Research Article

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Studies on genetic uniformity of Chowghat Green Dwarf and Malayan Green Dwarf varieties of coconut using molecular and morphometric methods

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Abstract

Two coconut varieties *viz*., Chowghat Green Dwarf (CGD) and Malayan Green Dwarf (MGD) were subjected to morphometric and molecular studies to assess their genetic uniformity. Since both these varieties possess traits for high yield and resistance to root (wilt) disease, they have already been released for cultivation in the root (wilt) disease prevalent tracts. Forty two CGD palms from 'disease hotspots' were analyzed using 43 simple sequence repeat (SSR) primers. Monomorphic bands were detected in all the CGD samples with 41 primers, which is an indication of its genetic uniformity. A single CGD palm showed polymorphism with two SSR primers. Forty eight MGD palms were analyzed using 24 SSR primers. The MGD palms clustered at 62 per cent similarity. Analysis of morphological and fruit component characters of CGD and MGD population revealed that both the populations were phenotypically uniform. Breeding behaviour studies revealed that both CGD and MGD were predominantly self pollinated, like other dwarf varieties of coconut. There was complete overlapping of male and female phases in almost 96 per cent of CGD palms. Almost 100 per cent self-pollination was ensured in these palms as male phase prolonged even after completion of female phase. However, only 60 per cent of the MGD palms showed complete overlapping and in the remaining 40 per cent palms, there was only partial overlapping of male and female phases. From the present study, it is inferred that breeding behavior and genetic uniformity could be highly correlated in coconut. Collection of seed nuts preferably from mother palms with overlapping of male and female phases could possibly ensure production of true to type progenies in dwarf varieties of coconut. Present study also indicated that molecular markers like SSRs may be used to identify genetically pure mother palms for varietal improvement programmes in coconut.

Keywords: Coconut, CGD, MGD, microsatellites, morphometric studies

Introduction

Root (wilt) disease is a very serious problem of coconut in Kerala State and adjoining districts of Tamil Nadu State in India. The disease caused an annual loss of 968 million nuts (Anon., 1985) and is contiguously prevalent in an area of about 0.4 million hectares affecting more than 25 million palms (Anon., 1996). The disease is caused by phytoplasma (Solomon *et al*., 1983) and transmitted by insect vectors (Mathen *et al*., 1987; Anon., 1991). Cultivation of resistant/ tolerant varieties is considered to be the only solution for management of the above disease.

The search for identifying coconut genotypes with resistance/tolerance to root (wilt) disease started during early 1930s (Varghese, 1934). Over the years, 84 cultivars and 68 hybrids were screened for resistance to root (wilt) disease and none of them had the desirable level of resistance (Jacob *et al*., 1998). Among the varieties screened, Chowghat Green Dwarf (CGD) was the only variety reported to be resistant to root (wilt) disease (Nair *et al*., 2004).

Based on an observation during 2005 in a seed production plot of five dwarf varieties of coconut *viz*. Malayan Green Dwarf (MGD), Malayan Yellow

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Dwarf (MYD), Malayan Orange Dwarf (MOD), Chowghat Green Dwarf (CGD), and Chowghat Orange Dwarf (COD), at Coconut Development Board Farm, Neriamangalam, Kalparaksha, a selection from MGD was identified as a promising resistant variety with high yield. Subsequently, during 2008, Kalparaksha was released for large-scale cultivation in the root (wilt) prevalent areas.

Similarly, survey in farmers' plots coupled with results from experimental trial at ICAR-CPCRI, Regional Station, Kayamkulam, revealed Chowghat Green Dwarf as a suitable variety for cultivation in the homesteads of root (wilt) disease prevalent tract (Nair *et al*., 2004). Ultimately, Kalpasree, a selection from Chowghat Green Dwarf population, was notified for release during 2012.

Genetic uniformity studies using molecular methods are essential to identify genetically pure mother palms of any variety for use in future breeding programme. The present paper reports results of genetic uniformity studies carried out on above two dwarf coconut varieties *viz*. CGD and MGD using molecular and morphometric techniques.

