

Inductive response of protective enzymes in coconut (*Cocos nucifera* L.) subjected to stress

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ABSTRACT

The coconut hybrids WCT x four exotic Talls viz., Philippines Ordinary (PHOT), Fiji (FIJT), Cochin China (CCNT), Strait Settlement Green (SSGT) as well as one indigenous tall- Kappadam (KPDT) were screened for tolerance to drought by using stomatal resistance and transpiration rate in the field as well as by the activities of the stress sensitive enzymes viz., malic dehydrogenase, peroxidase, super oxide dismutase and catalase under induced stress condition. Leaf water potential (Ψ leaf) was determined both *in situ* and induced stress conditions. Differences in the parameters studied did not give any significant variations among the hybrid combinations during non-stress or stress periods and significant variations were observed only between the two periods under *in situ* conditions. However, under induced stress condition, the hybrids behaved differentially in activities of the enzymes in response to the decreased Ψ leaf. Based on the increase or decrease in the water relation components as well as stress related enzyme activities as compared with the normal level the hybrids were ranked for tolerance to drought. Ranking of the hybrids based on Ψ leaf under induced stress condition as well as under field stress conditions gave similar results. Among the T x T combinations, WCT x SSGT was found to have relative tolerance to drought in terms of water relation components and enzyme activities. The results further established that rapid screening techniques can be employed with ease for screening large number of coconut cultivars / hybrids for tolerance to drought.

Key words : Protective enzymes, stress, drought, stomatal resistance, coconut.

INTRODUCTION

Coconut, a perennial tree crop of long life and continuous productivity adapts well to the warm humid tropical climate. Being a crop grown under rainfed conditions, growth and productivity of the palms are affected by frequent drought occurring with different intensities. Central Plantation Crops Research Institute at Kasaragod, the northern most part of Kerala, with the world's largest germplasm has over 181 indigenous and 132 exotic cultivars. Based on the water relation components (Rajagopal *et al.*, 12) and activities of stress sensitive enzymes (Shivashankar *et al.*, 13, 14; Chempakam *et al.*, 2; Kasturi Bai *et al.*, 5) large number of cultivars and hybrids were screened for tolerance to drought under field conditions. The genotypes were also ranked based on the above parameters. Based on the results obtained on the activities of stress sensitive enzymes with relation to leaf water potential changes, Rajagopal *et al.* (11) developed a rapid screening technique of the coconut cultivars for tolerance to drought by determining leaf water potential in the leaf at normal conditions and after 6 h stress induction by air desiccation. In the present paper performance of some of the Tall x Tall genotypes were assessed under field conditions as well as by employing

rapid screening technique along with stress related enzyme activities.

MATERIALS AND METHODS

Twenty five years old palms of WCT x four exotic Talls viz., Philippines Ordinary (PHOT), Fiji (FIJT), Cochin China (CCNT), Strait Settlement Green (SSGT) as well as one indigenous tall- Kappadam (KPDT) were the material selected for the evaluation. The palms were growing in laterite soil under unirrigated condition with normal cultural and agronomic practices. From each, 6 palms were used for the experimental purpose. Water relation components viz., stomatal resistance (sr), transpiration rate (E) and leaf water potential (Ψ leaf) were determined in the 6th leaf in the field during non-stress (just after the monsoon-August month) and stress (summer month-March) periods as per the method standardized by Rajagopal *et al.* (9 & 10) by using steady state porometer (Li-Cor 1600) and Scholander pressure chamber (Plant water console, model 3000). For the determination of Ψ leaf and enzyme activities under induced stress, leaflets from spindle leaf were used (Rajagopal *et al.*, 11). Observations were recorded immediately after leaf sampling (pre stress) as well as after air desiccating the samples for six hours (stress). Enzyme activities viz., peroxidase- POD and catalase- CAT (Kar and Mishra, 4), malic dehydrogenase- MDH (Kitto, 1,969),

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super oxide dismutase-SOD (Beauchamp and Fridovich, 1) were estimated as per standard methods. All the observations were carried out consecutively for two years and mean for the two years was taken for the interpretation of the results.

