The Planter, Kuala Lumpur, 82 (963): 407-413 (2006)

Performance of ECT x BSR Tolerant ECT Hybrid Coconut in High Inoculum Basal Stem Rot Soil of Tamil Nadu

KARTHIKEYAN G, RAGUCHANDER T, NATARAJAN C[1] AND S ARULRAJ[2]

The coconut cultivar East Coast Tall (ECT) was crossed with ECT tolerant to basal stem rot (Ganoderma) *disease collected from disease endemic area of Tamil Nadu (India) to evaluate their performance against* Ganoderma lucidum *in high inoculum basal stem rot soil. The progeny, ECT x BSR tolerant ECT was evaluated along with nine other cultivars against the disease. BSR tolerant ECT hybrid recorded a higher percentage of survival of 61.1 per cent at 15 years after planting and accordingly a higher cumulative mean nut yield of 126 nuts per palm. The biochemical analysis revealed that the hybrid, ECT x BSR tolerant ECT had a higher content of total phenol as compared to other genotypes. Similarly the activities of peroxidase and polyphenol oxidase were also higher in the hybrid ECT x BSR tolerant ECT compared to other genotypes.*

Keywords : *Coconut, basal stem rot, ECT x BSR tolerant ECT.*

Coconut is a traditional horticultural crop of the coastal agro eco-system of India. In India, coconut is grown in homestead gardens and plantations covering an area of 1.914 million hectares with an annual production of 12 141 million nuts and a productivity of 6 345 nuts per hectare per year (Ministry of Agriculture, India, 2003). Coconut is reported to be affected by several important diseases. Among them basal stem rot (BSR)/ *Ganoderma* disease caused by *Ganoderma lucidum* (Leys) Karst. has been recognised as a serious disease of coconut for many years, causing high economic losses. In India, *G lucidum* was first recorded in coconut palm in Karnataka state by Butler (1913). In Tamil Nadu, it was first reported from Thanjavur district in 1952 and named as Thanjavur wilt (Vijayan & Natarajan, 1972).

The symptomatology, host range, etiology and epidemiology of the disease were reviewed by Bhaskaran *et al.* (1984). Based on these studies several control measures were attempted to contain the disease. Various management practices like management of soil moisture regime, effect of organic manures, effect of fungicides and chemicals and also management through bio-control agents were attempted. However, these management practices were found effective only if the disease is detected in early stages (Bhaskaran *et al.*, 1996). The basal stem rot disease causes 15 to 25 per cent damage to roots and bole below the ground level by the time external symptoms are visible. Integrated disease management technologies without using much chemicals will bring a new cost effective and environment – friendly approach in the control and management of BSR disease in large coconut plantation. With this background, an

[1] *Tamil Nadu Agricultural University, Coimbatore – 641 003, Tamil Nadu, India*
[2] *Central Plantation Crops Research Institute, ICAR, Kasaragod – 671 124, Kerala, India*

attempt was made to develop BSR resistant/ tolerant coconut cultivar for the enhancement of coconut production in the BSR endemic areas.

MATERIALS AND METHODS

Performance of ECT x BSR tolerant ECT in BSR sick soil

The experiment was conducted at Thambikkottai village of Thanjavur district, Tamil Nadu (India), which is an endemic area for BSR of coconut with a high disease incidence of up to 46 per cent. Twenty palms out of 978 East Coast Tall palms were found to be completely free from BSR infection in a coconut garden in the village. These BSR tolerant palms were selected as pollen parent and were crossed with ECT palms at Coconut Research Station, Tamil Nadu Agricultural University, Veppankulam, Tamil Nadu (India). Seedlings obtained from this cross combination were evaluated against BSR disease along with other nine coconut cultivars *viz.*, San Ramon, Laccadive Ordinary, British Soloman Islands, Java Giant, Straight Settlement Green, WCT x COD, COD x WCT, VHC-1 (ECT x DG) and East Coast Tall (ECT) in the disease endemic area of the same village. The experiment was laid out during August, 1989. After 15 years of planting, observations on the survival percentage, disease intensity, number of functional leaves and nut yield were recorded in all the cultivars. The disease intensity was assessed as disease index with the following formula.

$$\text{Disease index} = 23.6 + 17.7h + 3.6r - 0.6l$$

Where,

- h - Height of bleeding patches from ground level in metres.
- l - Number of functional leaves.
- r - Reduction in leaf size based on 0 – 4 scale.