Materials and methods

Molecular analysis

i) DNA extraction

DNA was extracted from unopened spindle leaves of the above two varieties using the DNeasy mini kit (QIAGEN). The experimental population consisted of 42 palms of CGD and 48 palms of MGD. The palms of CGD variety were selected from farmers' plots in root (wilt) disease prevalent tracts located at Alappuzha, Kollam, Kottayam and Pathanamthitta Districts of Kerala State. Regarding MGD palms, 17 were selected from Seed Garden Complex, Munderi (Malappuram District) and 31 palms from DSP Farm, Neriamangalam (Ernakulam District).

ii) SSR analysis

Polymerase chain reaction (PCR) reaction was conducted in aliquots of 20 µL containing 35 ng genomic DNA, 0.2 µM each of forward and reverse primers (Sigma), 50 µM of each dNTPs (M/s Bangalore Genei Pvt. Ltd., Bangalore), 1X buffer (10 mM Tris-Hcl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$) and 1 Unit of *Taq* DNA polymerase (M/s Bangalore Genei Pvt. Ltd., Bangalore). Amplifications were performed using a gradient thermal cycler (Eppendorf) with a PCR profile of 94 ºC for 5 min followed by 29 cycles of 1 min at 94 ºC, 2 min at the 52 ºC and 2 min at 72 ºC with a final extension for 5 min at 72 ºC. The amplified products were run on 3 per cent agarose gel, stained with ethidium bromide and visualized using gel documentation system (Syngene).

iii) Data analysis

Each band generated by SSR primers was considered as an independent allele and only clearly resolved and unambiguous bands were used to generate binary data for calculating percentage polymorphism. Genetic associations between the palms were evaluated by calculating Dice similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers (Dice, 1945). Similarity matrix was generated using the NTSYS-PC software, version 2.0 (Rohlf, 1998). The similarity coefficients were used for cluster analysis followed by construction of dendrogram using Unweighted Pair-Group method (UPGMA; Sneath and Sokal, 1973).

Morphometric characterization

i) Morphological characters

For studies on important morphological and agronomic characteristics, 20 randomly selected palms of MGD and 80 randomly selected palms of CGD were studied. Observations were recorded based on standard morphological descriptors (Ratnambal *et al*., 1995). For observation on nut characteristics, 200 nuts of each variety were separately heaped and 20 nuts were randomly drawn. Observations were recorded following standard descriptors (Ratnambal *et al*., 1995).

ii) Breeding behaviour studies

Breeding behaviour was studied using randomly selected palms of CGD (25 nos.) and MGD (20 nos.). Intact inflorescences of selected palms were labeled and observations on opening, initiation and duration of male and female phases, were separately recorded. Duration in overlapping of male and female phases in each inflorescence was also recorded.

The data on morphological and nut characters were subjected to statistical analysis to work out measures of dispersion following standard procedures.

Results and discussion

SSR analysis

Monomorphic bands were detected in all the forty two CGD palm using forty one primers which indicated its genetic uniformity. However, one CGD palm accession showed polymorphism with two SSR primers (CNZ 40, CNZ 21).

Forty eight MGD palms were analyzed using 24 SSR primers. When considered together, the MGD palms from Munderi and Neriamangalam Farms were intermixed in the dendrogram (Fig. 1), forming two major clusters at 62 per cent similarity. Four palms (three from Munderi Farm and one from Neriamangalam Farm) formed a separate cluster, while the remaining 44 palms clustered together. Analysis of the MGD palms from Munderi Farm alone revealed clustering of the palms into two major clusters at 62 per cent similarity- three palms forming a cluster separate from the remaining 14 palms (Fig. 2). Maximum similarity was seen between three pairs of palms (92 per cent). The 31 MGD palms from Neriamangalam Farm formed two major clusters at 63 per cent similarity level (Fig. 3). Maximum similarity of 96 per cent was noticed. Three palms formed a separate cluster from the remaining 28 palms.

Although higher heterozygosity values were generally detected in tall accessions, higher frequencies of heterozygous loci were also detected in certain dwarf coconut cultivars indicating the possibility of out-crossing. Rivera *et al.* (1999) reported detection of heterozygous loci at quite high frequencies in some of the dwarf cultivars from Philippines, suggesting occurrence of out-crossing in the origin of these cultivars.

