

Years of Research on "Breeding for Resistance/Tolerance to Coconut Root (Wilt) Disease"





CENTRAL PLANTATION CROPS RESEARCH INSTITUTE (Indian Council of Agricultural Research) Kasaragod-671 124, Kerala Twenty-Five Years of Research on

"Breeding for Resistance/Tolerance to Coconut Root (Wilt) Disease"

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Compiled and edited by Regi Jacob Thomas M. Shareefa M.K. Rajesh R.V. Nair P.M. Jacob Anitha Karun George V. Thomas Front Cover Healthy WCT mother palm in the midst of diseased palms. Back Cover CPCRI Regional Station, Kayamkulam

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The coconut palm disease (rechristened as coconut root (wilt) disease) is believed to have made its appearance first in the Erattupetta area in Meenachil taluk of Kottayam district sometime between 1868 and 1878. It has since then spread from the epicenter of infection to various areas over a period of 135 years.

Establishment of CPCRI, Regional Station, Kayamkulam

The Central Coconut Research Station (CCRS) at Kayamkulam was established in 1948 by the Indian Central Coconut Committee with the mandate to investigate the etiology and to develop management practices for diseases of coconut with special emphasis on root (wilt) disease. The Indian Council of Agricultural Research took over the administrative control of the CCRS from April 1, 1966, consequent on the abolition of the Commodity Committees. With the establishment of the Central Plantation Crops Research Institute in 1970, CCRS, Kayamkulam became one of its Regional Stations.

ROOT (WILT) DISEASE

Symptoms & Spread

Flaccidity, yellowing and necrosis of leaflets are the major distinguishing symptoms of the root (wilt) disease. The disease is contiguously prevalent in the eight southern districts of Kerala from Thiruvananthapuram to Thrissur, and in isolated tracts in the remaining six



Root (wilt) diseased palm

northern districts (Solomon *et al.*, 2001). Sparse occurrence of this disease has also been reported from bordering areas of Tamilnadu such as Shenkotta, Coimbatore, Cumbum, Pollachi and Kulasekharam, Dakshina Kannada district of Karnataka and in Goa (Chandramohanan, 2010).

Etiology

Studies on the etiology of the disease suggested the association of phytoplasma (Solomon *et al.*, 1983a) transmitted through lace bug, *Stephanitis typica* (Mathen *et al.*, 1987) and the plant hopper, *Proutista moesta* (Rajan *et al.*, 2002). Association of phytoplasma with the root (wilt) disease of the coconut was further confirmed though Polymerase Chain Reaction (PCR) technology and phytoplasma belonging to the group 16 Sr XI is established to be associated with the root (wilt) disease of coconut in Kerala (Manimekalai *et al.*, 2010).

As phytoplasmal diseases cannot be controlled by conventional plant protection measures, development of disease resistant / tolerant variety is the most practical method for the management of this malady.

Breeding for Resistance/Tolerance to Coconut Root (Wilt) Disease

1.0. Efforts to locate resistance to root (wilt) disease

Butler (1908) surveyed the disease affected area and suggested identifying disease resistant variety as one of the possible solutions for the disease. The first such attempt was that of Varghese (1934) who surveyed about 4 sq. miles of highly diseased area around Kayamkulam, but he could not locate any resistant palm. Presence of healthy and high yielding palms in the midst of heavily diseaseaffected palms was reported by Davis (1953). The need for selecting such palms for breeding for resistance to root (wilt) disease was emphasized by Davis (1953).

Screening of available coconut germplasm by planting seedlings in the disease-affected farm at CPCRI Regional Station, Kayamkulam was initiated in 1961. However, all of them developed characteristic disease symptoms at various intervals after planting (Menon et al., 1981). Rawther and Pillai (1972) observed that Natural Cross Dwarfs (NCDs) exhibited higher tolerance to the disease as compared to West Coast Tall (WCT). Radha (1961) reported higher degree of resistance to both leaf rot and root (wilt) in Andaman Ordinary Tall and New Guinea Tall based on disease incidence. Mathai (1985) also reported higher degree of resistance to leaf rot and root (wilt) in Andaman Ordinary Tall, but his observation was based on disease index.

Results of large scale screening trials undertaken during 1972 at CPCRI Regional Station, Kayamkulam and also Technical Bulletin No. 72

in cultivator's gardens revealed that all the cultivars and hybrids evaluated contracted the disease. Davis (1970) suggested to take up planting of Malayan Dwarfs in root (wilt) affected areas, since it was reported to be tolerant to lethal yellowing disease in Jamaica. In 1982, a trial involving 27 cultivars, 10 hybrid combinations, F2 (OP) of D x T and progenies of elite WCT palms was laid out in cultivators' gardens. The results revealed that among the 27 cultivars, all except Kenthali Orange Dwarf had taken up the disease. Besides, all the cross combinations, F2 and progenies of elite WCT palms were affected by the disease (Jacob et al., 1998).

2.0. Genesis of 'hot spot' breeding programme

Based on the recommendations of the Ist International Symposium on Coconut Research & Development (ISOCRAD-I) held at CPCRI, Kasaragod during 27-31st December 1976, Iyer et al (1979) conducted a survey of natural population in root (wilt) disease affected areas during the period 1977-1981 to identify elite super palms exhibiting high vielding potential. A total of 12 elite palms were selected from the root (wilt) affected areas of Kollam, Alappuzha and Kottayam districts. Open pollinated seed nuts from these palms were raised and planted in root (wilt) disease affected areas. But all of them took up the disease in subsequent years.

With this background, a group meeting was organized at CPCRI, Kasaragod on 18th July 1988 to evolve a



Healthy WCT mother palm in disease hotspot.

programme on breeding for resistance/ tolerance to root (wilt) disease. Accordingly, a comprehensive breeding programme for evolving resistant/tolerant coconut varieties has been implemented at CPCRI Regional Station, Kayamkulam from 1988 (Nair et al., 1996). In an intensive survey in the hot spot districts of Alappuzha, Pathanamthitta, Kollam and Kottayam, 200 Chowghat Green Dwarf (CGD) palms were observed. It was found that 75% of them were disease-free indicating that this variety possessed higher level of resistance to root (wilt) disease compared to other varieties. Breeding for resistance to coconut root (wilt) disease was initiated primarily based on the following observations:

- 1. CGD is having higher level of resistance when compared to other varieties.
- 2. In the disease endemic areas, in the midst of heavily disease-affected WCT palms, disease-free and high yielding palms were found. These disease-free palms were identified as the base material for the breeding programme.
- 3. In disease endemic areas, a number of disease-free and high yielding Chowghat Orange Dwarf (COD) palms were also

found in the midst of heavily disease affected coconut palms. These were also included in the breeding programme to exploit the reported occurrence of high yield and tolerance to the disease in COD x WCT hybrids.

