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Short Communication

Morphological and molecular characterization of a large fruited unique coconut accession from Vaibhavwadi, Maharashtra, India

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Abstract

The morphological and molecular characterization of a large fruited coconut population was undertaken using twenty-three morphological traits, six tender nut traits and eight fruit component traits. Fruit component analysis of the nuts of this accession indicated that they have low husk to nut weight ratio typical of the *Niu vai* type cultivated in South East Asia. The microsatellite analysis indicated that Vaibhavwadi coconut population (MAHT) is generally close to the South East Asian coconut accessions and has proximity with dwarf accessions in the conserved germplasm of India. Though, genetic assignment test did not identify this accession specifically with any particular cultivar in the microsatellite database, the probable origin of this type could be identified as Borneo Tall (60% similarity) a cultivar which is known to produce large coconuts. It is suggested to conserve the MAHT in the National Coconut Gene Bank of India for its utilization in the breeding programme.

Key words: Coconut, microsatellite markers, genetic diversity and genetic assignment

Coconut is an important crop of India, grown in most coastal states and Islands. Coconut populations can be classified as tall or dwarf, based on the stature, *Niu vai* or *Niu kafa* based on the husk to nut weight ratio and Indo-African or Southeast Asian-Pacific based on the microsatellite markers. The cultivated coconuts in India could be classified to the *Niu Kafa* group with high husk to nut weight ratio with few exceptions like the Kappadam Tall - a large fruited coconut type found in low numbers in central Kerala. Generally, the coconut cultivars from western India are small fruited types and the region is not well known as a good traditional coconut growing area. However a special large fruited type of coconut grown in a farmer's garden in Vaibhavwadi Tehsil, Sindhudurg district of Maharashtra (western India) which was reported to be with large size of nuts was studied for morphological, fruit component traits and genetic assignment using microsatellite markers. The independent selection and domestication of these large fruited coconut types from the locally cultivated ones is improbable due to the recent history of cultivation in this region and the long time required for any coconut improvement. Hence it was hypothesised that they may have been introduced from elsewhere. A study was undertaken to characterize the type and to elucidate the probable origin using morphological and molecular markers.

The passport information and morphological data were recorded as per the standard procedures [1] for a sample population of selected coconut palms producing large sized fruits indicated by the farmer. The observations were recorded on the old palms of this type and their progeny palms planted in the same location. Ten leaf samples (3rd to 4th leaf from the top of the crown) each from the representative coconut palms were used for DNA extraction through the column purification method of the Himedia plant genomic DNA extraction kit (HIMEDIA). Eight

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polymorphic microsatellite loci [2] were analysed with the following PCR conditions. DNA template-25ng, 200 µM of dNTPs, 0.8 µm Primer, 1U *Taq* Polymerase and 2.5 mM $MgCl_2$ in a 30µl reaction volume and 34 cycles. In coconut, two different groups *viz* Pacific Ocean group and Indian Ocean group were distinguished by the Microsatellite Markers. The same results were obtained irrespective of the number of the microsatellites used in coconut (Baudouin and Lebrun, 2001; Perera et al, 2000, Devakumar *et al*., 2006). Hence, these eight microsatellite primers are considered sufficient enough to assign individuals in coconut. The microsatellite alleles were analysed with the 5% Polyacrylamide gel denaturing electrophoresis containing 7.5M Urea. The PCR amplified products were separated at a constant wattage of 50W (SQ3 Sequencer, Amersham Pharmacia). The bands were visualized using the silver staining technique [3] and the alleles were scored according to the size differences with reference to a 10 bp marker. The reference population includes fifteen accessions of tall coconut palms from India, eight accessions from South East Asian countries, four accessions from South Pacific Islands, two accessions from Sri Lanka, three accessions from Africa and one accession from South America. The dwarf palms include four accessions from India, one from Africa and three accessions from South East Asia.