Calorimetric analysis of total phenol, peroxidase and polyphenol oxidase activities

Total phenol content and of peroxidase and polyphenol oxidase activity in root tissues of coconut genotypes were analysed calorimetrically. Peroxidase activity was determined according to Hammerschmidt *et al.* (1982) and the polyphenol oxidase activity was determined as per Mayer *et al.* (1965). The total phenol content was estimated by the procedure of Swain and Hillis (1959).

Isozyme analysis of peroxidase

To study the expression pattern of different isoforms of peroxidase (PO) in different genotypes, activity gel electrophoresis was carried out. For the native anionic polyacrylamide gel electrophoresis, resolving gel of 8 per cent acrylamide concentration and stacking gel of 4 per cent acrylamide concentration were used. After electrophoresis, the gels were incubated in the solution containing 0.15 per cent benzidine in 6 per cent NH_4Cl for 30 minutes in dark. Then few drops of 30 per cent H_2O_2 was added with constant shaking till the appearance of bands. After staining, the gel was washed with distilled water and analysed for PO isoforms.

RESULTS AND DISCUSSION

Performance of ECT x BSR tolerant ECT in high inoculum BSR soil

Results revealed that the hybrid, ECT x BSR tolerant ECT registered a higher rate of survival

(61.1 per cent) as compared to other cultivars (*Table 1*) after 15 years of planting. The other cultivars recorded 0 to 40 per cent survival. The cultivars, Laccadive Ordinary and VHC-1, were totally distraught due to BSR incidence, whereas the cultivar ECT had 40 per cent survival rate. In ECT x BSR tolerant ECT hybrid, out of 11 surviving palms only one palm was found to be infected with BSR disease with a per cent infection of 9.1, while in ECT, out of four surviving palms two palms were found to be infected with BSR disease with a per cent infection of 50 (*Table 1*). The mean disease index was also low in ECT x BSR tolerant ECT (35.86) as compared to ECT (46.23). The outcome revealed that the hybrid, ECT x BSR tolerant ECT recorded a higher mean nut yield of 74 nuts per palm during the year 2003 – 2004, which also recorded a higher cumulative mean nut yield of 125.67 nuts per palm when compared to other genotypes (*Table 2*).

Calorimetric analysis of total phenol, peroxidase and polyphenol oxidase activities

The results of the calorimetric analysis in different genotypes of coconut revealed that the hybrid, ECT x BSR tolerant ECT had a

TABLE 1
PERFORMANCES OF COCONUT GENOTYPES IN HIGH INOCULUM BASAL STEM ROT SOIL

Genotype(s)	*No. of palms planted*	*No.of palms surviving after 15 years*	*Per cent survival*	*No. of palms infected**	*Per cent infection**	*No. of functional leaves*	*Disease index** (March 2005)*
San Ramon	15	2	13.3	2	100	31.5	23.38
Laccadive Ordinary	15	0	-	-	-	-	-
British Soloman Islands	12	1	8.3	0	-	29	-
Java Giant	15	2	13.3	0	-	36.5	-
Straight Settlement Green	15	4	26.7	2	50	32.3	41.17
WCT x COD	15	2	13.3	0	-	32.5	-
COD x WCT	15	2	13.3	0	-	34.0	-
VHC 1	10	0	-	-	-	-	-
East Coast Tall	10	4	40.0	2	50	29.0	46.23
ECT x BSR tolerant ECT	18	11	61.1	1	9.1	33.5	35.86

* Among surviving palms   ** Mean of infected palms

attempt was made to develop BSR resistant/ tolerant coconut cultivar for the enhancement of coconut production in the BSR endemic areas.