Selection criteria of mother palms for artificial pollination (i) West Coast Tall (WCT)

- a. Parental palms should yield 80 or more nuts per palm per year.
- b. Palms should be regular bearers and absolutely free from all diseases and pests.
- c. Palms should be more than 35 years old and surrounded by palms of which at least 80% are affected by the root (wilt) disease, in an endemic area.
- d. Tall mother palms should have typical WCT characters. Twenty-five open pollinated seedlings are grown from each mother palm and all progeny seedlings should show uniform vigour and colour characters of WCT. This progeny test is done to cull out hybrid mother palms, if any.
- e. Palms should be negative in their reaction to the root (wilt) antiserum (Solomon *et al.*, 1983b) and the serological tests are to be repeated every year.
- (ii)Chowghat Green Dwarf (CGD)/ Chowghat Orange Dwarf (COD)
- a. Palms should yield 100 nuts or more per year.
- b. Palms should be healthy and free from

all diseases and pests.

- c. Parental palms should be above 20 years of age and surrounded by palms of which atleast 80% are affected by the root (wilt) disease, in an endemic area.
- d. Palms should show typical characters with regard to stem, crown, leaf and nut.
- e. Palms should be negative in their reaction to the root (wilt) antiserum (Solomon *et al.*, 1983b) and the serological tests are to be repeated every year.

I. PROGRESS OF RESEARCH

(a) Hot spot breeding programme

Artificial pollination of mother palms were carried out in farmers' plots located in the hot spot districts (Alappuzha, Kollam, Kottayam and Pathanamthitta) of root (wilt) disease, with a view to produce artificially pollinated progenies which can be used either for developing a root (wilt) resistant/tolerant variety or for the production of parental materials for establishing seed gardens. Details regarding the varieties/ mother palms pollinated since 1998 are given in Table 1. The following cross combinations were carried out:

- 1. WCT (inter se)
- 2. WCT (self)
- 3. WCT x CGD
- 4. CGD (self/ inter se)
- 5. CGD x WCT
- 6. COD (self/ inter se)

Seedlings of the above cross combinations were under-planted since

Year	WCT	CGD	COD	Total
1988 - 89	22	3	÷	25
1989 - 90	30	6	-	36
1990 - 91	24	8	+	32
1991 - 92	40	8	÷	-48
1992 - 93	67	10	5	82
1993 - 94	104	5		109
1994 - 95	95	30		125
1995 - 96	85	20	4	109
1996 - 97	76	111	9	196
1997 - 98	76	119	9	204
1998 - 99	69	80	10	159
1999 - 00	63	97	4	164
2000 - 01	81	104	7	192
2001 - 02	71	79	7	157
2002 - 03	63	84	23	170
2003 - 04	69	85	25	179

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1991 in the normal spacing of 7.5 m x 7.5 m in different blocks of CPCRI (RS), Kayamkulam where more than 80% of the existing palms were affected by root (wilt) disease. Care was taken to see that the old diseased palms were retained at least for six to seven years after under planting of the seedlings, to provide sufficient inoculum for natural infection under field conditions. Heavy incidence of root (wilt) disease was observed in underplanted seedlings starting from the fourth year after planting. The under-planted seedlings were removed as and when they took up infection and the same pit was replanted with new seedling produced through artificial pollination.

(b) Studies on fruit setting

Artificial pollination on these selected mother palms located in the districts *viz*. Alappuzha, Kottayam, Pathanamthitta and Kollam were carried out over a period of 12 years from 1988 to 2000. The number of palms studied per year varied from 25 to 204. Results of artificial pollination of 1,09,551 buttons

Breeding for Resistance/Tolerance to Coconut Root (Wilt) Disease

belonging to 4,955 inflorescences resulted in a setting percentage of 26.1 (with a range of 14.8 to 38.5) over the years (Nair *et al.*, 2003b).

Table 2. Setting percentage in	various	cross combinations involving
WOT CODeed	COD	and for all margaret

Cross combi-nation	Jan	Feb	March	April	May	Nov	Dec	Avg. setting (%)
WCT (Self)	22.90	29.88	32.80	37,03	13.25	26.84	22.63	27.43*
WCT (inter se)	22.00	22.84	31.47	21.32	21.15	13:67	18.94	21.63 %
CGD (Self)	23.22	29.25	26.45	20.79	18.13	21.06	32.94	24.65 =
CGD (inter ie)	33.66	19.08	22.80	15.19	17.35	10.48	25.99	21.22 th
COD (Self)	50.76	40.44	40.38	39.20	38.48	26.42	29.20	39.54 *
WCT X CGD(TxD)	23.84	21.09	29,90	26.64	07.14	21.05	17.62	21.92 **
CGD X WCT(DXT)	20.39	19.91	21.53	18.37	18.11	13.51	18.72	19.16
COD X WCT(DxT)	29.23	36.99	24.16	32.01	03.45	06.67	38.37	26.12*
Average	27.62*	25.44*	28.73	24.83***	18.80	19.16*	22.98%	24.67

c) volume to lowered by the source lattest are not segmentary different by ANOVA (pc 0) CD to rease consistmentions) = 6.36. CD (Month) = 6.50.

Studies on fruit set in coconut have important implications on nut yield. A detailed study was carried out to determine the fruit set in coconut as influenced by variety, cross combination, climatic variables such as rainfall, temperature and relative humidity (Thomas et al., 2012). Artificial pollination was carried out on selected parental palms of West Coast Tall (WCT), Chowghat Green Dwarf (CGD) and Chowghat Orange Dwarf (COD) standing in farmer's plots over a period of five years commencing from 1996-2000. The various cross combinations tried among the three varieties, viz., WCT, CGD and COD were three selfing, two inter se and three crosses. The mean fruit set for the different cross combinations was 24.67% with a range of 19.16 - 39.54 (Table 2). The maximum fruit set (39.54%) was in COD (self) followed by WCT (self) and COD x WCT, and minimum in CGD x WCT (19.16%) indicating that COD variety as a female parent gave significantly higher fruit set compared to other varieties. Generally, the varieties WCT

and CGD under selfing gave a higher fruit set (27.43 and 24.65%) when compared to inter se (21.63 and 21.22%). Fruit set was maximum (28.73%) during March and minimum (18.80%) during May and the year-to-year variation was not significant. Fruit set was negatively correlated with bimonthly average relative humidity (-0.504), number of rainy days (-0.428) and rainfall (-0.395). Studies also revealed that there is a significant reduction in fruit set to the tune of 35%, when climatic conditions are not favourable. Investigations revealed that fruit set in coconut vary significantly due to genotype, cross combinations and climatic variables.