The microsatellite data were analysed for the genetic relationship between the large fruited coconut samples from Maharashtra and the rest of the major coconut accessions. The basic microsatellite analysis like heterozygosity and genetic diversity for the Vaibhavwadi samples were calculated using the Genalex software [4]. The genetic distance was calculated using the Nei's genetic distance [5] and a dendrogram was constructed with the UPGMA algorithm using the PowerSSR software [6]. To find out the likely origin of the coconut samples from Maharashtra, the microsatellite data was compared with the existing database of forty one accessions using Geneclass software [7]. The Baudouin and Lebrun [8] method implemented in Geneclass software is specifically designed to calculate the likely origin of the samples from any sexually reproducing species and the method specifically developed with coconut microsatellite data was used for this purpose. The reference data base (Tall-33 accessions and Dwarf-8 accessions) for the eight microsatellite primers reported earlier [9] was utilized for calculating the likely origin of the Vaibhavwadi samples.

Table 1. Morphological and fruit component traits of Vaibhavwadi coconut population (MAHT)

Parameters	Mean	CV
Plant height (cm)	2059.8	5.3
Girth of trunk (cm) at 1m height	85.8	8.0
Total leaves on the crown	19.8	7.6
Total length of leaf (cm)	484.0	8.4
Length of leaflet bearing portion (cm)	372.3	9.2
Girth of petiole (cm)	12.8	17.4
Number of leaflets	103.0	7.6
Breadth of leaflet (cm)	4.4	13.7
Length of leaflet (cm)	108.8	8.9
Number of leaf scars in 1m length	15.8	8.0
Length of inflorescence (cm)	105.0	2.1
Length of spikelet bearing portion (cm)	34.0	16.5
Girth of stalk (cm)	6.7	7.1
Length of spikelet (cm)	40.8	4.2
Number of spikelets/inflorescence	33.8	21.3
Number of female flowers/inflorescence	8.3	18.2
Wt. of tender nut (g)	1871.5	26.3
Quantity of water (ml)	348.3	33.0
TSS of tender nut water	6.75	4.85
pH of tender nut water	5.75	4.8
Weight of fruit (g)	1722.5	21.4
Length of fruit (cm)	21.0	3.2
Breadth of fruit (cm)	17.5	2.1
Weight of husked fruit (g)	975.8	13.6
Thickness of shell (mm)	4.45	0.6
Thickness of endosperm (mm)	11.0	0.8
Weight of endosperm (g)	462.9	11.5
Copra content (g)	190.8	16.5

Morphological observations indicated that the palms of Vaibhavwadi have spherical to semi-spherical crowns with sturdy trunk, large inflorescences of more than a metre length. The inflorescences are robust with an average of 36 spikelets and nearly 16 female flowers per inflorescence. The fruits are large and fruit shape ranges from round to oblong and oval and fruit colour ranges from green to shades of brown. The shape of the husked nut ranges from oval to round.

The grown up old palms of Vaibhavwadi are estimated to be about 75-80 years old and the average height of these palms was around 20 m and exhibited