The overall lower genetic diversity in dwarf coconuts when compared to talls has been attributed to their breeding habits (Lebrun, 1998). Tall coconuts are predominantly out-breeding and dwarf coconuts are predominantly inbreeding. The possibility of domestication of dwarf palms from a small number of tall palms also could not be ruled out as a causative factor for low levels of genetic diversity in dwarfs (Perera *et al*., 2000).

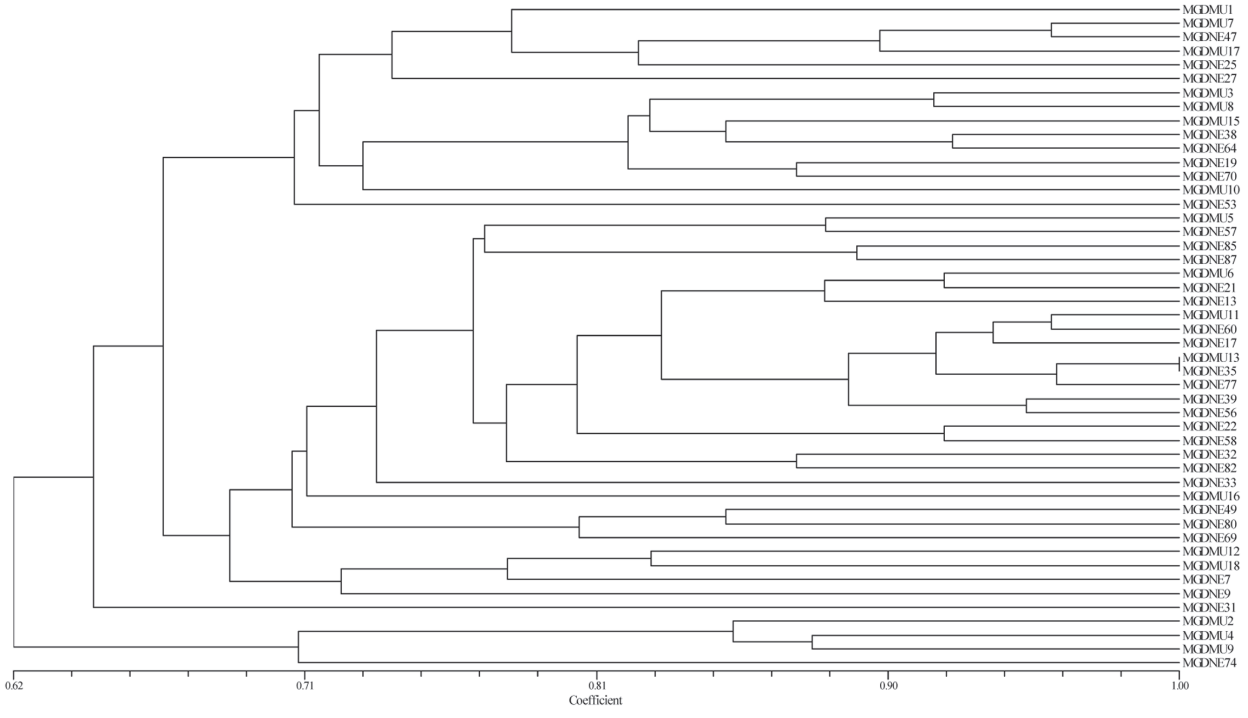

Fig. 1. Dendrogram showing the genetic relationship of MGD palms (17 at SGC, Munderi and 31 palms at DSP Farm, Neriamangalam) based on SSR analysis