(c) Development of software for studies on fruit setting

In addition, a Fox Pro based database software was designed (Ajithkumar *et al.*, 2003) (Coconut Artificial Pollination Database Management System) for analysis of data on fruit setting upon artificial pollination in coconut. This software was used to pool area wise, palm wise, year wise and month wise data on fruit set. It was helpful to refine the crossing programme in 'hot spots' for obtaining optimum fruit set upon artificial pollination.

(d) Evaluation of progenies

(i) WCT x WCT crosses

Progenies belonging to the following crosses of WCT were evaluated for root (wilt) disease resistance.

- 1. WCT x WCT (Inter se)
- 2. WCT (Self)
- 3. WCT (Mixed Pollen)
- 4. WCT (Open Pollinated)

All the seedlings were planted during 1992. Observations on disease incidence were recorded as and when the experimental palms developed initial symptoms of the disease. Initial symptoms of the disease viz., flaccidity of the young leaves started appearing four years after planting in all the cross combinations of WCT. Annual progression of the disease incidence is given in Table 3. Observations recorded twelve years after planting revealed that the disease incidence in the progenies raised from various cross combinations of WCT (inter se / self / mixed pollen) varied from 55.0% to 58.7%, indicating that there was no significant variation in the susceptibility to the disease among the different cross combinations of WCT (Nair et al., 2003a). In the case of the open pollinated progenies of WCT there was 80 % disease incidence. This showed the superiority of the artificially pollinated seedlings (full sibs) over the open pollinated seedlings (half sibs) with regard to their resistance to the disease. Studies on annual progression of disease incidence over the years indicated that the disease progression was similar in different crosses of WCT. This study finds application due to the fact that farmer's in the root (wilt) prevalent tracts traditionally plant open pollinated seedlings produced from high yielding and disease-free mother palms. Information regarding the disease incidence among the seedlings produced from disease-free mother palms will be of great use to farmers. Since the artificially pollinated seedlings are clearly superior to open pollinated seedlings with regard to disease resistance as established

Technical Bulletin No. 72 in the study, they are suitable especially for planting as parental materials in seed gardens.

Table 3. Progression of root (wilt) disease incidence in various cross combinations of West Coast Tall variety (Year of Planting: 1992)

SI No.	Cross-	Nos.			Perce	intage	discase	c incid	ence	1	_
	combination	planted	1996	1997	1998	1999	2000	2001	2002	2003	2004
1	WCT (Inter se)	87	6.9	13.8	40.2	47.1	50.9	54.0	38.2	57.5	58.7
2	WCT (Self)	37	27.0	29.7	43.2	43.2	47.4	51.4	56.8	56.8	56.8
1	WCT (MP)	118	15.3	17.0	39.8	41.5	49.1	51.7	53.4	55.0	55.0
4	WCT (OP)	20	25.0	25.0	55.0	60.0	65.0	70.0	70.0	75.0	80.0

Systematic evaluation trials at CPCRI Regional Station, Kayamkulam for developing varieties with resistance/ tolerance to root (wilt) disease has led to the release of three coconut varieties for the root (wilt) disease prevalent tract. The details of the varieties including one hybrid released are listed below.

(e) Identification of CGD as resistant/ tolerant to root (wilt) disease from screening trial



Chowghat Green Dwarf palm

In an intensive survey in large number of farmers' plots in the 'disease hot spots', 200 Chowghat Green Dwarf (CGD) palms were observed for root (wilt) disease incidence. Studies revealed that 75% of the observed palms

Table 4. Root (wilt) disease incidence and yield of Kalpasree and West Coast Tall palms in farmer's plots.

District	No. of		Kalpasree		West Coast Tall			
	plots	No. of palms	Disease incidence (%)	Avg. yield (nuts/palm)		Disease incidence (%)	Avg. yield (nuts/palm)	
Kollam	10	47	14.5	76.6	99	79.8	34.0	
Kottayam	35	80	14.2	75.5	206	88.8	37.8	
Alappuzha	23	167	15.8	78.8	205	82.5	40.6	
Pathan'thitta	43	198	13.5	92.5	154	85.6	40.5	
Total	126	551	14.5	76.5	664	85.2	39.0	

were disease-free indicating that CGD is having higher level of resistance to root (wilt) disease when compared to other varieties. Besides, a screening trial involving ten varieties was also laid out at CPCRI Regional Station, Kayamkulam in 1988. Observations recorded during 1994 based on percentage disease incidence revealed that CGD had the highest level of resistance (75) followed by Philippines Lono Tall (70.8), Zanzibar Tall (70.8) and Federated Malay States Tall (66.7) (Nair et al., 2004). Accordingly, CGD was identified as the source of resistance for the breeding programme.

Variety	Nut / palin/ year	Copra content (g/nut)	Copra/ palm (kg)	No. of palms/ ha	Copra/ ha (tonnes)	Oil content (%)	Oil/ha (tonnes)
Kalpasree Olesearch Status data)	90.0	96.3	8.667	236 (6.538.5 m	2.045	66.5	1.360
Kalpasree (Escreme's plat data!	76.5	96.3	7.367	236 (6.5X6.5 m structure)	1.739	66.3	1.156
WCT	48.7	210.1	10.232	175 (7.557.5 m spectral	1.791	67.0	1.200

Table 5. Nut, Copra and Oil yield of Kalpasree in comparison to West Coast Tall

Root (wilt) disease incidence and intensity of Chowghat Green Dwarf selection was further evaluated and compared with WCT in the disease prevalent tracts of Kerala. The studies confirmed the high yield and low incidence of root (wilt) disease in CGD (Tables 4 & 5) in hot spots of root (wilt) disease. Considering the high yield and low incidence of root (wilt) disease, selection made from Chowghat Green Dwarf was released under the name "Kalpasree" for

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cultivation in homesteads of the root (wilt) prevalent tract during the AICRP (Palms) workshop held during 2009 at Navsari, Gujarat.