RESULTS AND DISCUSSION

Water relation components viz., stomatal resistance (sr), transpiration rate (E) and leaf water potential (Ψ leaf) varied significantly between the non-stress and stress periods in the coconut hybrids under field conditions (Table 1). During non-stress period, sr did not vary much between the hybrids and ranged between 2.4 and 2.8 $\mu\text{g cm}^{-2}$, while during stress period sr increased in all the combinations. As compared to non-stress period the increase in sr ranged from 143.6% (WCT x PHOT) to 363.4% (WCT x SSGT). With the increase in sr during stress period, E rate showed a decrease. The decrease in E rate as

compared to non-stress period ranged between 57.6% (WCT x SSGT) and 68.9% (WCT x PHOT). Similarly Ψ leaf which was in the range of -5.1 to -6.2 bars during non-stress period showed a drastic reduction during stress period. The decrease in Ψ leaf during stress period as compared to non-stress period varied between 50.9% (WCT x SSGT) and 105.9% (WCT x KPDT). However, parameters studied did not show significant differences between the hybrid combinations during non-stress or stress periods and differences were observed only between the two periods.

The hybrid combinations were further evaluated during summer month by subjecting the leaflets to air desiccation and determining the Ψ leaf and activities of stress sensitive enzymes. As in field stress condition in the desiccated leaf also, Ψ leaf did not show significant differences between the cultivars. However, as compared to pre-stress level, Ψ leaf decreased by 169.7% (WCT x SSGT) to 203.8% (WCT x PHOT) in

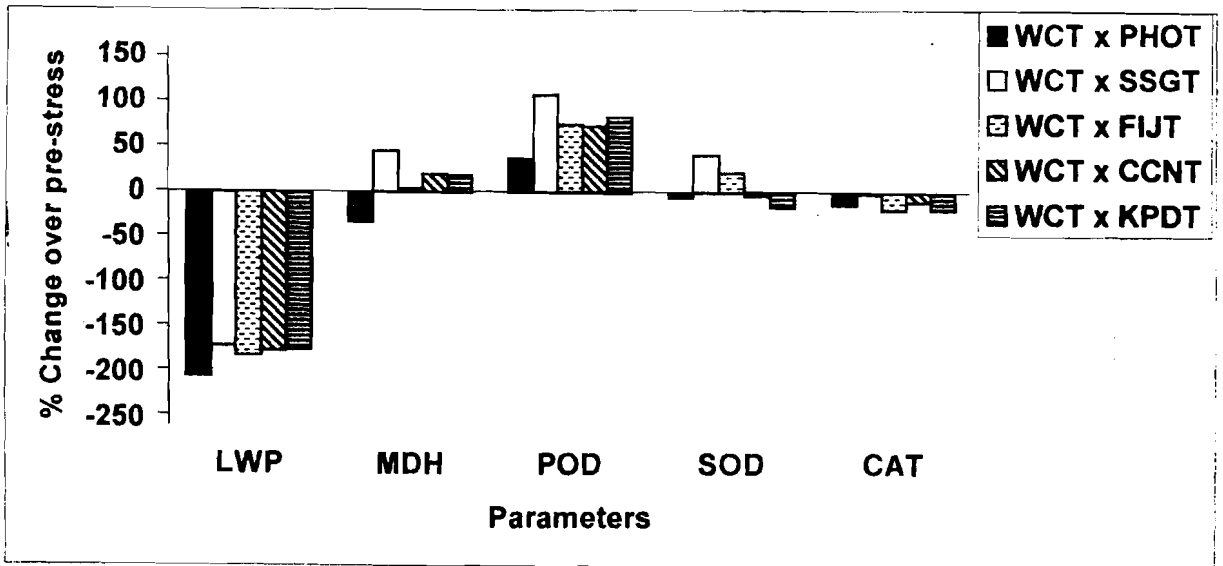


Fig. 1. Changes in Ψ leaf (LWP) and related enzyme activities during stress over pre-stress condition.

Table 1. Changes in stomatal resistance (sr), transpiration rate (E) and leaf water potential (Ψ leaf) of coconut hybrids during non-stress (NS) and stress (S) periods in the field conditions.

