Journal of Plantation Crops 24 (Supplement) : 548-554 (1996)

ASSAY OF ENZYMES IN COCONUT CULTIVARS AND HYBRIDS UNDER NON-STRESS AND STRESS CONDITIONS

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ABSTRACT

Coconut palms of three tall varieties (West Coast Tall (WCT), Philippines Ordinary (PO) and Andaman Ordinary (AO), two dwarf varieties (Malayan Yellow Dwarf (MYD) and Gangabondam (GB)) and three hybrids (WCT x Chowghat Orange Dwarf (COD), COD xWCTand WCT x WCT) were assayed for the activities of stress sensitive enzymes viz., acid phosphatase (APH), L-aspartate : 2 Oxoglutarate amino transferase (AOAT), Superoxide dismutase (SOD), Peroxidase (POD) and Polyphenol Oxidase (PPO) during the two distinct stages viz., non-stress (September / October) and Stress (February / March) of 1989 and 1990. In general, there was stress-induced increase or decrease in the activity of enzymes among the cultivars/hybrids, based on which they were ranked. The cultivar/hybrid with higher activities of SOD and POD and lower activities of PPO, APH and AOAT could be designated as drought tolerant. Accordingly, the order of tolerance was found to be WCT x WCT > WCT > PO = WCT x COD > AO > GB = COD x WCT > MYD.

INTRODUCTION

Coconut is a perennial tropical tree crop which thrives well in warm humid regions where annual precipitation ranges from 130 cms to 300 cms, temperature from 29°C to 34°C and radiation from 165 Wm^{-2} to 330 $W\ m^{-2}$. As the palms are mainly grown under rainfed condition it has to thrive on the stored soil moisture content during the summer months starting from January to May. Cocomitant with the increase in the atmospheric parameters and soil water deficit during this period, the coconut cultivars/hybrids were found to behave differently with respect to water relation components (Rajagopal *et. al.,* 1990), enzyme activities (Shivashankar *et. al.,* 1991), peroxidation of lipids (Chempakam *et. al.,* 1993) and yield (Bhaskara Rao *et. al.,* 1991).

Based on the water relation components Rajgopal *et. al.,* (1990) ranked 23 coconut cultivars and hybrids and classified them into drought tolerant and susceptible types. Although a number of studies have been conducted on the behaviour of the stress sensitive enzymes during induced stress (Rajagopal *et. al.,* 1988) as well as during the development of moisture stress in the field condition (Shivashankar *et. al.,* 1991), no attempt was made to rank the cultivars/hybrids based on enzyme activities except for nitrate reductase activity (Shivashankar, 1992). Hence, an attempt has been made in this paper to give a sound biochemical basis for the ranking of the cultivars/hybrids based on enzyme assay during non-stress and stress periods. The effect of water deficit on the activities of the hydrolytic and oxidative enzymes has been well established in the literature (Todd, 1972; Levitt, 1980). According to Todd (1972) the loss of an enzyme activity below a certain critical level is more dangerous than the reduction during stress condition.

The enzyme assayed during the present studies were acid phosphatase (APH, EC.3.1.3.2), L-aspartate : 2 oxoglutarate aminotransferase (AOAT, EC. 2.6.1.1), superoxide dismutase (SOD, EC. 1.15.1.1) peroxidase (POD, EC.1.11.1.7) and polyphenoloxidase (PPO EC. 1.10 3.1). These enzymes have been reported to be important stress sensitive enzymes in coconut (Rajagopal *et. al.*, 1988; Shivashankar *et. al.*, 1991; Chempakam *et. al.*, 1993).

MATERIALS AND METHODS

From the 23 coconut cultivars/hybrids screened for drought tolerance/susceptibility based on the water relation components (Rajagopal *et al.* 1990), the palms selected for the present studies were three talls - West Coast Tall (WCT), Philippines Ordinary (PO) and Andaman Ordinary (AO), two dwarfs - Malayan Yellow Dwarf (MYD) and Gangabondam (GB) and three hybrids - WCT X WCT; WCT X Chowghat Orange Dwarf (COD) and COD x WCT. These 18 year old Plams were planted in red sandy loam soil with good management under rainfed condition. The studies were conducted twice in an year i.e., non-stress (September/October) and stress (February/March) periods for two consecutive years, i.e., 1989 and 1990. From each cultivar/hybrid three palms were selected and each palm was treated as a single replication and the mean values of these replications as well as for the two years were used for the statistical evaluation.