(f) Evaluation of CGD x WCT hybrid



Kalpa Sankara hybrid

Observations on 31 CGD x WCT progenies, planted during 1991 indicated that the hybrid palms came to flowering in 40 to 50 months after planting. Disease incidence in CGD x WCT hybrids planted during 1991 is given in Table 6. Seventy percent of the hybrids took up disease 16 years after planting. Yield data of healthy and diseased hybrids over the years is

Year of observation	Nos. planted	Nos. diseased	Disease incidence
1999	31	10	32.3
2000	31	15	48.4
2001	31	16	51.6
2002	31	19	61.2
2003	31	20	64.5
2004	-31	21	67.7
2005	- 31	21	67.7
2006	31	21	67.7
2007	31	22	70.5

given in Table 7. It can be seen that even though majority of CGD x WCT hybrids were diseased, they gave a ten year cumulative average yield of 84 nuts/palm/ year indicating that this hybrid is tolerant to root (wilt) disease. Among the hybrids, disease-free hybrids gave an average

Table 7. Average nut yield of CGD x WCT hybrids (Year of planting: 1991)

Year	Hybrids (nuts/palm)	Avg. of healthy hybrids (nuts/palm)	Avg. of diseased hybrids (nuts/palm)
1999-00	112	128	79
2000-01	71	82	54
2001-02	85	95	65
2002-03	73	82	57
2003-04	71	79	64
2004-05	60.5	71	63
2005-06	85	82	78
2006-07	110	132	76.8
2007-08	103	123.2	77.4
2008-09	96	122.6	63.4
Cum. avg. yield	84	107	72

yield of 107 nuts/palm/year where as the disease-acquired hybrids gave 72 nuts/ palm/year. This yield is quite attractive especially when compared to the state level average of 35 nuts/palm/year for the root (wilt) prevalent tracts (Nair *et al.*, 2006a). Considering the performance of CGD x WCT in the root (wilt) disease prevalent tract it was released under the name "Kalpa Sankara" during the AICRP (Palms) workshop held during 2009 at Navsari, Gujarat. Kalpa Sankara yields 2.5 tonnes of copra and 1.69 tonnes oil per hectare.

(g) Identification of Malayan Green Dwarf as resistant to root (wilt) disease



Malayan Green Dwarf palm

Studies carried out during 2004 resulted in identification of another promising variety namely Malayan Green Dwarf as resistant to root (wilt) disease (Nair *et al.*, 2007). This observation was Technical Bulletin No. 72

Table 8. Incidence and intensity of root (wilt) disease on coconut varieties at CDB Farm, Neriamangalam

Varieties	eties Year of	Nos.	Numbe	Number diseased		incidence (%)	
	planting	observed	2007	2008	2007	2008	index
CGD	1993	261	25	52	9.6	19.9	10.5
MGD	1993/94	76	12	17	15.9	22.4	15.5
MYD	1993/94	202	43	62	21.3	30.9	25.0
MOD	1993/94	110	64	71	58.2	64.6	28.0
COD	1994	27	9	10	33.3	37.0	20.0
WCT		100	84	84	84.0	84.0	45.0

recorded from a seed production plot at Coconut Development Board Farm, Neriamangalam, planted with five dwarf varieties of coconut namely Malayan



Heavily diseased MOD plot adjacent to MGD plot

Green Dwarf (MGD), Malayan Yellow Dwarf (MYD), Malayan Orange Dwarf (MOD), Chowghat Green Dwarf (CGD), and Chowghat Orange Dwarf (COD). The popular cultivated variety West Coast Tall (WCT) was treated as the control. WCT showed 84 % disease incidence indicating the availability of sufficient inoculum for evaluation of resistance of the dwarf varieties. With regard to resistance, CGD showed maximum resistance with disease incidence of 19.9% followed by MGD with disease incidence of 22.4 %. Earlier studies in WCT clearly showed that the most susceptible stage of WCT palm to root (wilt) disease is the juvenile phase of the palms i.e., between 8 to 12 years after planting (Ramadasan et al., 1971; Nair et al., 2003b). Palms of different varieties

Varicties	Yield (nuts/ palm/year)	Copra content (g/nut)	Copra/palm (Kg)	Copra/ha (t/ha)	Oil/ha (t/ha)
Kalpasree	48.4	96.8	4.69	0.821	0,54
Kalparaksha	88.8	185.1	16.44	2.877	1.87
MYD	70.7	202.2	14.30	2.502	1.54
MOD	61.8	150.1	9.28	1.624	0.44
WCT	48.7	210.1	10.23	1.790	1.22

Table 9. Nut yield of dwarf coconut varieties at CDB Farm, Neriamangalam. Year of planting: 1993-94

included in this study had completed the most susceptible stage.

Studies on the intensity of root (wilt) disease among the varieties showed that the most of the diseased palms in MGD selection are in the early stage of the disease and progression of disease was slow in this variety (Table 8). MGD gave the highest nut yield (88.8) followed by MYD (70.7). MGD out performed WCT in terms of copra and oil yield mainly because of the higher nut yield and more bunches with nuts (Table 9). This high nut yield can again be attributed to its higher level of resistance to root (wilt) disease compared to WCT. Considering the high yield and resistance to root (wilt) disease, this MGD selection was released under the name "Kalparaksha" for cultivation in the root (wilt) prevalent tracts during the AICRP (Palms) workshop held during 2007 at Hyderabad, India (Nair et al., 2009).

(h) Establishment of seed gardens

Apart from breeding for resistance/ tolerance to root (wilt) disease, efforts were made for establishing nucleus seed gardens in the four disease-endemic districts for distributing quality planting materials of WCT, CGD, COD, CGD x WCT and COD x WCT hybrids (depending on demand) to the farmers (Nair et al., 2006b). Starting from 1995, a total of 4175

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Seed garden at CDB Farm, Neriamangalam

artificially pollinated seedlings were so far planted in the five nucleus seed gardens. This includes, 1936 at District Agricultural Farm-Mavelikkara (Alappuzha), 1190 at CPCRI - Kannara (Thrissur), 647 at Coconut Development Board Farm-Neriamangalam (Ernakulam) and 402 at State Coconut Nursery-Karunagappally (Kollam). In the case of seedling mortality due to biotic and abiotic stresses, they were removed and replanted annually another artificially pollinated with seedling of the same combination (T x T or D x D) for nearly eight years after initial planting. These seed gardens are presently being maintained by Department of Agriculture (Govt. of Kerala) and Coconut Development Board (Govt. of India).