MATERIALS AND METHODS

Performance of ECT x BSR tolerant ECT in BSR sick soil

The experiment was conducted at Thambikkottai village of Thanjavur district, Tamil Nadu (India), which is an endemic area for BSR of coconut with a high disease incidence of up to 46 per cent. Twenty palms out of 978 East Coast Tall palms were found to be completely free from BSR infection in a coconut garden in the village. These BSR tolerant palms were selected as pollen parent and were crossed with ECT palms at Coconut Research Station, Tamil Nadu Agricultural University, Veppankulam, Tamil Nadu (India). Seedlings obtained from this cross combination were evaluated against BSR disease along with other nine coconut cultivars *viz.*, San Ramon, Laccadive Ordinary, British Soloman Islands, Java Giant, Straight Settlement Green, WCT x COD, COD x WCT, VHC-1 (ECT x DG) and East Coast Tall (ECT) in the disease endemic area of the same village. The experiment was laid out during August, 1989. After 15 years of planting, observations on the survival percentage, disease intensity, number of functional leaves and nut yield were recorded in all the cultivars. The disease intensity was assessed as disease index with the following formula.

$$\text{Disease index} = 23.6 + 17.7h + 3.6\,r - 0.6\,l$$

Where,

h - Height of bleeding patches from ground level in metres.

l - Number of functional leaves.

r - Reduction in leaf size based on 0 – 4 scale.

Calorimetric analysis of total phenol, peroxidase and polyphenol oxidase activities

Total phenol content and of peroxidase and polyphenol oxidase activity in root tissues of coconut genotypes were analysed calorimetrically. Peroxidase activity was determined according to Hammerschmidt *et al.* (1982) and the polyphenol oxidase activity was determined as per Mayer *et al.* (1965). The total phenol content was estimated by the procedure of Swain and Hillis (1959).

Isozyme analysis of peroxidase

To study the expression pattern of different isoforms of peroxidase (PO) in different genotypes, activity gel electrophoresis was carried out. For the native anionic polyacrylamide gel electrophoresis, resolving gel of 8 per cent acrylamide concentration and stacking gel of 4 per cent acrylamide concentration were used. After electrophoresis, the gels were incubated in the solution containing 0.15 per cent benzidine in 6 per cent NH_4Cl for 30 minutes in dark. Then few drops of 30 per cent H_2O_2 was added with constant shaking till the appearance of bands. After staining, the gel was washed with distilled water and analysed for PO isoforms.

RESULTS AND DISCUSSION

Performance of ECT x BSR tolerant ECT in high inoculum BSR soil

Results revealed that the hybrid, ECT x BSR tolerant ECT registered a higher rate of survival

(61.1 per cent) as compared to other cultivars (*Table 1*) after 15 years of planting. The other cultivars recorded 0 to 40 per cent survival. The cultivars, Laccadive Ordinary and VHC-1, were totally distraught due to BSR incidence, whereas the cultivar ECT had 40 per cent survival rate. In ECT x BSR tolerant ECT hybrid, out of 11 surviving palms only one palm was found to be infected with BSR disease with a per cent infection of 9.1, while in ECT, out of four surviving palms two palms were found to be infected with BSR disease with a per cent infection of 50 (*Table 1*). The mean disease index was also low in ECT x BSR tolerant ECT (35.86) as compared to ECT (46.23). The outcome revealed that the hybrid, ECT x BSR tolerant ECT recorded a higher mean nut yield of 74 nuts per palm during the year 2003 – 2004, which also recorded a higher cumulative mean nut yield of 125.67 nuts per palm when compared to other genotypes (*Table 2*).

