

Studies on improvement of ‘West Coast Tall’ cultivar of coconut for yield and resistance to root (wilt) disease through recurrent selection

R.J. Thomas¹, M. Shareefa¹, R.V. Nair¹, P.M. Jacob¹, M. Sasikala¹, M.K. Rajesh² and A. Karun²

¹ICAR-Central Plantation Crops Research Institute, Regional Station, Kayamkulam, Kerala State, India; ²ICAR-Central Plantation Crops Research Institute, Regional Station, Kasaragod, Kerala State, India.

Abstract

In India, coconut is grown in 2.14 million hectares, and about 10 million people depend on coconut for their livelihood. Among the diseases affecting coconut, root (wilt) is the most serious. Cultivation of resistant varieties is the most ideal and practical solution for disease management. A comprehensive breeding programme for resistance to root (wilt) disease aimed at improving the local tall (‘West Coast Tall’) cultivar through recurrent selection was initiated in 1987. Disease-free, high-yielding palms found in the midst of heavily diseased palms and located in disease hotspots were selected as the base material for the breeding programme. The selected disease-free palms were selfed and inter se-mated since 1990 to produce two sets of first generation progenies (viz., self and inter se-mated). The first-generation progeny were planted during 1994-95 in experimental fields, which were hotspots of root (wilt) disease, in order to screen and select high yielding, disease-resistant palms. Observations recorded 18-19 years after planting revealed that the disease incidence in inter se-mated progenies was only 47% compared with 63% in selfed progenies. The inter se-mated progenies had 35-40% higher nut yields compared with selfed progenies and were also superior with regard to nut characteristics. A total of 40 high-yielding and disease-free palms belonging to the first generation were selected during 2009-10 to produce second-generation progenies for further evaluation, selection and crossing. Analysis of population structure of the mother palms and first-generation progeny using SSR markers, indicated higher values for inbreeding coefficient and homozygosity in subsequent generations. The inter se-mated progenies were superior to selfed progenies with respect to nut yield and resistance to root (wilt) disease.

Keywords: coconut, root (wilt) disease, resistance breeding, sib-mating inbreeding, genetic diversity, microsatellite

INTRODUCTION

The coconut palm (*Cocos nucifera* L.), referred to as the “Tree of Heaven”, is widely cultivated in the tropics. It is an important plantation crop, providing food and livelihood security to millions of people. Cultivation of coconut palm is important in sustaining the economy of several countries in the Asian and Pacific region. In addition to its importance as a source of healthy food and nutritional health drinks, it also provides an excellent natural fibre, shelter and gives an aesthetic appeal to landscapes. Coconut palm also sustains several fragile island ecosystems. In the context of climate change, coconut is a crop of the future due to its carbon sequestration potential. In India, diseases of coconut are a major concern, some of which are lethal, while others limit production.

Coconut root (wilt) disease is endemic in Kerala, India. Flaccidity, yellowing and necrosis of leaflets are reported to be the major diagnostic symptoms of the disease. According to a survey conducted in 1984, the disease was found to cause an annual loss of 968 million nuts (Anon., 1985). In the contiguously diseased area of Kerala, the vast majority of coconut palms have succumbed to the disease. Systematic investigations have shown an association of disease symptoms with a phytoplasma (Solomon et al., 1983). As the disease



could not be controlled by physical or chemical plant protection methods, the use of resistant or tolerant cultivars has been regarded as the most ideal and practical method for management of coconut plantations in disease-prevalent tracts.

Since 1988, a comprehensive breeding programme for resistance/tolerance to coconut root (wilt) disease has been implemented at the Regional Station, ICAR-Central Plantation Crops Research Institute (CPCRI), Kayamkulam. The mother palms for the breeding programme were selected based on strict criteria (Nair et al., 1996) from the approximately one in a million coconut palms that appeared to remain immune to the disease. Considering the better performance of the progeny produced from disease-resistant 'West Coast Tall' (WCT) palms, the Institute commenced establishing nuclear seed gardens by planting self- and sib-mated progeny of disease-resistant WCT, for large-scale production of quality coconut seedlings (Nair et al., 2002). The seedlings produced from the first-generation progeny are expected to have higher yields and a higher level of resistance to coconut root (wilt) disease.

An attempt was made to evaluate the performance of the self- and sib-mated progenies of disease-free WCT palms with regard to horticultural traits, nut yield and resistance to root (wilt) disease. Molecular analysis was carried out to study the extent of inbreeding in self- and sib-mated progenies after one cycle of recurrent selection.

MATERIALS AND METHODS

Base population

Mother palms from farmers' fields in hotspots of root (wilt) disease in southern districts of Kerala State, India, constituted the base population (S_0 population). The palms were selected based on strict criteria (Nair et al., 1996).

