCCTGGCACCCCTCTTCTT ChKGDESTI03 KU999092 (TA)3,3 ACGCCACCACTGCCCGTGTGAAC ChKGDEST103 KU999093 (TA)3,4 ACGCCACCATTGGGTCAGCG ChKGDEST196 KU999093 (TA)3,4 TCTGATGGCACCCGCATTGGAG ChKGDEST197 KU999099 (TA)3,4 ACGCCACACGTGTCTTTTGGCA ChKGDEST198 KU999099 (GA)3,4 AGGACCACACGTGCCCTTTTGGCA ChKGDEST198 KU999100 (TC)3,4 AGCTCCATGCTTTTTGGCACCACCACCACACACACACACA | ٠ ٧ | CnKGDEST96 | KU999091 | (TTC) | TGGGATAGACCTTGGTCTGTTGCTAT | TTCTCGCATCGTTCGTGATCCTG | Serine threonine protein | 57 |
| ChKGDESTI01 KX580071 (TC) ₁₃ AGGCCTGGCACCACTCTTCTT ChKGDEST103 KU999093 (TA) ₂₃ ACCCCAATGCCCGTGTGTGAAC ChKGDEST104 KU999093 (AT) ₂₁ ACCGCAACACTGGGTCAGAC ChKGDEST105 KU999099 (AT) ₂₁ TCTGATGGCACCAGTGTTGAGC ChKGDEST106 KU999099 (GT) ₂₁ TCTGATGGCACCAGTGTTTTTGGCA ChKGDEST198 KU999099 (GA) ₂₀ ACTTGTTGGCATCCTTTTGGCA ChKGDEST119 KU999100 (TC) ₂₁ TGTCCATGCTTTTTGGCACCACCACCACCACCACCACACACA | 9 | CnKGDEST100 | KU999092 | (GAGGCG), | TGGCCCTCAGCGAAAGGGAGAA | ACCGACGAGAATGGCGGTCTCT | 50s ribosomal protein 13 | 59 |
| ChKGDEST103 KU999093 (Th)20 ACCCCAATGCCCGTGTGTGAAC ChKGDEST95 KU999094 (TD)20 ACGGCACCAATTGGGTCAGAC ChKGDEST95 KU999094 (TD)21 TCTGATGGCACCACTTGGAG ChKGDEST90 KU999099 (TD)21 TGGATATCACAGCCTTTTGGAG ChKGDEST98 KU999099 (GA)20 TGGATATCACAGCCTTTTTTTGGCA ChKGDEST19 KU999099 (GA)20 ACTTGTTGGGATGGCACCACTCATTTTTTTTTTTTTTTT | 7 | CnKGDEST101 | KX580071 | (TC), | AGGCCTGGCACCACCTCTTCTT | ATCGAGGCAGCCCACCTTACT | bhlh transcription factor-like protein | 59 |
| ChKGDEST195 KU999094 (TC)20 ACGGCACCAATTGGGTCAGACG ChKGDEST106 KU999095 (AT)21 TCTGATGGCACCCGCATTGGAG ChKGDEST108 KU999096 (CT)21 TGGATATCACAGCCCTTCCATGCT ChKGDEST198 KU999097 (TA)20 GGCACAACCAGTGTCTCTTTTGGCA ChKGDEST198 KU999099 (GA)20 AGACCCTCATGCACTCTTTTTGGCA ChKGDEST119 KU999100 (TC)21 TGTCCATGCTTTTTGTGGATGTGGG ChKGDEST119 KU999100 (TC)21 TGTCCATGCTTTTTGTGGATGTGGG ChKGDEST113 KX880072 (TC)20 TGTCCATGCTTTTTGTGGATGGGG ChKGDEST128 KU999100 (TC)20 ACTTGTTGCTGCTGCTGCACCAGGG ChKGDEST113 KX880072 (TC)20 ACTCTGCTGCTCTCCAGGAGGT