Calorimetric analysis of total phenol, peroxidase and polyphenol oxidase activities

The results of the calorimetric analysis in different genotypes of coconut revealed that the hybrid, ECT x BSR tolerant ECT had a

TABLE 1
PERFORMANCES OF COCONUT GENOTYPES IN HIGH INOCULUM BASAL STEM ROT SOIL

Genotype(s)	*No. of palms planted*	*No.of palms surviving after 15 years*	*Per cent survival*	*No. of palms infected**	*Per cent infection**	*No. of functional leaves*	*Disease index** (March 2005)*
San Ramon	15	2	13.3	2	100	31.5	23.38
Laccadive Ordinary	15	0	-	-	-	-	-
British Soloman Islands	12	1	8.3	0	-	29	-
Java Giant	15	2	13.3	0	-	36.5	-
Straight Settlement Green	15	4	26.7	2	50	32.3	41.17
WCT x COD	15	2	13.3	0	-	32.5	-
COD x WCT	15	2	13.3	0	-	34.0	-
VHC 1	10	0	-	-	-	-	-
East Coast Tall	10	4	40.0	2	50	29.0	46.23
ECT x BSR tolerant ECT	18	11	61.1	1	9.1	33.5	35.86

* Among surviving palms ** Mean of infected palms

TABLE 2
NUT YIELD OF COCONUT CULTIVARS

Cultivar(s)	Nut yield/palm	
	2003 - 2004	Cumulative mean (1995 – 2004) of nine years
San Ramon	57.3	57.37
Lakshadweep Ordinary	-	-
British Soloman Islands	60.0	99.22
Java Giant	65.5	84.17
Straight Settlement Green	63.5	80.37
WCT x COD	68.5	94.87
COD x WCT	59.7	121.86
VHC 1	-	-
East Coast Tall	70.7	98.52
ECT x BSR tolerant ECT	74.0	125.67

higher content (16.2 mg/g) of total phenol when compared to other genotypes (*Table 3*). Rapid incorporation of phenolic materials into plant cell walls either by esterification of hydroxyl cinnamic acid derivatives or by peroxidase catalysed polymerisation of phenyl propane alcohols to yield lignin is often associated with resistance (Friend, 1981; Matern & Kneusel, 1988). Phenols and their oxidative products have been reported as defence chemicals against many pathogens (Ohato & Kubo, 1974; Sathianathan & Vidhyasekaran, 1981).

Similarly the activities of peroxidase and polyphenol oxidase were also more in the hybrid ECT x BSR tolerant ECT when compared to other genotypes (*Table 3*). Plant development and environmental changes including biotic stress are often followed by dramatic changes in peroxidase activity and in the number of isoperoxidase present in specific tissues.

TABLE 3
TOTAL PHENOL, PEROXIDASE AND POLYPHENOL OXIDASE ACTIVITIES IN COCONUT GENOTYPES

Genotype(s)	Total phenol (mg/g)	Peroxidase activity A_{420} nm/min/g	Polyphenol oxidase activity A_{495} nm/min/g
San Ramon	12.4	0.432	0.218
British Soloman Islands	14.3	0.508	0.292
Java Giant	9.1	0.392	0.199
Straight Settlement Green	12.8	0.451	0.263
WCT x COD	11.4	0.404	0.206
COD x WCT	12.6	0.456	0.242
ECT	15.1	0.478	0.311
ECT x BSR tolerant ECT	16.2	0.589	0.398

Peroxidase participates in a variety of plant defence mechanisms (Mareschbacher *et al.*,1986) in which H_2O_2 often supplied by an oxidative burst, a common event in defence responses (Dixon & Lamb, 1990). The cell wall of plants appear to be a major site for defence related peroxidase polymerisation reaction such as lignification (Hammerschmit & Kuc, 1982), suberisation (Espelie *et al.*, 1986) and cross linking of structural cell wall proteins (Fry, 1986).