Experimental population

A crossing programme for development of an improved WCT cultivar, by inter se mating/selfing of selected disease-free WCT palms, was initiated during 1992-93. A total of 1250 selfed/inter se-mated progeny of disease-free mother palms were planted during 1994-96 at ICAR-Central Plantation Crops Research Institute (CPCRI), Regional Station, Kayamkulam, Kerala, India. Selections were made during 2008-09 from within the progeny planted during 1994-96. Initially, a total of 80 disease-free palms were selected based on visual appearance (disease-free nature) and finally 40 selfed/inter se-mated palms were selected after confirmation by ELISA test (Sasikala et al., 2001).

Plant and fruit characteristics

Observations on morphological characters were recorded from 10 palms of each population (viz., mother palms, selfed progenies and inter se-mated progenies) based on standard procedures (COGENT, 2007). Two nuts each from 10 palms were collected for nut character studies. The data on various characters were analysed statistically for estimation of means and standard errors. A random population of 100 palms each from the self and inter se populations was taken for recording disease incidence, disease index and 25 palms of each population were used for measuring nut yield.

Disease incidence and index

Two parameters were scored for the evaluation of resistance: (i) disease incidence, calculated based on percentage of root (wilt) affected palms in each cultivar; and (ii) disease index/intensity, calculated based on the modified method suggested by Nambiar and Pillai (1985). The following criteria were adopted for disease indexing: disease index 0 = total absence of all symptoms; below 20 = disease early stage; 20-50 = disease middle stage; above 50 = disease advanced stage. In addition, all palms with doubtful symptoms were subjected to ELISA test to confirm the disease-free nature of the palms (Sasikala et al., 2001).

Molecular analysis

Tissue for DNA extraction was taken from the youngest leaf (unopened spear or spindle) of each selected palm. A total of nine highly polymorphic SSR primer pairs were used. PCR was conducted following the procedure of Rajesh et al. (2008). The pattern of amplified products across the samples was resolved by silver staining following the procedure of Panaud et al. (1996). Microsatellite data were analysed using version 1.31 of the software POPGENE (Yeh et al., 1999) and heterozygosity values were calculated using the software GDA (Lewis and Zaykin, 2002).

RESULTS AND DISCUSSION

Resistant mother palms occur sporadically, and may be distinct genotypes evolved through natural selection/cross combinations. Recurrent selection is the breeding strategy adopted in heterozygous crops such as coconut, because the focus is on population improvement rather than individual improvement (Baudouin et al., 1997). As expected, plant and fruit character studies conducted in the base and experimental populations reveal that there is distinct inbreeding depression in the selfed population compared with the base population with respect to most of the morphological and fruit characters (Tables 1 and 2). In coconut, inbreeding depression in the form of low nut set and disease susceptibility upon selfing and sib-mating has also been observed elsewhere (Devakumar et al., 2011). However, in the sib-mated population, heterosis is expressed for most of the characters, and occurred in crossing between two tall genotypes (high-yielding WCT palms). Crossings were also attempted between WCT palms growing in diverse locations. At times, pollen from the few high-yielding palms in one district was used to pollinate the mother palms located in another district. The diversity of the parental palms can be noticed from higher values of observed heterozygosity of the parental palm population. It can also be noted that the coefficient of variation (%) values for most of the plant and fruit characters in the selfed population is higher than that in the sib-mated population. This can be due to segregation upon selfing, as coconut is highly heterozygous.

Table 1. Plant characters of the experimental and base populations.

Morphological character	Self	CV (%)	Inter se	CV (%)	Base	CV (%)
Total number of leaves	30.33	12.45	33.25	6.78	29.20	15.90
Length of petiole (cm)	114.23	30.64	137.42	7.67	107.80	13.80
Length of leaf-bearing portion (cm)	345.44	15.00	433.33	7.07	349.30	8.80
Number of leaflets	107.10	51.90	114.08	6.42	114.50	9.90
Length of leaflets (cm)	5.36	13.98	6.58	7.87	6.40	10.10
Breadth of leaflet (cm)	110.40	13.95	138.75	6.04	110.90	13.90
Number of leaf scars in 1 m	13.22	21.23	12.92	11.48	10.10	14.30
Length of internodes (cm)	8.45	21.54	6.63	13.23	9.50	13.50

Table 2. Reproductive characters of the experimental and base populations.