The enzyme activity was determined in the sixth leaf which is the standard leaf for water relation studies (Rajagopal *et. al.*, 1986). The enzymes were extracted by homogenizing the leaf tissue with 0.1. M sodium phosphate buffer, pH 7.6 containing 0.5% β-mercaptoethanol and 5% Polyvinyl poly-pyrolidone. The homogenate was squeezed through four layers of cheese cloth and centrifuged at 10,000 r.p.m for 20 minutes at 5°C. The precipitate formed after the purification of the clear supernatant with solid ammonium sulphate (final concentration 90%) was dissolved in 3 ml of the extraction buffer and was dialysed overnight in cold and centrifuged. The suitably diluted aliquots of the clear supernatant was used for all the enzyme assays. A blank was run with the denatured enzyme along with each enzyme assay. APH was assayed according to the method of Linhardt and Walter (1963) and AOAT as per the method of Bergmeyer (1963). SOD activity was determined by measuring its ability to initiate photochemical reaction of nitroblue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971) and both POD and PPO were assayed by the method of Kar and Mishra (1976).

RESULTS AND DISCUSSION

The non-stress period was characterised by high rainfall (117.5 cms.) with low radiation (141.7 Wm^{-2}), temperature (33.3°C) pan evaporation rate (3.6 mm day^{-1}), whereas the stress period with meagre rainfall (4.3 cms.), high radiation (314.1 Wm^{-2}) coupled with high temperature (36.1°C) and high pan evaporation rate (5.3 mm day^{-1}).

In coconut, with decrease in available soil water as well as leaf water potential activities of some of the hydrolytic and oxidative enzymes were found to decrease/increase to different degrees (Shivashankar *et. al.*, 1991). APH which is a hydrolytic enzyme usually gets released from the chloroplast into cytoplasm due to the drought induced membrane damage (Vieira da Silva, 1976). Although increased activity was observed during stress period the increase was smaller in WCT, PO, WCT X WCT and WCT X COD (9.5% to 24.4%) than in the rest (45.8% to 58.6%) (Table I). This indicates that the moisture stress-induced membrane damage is higher in the latter than the former. Similarly AOAT which play an important role in the synthesis of secondary metabolites such as proline (Fruton and Simmonds, 1965) showed an increase in the activity by 18.3 per cent (WCT) to 163.4 percent (GB) during stress period as compared with the non-stress period (Table I). The highest activity observed in MYD and GB indicate that they are more susceptible to stress than the other types.

Table I. Activities of enzymes in Coconut cultivars / hybrids during non-stress (NS) and stress (S) periods.

Cultivar / hybrid	APH (μ mol h^{-1} mg^{-1} protein)		AOAT (μ g h^{-1} mg^{-1} protein)	
	NS	S	NS	S
TALLS				
WCT	91.80	103.2(12.4)	1.0	1.2(18.3)
PO	86.30	97.3 (12.7)	1.80	1.5 (52.0)
AO	59 .60	94.50 (58.6)	0.90	1.8 (107.9)
DWARFS				
MYC	82.8	122.7 (102.8)	0.9	2.2 (140.9)
GB	88.9	129.6 (109.3)	0.8	2.2 (163.4)
HYBRIDS				
WCT X WCT	81.8	89.6 (9.5)	0.9	1.4 (54.3)
WCT X COD	91.5	113.8 (24.4)	0.9	1.6 (68.1)
COD X WCT	82.6	(129.9 (106.3)	0.9	1.9 (113.8)
Gen. mean	96.6		1.3	
SE plot -1	13.0		0.2	
CV (%)	13.5		16.0	
CD (P<0.05)				
For cultivar/hybrid	15.3		0.25	
NS vs S	7.7		0.13	
Interaction	NIL		0.36	

Figures in bracket indicate percentage increase over non-stress.

Table II. Activities of enzymes in Coconut cultivars / hybrids during non-stress (NS) and stress (S) periods.

Cultivar / hybrid	SOD (Units)		POD (Δ m^{-1} mg^{-1} protein)		PPO (Δ Am^{-1} mg^{-1} protein)	
	NS	S	NS	S	NS	S
TALLS						
WCT	2.6	4.1 (59.0)	31.90	41.2 (29.3)	39.6	38.3 (-3.4)
PO	2.6	3.1 (20.7)	32.60	47.9 (47.1)	38.1	27.30 (-28.3)
AO	1.7	1.8 (8.2)	25.2	30.00 (18.7)	31.80	44.6 (+40.5)
DWARFS						
MYD	1.9	2.1 (7.8)	25. 9	27.2 (4.8)	23.4	51.40 (+119.4)
GB	2.0	2.4 (21.9)	27.7	32.0 (15.6)	27.9	42.1 (+51.0)
HYBRIDS						
WCT X WCT	2.3	4.3 (85.8)	19.3	44.7 (131.4)	34.7	32.9 (-8.6)
WCT X COD	3.1	4.7 (51.9)	27.1	41.3 (52.3)	47.2	29.2 (-16.9)
COD X WCT	1.7	2.3 (35.3)	34.4	37.0 (7.6)	32.8	65.5 (+99.6)
Gen. mean	2.7		32.8		38.5	
SE plot -1	0.4		5.2		4.6	
CV (%)	15.4		15.8		11.9	
CD (P<0.05)						
For cultivar/hybrid	0.48		6.12		5.41	
NS vs S	0.24		3.06		7.65	
Interaction	0.68		NIL		NIL	

Figures in bracket indicate percentage increase over non-stress.

