Characterization of a spicata mutant of coconut palm in India

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(Manuscript Received:12-07-13, Revised: 06-08-13, Accepted: 05-10-13)

Keywords: Coconut, ethnobotany, microsatellites, morphometric, Spicata

In coconut palm (*Cocos nucifera* L.), the number of female and male flowers per spadix varies considerably with variety, age of the tree, genotype, season and the conditions in which they are grown. The spicata palms exhibit unique and distinct floral characters - they produce a large number of female flowers on the unbranched spadix with a conspicuous reduction in the number of male flowers. One of the characteristic feature of the unbranched type of spicata is that female flowers are attached throughout the main rachis of the inflorescence, resulting in closely set fruits compared to normal inflorescence (Fig. 1). The spicata palms are known to occur in most of the coconut growing countries (Sugimura *et al.,* 1994). A single gene in heterozygous state (*Ss*) controls this spicata trait. Cytological evidence showed that spicata palms exhibit several aberrations during meiosis and that meiosis in them was aberrant (Ninan *et al.,* 1960). Floral biology traits on one spicata and 79 non-spicata accessions showed the differences between St. Kudat (an Indonesian spicata variety) with that of others was due to the short male phase and low fruit setting percentage (Ratnambal *et al.,* 2003). The ratio of male and female flowers is 0.25: 1 in spicata palms compared to 1:0.0036 in ordinary talls.

Fig. 1. Spicata bunch (A) compared to a normal bunch (B) of coconut

Molecular markers have also been employed in determining the relationship of spicata with other varieties. While Rivera *et al.* (1999) and Perera *et al.* (2003) had reported clustering of spicata tall to other tall cultivars from the Philippines and Sri Lanka respectively; Meerow *et al*. (2003) reported that 'Red spicata' from Florida was more closely related to 'Fiji dwarf'.

Characterization of coconut varieties was generally done using morphometric traits (Sugimura *et al.,* 1997) and molecular tools such as RAPD (Ashburner *et al.,* 1997); ISTR (Rohde *et al.,* 1995) and SSRs (Perera *et al.,* 2003). Importance of mutants in coconut improvement has already been reported (Arunachalam *et al.,* 2001). The present study has been undertaken with the objective of understanding the nature of spicata mutant from east coast of India using the multiple tools of ethnobotany, morphometric, fruit component traits and microsatellite DNA markers.

The work was undertaken at Poornankuppam village of Ariyankuppam commune of Pondicherry Union territory, India. Spikelet-less mutant coconut palms present in the village were used in the study. Farmers, housewives and toddy tappers were

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interviewed during the study using the method after Mulller and Scherr (1990) and Eyzaguirre and Lipman (1999) to collect ethnobotany data.

Five representative spicata palms located in the village with typical spikelet-less inflorescence were sampled from four farmers' plots. These palms were characterized as described in Sugimura *et al*. (1997). Frequency distribution of 18 morphological traits recorded on stem, leaf and inflorescence was used to work out Shannon-Weaver index as below:

$$H' = \sum_{i=1}^{n} P_i \log_2 P_i ,$$

where p_i is the proportion of plants in i^{th} class interval.

Total value obtained for the index for a trait is divided $\log_2 n$, where, n is the number of classes. Mature 11-month old nuts were harvested, pooled and a sample of eight fruits was randomly drawn and their composition worked out.

Total DNA was extracted from spindle leaves using a modified SDS-protocol. PCR was carried out as per the procedures of the microsatellite kit developed at CIRAD (Baudouin and Lebrun, 2002; Rajesh *et al*., 2008). A sample size of seven palms was used for the SSR study using 14 primer pairs. Nei's gene diversity and proportion of heterozygotes were worked out for all the 14 microsatellite loci.

