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SSR markers indicate a common origin of self-pollinating dwarf coconut in South-East Asia under domestication

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ABSTRACT

The commercial cultivation of dwarf coconut is rare in the world, representing about 5% of global population. However, Dwarfs are currently receiving more attention, particularly for the harvest of tender nut water. Dwarfs are distinguished from tall coconuts primarily by their short height with an absence of a bole at the base of the stem, their early setting of nuts, their predominantly self fertilizing mating system and by large numbers of relatively small nuts. To date, the origin and domestication of Dwarfs has not been established. This study investigates the origin and domestication of dwarf coconut using molecular markers, mainly microsatellite (SSR) data. The inheritance of height and the presence of a bole was investigated in the F_2 of a cross between Dwarf and Tall palms. The data suggest that the presence of a bole results from a single codominant locus. There was no strong association between the presence of a bole and height, with height also depending on a single codominant gene. However genetic and environmental factors make it difficult to assign individuals a definite genotype. SSR allele frequency differences between dwarf and tall accessions, ethno botanical and geographic information indicate that dwarf coconut originated from a typical domestication event in Southeast Asia.

1. Introduction

The most popular classification of coconut is based predominantly on height and pollination behaviour, resulting in two broad categories; Tall and Dwarf coconuts, also known as'typica' and 'nana' respectively (Menon and Pandalai, 1958). Tall coconut is widely grown commercially. It is fast growing, has a swelling (a bole) at the base of the stem, bears fruit late, thrives in diverse environments, produce medium to large nuts and is predominantly outcrossing. However, self-pollination (geitonogamy) through inter-spadix pollination has been observed in some Tall populations (Bourdeix, 1988; Bourdeix et al., 1990; unpublished data in Sri Lanka).

Dwarf coconuts represent about 5% of global population and are usually found close to habitation. They share a number of characteristics which distinguishes them from Talls; short internodes, slow growth in height, predominant self-pollination, short leaves with a small number of leaflets, large numbers of relatively small fruits, early fruit set and a relatively short life span. Most Dwarfs have no bole and their nuts have a sweet and pleasant- to-drink water (liquid endosperm). Dwarfs are diverse for a small number of traits such as colour and appearance of the nut, shape of the inflorescence and of the crown. It is worth noting that the Niu Leka Dwarf coconut, with its short stature and short leaves has a markedly different phenotype, often called "compact Dwarf" to differentiate it from the usual Dwarfs. It is an out-breeder and the phenotype is dominant. In this paper we deal only with the self-pollinating Dwarfs.

Dwarf coconut is usually cultivated in small numbers close to houses. It is appreciated for its short stature and for the sweet and pleasant taste of its coconut water. Due to below average copra quality and yield it is rarely grown commercially for oil production though it is increasingly planted on a large scale for coconut water production, especially in Brazil. It is also used to produce commercial F_1 Dwarf × Tall hybrids. These are produced by emasculating the inflorescences of Dwarf coconuts planted in isolation and applying pollen from selected Talls (De Nucé De Lamothe and Rognon, 1973). Alternatively, a few Tall coconuts can be inter-planted with Dwarfs for natural pollination. Compared to their parents, the phenotype of the hybrids is intermediate, though with a tendency to resemble the Tall parent for presence of bole, height, growth rate, reproductive pattern and fruit size, but to resemble the Dwarf for

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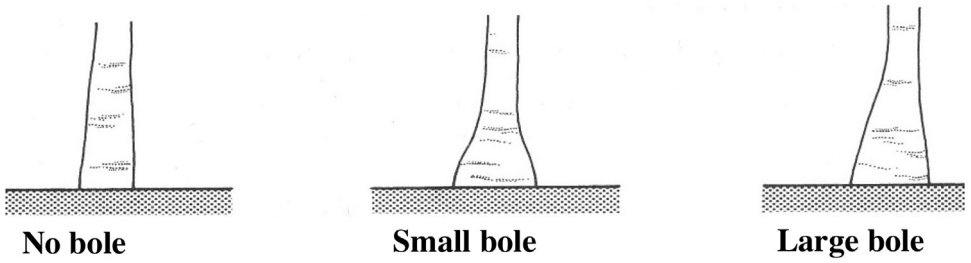

Fig. 1. Bole ranking based on size shape and height.

precocity and fruit number (De Nucé De Lamothe and Rognon, 1973). Height, time to fruit and pollination behaviour segregates independently in the F_2 (Fernando and Perera, 1997).

The origin and genetic control of dwarfism has been discussed (Ninan and Satyabalan, 1964) but is sometimes obscured by imperfect control of pollination. Swaminathan and Nambiar (1961) suggested that Dwarf coconuts originated as a result of inbreeding Talls. Purseglove (1985) stated that Dwarfs were mutations while Harries (1978) included the Dwarfs in the "domesticated" coconut type based on traits such as early germination, precocity, distinctive bright fruit colour, the proportion of husk to nut, and for some forms the high degree of resistance to lethal yellowing disease. However, to date no conclusive evidence has been presented about the origin and domestication of Dwarfs. This study investigates the origin of Dwarf coconut and its domestication.