Enhanced activity of APH and AOAT during induced stress as well as with the development of moisture stress under field condition in the tolerant than the susceptible types has been reported in coconut earlier (Rajagopal *et al.* 1988; Shivashankar *et. al.*, 1991). Similar observation on APH activity has been reported in many annual crops (Gupta and Sheoran, 1979).

The enzymes SOD and POD normally known as scavenging enzymes, exhibited higher activities during the stress period as compared with the non-stress period. However the increase was much lower in AO, MYD, GB and COD X WCT than in the others (Table II). Higher increase in the SOD activity was observed in WCT (59%), WCT X WCT (85.8%) and WCT X COD (51.9%) during stress period as compared with the non-stress period. The lower activity of the enzyme can result in the formation of superoxide radical $(O_2)^+$ and H_2O_2. Increased activity of the enzyme in the tolerant than the susceptible types with respect to lower lipid peroxidation levels has been reported by Chempakam *et. al.* (1993).

Similarly, higher increase in the POD activity was observed in WCT X WCT (131.4%) and WCT X COD (52.3%) than in MYD (4.8%) and COD X WCT (7.6%) during stress period as compared with the non- stress period (Table II). This indicates that the accumulation Of H_2O_2 enhancing peroxide formation (Kalir *et. al.*, 1984) is more in the latter than the former. From the foregoing it is obvious that both SOD and POD play important roles in the scavenging of the toxic intermediates of the incomplete oxidation of tissues (Dhindsa *et. al.*, 1982), thus maintaining cell membrane integrity (Elstner *et. al.*, 1988). The negative correlation observed between lipid peroxidation and the activities of the two enzymes in coconut (Chempakam *et. al.*, 1993) thus corroborate with the above view. All the cultivars/hybrids exhibited higher activities during stress period as compared with the non-stress period which implies the activation of protective enzymes during water stress, as reported by Elstner *et. al.*, (1988).

PPO and POD are inter-related enzymes such that the increased levels of hydroperoxides associated with drought susceptibility can be attributed to the higher activity of PPO and lower activity of POD. Since PPO is a plastid enzyme which is in inactive form gets activated only when released into the cytoplasm following cell injury (Vaughn *et al.* 1988). The higher level of activity observed in AO, MYD, GB and COD X WCT during stress period indicate higher cell membrane damage (Table II). Chempakam *et. al.* (1993) reported a positive correlation between the levels of peroxidation of lipids and PPO activity in coconut during stress period.

From the foregoing it is obvious that APH, AOAT, SOD, POD and PPO are all stress sensitive enzymes and got activated during stress period and the activities varied greatly among the cultivars / hybrids. Based on the variation in the activities during stress, as compared with the non-stress period an attempt was made to rank the cultivars / hybrids (Table III). For APH and AOAT rank was given from lower increase to higher, for SOD and POD from higher increase to lower, whereas for PPO from decrease to increase. The tolerant types are between 1st and 3rd rank while the susceptible ones fall between 4th and 6th. Ranksum is a clear indication of the distinct differences between the tolerant and susceptible groups. WCT X WCT is relatively more tolerant to stress than the other three in the group. WCT X COD turned out to be highly superior to COD X WCT. Among the talls WCT is superior to PO and AO whereas between the dwarfs MYD is more susceptible than GB. This is in agreement with the earlier report on ranking of the coconut cultivars/hybrids based on the water relation components viz., stomatal resistance, leaf

Table III. Ranking of Coconut cultivars and hybrids based on enzyme activities.

Cultivar / Hybrid	APH	AOAT	SOD	POD	PPO	Rank sum	Rank
Talls							
WCT	2	1	2	4	1	10	2
PO	2	2	4	3	4	15	3
AO	6	5	7	5	5	28	4
Dwarfs							
MYD	4	7	8	8	8	35	6
GB	4	8	6	6	6	30	5
Hybrids							
WCT x WCT	1	3	1	1	2	8	1
WCT x COD	3	4	3	2	3	15	3
COD x WCT	5	6	5	7	7	30	5

water potential and wax content (Rajagopal *et al.* 1990). That study also placed WCT X WCT in the first rank and MYD the lowest rank. Thus the present observations gave a sound biochemical basis for tolerance to drought in coconut based on enzyme assay.

ACKNOWLEDGEMENTS

The authors wish to express their sincere gratitude to Dr. M.K. Nair, Director, CPCRI, Kasaragod for providing the necessary facilities for the work. Thanks are also due to the Division of Crop Improvement for sparing the palms for the work and also to Mr. M.V. George, Principal Scientist for the statistical analysis of the data.

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