Indian J. Genet., 72(4): 472-474 (2012)

Short Communication

Genetic purity assessment of D x T hybrids in coconut with SSR markers

M. K. Rajesh[1,*], R. J. Thomas[2], J. Rijith[1], M. Shareefa[2] and P. M. Jacob[2]

[1]Divison of Crop Improvement, Central Plantation Crops Research Institute, Kasaragod 671 124
[2]CPCRI (Regional Station), Kayamkulam 690 533

(Received : July 2012; Revised : September 2012; Accepted : October 2012)

Abstract

Assessment of genetic purity in coconut hybrids was done using molecular markers. A set of 50 hyperpolymorphic coconut SSR markers were utilized to characterize parental lines Chowghat Green Dwarf (CGD) and West Coast Tall (WCT) used for hybrid coconut production. A panel of 17 informative SSR markers capable of distinguishing these parental lines was identified and these markers were utilized in DxT hybrid seedling purity assessments in coconut nurseries.

Key words: Coconut, hybrids, genetic purity, microsatellite markers

The success of hybrid coconut based farming depends on the timely production and adequate supply of genetically pure hybrid seedlings to the farmers. In the coconut root (wilt) disease affected tracts of Kerala, Chowghat Green Dwarf x West Coast Tall (CGD x WCT) hybrids have performed consistently well with a cumulative average yield of 84 nuts/palm for the past 10 years and showing tolerance to the disease [1].

The CGD x WCT hybrid seedlings are produced commercially by carrying out pollination on CGD mother palms located in farmers' plots. Pollen collected from disease-free WCT palms is used for pollination. A set of morphological descriptors are currently used for varietal identification, description and seed purity assessment. Petiole colour is the most widely used marker to select hybrid seedlings in the nursery stage. Selection of hybrids by petiole colour is reliable only if the progenitors are homozygous for yellow, red or green petiole. Though widely adopted and practised, purity assessments based on morphology is often affected by environment, besides time and resources. Further, many of the varieties and hybrids are phenotypically less distinct than making morphology based distinction difficult. Identification of genuine CGD x WCT hybrids in the nursery stage is the most difficult task in hybrid production as both the progenitors are of the same petiole colour. Also, morphological traits are subjected to environmental effect further limiting their use. Therefore, an alternative technique that offers efficient, quick and reliable assessment of genetic purity is necessary.

The objectives of the present study were (i) to characterize CGD and WCT parental lines used for DxT hybrid coconut production using a set of hyper-polymorphic coconut SSR markers, (ii) to identify informative SSR markers capable of distinguishing the parental lines and (iii) to utilize the selected markers in hybrid seedling purity assessments.

The mother palms of WCT and CGD in the farmer's plots were selected, based on strict selection criteria [2]. Artificial pollination of CGD mother palms, with pollen of WCT, was carried out during January-March 2007 for the production of F_1 hybrid. The mature seed nuts were harvested during 2008, and sown in the nursery. The selected one-year-old hybrid seedlings were planted at CPCRI (RS) Kayamkulam at a spacing

*Corresponding author's e-mail: mkraju_cpcri@yahoo.com
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

of 7.0 meters (plot-to-plot) during August 2009. The parents and progenies from cross D_1xT_1 (PG1xPTA 96) and D_2xT_2 (PG2xPTA99) were used in the present study. A total of 12 progeny plants of D_1xT_1 and 10 of D_2xT_2 were evaluated for hybridity. Four selfed progenies, two plants from each cross, were also included in this study.

Total DNA was extracted from spindle leaves of parents and hybrids. A set of 50 hyperpolymorphic coconut SSR markers (Table 1), distributed in different coconut chromosomes were used for distinguishing between CGD and WCT parental lines used for hybrid coconut seedling production. Microsatellite analysis was carried out as described in Rajesh *et al*. [3]. The polymorphic primers distinguishing the parental lines were then selected and utilized to survey their F_1 hybrids for parentage confirmation. Out of the 50 microsatellite markers tested, 29 markers could unambiguously differentiate the tall and dwarf parents (Table 1, Fig. 1). Seventeen markers could unambiguously clearly differentiate the two WCT parental lines. These selected markers, which exhibited amplification of allele's specific to a parental line, were then used to screen the 22 hybrids and four selfed progenies of the two crosses in order to test the reliability of the SSR markers. Microsatellites being co-dominant, a true hybrid would possess the banding pattern of both the parents. All the

Table 1. Details of SSR primers used in the present study, those showing polymorphism between WCT and CGD palms and also, showing polymorphism between the two WCT parents

S.No.	Primers	Polymorphism tall/dwarf (WCT/CGD)	Polymorphism between WCT parents
1.	CnCir 14	-	-
2.	CnCir H11	-	-
3.	CnCir A3	+	-
4.	CnCir H4	+	-
5.	CnCir C12	-	-
6.	CnCir B12	+	-
7.	CnCir E1	-	-
8.	CnCir A4	-	-
9.	CnCir F3	-	-
10.	CnCir K8	-	-
11.	CnCir G11	+	+
12.	CnCir 74	-	-
13.	CnCir B4	+	+
14.	CnCir E11	-	-
15.	CnCir 87	+	-
16.	CnCir C9	-	-
17.	CnCir E4	+	-