ChKGDEST113 KX999100 (TC)20 ACTCTGCTGCTCTCCAGGAGGT ChKGDEST13 KU999100 (TC)20 TCTTCTTCCTCGTCACCAGGAGGT ChKGDEST13 KX880070 (TC)20 TCTTCTTCCTCGTTCACAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG | ∞ | CnKGDEST103 | KU999093 | (TA) ₃₃ | ACCCCAATGCCCGTGTGTGAAC | AGGGCTAAGATTGCAGTGACCCT | Midasin | 59 |
| ChKGDEST106 KU999095 (AT)21 TCTGATGGCACCCGCATTGGAG ChKGDEST85 KU999096 (CT)21 TGGATATCACAGCCCTTCCATGCT ChKGDEST90 KU999097 (TA)20 GGCACAACCAGTGTCTTTGGCA ChKGDEST19 KU999099 (GT)21 AGACCCTCATGCACTGGCCAC ChKGDEST119 KU999100 (TC)21 TGTCCATGCTTTTGTGGATGTGGC ChKGDEST119 KU999101 (GA)21 GCATTGTTGGGATGTGGCA ChKGDEST112 KU999101 (TG)21 TGTCCATGCTTTTTGTGGATGTGGGT ChKGDEST112 KU999101 (TG)21 ACTCTGCTGCTTTTTGTGGATGTGGGT ChKGDEST113 KU999102 (TG)21 ACTCTGCTGCTTTTTCCAGCAAAA ChKGDEST136 KU999103 (AG)22 ACTCTGCTGCTCTCCACCCAGGGTG ChKGDEST136 KU999104 (TC)21 TCTCTTCCTCCACCCAGGGTG ChKGDEST136 KU999104 (TC)21 TGCACGGGGCGTTCACAGACACCACACACACACACACACA | 6 | CnKGDEST95 | KU999094 | $(TC)_{20}$ | ACGGCACCAATTGGGTCAGACG | TTGGTGCAGTTTCTTGGCCCCC | Phosphomethyl pyrimidine synthase, chloroplastic | 28 |
| ChKGDEST85 KU999096 (CT)21 TGGATATCACAGCCCTTCCATGCT ChKGDEST90 KU999097 (TA)22 GGCACAACCAGTGTCTCTTTGGCA ChKGDEST198 KU999098 (GT)22 AGACCCTCATGCACTAGGCCAC ChKGDEST199 KU999100 (TO)21 ACTTGTTGGGATGGGTGCGCAC ChKGDEST119 KU999101 (TO)22 TGTCCATGGTTTTTGTGGATGGGT ChKGDEST119 KU999101 (TO)22 ACTCTGCTGCTTTTTGTGGATGGGT ChKGDEST1126 KU999102 (TG)22 ACTCTGCTGCTTTTTCCAGACAGGT ChKGDEST1126 KU999103 (TO)22 ACTCTGCTGCTTTTTCCAGACAGGT ChKGDEST1126 KU999103 (TC)22 ACTCTGCTGCTTTTTCCAGACAGGT ChKGDEST1126 KU999103 (TC)22 TGCATCAACTGCTCCCCCAGGAGTG ChKGDEST1127 KU999103 (TC)22 TGCATCAACCCCCAGGAGTG ChKGDEST113 KU999104 (TC)23 TGCAGCACATGCGTTCACCACACACACACACACACACACA | 10 | CnKGDEST106 | | $(AT)_{21}$ | TCTGATGGCACCCGCATTGGAG | TCATCCAAGACTGCCACACGCC | ACT domain containing protein | 59 |