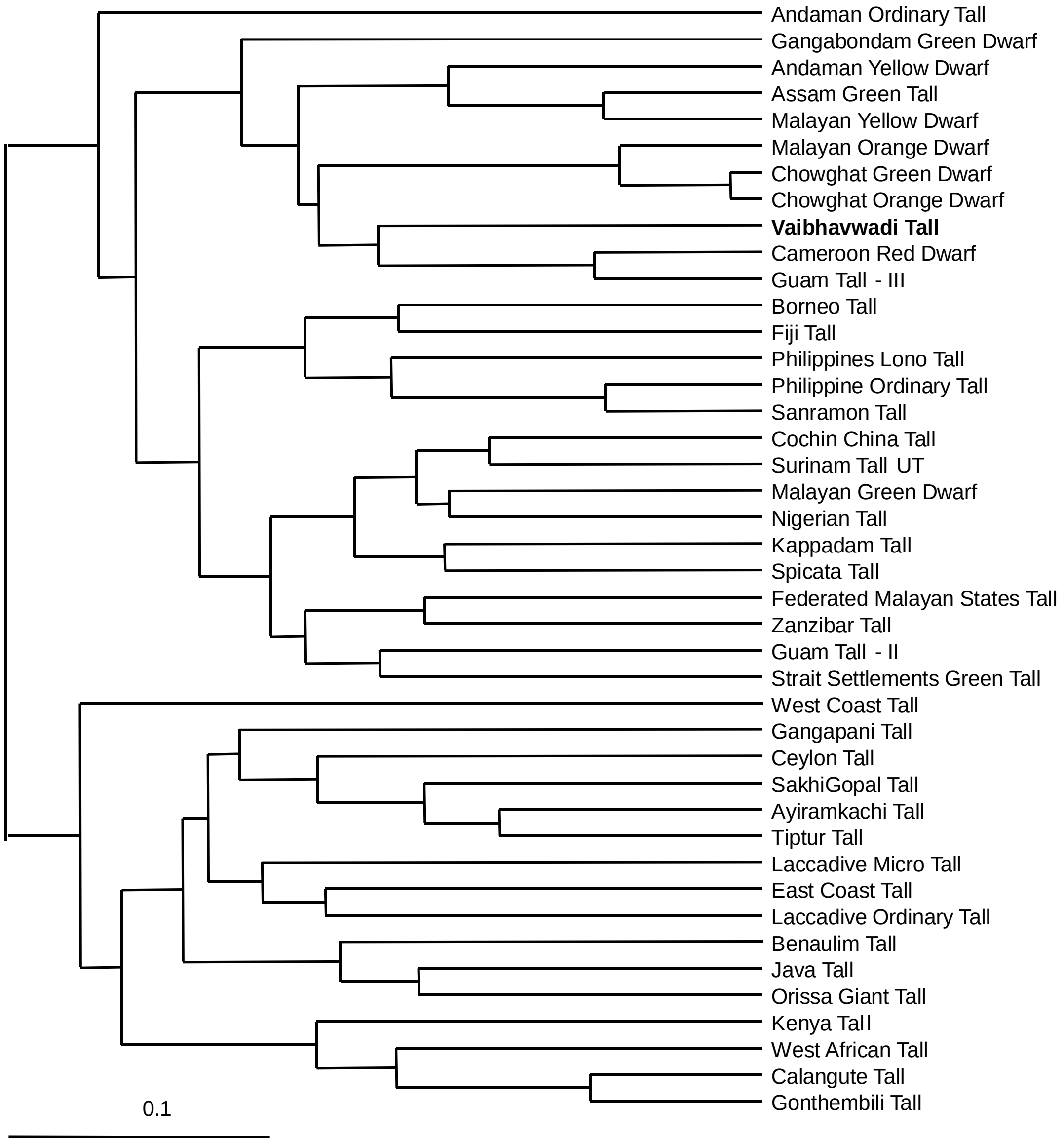

Fig. 1. Relationship among Vaibhavwadi Tall (MAHT) with the major coconut populations of the world

19-22 leaves on the crown. Observations recorded on the second generation from the original population which was planted at this location, revealed that the progenies of the old palms were also bearing large sized fruits. The height of these palms ranged from 11.5 to 16.8m. The results indicated that the progenies are also superior for nut traits as evidenced through higher mean values for tender nut traits and also comparable performance for inflorescence traits. The crown is predominantly semi-spherical in the progenies with about 25 leaves and the trunk is sturdy with a prominent bole. The inflorescences of the progenies are large and robust with an average of 37 spikelets and 20 female flowers per inflorescence. The quantity of tender nut water ranged from 200 ml to 700 ml with an average of 425 ml. Similar large fruited *Niu Vai* coconut populations are also available in the National Coconut Gene Bank maintained at CPCRI, *viz.,* Borneo Tall, San Ramon Tall, Fiji Longtongwan Tall, Federated Malay States Tall and Markham Valley Tall. The fruits can be considered as large sized as the average fresh fruit weight of mature fruits is about 1720 g whereas it was reported to be around 1200 g in case of West Coast Tall (WCT) which is the predominant cultivar in the West Coast region of India [10]. The average weight of the husked fruit was recorded as around 976 g (in case of WCT it was about 570g) and shape of the husked nut ranges from oval to round. The average thickness of the endosperm is around 1.10 cm with average shell thickness of 0.44 cm. The copra content is around 190.83 g. The morphological and fruit component traits are given in Table 1. The percentage of husk to fresh fruit weight in MAHT is comparatively low (around 42.3 %), indicating that the fruits belong to the *Niu Vai* type.