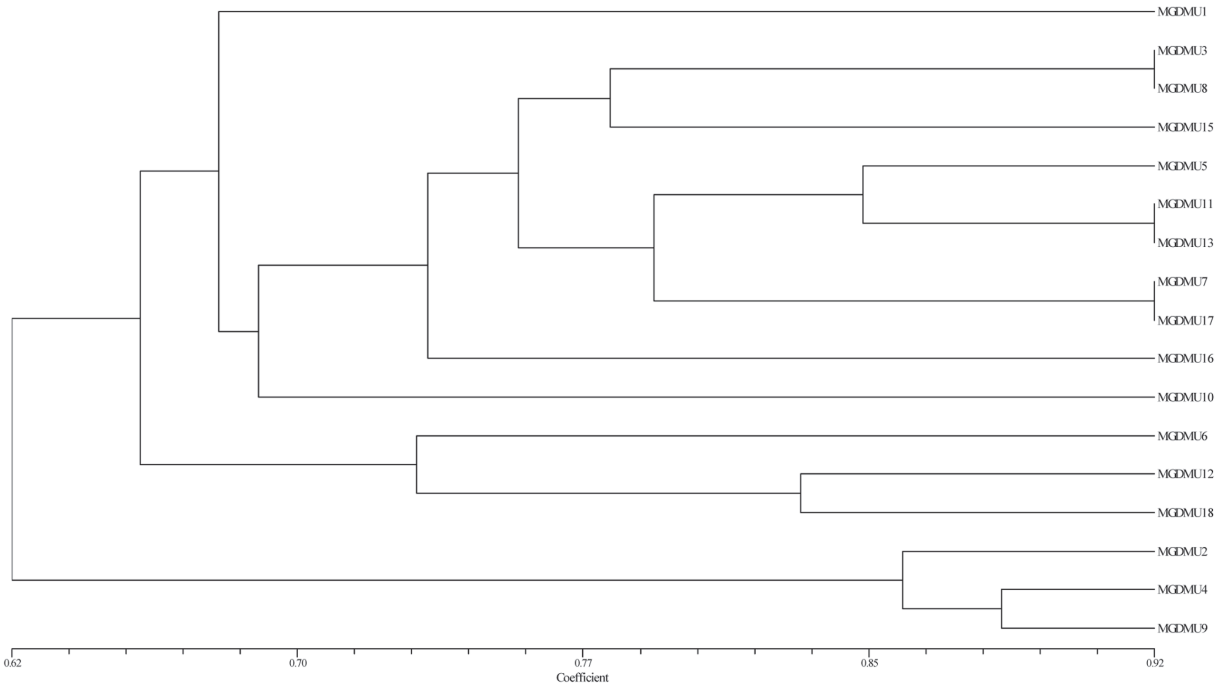

Fig. 2. Dendrogram showing the genetic relationship of MGD palms (located at SGC, Munderi) based on SSR analysis