2. Material and methods

2.1. Place of dwarfs within coconut diversity

To study diversity and among Tall and Dwarf coconuts, we calculated allele frequencies at 12 microsatellite loci (CAC2, CAC3, CAC4, CAC6, CAC8, CAC10, CAC13, CAC20, CAC52, CAC56, CAC65, CAC68) using ARLEQUIN (Version 1.0) for 51 Tall and 43 Dwarf coconut varieties. These were collected from all over the world based and are described in Perera et al. (2003). The PCR conditions were as follows: denaturing at 94 °C for 3 min, annealing at 65 °C for 1 min followed by a seven-step touchdown decreasing by 1 °C at each step to 58 °C and an extension step at 72 °C for 2 min. Conditions for the final 27 cycles were 94 °C for 1 min, 58 °C for 1 min and 72 °C for 2 min. PCR was performed in a total volume of 20 μl containing 1 x PCR buffer (10 mM Tris-HCl, 1.5 mM MgCl2, 50 mM KCl, pH 8.3), 200 mM dNTPs, 10 pmol ^{32}P end-labeled forward primer, 10 pmol reverse primer, 0.1 U Taq polymerase and 20 ng of genomic DNA. Reaction products were separated on 6% polyacrylamide gels in 1 × TBE buffer and visualized by autoradiography.A standard M13 sequence reaction was run with each primer pair for the purpose of accurate allele sizing.

2.2. Microsatellite diversity among Dwarfs

We analyzed 246 individuals representing 28 Dwarf varieties (Table 1) with 13 microsatellite markers (CnCirA3, CnCirA9, CnCirB6, CnCirB12, CnCirC7, CnCirC12, CnCirE2, CnCirE10, CnCirE12, CnCirF2, CnCirG11, CnCirH4′ and CnCirH7). The SSR analyses were performed on an automatic sequencer Li-Cor IR2 (Lincoln, Nebraska). For each SSR locus, one of the primers was designed with a 5′-end M13 extension. For the PCR amplification, 25 ng of DNA was used in a 10 μl final volume, containing 0.08 μM of the M13 labeled primer, 0.1 μM of the other primer and 0.06 μM of M13 primer-fluorescent dye IR700 or IR800 (Biolego, The Netherlands). The PCR mix contained 1 × Buffer (10 mM Tris–HCl pH 8, 50 mM KCl and 2 mM MgCl2), 200 μM DNTP and 1 U Taq DNA polymerase. The PCR program started with an initial denaturation at 94 °C for 5 min, then 35 cycles of 94 °C for 30 s, 51 °C for 1 min 15 s and 72 °C for 1 min 30 s, and stopped after a final elongation at 72 °C for 5 min. Each mix of the PCR products contained one or two IR700 and IR800 labeled M13 reverse complement extensions, diluted to one-fourth with formamid blue; 0.8 μl of the final mix was loaded on a 6.5% polyacrylamide gel and then detected by the IR fluorescence scanning system of the sequencer (Baudouin et al., 2006).

Data on markers and genotypes are publicly available in TropGENE DB (http://tropgenedb.cirad.fr/tropgene/JSP/index.jsp). Data were analyzed by computing the pairwise Euclidean distance for each locus and summing over all loci. This ensures that the distance between completely homozygotes varieties is equal to the number of allele substitutions. The results are presented in the form of a weighted neighbour joining dendrogram using DARwin 6.0.4 (Perrier and Jacquemoud-Collet, 2006).

2.3. Height measurements

To study the inheritance of height and the presence of bole (an enlargement at the base of the stem), we measured the distribution of these traits in 43 years old seventy individuals of a F_2 population derived from the F_1 between Sri Lanka Green Dwarf and Sri Lanka Tall, planted at Bandiripuwa estate, Lunuwila, Sri Lanka. Palms were classified as having no bole, low bole and high bole based on the size, shape and height of the bole (Fig. 1). The palm height was measured from the ground level to the base of the oldest living frond (Santos et al., 1996).

3. Results

3.1. Place of Dwarfs in the global coconut diversity

The allele frequencies for each locus and each group (Tall and Dwarf) are presented in Table 2. The allele frequency distributions of Talls and Dwarfs at locus CAC2 is presented in Fig. 2. The Dwarfs have reduced numbers of alleles with only 42 alleles out of a total of 85. Only one of these alleles (from CAC8) was not observed in Talls (Table 2). In many cases, the most frequent allele was the same for both Tall and Dwarf: CAC2, CAC3, CAC4 (186 bp), CAC6, CAC10, CAC13, CAC52 and CAC65. This is however not always the case and at four loci the most frequent allele in the Dwarf was rare in the Talls: CAC4 (212 bp), CAC8, CAC20 and CAC56. Conversely, at two loci the most frequent alleles of the Tall were not present in the Dwarfs: CAC8 and CAC68. A On splitting the Talls into Indo-Atlantic and Pacific groups, the Dwarfs share 43 alleles with the latter but only 22 with the former, out of a total of 43 alleles.

Fig. 3 reproduces the neighbour joining dendrogram of Perera et al. (2003) using the same 51 Tall and 43 Dwarf varieties. showing two main and distinctive clusters of Tall coconuts, one consists of all Tall ecotypes sampled from Southeast Asia and the Pacific and the other consists of samples collected from South Asia and