Isozyme analysis of peroxidase

The isozyme analysis of peroxidase in BSR resistant genotype (ECT x BSR tolerant ECT) and susceptible genotype (ECT) revealed that an isoform PO1 was induced only in the resistant genotype. The isoform PO2 was induced in both resistant and susceptible genotypes. But PO2 was intensified in the resistant genotype (*Figure 1*). Most of the higher plants possess a number of different isozymes and/or isoforms of peroxidase. These isoforms can be distinguished by their isoelectric point (pI) or elution profile into three sub-groups anionic, neutral and cationic peroxidases (Van Hustee & Cairns, 1990; Shinmyo *et al.*, 1993). Peroxidase (PO) and polyphenol oxidase (PPO) mainly catalyse the oxidation of phenolic compounds through PO-PPO-H_2O_2 system (Srivastava, 1987). PPO and PO oxidise the phenols to more toxic phenol derivatives. PO seems to be associated with resistance by catalysing the final polymerisation step of lignin synthesis (Hammerschmidt & Kuc, 1982).

In the present study, the resistance against BSR in the crossed progeny may be due to the

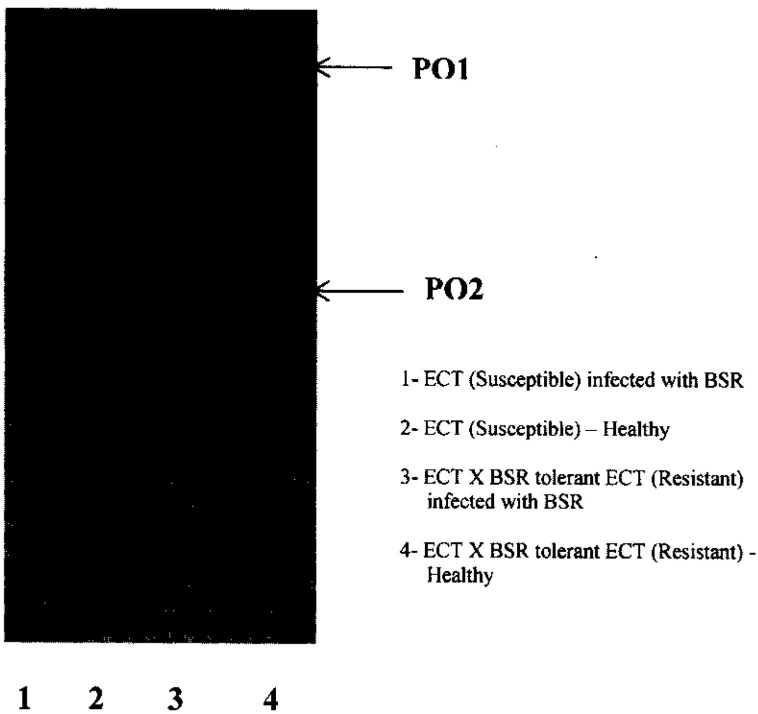

Figure 1 Induction of peroxidase isoforms in BSR resistant and susceptible genotypes of coconut

existence of phenolic polymers such as lignin. Lignin acts as a physical barrier to infection by the pathogen. Lignification occurs at the site of fungal penetration and this barrier is resistant to cellulolytic and macerating enzymes of the pathogens. Similar results were reported by Karthikeyan *et al.* (2000) as the higher level of total phenols, optical density (O.D.) phenols, lignin and aminonitrogen contents in ECT x BSR tolerant ECT coconut cultivar might be responsible for the resistance against basal stem rot disease.

ACKNOWLEDGEMENT

The authors would like to thank All India Co-ordinated Research Project on Palms (ICAR, New Delhi) for funding the research work.

REFERENCES

BHASKARAN, R; RAJAMANNAR, M and KUMAR, S N S. 1996. Basal stem rot disease of coconut. *Technical Bulletin No. 30*, Central Plantation Crops Research Institute, Kasaragod. p.15.

BHASKARAN, R; RAMANATHAN, T and RAMIAH, M.1984. Chemical control of Thanjavur wilt of coconut. In: *Proc. All India Symp. On Coconut Diseases.* Coconut Research Station, Veppankulam. 22-24.