Hybrid	sr (S.m^{-1})		E ($\text{mg.cm}^{-2}.\text{s}^{-1}$)		Ψ leaf (bars)	
	NS	S	NS	S	NS	S
WCT x PHOT	2.83	11.50	3.91	1.38	-5.62	-11.30
WCT x SSGT	2.45	10.53	3.79	1.61	-6.05	-9.05
WCT x FIJT	2.40	10.51	3.69	1.32	-5.07	-10.48
WCT x CCNT	2.49	12.58	4.24	1.41	-5.07	-10.50
WCT x KPDT	2.63	11.69	3.78	1.44	-6.18	-10.92
Mean	2.56	11.36	3.89	1.43	-5.62	-10.45
CD: NS vs S (P = 0.05)		0.858		0.371		0.710

Table 2. Leaf water potential (ψ leaf) and enzyme activities during pre-stress (N) and stress (S) conditions.

Hybrid	ψ leaf (-bars)		POD (C m ⁻¹ mg ⁻¹ protein)		MDH (m ⁻¹ mg ⁻¹ protein)		SOD (Units)		CAT (mM H ₂ O ₂ consumed m ⁻¹)	
	N	S	N	S	N	S	N	S	N	S
WCT x PHOT	-5.66	-17.22	0.056	0.077	0.693	0.469	4.3	4.06	3.29	2.84
WCT x SSGT	-5.88	-15.87	0.046	0.095	0.436	0.633	3.62	5.09	3.46	3.41
WCT x FIJT	-6.0	-16.85	0.061	0.106	0.652	0.680	4.41	5.32	4.77	3.86
WCT x CCNT	-6.01	-16.53	0.061	0.106	0.579	0.694	3.74	3.61	3.21	2.87
WCT x KPDT	-5.73	-15.72	0.050	0.091	0.476	0.563	3.91	3.28	4.39	3.56
Mean	-5.86	-16.44	0.055	0.095	0.567	0.608	3.99	4.27	3.82	3.31
CD: NS vs S (P = 0.05)		0.33		0.01		0.04		0.21		0.26

Table 3. Ranking of coconut hybrids based on (%) increase/ decrease in Ψ leaf, enzyme activities (under and water relation components (under field stress).

Hybrid	Induced stress						Field stress				Rank
	ψ leaf	Enzyme activities				ψ leaf	sr	sr	E	Rank	
		POD	MDH	SOD	CAT						
WCT x PHOT	4	5	5	3	4	17	3	5	4	12	V
WCT x SSGT	1	1	1	1	1	4	1	1	1	3	I
WCT x FIJT	3	3	4	2	3	12	4	3	4	11	IV
WCT x CCNT	2	4	3	4	2	13	2	4	3	9	III
WCT x KPDT	2	2	2	5	3	12	5	2	2	9	II

the stressed leaflet after 6 h air desiccation. Changes in the activities of the enzymes were also observed among the cultivars at pre-stress level as well as stress level parallel with decrease in the ψ leaf (Table 2). In general, with a decrease in ψ leaf by 180.7%, there was an increase in the activities of POD (73.4%), MDH (7.2%) and SOD (6.86%) and decrease in CAT (13.5%) in the stressed sample as compared to the pre-stress sample.

The increase or decrease in the activities of the stress sensitive enzymes under desiccation as compared to the pre-stress level indicated the differential adaptability of the coconut palms with change in leaf water potential (Fig. 1). Adaptive mechanism in coconut palms under field stress conditions have been reported by Shivashankar *et al.* (13), Chempakam *et al.* (2) and Kasturi Bai *et al.* (5). Higher MDH activity in the cell is required for regeneration of the NADH in the cell (Kun, 7). Earlier, Shivashankar *et al.* (13) have reported higher MDH activity in the palms under irrigation than in un-irrigated palms. Between the hybrids, higher increase in MDH activity has been observed in WCT x SSGT (45%) than the others. However, CAT activity was found to decrease under induced stress. The lower activities in CAT can be attributed to the stress impact on the quaternary structure of the enzymes thus decreasing

the total concentration of the active enzymes. If the palms are tolerant to drought, the destruction of the enzyme will be lower. Lee *et al.* (8) reported decrease of CAT activity in rice under salt stress. Among the hybrid combinations lower reduction in CAT activity has been observed in WCT x SSGT. Similarly, SOD and POD that protect the cell membrane by detoxifying the super oxide radicals as well as peroxide formed by the incomplete metabolism in the cell (Dhindsa *et al.*, 3) showed an increase under stress induction indicating the maintenance of cell membrane integrity during severe stress periods. Chempakam *et al.* (2) reported the relationship between activities of scavenging enzymes and lipid peroxidation in coconut. Higher increase in POD and SOD activity has been observed in WCT x SSGT (107.3 and 40.7%, respectively) than the others.