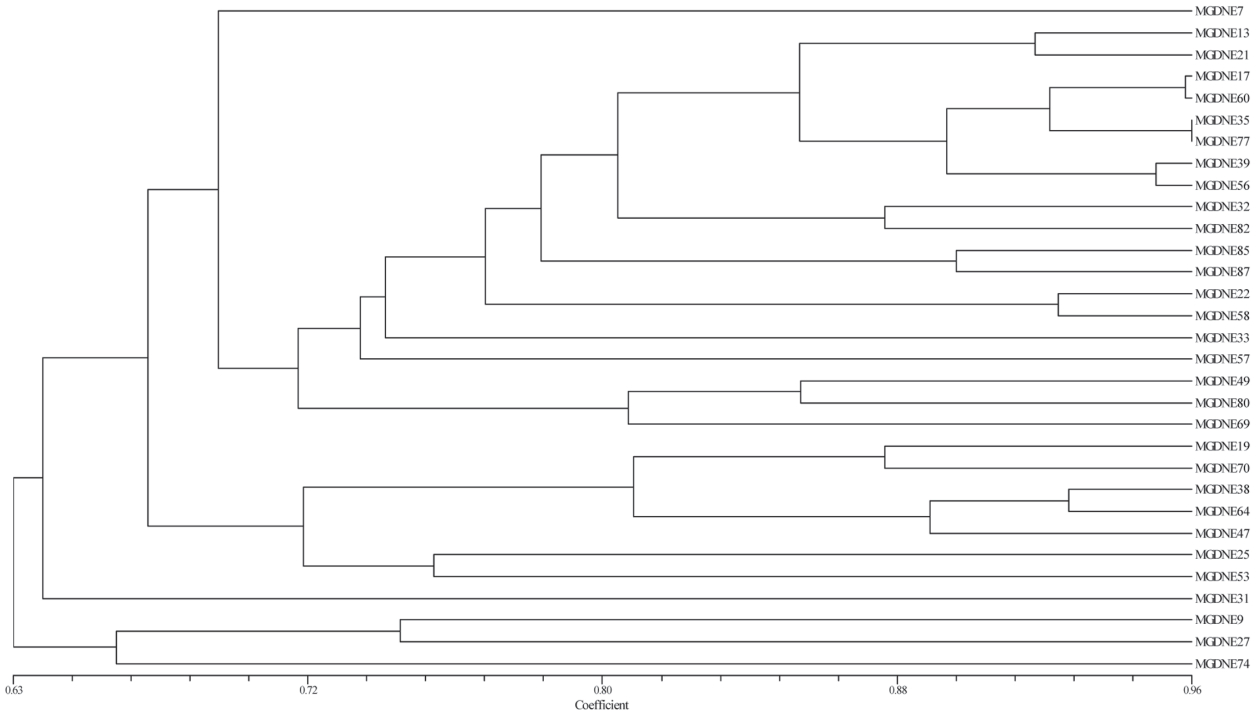

Fig. 3. Dendrogram showing the genetic relationship of MGD palms (located at DSP Farm, Neriamangalam) based on SSR analysis

The dwarf coconut varieties have been considered to have originated from taller ones (Ninan and Satyabalan, 1964; Swaminathan and Nambiar, 1961). Reduction, fusion and change in symmetry are the three types of karyo-morphological changes taking place during the evolutionary specialization in vascular plants (Stebbins, 1950). The overall reduction in phenotypic features in the dwarf coconuts (such as plant height, longevity, size of vegetative organs, fruits, seeds etc.) as compared to talls showed that they were more specialized. This was also accompanied by a change in the breeding system from out-crossing in taller varieties to almost complete selfing in some dwarf varieties (Raveendranath and Ninan, 1973).

According to Lebrun *et al.* (1998), it was likely that all the dwarfs appeared at the same time, or at least within a single population. The autogamy of dwarfs resulted in total absence of intermediate frequency bands and a very low heterozygosity rate. The appearance of 13 RFLP markers shared by almost all the dwarfs and three of these markers being in minority in talls seemed to favour an appearance at the same time. Dwarf coconut palms are rarely found in large, uniform stands. Dwarfism, could therefore, be regarded as the last stage of coconut palm domestication, in which the determining factor was the appearance of autogamy, and the subsequent possibility of reproducing true-to-type palms (Lebrun *et al.*, 1998). The characters sought would have included slow growth, precocity, the excellent taste of water in immature fruits; their characteristic shape and the palm growth habit are effective identification factors (Samsudeen *et al*., 2006).

Previous studies of SSR variation in coconuts (Perera *et al*., 1999, 2000; Rivera *et al*., 1999; Teulat *et al*., 2000; Merrow *et al*., 2003; Rajesh *et al*., 2008; Thomas *et al*., 2013) have all concluded that tall and dwarf varieties can be distinguished by the higher heterozygosity and gene diversity of the talls compared to dwarfs.

Morphological characters

Morphological characteristics of the two varieties are given in Table 1. Based on various morphological parameters of the varieties recorded in this study, it is observed that CGD possesses more dwarfish traits when compared to MGD. Regarding vegetative and floral characters MGD displayed more robust nature compared to CGD. Standard deviation and coefficient of variation of various morphological parameters of CGD and MGD were within standard limits as prescribed for stable varieties of coconut.