(i) Molecular markers for tagging gene(s) imparting resistance to coconut root (wilt) disease

The Biotechnology group at CPCRI, Kasaragod have standardized the AFLP, SSR and DAF protocols for tagging gene(s) imparting resistance to coconut root (wilt) disease (Rajesh et al., 2004; Jayadev et al., 2005). The population structure and genetic relatedness among root (wilt)

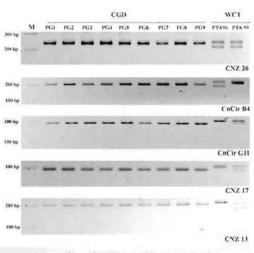
disease resistant and susceptible West Coast Tall coconut palms were analyzed using microsatellite markers (Devakumar et al., 2011). The pair-wise population differentiation estimate (Fst) between the resistant and susceptible populations was 0.021. Two major populations and a subpopulation cluster were identified among the resistant palms. The analysis of genetic relatedness between the resistant mother palms showed that most of the palms located in a single locality shared sib relationship among them. The existence of close genetic relationship among resistant palms from Chengannur, Thiruvalla, Kottayam and Pavukkara localities in Kerala has been reported.

Molecular analysis using microsatellite markers have been conducted to study the genetic uniformity of Kalpasree and Kalparaksha populations (Thomas et al., 2010). Forty two Kalpasree palms from 'disease hot spots' have been analyzed using 43 SSR primers. Monomorphic bands were detected in all the Kalpasree samples with 41 primers. A single Kalpasree palm showed polymorphism with two SSR primers. Forty eight Kalparaksha palms (17 from Seed Garden Complex, Munderi and 31 palms from DSP Farm, Neriamangalam) were analyzed using 24 SSR primers. The Kalparaksha palms clustered at 70% similarity.

(j) Genetic purity assessment of D x T hybrids using microsatellite markers

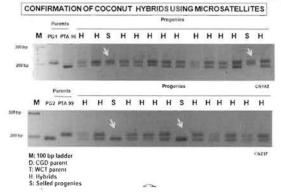
Farmers in the root (wilt) diseased tract of Kerala prefer dwarf/hybrid varieties of coconut for replanting, as these Technical Bulletin No. 72

palms are precocious, short in stature and possess higher level of resistance tolerance to the disease. In the root (wilt) diseased tracts, Chowghat Green Dwarf x West Coast Tall (CGD x WCT) hybrid has performed consistently giving a cumulative average yield of 84 nuts/ palm/ year. 'Kalpa Sankara' hybrid is produced by hybridizing Chowghat Green Dwarf with pollen collected from disease-free West Coast Tall palms located in the 'hot spots' of root (wilt) disease. The success of hybrid coconut technology depends on higher nut set upon artificial pollination and identification of genetically pure hybrids in the nursery. Identification of genuine hybrids in the nursery stage is the most difficult task in hybrid production. A set of morphological descriptors are currently used for varietal identification, description and seed purity assessment. Though widely adopted and practiced, purity assessments based on morphology is often affected by environment. Furthermore, many of the varieties and hybrids are phenotypically less distinct making morphological



SSR profiles of CGD & WCT parental palms

Maluation more difficult. Therefore, an alternative technique that offers efficient, quick and reliable assessment of genetic purity is urgently needed. Molecular markers offer an attractive option instead of morphological markers in coconut hybrid production programmes. Among the molecular markers currently available, microsatellites or simple sequence repeats (SSR) are popular because of their codominance, abundance and their capacity to detect high levels of allelic diversity. A set of 50 hyperpolymorphic coconut SSR markers were used to characterize CGD and WCT parental lines used for hybrid coconut production. From these SSRs, a panel of 17 informative SSR markers capable of distinguishing these parental lines were identified and these markers have been utilized in D x T hybrid seedling



purity assessments in coconut nurseries (Rajesh *et al.*, 2012).

(k) Isolation and characterization of Resistance Gene Analogues (RGAs) in coconut

Two sequences, showing homology to known RGAs from other plants, have been isolated from coconut using the degenerate primer-PCR strategy. The Technical Bulletin No. 72

first sequence [NBS-LRR resistance protein gene (RGA1); FJ666396] showed homology the NBS-LRR to type resistance protein gene from other species like Musa acuminata, Lolium perenne, Mangifera indica, Corylus avellana and Oryza sativa. Multiple alignment of deduced amino acid sequence of coconut RGA with related sequences revealed high sequence similarity at the kinase-2 domain, LLVLDDV. The second sequence [NBS-LRR resistance protein-like (RGA2) geneGQ856288] also showed homology to the NBS-LRR type resistance protein gene from Musa spp. and Elaeis guineensis.

(l) Histological studies of leaf tissues of dwarfs

Six varieties of coconut (CGD, COD, MGD, MOD, MYD & WCT) were subjected to anatomical studies for studying the morphological / structural characters associated with disease resistance. In each variety, samples representing three maturity levels viz., (i) mature leaflets (from outside the crown), (ii) middle (middle of the crown) and (iii) young (spindle portion). These leaves were collected from CDB Farm, Neriamangalam fixed in Carnov's B fixative (60% absolute alcohol, 30 % chloroform and 10 % acetic acid) for 24 hours and subsequently transferred to 70 % alcohol. These leaf samples upon dehydration were subsequently embedded in the paraffin waxblocks. Sections of 10 µm were taken with help of Leica microtome and these sections were de-paraffinized with xylene and butanol series and stained with PAS reagent and observed the section under microscope attached to the image

analyzer. The following observations were taken i) thickness of the leaf, ii) width of parenchyma iii) cuticle thickness, (iv) distance between the stomata and phloem etc. It was observed that lower cuticle thickness was more in MGD (10.27 um) compared to other varieties. Younger leaves of CGD had higher values for leaf thickness (1077.65 µm), width of parenchyma (47.29 µm) (between the vascular bundle and epidermis), and larger distance between the stomata to phloem tissues (88.25 µm) making it difficult for the vector to penetrate and inoculate the pathogen. These morphological/structural features may be the reason for higher level of resistance reported in CGD and MGD.