Based on the increase or decrease in water relation components under field conditions as well as enzyme activities under stress as compared to pre-stress condition, the hybrids were ranked (Table 3). Ranking for the parameters was given based on the intensity of change as compared with the pre-stress level. For sr, SOD, POD and MDH rank was given from higher increase to lower increase, for E, from higher reduction to lower reduction and for ψ leaf, and CAT from lower reduction to higher reduction. Depending on the rank

sum, the hybrids were ranked for tolerance to drought which showed that WCT x SSGT is more adapted and relatively tolerant to stress condition than WCT x KPDT < WCT x CCNT < WCT x FIJT < WCT x PHOT in that order. From the rank sums, it is clear that ranking of the hybrids based on ψ leaf under induced stress condition was on par with the ranking based on enzyme activities under stress as well as water relation components under *in-situ* conditions. Thus, the results of the present study further confirm that rapid screening techniques developed by Rajagopal *et al.* (11) based on ψ leaf changes can be employed with ease for screening large number of coconut cultivars/hybrids for tolerance to drought.

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REFERENCES

1. Beauchamp, C.O. and Fridovich, I. 1971. Superoxide dismutase: Improved assay and an assay applicable to acrylamide gels. *Anal. Biochem.* **44**: 276-87.
2. Chempakam, B., Kasturi Bai, K.V. and Rajagopal, V. 1993. Lipid peroxidation in relation to drought tolerance in coconut (*Cocos nucifera* L.). *Pl. Physiol. Biochem.* **20**: 5-10.
3. Dhindsa, R.S., Dhindsa, P.L. and Reid, D.M. 1982. Leaf senescence and lipid peroxidation. Effect of some phytohormones and scavengers of free radicals and single oxygen. *Physiol. Plant.* **56**: 453-57.
4. Kar, M. and Mishra, D. (1976). Catalase, peroxidase and poly phenoloxidase activities during rice leaf senescence. *Plant Physiol.* **57**: 315-19.
5. Kasturi Bai, K.V., Rajagopal, V., Chempakam, B. and Prabha, C.D. 1996. Assay of enzymes in coconut cultivars and hybrids under non stress and stress conditions. *J. Plantn. Crops* **24** (Supplement): 548-54.
6. Kitto, G.B. 1969. Intra- and extra-mitochondrial malate dehydrogenase from chicken and tuna heart. In: *Methods in Enzymology*, XIII, J.M. Lowenstein, (Ed.), pp.106-16. Academic Press, New York.
7. Kun, E. 1963. *The Enzymes*, VII. P.D. Boyer., H. Lardy, and K. Myrback (Ed.), pp. 149. Academic Press, New York.
8. Lee, D.H., Kim, Y.S. and Lee, C.B. 2001. The inductive responses of antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *J. Pl. Physiol.* **158**: 737-45.
9. Rajagopal, V., Patil, K.D. and Sumathykutty Amma, B. 1986. Abnormal stomatal opening in coconut palms affected by root (wilt) disease. *J. Exp. Bot.* **37**: 1398-1405.
10. Rajagopal, V., Samathykutty Amma, B. and Patil, K.D. (1987). Water relation studies in coconut palms affected with root (wilt) disease. *New Phytologist* **105**: 289-93.
11. Rajagopal, V., Shivashankar, S., Kasturi Bai, K.V. and Voleti, S.R. (1988). Leaf water potential as an index of drought tolerance in coconut (*Cocos nucifera* L.). *Pl. Physiol. Biochem.* **15**: 80-86.
12. Rajagopal, V., Kasturi Bai, K.V. and Voleti, S.R. 1990. Screening of coconut genotypes for drought tolerance. *Oleagineux.* **45**: 215-23.
13. Shivashankar, S., Kasturi Bai, K.V. and Rajagopal, V. 1991. Leaf water potential, stomatal resistance and activity of enzymes during the development of moisture stress in coconut palm. *Trop. Agric.* **68**: 106-10.
14. Shivashankar, S. 1992. Thermal stability of nitrate reductase in relation to drought tolerance in coconut. *J. Planation Crops* **20**(Suppl.): 267-73.

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