| ChKGDEST90 KU999097 (Th)20 GGCACAACCAGTGTCTCTTTGGCA ChKGDEST198 KU999098 (GT)23 AGACCCTCATGCACTAGGCCAC ChKGDEST119 KU999100 (TC)21 TGTCCATGCTTTTGTGGATGGGT ChKGDEST112 KU999101 (GA)21 CGCATGGGAGCTGAGGCAAAA ChKGDEST112 KU999101 (TC)20 GCAGTTTGACTGCTGCATTTGC ChKGDEST112 KU999102 (TC)21 AGCTCCAGTCCACTGCATTTGC ChKGDEST113 KU999103 (AG)22 AGCTCCAGTCCACTGCATTTGCC ChKGDEST113 KU999104 (TC)23 TCCACGGTGCCCTATTGGTCA ChKGDEST113 KU999104 (TC)23 TCCACGGTGCCCTATTGGTCA ChKGDEST113 KU999106 (TC)23 TCTTCTTCCTCGTCTCCTCCTCCCCCCCCCCCCCCCCC | Ξ | CnKGDEST85 | KU999096 | $(CT)_{21}$ | TGGATATCACAGCCCTTCCATGCT | GTTGCAGTTTGGTGCATGTGAAAGAT | Squalene synthase | 57 |
| ChKGDEST98 KU999098 (GT) ₂₁ AGACCCTCATGCACTAGGCCAC ChKGDEST199 KU999100 (TC) ₂₁ ACTTGTTGGGATAGGCCGC ChKGDEST110 KU999100 (TC) ₂₁ TGTCCATGCTTTTGTGGATGGGT ChKGDEST112 KX580072 (TC) ₂₀ GCAGTTTGACTGCTGCACTTTGCC ChKGDEST126 KU999102 (TG) ₂₁ ACTCTGCTGCTTTTCCAGACAGGT ChKGDEST136 KU999104 (TC) ₂₀ ACTCTGCTGCTTTTCCAGACAGGT ChKGDEST136 KU999104 (TC) ₂₀ ACTCTGCTGCTCCACCCAGGAAT ChKGDEST136 KU999107 (ACAAAACA) ₆ TGCAGAAACCAGGAAT ChKGDEST137 KU999106 (TC) ₂₀ TCTTCTTCCTCGTCCTCCTCCCCCCCCCCAGGAAT ChKGDEST117 KX580073 (TGGAGCA) ₆ TCTTCTTCCTCGTTCCTCCCCCCCCCCCCCCCCCCCCC | 12 | CnKGDEST90 | KU999097 | $(TA)_{20}$ | GGCACAACCAGTGTCTCTTTGGCA | GTGGCTTTGCCTCCCCATGCTT | F-box protein SKP2A-like | 59 |
| Chrigdestig KU99909 (GA) ₂₀ ACTTGTTGGGATAGGGTGCGGC Chrigdestig KU999100 (TC) ₂₁ TGTCCATGCTTTTGTGGATGTGGT Chrigdestig KU999101 (GA) ₂₁ CGCATGGGAGCTGAGGCAAAA Chrigdestig KU999102 (TC) ₂₀ GCAGTTTGACTGCTGCACTTTGCC Chrigdestig KU999102 (TG) ₂₁ ACTCTGCTGTTTTCCAGACAGGT Chrigdestig KU999103 (AG) ₂₂ ACTCTGCTGCTTTTCCAGAAGGT Chrigdestig KU999104 (TC) ₂₀ TGCATAAACGGGCAGTG Chrigdestig KU999106 (TC) ₂₀ TGCATAAACCGGGCAGTG Chrigdestig KU999106 (TC) ₂₀ TGCATAAACCGGGCAGTGGTG Chrigdestig KU999106 (TC) ₂₀ TGTATCTTCCTCCTCCTCCTCCCCCCCCCCCCCCCCCCC | | CnKGDEST98 | KU999098 | $(GT)_{23}$ | AGACCCTCATGCACTAGGCCAC | TGGCCAACACCAGGAATTTGT | Isovaleryl-CoA dehydrogenase, mitochondrial | 58 |