Farmers refer to spicata as a 'palmyrah coconut' and they preferred due to its showy inflorescence and production of large number of female flowers and lower level of mite infestation. Low incidence of eriophyid mite infestation in spicata palms was reported in earlier studies (Levin and Mammotty, 2003; Muthiah and Natarajan, 2004). Round nut shape and tightness of perianth lobes are known to be the traits associated with tolerance to mite (Moore and Alexander, 1990; Moore, 1986). However, farmers do not prefer spicata due to very low fruit set during dry season, alternate bearing habit, difficulties in dehusking owing to tough fibres, and lower yield of inflorescence sap.

The morphometric variations in spicata palms are presented in Table 1. The spicata palms showed high CV of 54 per cent for number of female flowers in our study. Progenies from varietal cross between

Table 1. Shannon's diversity index, mean and variation of morphometric traits of spicata palms

Sl.No	Trait	H'	Mean	Range	CV(%)
1.	Girth of stem at 1 m height (cm)	0.655	129.4	90-165	25
2.	Number of leaves	0.685	29.2	24-36	16
3.	Length of petiole (cm)	0.685	117.0	100-140	13
4.	Length of leaflet bearing portion (cm)	0.361	340.8	315-370	6
5.	Length of leaf (cm)	0.485	457.8	430-490	6
6.	Number of leaflets on one side	0.761	103.8	92-114	9
7.	Width of leaflet (cm)	0.361	4.9	4-5.3	11
8.	Length of leaflet (cm)	0.361	110.0	100-120	6
9.	Number of leaf scars per m	0.361	5.2	4-7	21
10.	Internodal length (cm)	0.685	19.9	14.3-25	19
11.	Length of inflorescence (cm)	0.761	107.6	75-133	21
12.	Length of spike portion (cm)	0.761	59.6	45-70	19
13.	Length of stalk (cm)	0.961	48.0	25-63	31
14.	Number of female flowers per inflorescence	0.685	107.0	61-200	54
15.	Number of bunches per palm	0.960	11.6	5-16	36
16.	Number of nuts per bunch	0.761	10.0	3-16	55
17.	Number of bunches with buttons	0.613	4.6	3-6	25
18.	Number of bunches with nuts	0.960	7.0	2-10	48
	Mean	0.593			

H': Shannon's diversity index**;** CV: Coefficient of variation tall and spicata inherit the spicata trait of high degree number of female flowers (Nambiar, 1971). Progenies from varietal cross between dwarf and tall accessions also exhibit high female flower production indicating the heritable nature of high female flower production. Hence, dwarfs and spicata could be exploited in coconut breeding programmes for high female flower production. Length of inflorescence stalk, total number of bunches and number of bunches having only mature nuts exhibited very high level of Shannon's diversity (>0.95). Spicata mutant showed low diversity for leaflet length and width, leaf lamina length and number of leaf scars m^{-1}, but high diversity for traits such as length of inflorescence stalk, number of bunches with mature nuts and total number of bunches. Number of female flowers, number of nuts per bunch and number of bunches having mature nuts were the highly varying traits (CV >45%). Fruit component studies (Table 2) reveal that fruits of spicata palms are medium-sized and elongate with high husk content (68.3%), low kernel (13.2%) and shell (13.8%) content.

Mean allelic diversity of this variety at 14 microsatellite loci was found to be low (0.258). A mean number of three alleles per loci were detected in this mutant (Table 2). Mean proportion of heterozygotes was 0.467 and was nil in four (CnCir E12, CnCir A3, CnCir A9 and CnCir H7) microsatellite loci. Allelic diversity was absent in two of 14 loci (CnCir E12 & CnCir A3) studied. High level of either allelic diversity or proportion of heterozygotes (>0.7) is observed in five loci (CnCir C3b, CnCIR C7, CnCIR E2, CnCir E10 and CnCir G11) (Table 3). At two loci (CnCir A9 and CnCir C3b), allelic diversity and heterozygosity values differed multifold. This shows that spicata exhibit deviation from Hardy-Weinberg equilibrium at these two loci. Deviation from Hardy Weinberg equilibrium occurs due to selection, introduction or mutation. As there is no selection or introduction the deviation in spicata could be attributed to mutation at these marker loci. The locus CnCir A9 has been earlier shown to possess tight linkage with one of the QTLs linked to nut/fruit (%) (Baudouin *et al*., 2006).