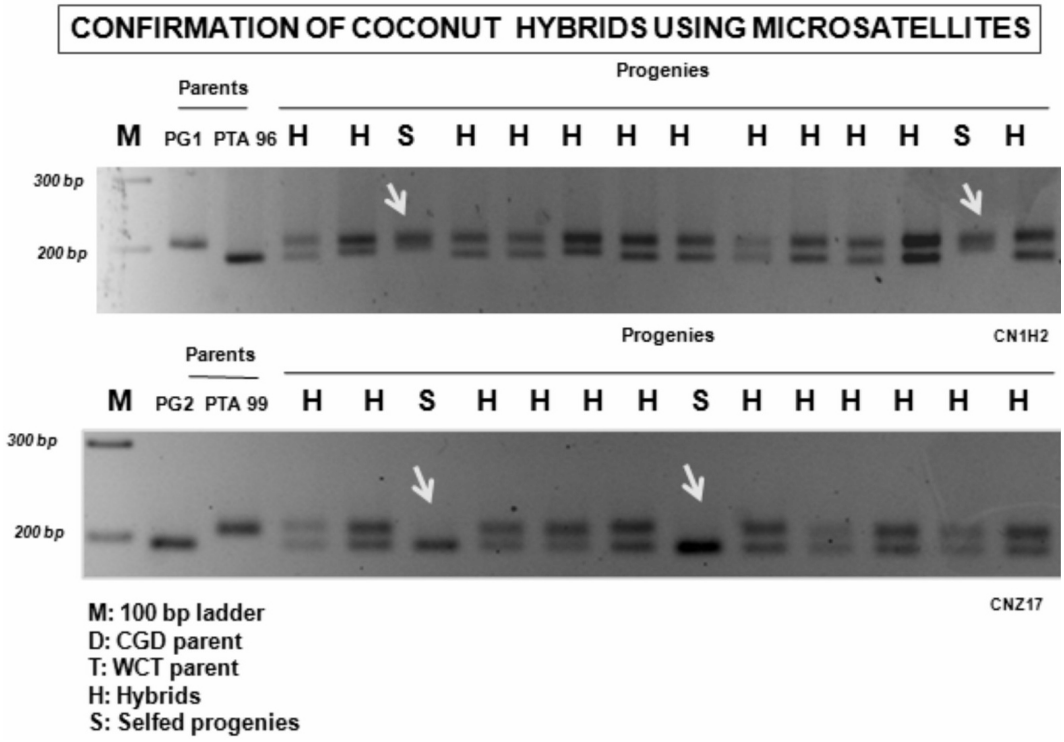

Fig. 1. SSR profiles of parental lines and hybrids

18.	CnCir 2	+	-
19.	CnCir C_7	-	-
20.	CnCirH_7	-	-
21.	CnCir73	+	-
22.	CnCir86	+	+
23.	CnCirH9	+	+
24.	CnCirE_7	+	+
25.	CnCirG_4	+	-
26.	CnCir56	+	-
27.	CNZ 32	-	-
28.	CNZ 17	+	+
29.	CNZ 21	+	+
30.	CNZ 29	-	-
31.	CNZ 13	+	+
32.	CNZ 26	+	-
33.	CNZ 31	-	-
34.	CNZ1	+	-
35.	CNZ4	+	+
36.	CNZ5	+	+
37.	CNZ2	+	+
38.	CNZ6	-	-
39.	CNZ10	+	+
40.	CAC 71	-	-
41.	CAC 72	-	-
42.	CAC 77	-	-
43.	CAC8	+	+
44.	CAC10	+	-
45.	CAC13	-	-
46.	CAC3	-	-
47.	CAC4	+	+
48.	CAC6	+	+
49.	CAC2	+	+
50.	CN1H2	+	+

17 microsatellites selected conclusively displayed the complementary banding patterns of both the parents (Fig. 1), confirming the role of microsatellites as a promising approach in purity monitoring of coconut hybrids. Selfed progenies possessed the banding patterns of only the maternal parent (CGD).

Acknowledgements

The authors thank Dr. George V. Thomas, Director, CPCRI for his guidance and facilities provided.

References

1. **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.
2. **Ratnambal M. J., Nair M. K., Muralidharan K., Kumaran P. M., Rao E. V. V. B. and Pillai R. V.** 1995. Coconut Descriptors-Part I. Central Plantation Crops Research Institute, Kasaragod, Kerala: 197.
3. **Rajesh M. K., Arunachalam V., Nagarajan P., Lebrun P., Samsudeen K. and Thamban C.** 2008. Genetic survey of ten Indian coconut landraces by simple sequence repeats (SSRs). Sci. Hortic., **118**: 282-297.