Like any coconut population, Vaibhavwadi Tall population also included low, medium and high yielding palms. By application of mother palm selection criteria, it should be possible to identify the high yielding mother palms for development of a superior population for desirable traits such as copra content. In this study, eight microsatellite primers were used and this set of microsatellite primers has shown probable evidences on the origin of the Vaibhavwadi Tall. This particular set of eight microsatellite primers is robust enough to distinguish the coconut cultivars into two broad categories indicating the ability of the markers in detecting the conserved region of DNA. The observed heterozygosity is 0.163 while the expected value (Nei's diversity) is 0.296. This reflects a deficit of heterozygotes that may result from heterogeneity in the population. The inbreeding coefficient was 0.33. The observed mean diversity for the Vaibhavwadi Tall was only 0.258. Both the CAC10 and CAC2 primer showed the highest diversity of 0.54. The lowest gene diversity was observed for the CAC11 primer.

Clustering pattern of Vaibhavwadi Tall was observed through the UPGMA dendrogram constructed with the reference samples conserved at the National Coconut Gene Bank (Fig. 1). The majority of the dwarf types and the South East Asian Talls sorted along with the Indian accessions such as Kappadam Tall and Spicata Tall which formed one major group and the other group was formed by the Indian, Sri Lankan and African accessions. The Vaibhavwadi Tall coincided with the first group along with the dwarf types and Tall accessions from the South East Asia and South Pacific zones. The reference database [9]) was strengthened by removing six accessions *viz*., Andaman Yellow Dwarf (AYD), Malayan Green Dwarf (MGD), Federated Malayan Straits (FMS), Nigerian green Dwarf (NIGD), Java Tall (JVT) and Suriname Brown Dwarf (SUBD) that had null alleles at more than one loci. The genetic assignment test of the group wise analysis (10 samples) of Vaibhavwadi Tall with the reference database ranked Borneo Tall with a 60% and Laccadive Micro Tall with a 40% probability respectively.

Considering the clustering pattern, the MAHT may be considered close with dwarf accessions and related to Guam Talls which are known for their long fruited *Niu kafa* type nuts and Borneo Tall, a large fruited type, both of which have originated from South East Asia [10]. While the dendrogram gives a broad picture of the relatedness between the individual populations, the genetic assignment test identifies the likely origin of the test population against the reference population. This is the first time when genetic assignment test is used to successfully assign the unknown coconut samples from India. Previously, Martinez *et al.* [11] utilized the genetic assignment test to ascertain the Indo Atlantic origin of the Dominican Republic coconut samples. The coconut is a highly cross pollinated crop and no two individuals in the same population have the same genetic markers. In our study, the assignment test identified two cultivars, Borneo Tall and Laccadive Micro Tall with 60% and 40% probability using the Bayesian statistics. Earlier reports suggest the presence of large fruited coconut types in Lakshadweep islands of Indian Ocean which is located in the western region to Indian mainland [12], as well as Andaman Islands of India located in the eastern region to the main land [9]. The occurrence of large fruited type at Vaibhavwadi may have resulted either from direct introduction from Andaman and Nicobar Islands or Lakshadweep Islands and or through subsequent movement of coconut within mainland India. Considering the superiority of Vaibhavwadi Tall for desirable morphological and fruit component traits such as lesser height increment, large fruit size and higher copra content, it needs to be conserved in the National Coconut Gene Bank of India for further evaluation for nut yield and possible utilization in the breeding programmes.

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