BUTLER, E J. 1913. Report of Imperial Mycologist, Report of Agricultural Research Institute and College, Pusa, 1911-12. p. 60.

DIXON, R A and LAMB, C J. 1990. Molecular communication in interactions between plants and microbial pathogens. *Annu. Rev. Plant Mol. Biol.* **41**: 339-367.

ESPELIE, K E; FRANCESHI, V R and KOLATTUKUDY, P E. 1986. Immunocytochemical localisation and time course of appearance of an anionic peroxidase associated with suberisation in wound healing potato tuber tissue. *Plant Physiol.* **81** : 487-492.

FRIEND, J.1981. Plant phenolics, lignification and disease resistance. *Prog. Phytochem:* **7** : 197-261.

FRY, S C. 1986. Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annu. Rev. Plant Physiol.*, **37** : 165-183.

HAMMERSCHMIDT, R and KUC, J. 1982. Lignification as a mechanism for induced systemic resistance in cucumber. *Physiol. Plant Pathol.* **20** : 61-71.

HAMMERSCHMIDT, R; NUCKLESS, E M and KUC, J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium. Physiol. Plant Pathol.* **20** : 73-82.

KARTHIKEYAN, A; BHASKARAN, R and KARTHIKEYAN, M. 2000. Development and performance of basal stem rot (*Ganoderma*) disease resistant coconut genotype in endemic area. *CORD* **XVI** (1) : 50 – 55.

MARESCHBACHER, B M; KOGEL, K H; NOLL, U and REISENER, H J. 1986. An elicitor of the hypersensitivity lignification response in wheat leaves isolated from the rust fungus, *Puccinia graminis* f.sp. tritici.I. Partial purification and characterisation. *Z. Naturforschung* **41**: 830-838.

MATERN, U and KNEUSEL, R E. 1988. Phenolic compounds in plant disease resistance. *Phytoparasitica* **16** : 158-170.

MAYER, A M; HAREL, E and SHAUL, R B. 1965. Assay of catechol oxidase, a critical comparison of methods. *Phytochemistry* **5** : 783-789.

MINISTRY OF AGRICULTURE, INDIA. 2003. *All India Final Estimates of Coconut, 2002-2003.* Directorate of Economics and Statistics, Ministry of Agriculture, Government of India.

OHATO, K and KUBO, C. 1974. Studies on the mechanism of disease resistance of rice varieties to *Cochliobolus miyabeanus. Bulletin of the Shikoku Agricultural Experiment Station* **28** : 17-57.

SATHIANATHAN, S and VIDHYASEKARAN, P. 1981. Ethylene-controlled infection of phenylalanine-ammonia lyase in citrus fruit peel. *Plant Pathol.* **44**: 631-635.

SHINMYO, A; FUGIYAMA, K; KAWAOKA, A and INTAPRUK, C. 1993. Structure and expression of peroxidase isozyme genes in horseradish and *Arabidopsis*. In : *Plant Peroxidases : Bio-chemistry and Physiology* (K G Welinder, S K Rasmusses, C Panel and H Greppin, eds.). University of Geneva, Geneva. 221 pp.

SRIVASTAVA, S K. 1987. Peroxidase and polyphenol oxidase in *Brassica juncea* infected with *Macrophomina phaseolina* (Tassi) Goid. and their implications in disease resistance. *J. Phytopathol.* **120** : 249-254.

SWAIN, T and HILLIS, W E. 1959. The phenolic constituents of *Prunus domestica* L. The quantitative analysis of phenolic constituents. *J. Sci. Food Agric.*

10 : 63-68.

VAN HUSTEE, R B and CAIRNS, W L. 1990. Appraisal of studies on induction of peroxidase system by phenolic compounds. *Phytopathology* **65** : 686-690.

VIJAYAN, K M and NATARAJAN, S. 1972. Some observations on the coconut wilt disease of Tamil Nadu. *Coconut Bulletin* **n2** (12) : 2-4.