| CnkGDEST107 KU999100 (TC)21 TGTCCATGCTTTTGTGGATGTGGT CnkGDEST119 KU999101 (GA)21 CGCATGGGAGCTGAGGCAAAA CnkGDEST123 KX580072 (TC)20 ACTCTGCTGCTGTTTCCAGACAGGT CnkGDEST136 KU999102 (TG)21 ACTCTGCTGCTTTTCCAGACAGGT CnkGDEST139 KU999103 (AG)22 ACTCTGCTGCTTTTCCAGACAGGT CnkGDEST142 KU999104 (TC)23 TGCAGAAACCGGGCAGTCGCCCAGGGAAT CnkGDEST143 KU999104 (TC)20 TGCATAAACCGGGCAGTCGCCCAGGGAAT CnkGDEST143 KU999106 (TC)20 TGCATAAACCGGGCAGTCGCTCCCCCACCCACCCACCCAC | 14 | CnKGDEST199 | KU999099 | $(GA)_{20}$ | ACTTGTTGGGATAGGGTGCGGC | TCCACCATGCCCACAACAGTGC | Glutamate receptor 3.5 isoform X1 | 59 |
| CnkGDEST119 KU999101 (GA)21 CGCATGGGAGGCTGAGGCAAA CnkGDEST123 KX580072 (TC)20 GCATTTGACTGCTGCACTTTGCC CnkGDEST126 KU999102 (TG)21 ACTCTGCTGCTTTTCCAGACAGGT CnkGDEST136 KU999103 (AG)22 ACTCTGCTGCTTTTCCAGACAGGT CnkGDEST137 KU999104 (TC)23 TGCATAAACCGGGCAGTCGGTG CnkGDEST142 KU999106 (TC)20 TGTTCTTCCTCGTCTCCTCCTCCTCCTCCTCCTCCTCCTC | | CnKGDEST107 | KU999100 | $(TC)_{21}$ | TGTCCATGCTTTTGTGGATGTGGGT | TGTGTGTGTGTGTGTGTGT | Serine/arginine-rich splicing factor SR30 |) 59 |
| CnKGDEST123 KX580072 (TG) ₂₀ GCAGTTTGACTGCTGCACTTTGCC CnKGDEST136 KU999102 (TG) ₂₁ ACTCTGCTGCTTTTCCAGACAGGT CnKGDEST139 KU999103 (AG) ₂₂ AGCTCCAGTCCACCCAGGAAT CnKGDEST142 KU999104 (TC) ₂₃ TCCACGGTGCCCTATTGGTCA CnKGDEST143 KU999106 (TC) ₂₀ TCTTCTTCCTCGTCTCCCCACC CnKGDEST143 KU999107 (AAAAACA) ₃ TGCAGCCACTGGGTGCACC CnKGDEST115 KU999108 (TGAG TGCAGCCACTGGTTCACACACC CnKGDEST117 KX580073 (TGGAGC) ₇ AGGTTGGTTGAGCGTTCACCACACCCACCCACCCACCCAC | | CnKGDEST119 | KU999101 | $(GA)_{21}$ | CGCATGGGAGGCTGAGGCAAAA | AAGGGCCTCTTCCCATGCCTT | Ethylene-responsive transcription factor ERF113 | 89 |
| CnkGDEST126 KU999102 (TG)21 ACTCTGCTGCTGCTTTCCAGACAGGT CnkGDEST136 KU999103 (AG)22 AGCTCCAGTCCACCCAGGAAT CnkGDEST139 KU999104 (TC)23 TCCACGGTGCCCCTATTGGTCA CnkGDEST142 KU999105 (GGCGGA)6 TCTCTCTCCTCGTCTCCACCC CnkGDEST143 KU999106 (TC)20 TCTTCTTCCTCGTCTCCACCC CnkGDEST115 KU999107 (AAAAAACA)5 TGCAGCCACATGCGTTCACAGA CnkGDEST115 KU999108 (TGAGC)7 AGGTTGGTTGAGCACACACACACACACACACACACACACA | 17 | CnKGDEST123 | KX580072 | $(TC)_{20}$ | GCAGTTTGACTGCTGCACTTTGCC | ACACACACACACACACACACACA | Early flowering 3-like isoform X1 | 59 |