For various fruit component characters also MGD showed robustness compared to CGD

Table 1. Morphological characters of CGD and MGD varieties of coconut

Plant morphology	CGD					MGD				
	Mean	Min	Max	CV (%)	SD	Mean	Min	Max	CV (%)	SD
Girth of trunk (cm)	59.9	48.0	70.0	7.5	4.5	69.8	58.0	87.0	11.7	8.2
Total no. of leaves	25.3	13.0	36.0	18.8	4.7	29.8	26.0	34.0	8.9	2.7
Length of petiole (m)	1.1	0.8	1.6	15.7	17.4	1.3	1.2	1.5	8.5	0.1
Length of leaf bearing portion (m)	3.2	2.2	4.3	13.0	41.1	3.6	3.2	3.9	7.5	0.3
No. of leaflets	105.2	88.0	129.0	9.3	9.8	99.1	93.0	108.0	4.1	4.0
Breadth of leaflet (cm)	4.3	2.4	6.0	19.1	0.8	5.1	3.0	6.0	18.9	1.0
Length of leaflet (cm)	101.9	70.0	126.0	12.0	12.2	100.8	83.0	127.0	12.4	12.4
No. of leaf scars in 1 m length	40.2	22.0	57.0	18.6	7.5	25.6	22.0	31.0	13.7	3.5
Length of inflorescence (cm)	59.0	52.0	65.0	7.1	4.2	89.7	72.0	110.0	12.3	11.1
Length of spikelet bearing portion (cm)	27.3	22.0	35.0	14.9	4.1	39.5	31.0	50.0	15.9	6.3
Length of stalk (cm)	31.7	22.0	43.0	17.7	5.6	48.2	32.0	69.0	22.9	11.1
Length of spikelet (cm)	29.6	25.0	37.0	12.5	3.7	32.2	24.0	42.0	17.6	5.7
No of spikelet / inflorescence	26.3	23.0	32.0	11.3	3.0	35.9	29.0	42.0	12.4	4.4
Avg. no. of female flowers	25.3	14.0	29.0	19.9	3.9	22.2	12.0	38.0	31.1	6.9

Table 2. Nut characters of CGD and MGD varieties of coconut

Nut characters	CGD					MGD				
	Mean	Min	Max	CV (%)	SD	Mean	Min	Max	CV (%)	SD
Length of fruit (cm)	17.0	14.5	19.0	5.8	1.0	19.9	18.0	21.0	5.1	1.0
Breadth of fruit (cm)	12.0	10.5	13.5	6.6	0.8	14.8	13.5	16.0	5.5	0.8
Weight of fruit (g)	683.5	375.0	1000.0	18.9	129.3	963.9	750.0	1250.0	12.8	122.9
Thickness of husk (cm)	4.2	2.0	5.5	19.5	0.8	1.4	0.7	2.0	26.3	0.4
Weight of dehusked nut (g)	349.5	225.0	475.0	19.1	66.6	635.0	520.0	880.0	13.5	85.6
Percentage of husk to whole fruit weight	47.5	24.0	68.4	3.2	10.5	33.8	12.5	42.1	21.1	7.1
Thickness of kernel (cm)	1.0	0.5	1.3	14.4	0.1	1.0	1.0	1.2	6.8	0.1
Copra content (g)	96.8	65.1	138.3	18.2	17.6	185.1	126.9	232.0	18.3	33.8
Weight of shell (g)	85.2	51.0	130.0	25.6	21.8	112.1	86.9	137.4	15.7	17.7
Quantity of water (mL)	82.2	8.0	170.0	50.9	41.8	196.8	142.0	248.0	16.4	32.3

(Table 2). This was very evident for characters like size and weight of fruit, kernel thickness, shell weight and copra content. However, the husk of CGD nut was thicker (4.18 cm) compared to MGD (1.35 cm). The husk thickness variation was reflected in the ratio of husk to whole fruit weight where 47.5 per cent of CGD nut weight was contributed by husk whereas in MGD, husk contributed only 33.8 per cent of fruit weight. The coefficient of variation and standard deviation values for the different nut characters were within the prescribed limit for a stable variety.