Morphological character	Self	CV (%)	Inter se	CV (%)	Base	CV (%)
Length of inflorescence (cm)	108.11	20.35	112.08	18.46	93.10	12.80
Length of spikelet-bearing portion (cm)	52.00	20.39	41.75	25.67	34.60	20.24
Length of stalk (cm)	40.00	19.44	49.33	8.94	37.10	16.13
Length of spikelet (cm)	33.90	16.14	40.00	14.95	32.10	13.92
Number of spikelets	16.50	55.33	34.33	77.46	28.50	23.57
Number of female flowers	16.78	12.31	16.00	11.34	14.30	6.96
Bunches with buttons	5.22	26.66	4.92	20.34	4.40	21.32
Bunches with nuts	13.56	25.52	8.92	17.70	9.20	20.93
Average nut yield	119	-	165	-	140	-

Observations recorded after 18-19 years of planting revealed that the disease incidence in inter se-mated progenies was only 47% compared with 63% in selfed progenies. Among the diseased palms of the selfed population, the number in the disease middle category is significantly higher (28) compared with the inter se-mated population (14). The inter se-mated progenies had 35-40% higher nut yield compared with selfed progenies and was superior with regard to nut characters (Table 3). The average copra content in the nuts of inter se progeny was also higher (214 g) compared with the selfed population (165 g).

Table 3. Nut characters of the experimental and base populations.

Nut character	Self	CV (%)	Inter se	CV (%)	Base	CV (%)
Length of fruit (cm)	16.97	8.35	21.00	15.22	20.50	7.77
Breadth of fruit (cm)	13.92	11.54	14.70	29.27	14.20	11.06
Weight of fruit (g)	1163.30	31.66	1247.00	30.54	1033.50	15.00
Thickness of husk (cm)	2.47	15.31	2.48	26.82	2.70	17.98
Weight of dehusked nut (g)	513.30	28.59	694.40	32.89	614.50	19.40
Husk to whole fruit weight (%)	51.48	6.28	46.04	19.14	40.30	20.56
Thickness of kernel (cm)	1.55	2.15	1.51	14.38	1.24	15.64
Weight of copra (g)	166.67	25.00	213.82	26.39	177.00	22.26
Thickness of shell	0.208	4.72	0.209	4.77	0.320	18.49
Weight of shell (g)	125.00	28.17	164.30	28.30	137.20	15.74
Quantity of water (mL)	100.00	52.15	173.30	48.23	130.00	47.96

Observations on nut yield (Table 3) and disease incidence (Table 4) in the selfed population compared with the inter se-mated population give a clear indication as to the strategy to be adopted while carrying out recurrent selection in coconut, especially for disease resistance. The strategy recommended is to adopt inter se/sib mating instead of selfing. However, further studies in this direction are suggested in order to confirm the results.

Table 4. Disease incidence and index in selfed and inter se-mated progeny.

Character	Self (%)	No. of palms	Inter se (%)	No. of palms
Disease incidence	63.0	-	47.0	-
Disease early	49.0	31	57.0	27
Disease middle	45.0	28	30.0	14
Disease advanced	6.0	4	13.0	6

Selfing in a heterozygous crop like coconut can lead to random loss of favourable genes. By contrast, inter se mating/crossing between disease-free palms would give a greater chance for recombination of favourable genes (Allard, 1960). Indeed, a higher percentage of disease-free palms in the inter se/sib-mated progeny (Table 4) supports this approach. In a segregating population, production of less healthy progeny can also be expected as a consequence of inbreeding depression. Disease resistance per se is a complex phenomenon and is closely linked with health of the host plant. Inbreeding and loss of genetic diversity reduced the ability of *Drosophila* populations to cope with disease caused by infection of both *Bacillus thuringiensis* and *Serratia marcescens* (Spielman et al., 2004). There is experimental evidence in both plant and animal breeding that inbred lines obtained under milder inbreeding with selection was not much different from the results with selfing (Allard, 1960).

Nine polymorphic SSR markers were used to analyse the populations (Table 5). The proportion of polymorphic loci was 1.00 (P), with all the analysed loci being highly polymorphic (100%). The number of alleles (A) ranged from 8 to 12, with an average of 9.22

alleles per locus. The loci CNZ26 and CnCirB6 have the highest number of alleles (12). The markers had polymorphic information content (PIC) values between 0.12 (CnCir B12) and 0.73 (CnCirB6), with an average of 0.39 (Table 6).

Table 5. Sequence and melting temperatures (T_m) of SSR markers.