(m) Plumule culture for raising planting material for establishing seed garden

Vegetative propagation of coconut is of prime importance for rapid multiplication of promising genotypes obtained through conventional breeding. Among the various explants tried, plumular tissue was found to be the most responsive explant for vegetative propagation of coconut. Research on plumule culture was initiated at CPCRI during 1998 and the potential of using this protocol for increasing the planting material production upto five to ten fold has already been reported (Karun *et al.*, 2008 and Rajesh *et al.*, 2005).

Since the embryos are obtained by selfing selected dwarf parental palms, the clones from these embryos are expected to be true-to-type. SSR analysis has confirmed that the plantlets (upto 15



Regeneration of somatic embryo from MGD cultures

plants) derived from a single plumule are genetically uniform. As plumule culture technique has relevance in the establishment of nucleus seed garden of dwarf coconut varieties released for the root (wilt) diseased tract, it is necessary to increase the number of clones obtained from a single plumule by modifying the CPCRI protocol.

The protocol was refined to get maximum plantlet regeneration from plumule derived callus of coconut. Initially the cultures were maintained in media supplemented with 2,4-dichlorophenoxy aceticacid (16.5mg/L) with its gradual reduction (16.5>10>5mg/L) in the later stages. Spermine (50 µM/L) and Thidiazuron (1mg/L)were also incorporated in the initial medium for better callus multiplication. This protocol was further validated with selected dwarf varieties (MGD, COD, CGD and GBGD). In all varieties, palm to palm and nut to nut variation was observed with regard to callus initiation, multiplication, embryogenic competence and complete recovery of plantlet.

In the case of MGD, embryos were collected from ten month old self pollinated nuts of 22 typical palms

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Plantlet formed from MGD palms

planted at CDB Farm, Neriamangalam. Even though callus production was achieved in all MGD palms, only 12 palms responded optimally for further growth and development into plantlets.

Embryogenic calli were obtained uniformly in all the dwarf varieties. On an average 12 somatic embryos/plumule were obtained. Shoot induction from callus was initiated after four months of culture initiation. Incorporation of BAP (5mg/L) along with GA₃ (1mg/L) and Glutamine (5mg/L) in the regeneration liquid medium in rotary shaker resulted in the initiation of shoots from somatic embryo. Gradual increase in the concentration of BAP (15-20 mg/L) resulted in recovery of normal plantlets. Currently, on an average four plantlets are obtained from a single plumule.

Abnormalities like compact calli, abnormal somatic embryoids, rudimentary shoots with multiplied roots and elongated scale leaves were observed. Anatomical studies revealed the presence of intact cotyledonary leaves which seemed to inhibit the apical meristem development of the somatic embryoids. Presence of vascular bundles in the early stages of Technical Bulletin No. 72

callus formation has been noticed, which may lead to the direct formation of meristemoids.

(n) Standardizing nursery management techniques in the root (wilt) prevalent tract

Suitability of using eriophyid mite (Aceria guerreronis Keifer) infested seed nuts for raising quality coconut seedlings

Considering the wide spread apprehension regarding suitability of miteinfested seed nuts for raising seedlings, a study was undertaken to evaluate the growth and vigour of coconut seedlings raised from mite-infested seed nuts of different intensities (Grade 0, 1, 2, 3 and 4). The study revealed that mite infestation significantly affected seedling characters like germination at five months, height at 12 months, collar girth at nine months, weight of seedling and percentage of seedlings with 'Z' score of above 22. (Z=-1.24X₁+3.18X₂+2.83X₃+X₄; X₁-collar girth, X2-no. of leaves, X3-seedling weight, X4 no of thick roots, Vijayakumar et al., 1991) The maximum values for all the growth characters were recorded in seedlings raised from grade-3 infested seed nuts (Table 10). However, higher percentage

Treatment	Height (cm)	No. of leaves	Collar girth (cm)	% of seedlings with split leaves	No. of thick roots	Weight of seedling (kg)	% of seedlings with Z score 22 and above
Grade 0	110.95 ^{ab}	11.02	6.09	34.45 (35.56)	13.52	1.756*	48.93 ⁴ (43.99)
Grade 1	102.97**	10.13	5.97	30.35 (31.40)	12.53	1,301 ⁶	28.27 ^a (26.41)
Grade 2 ·	95.20	9.76	5.61	26.12 (30.32)	12.04	1.218%	21.67* (23.74)
Grade 3	117.65*	10.96	6.38	42.97 (40.59)	13.96	1.3729	41.03 ⁴⁵ (37.52)
Grade 4	101.76 ^{5e}	10.61	6.22	30.62 (33.28)	12.75	1.120%	22.57 ⁰ (26.21)
Mean	105.71	10.50	6.05	32.90 (34.33)	12.96	1.353	32.49 (31.57)
CD (p=0.05)	13.22	NS	NS	NS	NS	0.235	13.83

Table 10. Growth and vigour of seedlings in relation to intensity of

columns, values followed by some alphabet(s) are not significantly different

of vigorous seedlings (with 'Z' score of 22 and above) was obtained in seedlings raised from uninfested/healthy nuts. The seedlings raised from grade-2 infested nuts recorded the minimum values for all the growth characters studied. The faster germination recorded in grade-3 infested nuts and their subsequent better establishment may be the reasons for their superior performance (higher values for growth characters) of seedlings raised (Thomas et al., 2004). Therefore, it is recommended that mite infested seed nuts should be sorted into lots, according to severity of infestation, and nursery raised separately for each lot so that competition between seedlings, raised from different grades of mite infested nuts, can be minimized.

Although some of the dwarf varieties such as Kalpasree and Kalparaksha are more resistant to root (wilt) disease, the germination percentage is very low (60) and hence nursery techniques needs to be standardized in dwarfs. Efforts have been initiated to standardize the seed nut storage technique in dwarf varieties and to study the extent of vivipary in dwarf cultivars.

(o) Evaluation trial involving dwarfs and its hybrids

A new screening trial was initiated at CPCRI, Regional Station, Kayamkulam to evaluate the reaction of following dwarfs and their hybrids to root (wilt) disease of coconut.

1. MGD (Self)	2. MYD (Self)
3. MOD (Self)	4. CGD x MGD
5. MYD x WCT	6. MGD xWCT
7. CGD x WCT	8. WCT



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Evaluation trial of dwarfs and its hybrids

The experiment was laid out during August 2009 in Randomized Block Design with three replications for each treatment and fifteen palms per replication. Observations after three years of planting showed that 2-3 seedlings of MOD and the hybrid involving MYD alone took up the disease indicating their highest level of susceptibility to the disease. The performance of MGD, CGD x MGD and MGD x WCT is encouraging. In terms of hybrid vigour MGD x WCT and MYD x WCT are superior compared to CGD x WCT.