Breeding behaviour

Observation on breeding behaviour of the two varieties is consolidated in Table 3. Essentially, there were four categories for both CGD and MGD populations. Palms of variety CGD showed the typical breeding behaviour of dwarfs marked by complete overlapping of male and female phases. The male phase was initiated on the day or one day after bunch opening and female phase started within 9 days in 40 per cent of CGD palms. The female phase extended up to 15 days and male phase extends upto 11 to 23 days in 96 per cent of palms. Although MGD also showed overlapping of male and female phase, there was marked difference from that of CGD for initiation and duration of female phase (Table 3). Though male phase initiation in MGD was similar to CGD, it extended for more periods, sometimes even upto four weeks after inflorescence opening. But the variation was more

Table 3. Breeding behaviour in CGD and MGD varieties of coconut

Variety	Initiation of male phase	Duration of male phase (days)	Initiation of female phase (days after in florescence opening)	Duration of female phase (days)	Overlapping duration (days)	% of palms
CGD Category 1	Same day	10	6	6	5	4.00 (PO)
CGD Category 2	Same day	11-14	6-9	5-6	5-6	8.00 (CO)
CGD Category 3	0-1 day after inflorescence opening	17-20	7-9	9- 10	9-10	28.0 (CO)
CGD Category 4	0-1 day after inflorescence opening	21-23	8-15	7-10	7- 10	60.0 (CO)
MGD Category 1	1 day after inflorescence opening	17-18	15-16	3	3	10.0 (CO)
MGD Category 2	1 day after inflorescence opening	17-19	15-16	4-6	3-5	25.0 (PO)
MGD Category 3	1 day after inflorescence opening	20-22	17-18	4-6	3-5	15.0 (PO)
MGD Category 4	1 day after inflorescence opening	24-25	16-18	7-8	7-8	50.0 (CO)

CO: Complete Overlapping; PO: Partial Overlapping

CGD: CO-96 % palms & PO- 4% palms; MGD: CO-60 % palms & PO- 40 % palms

evident in female phase initiation which started only after two weeks and extended only for a period of 3 to 8 days by which time the male phase in MGD was completed.

Morphometric characters

Dwarf varieties were proposed to have evolved from taller ones through mutation (Swaminathan and Nambiar, 1961). The SSR analysis showed that the experimental population of CGD was uniform and the breeding behaviour studies showed that CGD was predominantly self-pollinated. The uniformity in CGD population could be due to self-pollination which was evident from the breeding behaviour studies.

Morphological and fruit component data of MGD and their corresponding statistical parameters indicated that it was a phenotypically uniform population. However, molecular data and UPGMA dendrogram showed that MGD palms clustered only at 62 per cent similarity which could be attributed to its breeding behaviour. Among MGD population, only 60 per cent showed complete overlapping of male and female phases which meant that in the rest 40 per cent palms there was more chance for out-crossing. Out crossing can be from pollen from the same variety or pollen from another variety. If the pollen from another variety was from a green petiole/ nut coloured variety, it would be difficult to identity the out crossed progenies. Dwarfs are generally considered to breed true-to-type (Lebrun, 1998). Out-crossing could lead to production of off-type progenies and identification of off-types might be difficult if the pollen is from another variety with same petiole/nut colour as that of the female parent. Collections of seednuts preferably from mother palms with overlapping of male and female phases could possibly ensure production of true-to-type progenies in dwarf varieties like MGD. Heterozygosity detected at molecular level in MGD could be due to its breeding behaviour which promoted some amount of out-crossing.

Green colored dwarf varieties are generally considered to be more dwarfish that other dwarf types like yellow, orange and red. All green dwarfs (Srilankan Green Dwarf, Brazilian Green Dwarf, Chowghat Green Dwarf, Nigerian Green Dwarf) other than Malayan Green Dwarf have typical dwarfish characters. This was reflected in its stature, growth rate, breeding behaviour and various other attributes. Stature wise, MGD has been considered to be semi-tall and more robust compared to other green dwarfs. The autogamy of dwarfs resulted in a very low heterozygosity rate (Lebrun, 1998). However, the slightly higher heterozygosity rate in MGD as evident from the SSR analysis in the present study could be attributed to its breeding behaviour. Only in 60 per cent of the MGD population there was complete overlapping of male and female phases.

Presently, genetically pure mother palms in coconut varieties are selected based on morphological descriptors of that variety. Molecular analysis using SSR markers is a robust method in identification of genetically pure palms of any variety. However, in the absence of molecular marker data, accurate morphological characterization using stable descriptors and precise information on breeding behaviour should be used to select coconut palms for genetic uniformity.

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