| CnKGDEST136 KU999103 (AG)22 AGCTCCAGTCCACCCACGGAAT CnKGDEST139 KU999104 (TC)23 TCCACGGTGCCCCTATTGGTCA CnKGDEST142 KU999105 (GGCGGA)6 TGCATAAACCGGGCAGTCGGTG CnKGDEST143 KU999106 (TC)20 TCTTCTTCCTCGTCTCCTCCACCC CnKGDEST115 KU999107 (AAAAAACA)5 TGCAGCCACATGCGTTCACAGA CnKGDEST117 KX580073 (TAGA)10 CCGCCTCGGTTCAACAAAACCA CnKGDEST117 KX580074 (CATA)10 CCGCCTCGGTTCAACAAAACCA CnKGDEST122 KX580074 (CATA)10 CCGCCTCGGTTGGAAGCCT CnKGDEST124 KX580076 (AG)21 ACACACACACACACACACACACACACACACACACACAC | 18 | CnKGDEST126 | KU999102 | $(TG)_{21}$ | ACTCTGCTGCTTTTCCAGACAGGT | AGTTAACAGAATCACATTGGCGGACA | UV-damaged dna-binding | 58 |
| ChKGDEST139 KU999104 (TC)23 TCCACGGTGCCCCTATTGGTCA ChKGDEST142 KU999105 (GGCGGA)6 TGCATAAACCGGGCAGTCGGTG ChKGDEST143 KU999106 (TC)20 TCTTCTTCCTCCTCCTCCTCCACCC ChKGDEST115 KU999107 (AAAAAACA)3 TGCAGCCACTGCGTTCACAGA ChKGDEST117 KX580073 (TGGAGC)7 AGGTTGGTTGAGCACACACACACACACCAACCCAACCCA | 19 | CnKGDEST136 | KU999103 | $(AG)_{22}$ | AGCTCCAGTCCACCACGGAAT | TCCTTCCCTCCATCATTCCCTCTCT | Abscisic acid 8-hydroxylase | 58 |
| CnkGDEST142 KU999105 (GGCGGA) ₀ TGCATAAACCGGGCAGTCGTG CnkGDEST143 KU999106 (TC) ₂₀ TCTTCTTCCTCGTCTCCTCCACC CnkGDEST115 KU999107 (AAAAACA) ₅ TGCAGCCACATGCGTTCACAGA CnkGDEST117 KX580073 (TGGAGC) ₇ AGGTTGGTTGAGCGTACACAAACCCA CnkGDEST117 KX580074 (CATA) ₁₀ CCGCCTGGTTCAACAAACCCA CnkGDEST127 KX580074 (TC) ₂₅ TATGCTGGTAAGCGTTGGAATGTCCT CnkGDEST124 KX580076 (AG) ₂₁ ACACACACACACACACACACACACACACACACACACAC | | CnKGDEST139 | KU999104 | $(TC)_{23}$ | TCCACGGTGCCCCTATTGGTCA | A CGAGAAAGGAGACAAAATGGGGAAAA | Riboflavin biosynthesis protein riba | 59 |
| ChKGDEST143 KU999106 (TC) ₂₀ TCTTCTTCCTCGTCTCCCCCCC ChKGDEST91 KU999107 (AAAAAACA) ₅ TGCAGCCACATGCGTTCACAGA ChKGDEST115 KU999108 (TGGAGC) ₇ AGGTTGGTTGAGGACTTCACAGA ChKGDEST117 KX580073 (TGAA) ₁₀ CCGCCTCGGTTCAACAAACCCA ChKGDEST124 KX580074 (CATA) ₁₀ CCGCTTGGTAAGCGTTGGAATGTCCT ChKGDEST124 KX580076 (AG) ₂₁ ACACACACACACACACACACACACACACACACACACAC | 21 | CnKGDEST142 | KU999105 | (GGCGGA), | TGCATAAACCGGGCAGTCGGTG | CCCCGCCCATTGAAATCGGAA | Methyltransferase- like protein | 59 |