(p) Recurrent selection programme / Evaluation of S, and S, progenies

Crossing programme for development of an improved WCT variety, by *inter se* mating/selfing of selected disease-free WCT palms was initiated during 1991-92. A total of 1,250 selfed/ *inter se* mated progenies of disease-free mother palms were planted during 1994-96 at CPCRI Regional Station, Kayamkulam. Selections were made within the progenies planted during 1994-96. During 2009, 80 disease-free S_1 progenies were selected based on visual appearance (diseasefree nature) and finally 40 S_1 palms were

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selected after confirmation by ELISA test. Subsequently crossing programme was carried out during 2009-10 on 40 S_1 palms for generating S_2 progenies through selfing. Currently 600 S_2 progenies are ready for planting and further evaluation will be carried out on the S_2 progenies to develop an improved WCT variety with high level of resistance and higher yield.

II. EXTENSION ACTIVITIES / ACHIEVEMENTS

(a) Production of quality coconut seedlings



Coconut Nursery

During the twenty year period (1991-2012), a total of 91, 958 seedlings belonging to different cross combinations of WCT, CGD and COD were produced utilizing the disease-free mother palms selected in disease-hot spots. During the



Dr. H.P. Singh, DDG (Hort.) releasing the DVD on production of quality seedlings in coconut

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above period, a total of 11,891 seedlings were used for field experiments at CPCRI Regional Station, Kayamkulam and for establishment of seed gardens. The remaining 80,067 seedlings were distributed to farmers belonging to the eight root (wilt) disease prevalent districts of Kerala State (Table 11).

Table 11. Details of seedlings produced and distributed

Year	Planted in seed garden (Nos.)	Distributed to farmers (Nos.)	Total seedlings (Nos.)
1991-94	2,725	1,200	3.925
1995-02	4,175	5,100	9,275
2002-03	869	1,771	2,640
2003-04	785	2,791	3,376
2004-05	471	2.310	2,781
2005-06	772	2,400	3,172
2006-07	408	2,038	2,446
2007-08	438	8,362	9,000
2008-09	607	10,170	10,777
2009-10	116	17,450	17,566
2010-11	475	19,625	20,100
2011-12	50	6.650	6,700
Total	11,891	80,067	91,958

(b) Development of large-scale coconut nurseries by progressive farmers in the root (wilt) prevalent tract

Progressive farmers were identified and trained for identification of mother palms and raising coconut nurseries in collaboration with Coconut Development Board and Department of Agriculture, Govt. of Kerala. Nearly one lakh seedlings were produced by private nurseries during 2004-05 and as a result of which there was surplus production of quality seedlings in Alappuzha District, where there was serious shortage of quality seedlings earlier. Accreditation standards for private coconut nurseries were developed in consultation with Coconut Development Board for large scale multiplication of root (wilt) resistant/tolerant coconut planting materials.

(c) Accreditation of coconut nursery

The team constituted by the National Horticultural Board (NHB) visited coconut nursery at CPCRI Regional Station, Kayamkulam to assess and grade the nursery under the scheme on accreditation of horticultural nurseries. The team headed by Dr. Shyam Singh, formerly Director, NRC Citrus, Nagpur visited the station on 8th June, 2011 and inspected the nursery beds, infrastructure facilities and mother palms for the production of quality planting materials in coconut for the root (wilt) endemic areas. CPCRI Regional Station, Kayamkulam is the first to obtain NHB accreditation for coconut nursery in the root (wilt) disease prevalent tract.

(d) Efforts for decentralized hybrid seedlings production

The two key components required for production of Kalpa Sankara hybrid seedlings are the availability of Chowghat Green Dwarf mother palms and diseasefree West Coast Tall male parental palms. Chowghat Green Dwarf mother palms are available in farmer's plots and such palms can be used for production of D x T hybrids. Since these palms are located in far off places in the disease affected tracts, largescale production of D x T hybrids cannot be achieved through a centralized system. Hence, a decentralized method of D x T hybrid production is proposed wherein 20 hybridization units (located in 20 Krishi Bhavan areas) will be established for production of hybrids. CPCRI will identify

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the male WCT parental palms and male flower processing will be done at CPCRI to collect the pollen from disease-free WCT palms which will be stored in deep freezer/ liquid nitrogen. The stored WCT pollen will be transported and kept in desiccators which will be maintained at each Krishi Bhavans. Pollen will be replenished at weekly intervals. Through this centralized mechanism of pollen processing, diseasefree status of the male parental palms and quality of the pollen will be assured. Under each hybridization unit, 25 CGD mother palms can be hybridized under the supervision of a progressive farmer. Decentralized hybridization programme will enhance the hybrid seedling production in the root (wilt) disease prevalent tract.

III. TRAININGS/ HUMAN RESOURCE DEVELOPMENT

The technology for selection of disease-free mother palms in the root (wilt) disease prevalent tract for seed nut procurement and raising quality seedlings was accepted by Department of Agriculture, Govt. of Kerala. Based on the demand from Department of Agriculture, 16 batches of training programmes (three days and day) were organized by CPCRI Regional Station, Kayamkulam during the period 2003-2008. A total of 600 officials of Department of Agriculture (Agricultural Officers and Agricultural Assistants) have been trained in 'Elite seedling production in coconut for the root (wilt) diseaseaffected region'.

SUMMER/ WINTER SCHOOL Conducted

A 21 days ICAR-sponsored winter school on "Breeding for resistance to diseases and insect pests in plantation crops" was conducted at CPCRI Regional Station, Kayamkulam during October 16- November 6, 2006. There were 25 participants from the states of Orissa, Karnataka, Andhra Pradesh, Tamil Nadu and Kerala. The participants were in the grade of Asst. Professors/Assoc. Professors working in ICAR Institutes/ State Agricultural universities. Dr. R.V. Nair, Head, Division of Crop Improvement, CPCRI was the Course Director and Dr. Regi Jacob Thomas, Senior Scientist was the Course Coordinator. Lectures notes of the winter school were compiled and published as an Institute publication entitled "Breeding for resistance to diseases and insect pests of plantation crops".