Due to the reduction in male flower production, spicata has high possibility of cross-pollination. But the mean allelic diversity level was very low as found in autogamous dwarfs of coconut (0.258).

Table 2. Fruit composition of spicata palms

Sl. No.	Trait	Mean	Range
1.	Weight of the fruit (g)	1286	900-1600
2.	Husked fruit weight (g)	409	150-602
3.	Polar circumference of fruit (cm)	58.40	40-75
4.	Equatorial circumference of fruit (cm)	32.10	27-39
5.	Polar circumference of husked fruit (cm)	41.00	30-47
6.	Equatorial circumference of husked fruit (cm)	27.30	25-30
7.	Thickness of husk at perianth end (cm)	7.60	5.5-11
8.	Thickness of husk at middle (cm)	1.64	1.2-2.5
9.	Thickness of husk at stylar end (cm)	3.68	1.5-7
10.	Length of the long husk fibre (cm)	20.00	15-26
11.	Thickness of the long husk fibre (mm)	0.17	0.02-0.82
12.	Thickness of kernel (cm)	1.27	1.1-1.5
13.	Thickness of shell (cm)	0.37	0.2-0.5
14.	Diameter of cavity (cm)	5.11	4.8-5.5
15.	Volume of cavity (ml)	94.30	60-130
16.	Weight of kernel/nut (g)	170	150-250

Table 3. Diversity of Spicata palms at 14 microsatellite loci

Sl. No.	Microsatellite loci	Proportion of heterozygotes	Allelic diversity	Number of alleles
1.	CnCir E12	0	0	1
2.	CnCir A9	0	0.659	3
3.	CnCir B12	0.286	0.264	2
4.	CnCir C3b	0.143	0.714	4
5.	CnCir A3	0	0	1
6.	CnCir C7	0.857	0.648	4
7.	CnCir H4b	0.143	0.385	3
8.	CnCir E2	0.571	0.758	4
9.	CnCir F2	0.429	0.505	5
10.	CnCir H7	0	0.440	2
11.	CnCir B6	0.429	0.626	3
12.	CnCir E10	0.714	0.495	2
13.	CnCir G11	0.714	0.780	6
14.	CnCir C12	0.286	0.264	2
	Mean	0.467	0.258	3

Meerow *et al*. (2003) also reported similar observation in red spicata from Florida.

Secretaria *et al.* (2002) recorded the length of spadix and number of leaves on the crown as important traits having positive and significant correlation with toddy yield. Shannon's diversity index scores in this mutant for the number of leaves (0.685) and inflorescence length (0.761) was found to be high. So, there is scope to exploit these two traits to indirectly increase sap yield.

Thus, the present study supplements new information to the existing knowledge of spicata having high female flowers production, short leaflets, elongated fruit shape, lesser duration of male phase, low fruit set (%) and tolerance to eriophyid mite. The spicata palms were found to have low sap yield and tight husk. High variation was noticed in floral and stem traits (CV>19%). This mutant also possess medium sized fruits, long husk thickness at perianth end, long and thick husk fibres with low kernel but large proportion of husk and very high level of diversity in length of inflorescence stalk, number of bunches and number of bunches with nuts.

Acknowledgement

We thank the Ariyankuppam Commune Coconut Farmers Association and the Department of Agriculture, Government of Pondicherry. We are grateful to IPGRI-COGENT/DFID for the financial support. Our sincere thanks are due to Director, CPCRI, and Dr S. Arulraj, Formerly, Head, Division of Social Sciences, CPCRI, for their guidance. We are grateful to Dr. L. Baudouin and Ms. Patricia Lebrun, CIRAD, Montpellier for all their help and support in this work. First two authors are grateful to IPGRI/ COGENT/ BUROTROP/ CIRAD for the training. The authors are also grateful to Dr R.D. Iyer, Former Head Crop Improvement, CPCRI, Kasaragod for the suggestions on improving the manuscript.

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