| ChKGDEST91 KU999107 (AAAAACA) ₅ TGCAGCCACATGCGTTCACAGA ChKGDEST115 KU999108 (TGGAGC) ₇ AGGTTGGTTGAGCAGACTT ChKGDEST117 KX580073 (TAGA) ₁₀ CCGCCTCGGTTCAACAAACCCA ChKGDEST127 KX580074 (CATA) ₁₀ CC(CATTGGTAAGCGTTGGAATGTCCT ChKGDEST124 KX580075 (TC) ₂₅ TATGCTGGTGCGGAGATGGGA ChKGDEST129 KX580077 (AG) ₂₁ ACACACACACACACACACACACACACACACACACACAC | 22 | CnKGDEST143 | KU999106 | $(TC)_{20}$ | TCTTCTTCCTCGTCTCCTCCACCC | AGAGCTCTCCAGTGGCGACCAA | COP1-interacting protein-related | 58 |
| CnKGDEST115 KU999108 (TGGAGC), AGGTTGGTTGAGGCCGGAGCTT CnKGDEST117 KX580073 (TAGA) ₁₀ CCGCCTCGGTTCAACAAACCCA CnKGDEST137 KX580074 (CATA) ₁₀ CCGCCTGGTTCAACAACCCA CnKGDEST122 KX580075 (TC) ₂₅ TATGCTGGTGCGGAGATGGGA CnKGDEST124 KX580076 (AG) ₂₁ ACACACACACACACACACACACACACACACACACACAC | 23 | CnKGDEST91 | KU999107 | (AAAAACA) ₅ | TGCAGCCACATGCGTTCACAGA | AGCTGGGATGGAAAGCAAAGGGC | Peroxisome biogenesis protein 22-like | 59 |
| CnkGDEST117 KX580073 (TAGA) ₁₀ CCGCCTCGGTTCAACAAACCCA CnkGDEST137 KX580074 (CATA) ₁₀ CC(CA) ₂₁ GCCTTGGTAAGCGTTGGAATGTCCT CnkGDEST122 KX580075 (TC) ₂₅ TATGCTGGTGCGGAGAGTGCGA CnkGDEST124 KX580076 (AG) ₂₁ ACACACACACACACACACACACACACACACACACACAC | 24 | CnKGDEST115 | KU999108 | $(TGGAGC)_{7}$ | AGGTTGGTTGAGGCCGGAGCTT | AGGTGCAACGGGAGCCTCATCT | Ubiquitin receptor RAD23b-like | 09 |
| CnKGDEST137 KX580074 (CATA) ₁₀ CC(CA) ₂₁ GCCTTGGTAAGCGTTGGAATGTCCT CnKGDEST122 KX580075 (TC) ₂₅ TATGCTGGTGCGGAGAGTGGA CnKGDEST124 KX580076 (AG) ₂₁ ACACACACACACACACACACACACACACACACACACAC | 25 | CnKGDEST117 | KX580073 | $(TAGA)_{10}$ | CCGCCTCGGTTCAACAAACCCA | TCCCCACCCACAACCA | Inactive β-amylase 9 | 59 |
| CnKGDEST122 KX580075 (TC) ₂₅ TATGCTGGTGCGGAGAGTGGGA CnKGDEST124 KX580076 (AG) ₂₁ ACACACACACACACACACACACACACACACACACACAC | 56 | CnKGDEST137 | KX580074 | (CATA) ₁₀ CC(CA) ₂₁ | \circ | AACCACATGGCCCCTACTCCCA | Sym-1 like protein | 59 |