SL. no.	Primer name	Sequence (5'-3')	T_m (°C)
1	CN1H2 F	TTGATAGGAGAGCTTCATAAC	54
	CN1H2 R	ATCTTCTTTAATGCTCGGAGT	
2	CnCir 87F	ATAACATCCTCCAACCTG	58
	CnCir 87R	GACTGAATCCAACCTT	
3	CnCir E4F	GCATGGTATTCCGATTTG	54
	CnCir E4R	ATGGTTCAGATTTGGACAGT	
4	CNZ 26 F	CTAGGCTCCCATGTGTTTTT	58
	CNZ 26 R	CACTGCTGTTGTACACCTCCA	
5	CnCir B12 F	GCTCTTCAGTCTTTCTCAA	58
	CnCir B12 R	CTGTATGCCAATTTTTCTA	
6	CnCir B6 F	GAGTGTGTGAGCCAGCAT	59
	CnCir B6 R	ATTGTTACAGTCCTTCCA	
7	CnCir E10F	TTGGTTCCATTTCTTCTCTCATC	59
	CnCir E10R	GCTCTTTAGGGTTCGCTTTCTTAG	
8	CnCir F2F	GGTCTCCTCTCCCTCCTTATCT	56
	CnCir F2R	CGACGACCCAAAACCTGAACAC	
9	CAC 10F	GATGGAAGGTGGTAATGCTG	56
	CAC 10R	GGAACCTCTTTGGGTCATT	

Table 6. Descriptive statistics (by locus).

Locus	P	A	PIC
CN1H2	1.000000	8.000000	0.384058
CnCirE4	1.000000	9.000000	0.514706
CNZ26	1.000000	12.000000	0.189781
CnCir87	1.000000	11.000000	0.467626
CnCirB12	1.000000	7.000000	0.126984
CnCirB6	1.000000	12.000000	0.731884
CnCirE10	1.000000	7.000000	0.258993
CnCirF2	1.000000	9.000000	0.248175
CAC10	1.000000	8.000000	0.661765
All	1.000000	9.222222	0.398219

P, Proportion of polymorphic loci; A, number of alleles; PIC, polymorphic information content value.

For all populations, the observed heterozygosity was less than expected (Table 7), indicating inbreeding and a deficit of heterozygotes. Selfing and sib mating/inter se mating could have reduced the heterozygosity. The lowest value for observed heterozygosity was obtained for selfing compared with inter se mating and random mating. The divergence between expected and observed heterozygosity for all individuals, as reflected in the F_{IT} parameter, had a mean of 0.52 for all loci, and ranged for the different markers between 0.18 (CnCirB6) and 0.82 (CnCirB12), indicating inbreeding (Table 8). Mean F_{ST} was 0.17, indicating that the sub-populations are highly differentiated (alleles fixed). For all loci, the value of F_{IS} was positive, indicating heterozygote deficiencies in these loci. Gene flow (N_m) among populations equalled 1.15, which gives an estimate of the average number of migrants between all studied populations per generation (Table 8).

Table 7. Details of expected (He) and observed (Ho) heterozygosity and fixation index (f).

Population	He	Ho	f
Base	0.845725	0.573545	0.312893
Selfed 1	0.719883	0.300000	0.596346
Selfed 2	0.723923	0.301235	0.596874
Inter se mated 1	0.799530	0.501235	0.386296
Inter se mated 2	0.658136	0.358025	0.469521
Open pollinated 1	0.761916	0.361728	0.539165
Open pollinated 2	0.663158	0.400000	0.409836
Mean	0.730193	0.400677	0.465227

Table 8. Summary of F statistics and gene flow for all loci.

Locus	F _{IS}	F _{IT}	F _{ST}	Nm ¹
CN1H2	0.4164	0.5356	0.2042	0.9743
CnCirE4	0.3210	0.3968	0.1116	1.9900
CNZ26	0.7115	0.7753	0.2210	0.8812
CnCir87	0.3418	0.4554	0.1726	1.1985
CnCirB12	0.7506	0.8225	0.2884	0.6170
CnCirB6	0.1029	0.1872	0.0939	2.4115
CnCirE10	0.6143	0.6872	0.1889	1.0734
CnCirF2	0.6140	0.7117	0.2530	0.7381
CAC10	0.1374	0.2091	0.0832	2.7562
Mean	0.4218	0.5244	0.1774	1.1589

¹Nm, Gene flow estimated from $F_{ST}=0.25(1-F_{ST})/F_{ST}$.

CONCLUSION

Selfing in coconut has been observed to lead to inbreeding depression, expressed in both plant and fruit characteristics. Molecular analysis has shown that selfing reduced genetic diversity, leading to a random loss of favourable genes. A higher percentage of disease-free palms in the inter se-mated progeny indicates that milder inbreeding (intercrossing of more than one disease-free palm) would give a greater chance for recombination of favourable genes. Hence, such milder inbreeding is suggested for implementation in breeding coconut for disease resistance.

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