IV. TECHNOLOGY GENERATED / VARIETIES RELEASED

 Kalparaksha: A new coconut variety, Kalparaksha (selection from Malayan Green Dwarf) was recommended for release as a high yielding variety with resistance (field resistance) to root (wilt) disease during the XVIIIth AICRP (Palms) biennial workshop held at ANGRAU, Hyderabad during 27-29th Nov, 2007. 'Kalparaksha' has large sized nut with good quality of copra and oil. It is also excellent as a tender nut variety. Semi tall habit of this variety makes it easy for climbing, thus reducing the cost of cultivation since climbing for harvest and plant protection operation, is expensive. With all the above desirable attributes, this variety is expected to boost coconut production and productivity in the root (wilt) prevalent tracts. This is first coconut variety released for cultivation in the root (wilt) diseased tract of Kerala after taking into consideration the high yield and disease resistance. The persons involved in developing this variety are: R. V. Nair, Regi J. Thomas, M. Thomas Mathew, C. P. R. Nair, V. Niral, B Augustine Jerard, K. Samsudeen, R. V. Pillai, M. Sasikala, P. K. Koshy, P. M. Jacob, George V. Thomas, Minnie Mathew and S. Arulraj.

The details of release of the new coconut variety, Kalparaksha (CCS-7, selection from Malayan Green Dwarf) was notified in The Gazette of India (Printed by The Manager, Govt. of India Press, New Delhi and published by the Controller of Publications, New Delhi) as Notification of Ministry of Agriculture (Department of Agriculture and Co-operation) S.O.1714E dated 18th July 2008.

2. Kalpasree: This variety was developed by selection made from Chowghat Green Dwarf located in hot spots of root (wilt) disease. Kalpasree was recommended for release as a variety suitable for cultivation in homesteads in the root (wilt) diseased tract. The persons involved in developing this variety are: R. V. Nair, Regi J. Thomas, P. M. Jacob, P. K. Koshy, S. Naresh Kumar, M. K. Nair, R. D. Iyer, M. Sasikala,

P. Rajan, M.K. Rajesh and George V. Thomas.

The details of release of the new coconut variety, Kalpasree (selection from Chowghat Green Dwarf) was notified in The Gazette of India (Printed by The Manager, Govt. of India Press, New Delhi and published by the Controller of Publications, New Delhi) as Notification of Ministry of Agriculture (Department of Agriculture and Co-operation) S.O. 456(E) dated 16th March 2012.

3. Kalpa Sankara: This is the first coconut hybrid released for cultivation in the root (wilt) disease prevalent tract. Kalpa Sankara was developed by crossing root (wilt) disease-free Chowghat Green Dwarf as female parent and root (wilt) disease-free West Coast Tall as male parent. Parental palms were selected from hot spots of root (wilt) disease. The persons involved in developing this hybrid are: P. M. Jacob, Regi J. Thomas, R. V. Nair, P. K. Koshy, M. K. Nair, M. Sasikala, R. D. Iyer and George V. Thomas.

The details of release of the new coconut variety, Kalpa Sankara (CGD x WCT) was notified in The Gazette of India (Printed by The Manager, Govt. of India Press, New Delhi and published by the Controller of Publications, New Delhi) as Notification of Ministry of Agriculture (Department of Agriculture and Co-operation) S.O.456(E) dated 16th March 2012.

Technical Bulletin No. 72 V. AWARDS/ APPRECIATIONS / U EXTERNAL FUNDED PROJECTS

Name of the Award	Awarding Organization	Year 2003-04	
Best Scientific Team Research Award	CPCR1, Kasaragod		

Team: R. V. Nair, Regi J. Thomas, P.M. Jacob, P.K. Koshy, M. Sasikala, M.K. Rajesh and K. Devakumar.

- Four Sri Lankan delegates including Chairman, Coconut Research Institute, Sri Lanka visited CPCRI Regional Station, Kayamkulam on 20th August.2010. The team also visited various farmers' plots in 'disease hot spots' in Alappuzha and Pathanamthitta Districts as a part familiarization visit & training on 'Selection of root (wilt) disease-free mother palms'.
- A high level Sri Lankan delegation (including two cabinet ministers, Chairman and Director, Coconut Research Institute) visited CPCRI. Kayamkulam during 26-27th July 2011. The team also visited nearby farmers' plots to take note of the performance of high yielding, disease-free WCT palms and CGD in the 'disease hot spots'.

Project Title	Period	Budget	Funding agency
Hot spot survey for elite disease-free coconut palms.	1992-05	2.8 lakhs	ICAR AP Cess Fund
Multiplication of high yielding disease resistant coconut planting materials.	1995-00	30.4 lakhs	ICAR AP Cess Fund
Production of high yielding root (wilt) resistant / tolerant planting materials for the management of root (wilt) disease.	2000-03	41.7 lakbs	NATP, ICAR NATP-CGP
'Molecular studies for tagging root (wilt) resistant genes in coconut'.	2000-03	31.6 lakhs	NATP, ICAR
Production of root (wilt) resistant/ tolerant coconut seedlings for root (wilt) disease prevalent tracts.	2005-09	44.4 lakhs	TMOC, CDB
Seed Production in coconut, arecanut and cocoa	2007-12	10.0 lakhs	ICAR-Network Project

EXTERNAL FUNDED PROJECTS

VI. PUBLICATIONS

a) Research papers in refereed journals

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populations of West Coast Tall variety of coconut using micro satellite markers. *Journal of Plantation Crops* **34** (Supp): 587-590

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Scientists associated with the project "Breeding for resistance/tolerance to coconut root (wilt) disease"

(Period: 1987-2012)

Sl. No.	Name of the Scientist	Duration	Period
1	R. V. Nair	23 years	1988-2011
2	M. Sasikala	22 years	1987-2009
3	P. M. Jacob	18 years	1987-1999, 2007-2012
4	Regi J. Thomas	12 years	2000-2012
5	P. K. Koshy	6 years	1995-2001
6	M. P. Govindankutty	5 years	1987-1992
7	A. Muralidharan	4 years	1987-1991
8	T. S. S. Rawther	4 years	1987-1991
9	N. G. Pillai	4 years	1991-1995
10	P. Rajan	4 years	1999-2003
11	R. Chandramohanan	2 years	2003, 2004
12	K. Nihad	2 years	2009, 2010
13	N. Srinivasan	2 years	2010, 2011
14	M. Shareefa	2 years	2010-2012
15	K. Mathen	1 year	1987-1988
16	K. D. Patil	1 year	1987-1988
17	J. J. Solomon	1 year	1987-1988
18	Anitha Karun	1 year	2011-2012
19	A. Josephrajkumar	1 year	2011-2012

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