| CnKGDEST124 KX580076 (AG) ₂₁ ACACACACACACACACACACACACACACACACACACAC | 27 | CnKGDEST122 | KX580075 | $(TC)_{25}$ | Ε | CGCGTAATTGGCAACTGACATGGG | 2-Hydroxyacyl-CoA lyase-like | 59 |
| ChKGDEST129 KX580077 (AG) ₂₂ AGCTGCAGCACCGAGGATGAGA ChKGDEST130 KX580078 (CT) ₂ GCCATTGGAGGCATGGAAGCCA | 28 | CnKGDEST124 | KX580076 | $(AG)_{21}$ | ACACACACACACACACACA | TGGTTGTCCTTGGATTGCACATGGT | ADP-ribosylation factor GTPase-activating protein AGD13 | 59 |
| ChKGDEST130 KX580078 (CT). GCCATTGGAGGCATGGAAGCCA | | CnKGDEST129 | KX580077 | $(AG)_{22}$ | AGCTGCAGCACCGAGGATGAGA | TGCGTTTCATGCACTCACAAAAGG | Transcription factor MYB3- like | 59 |
| | | CnKGDEST130 | KX580078 | (CT),, | GCCATTGGAGGCATGGAAGCCA | TCTCCTGCTGCCCTCTTCCCTT | GRF1-interacting factor like | 59 |

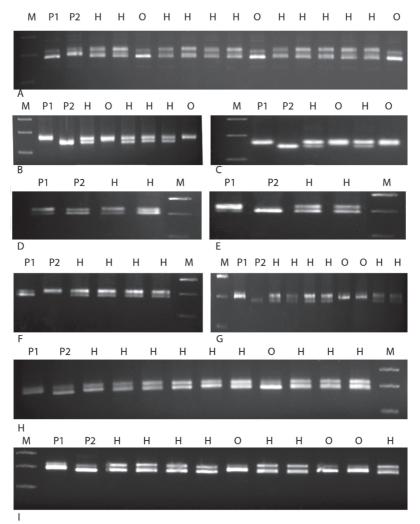


Fig. 1. Gel profile of coconut parents and their hybrids (M: 100bp ladder, P1: Female parent, P2: Male parent, H: Hybrids, O: Offtypes, A: CGD x WCT, B: MYD x TPT, C: COD x WCT, D: GBGD x PHOT, E: GBGD x LCOT, F: LCOT x CCNT, G: GBGD x FJT, H: WCT x COD, I: LCOT x COD)

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 F_1 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 F_1 progenies (Fig. 2H). In the cross between GBGD

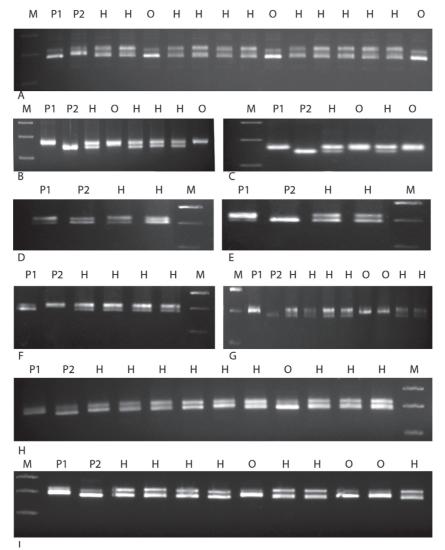


Fig. 2. Gel profile of parents and hybrids
(M: 100bp ladder, P1: Female parent, P2: Male parent, H: Hybrids, O: Offtypes, A: COD x CCNT, B: CRD x CCNT, C: MYD x CCNT, D: CGD x CCNT, E: MYD x SNRT, F: MOD x SNRT, G: MGD x CCNT, H: COD x SNRT, I: GBGD x SNRT)

and SNRT, when tested with the primer CnKGDEST117, a total of three selfed progenies were detected among the four F₁ 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 invovled 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 crosstransferability 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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