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Lipid Peroxidation in Relation to Drought Tolerance in Coconut (*Cocos nucifera* L.)

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Lipid peroxidation levels and activities of superoxide dismutase, catalase, peroxidase and polyphenol oxidase were determined in eight coconut cultivars/hybrids differing in their degree of drought tolerance. Tolerant ones showed lower levels of peroxidation and higher activities of superoxide dismutase, catalase and peroxidase. Polyphenol oxidase activity was found to be lower in tolerant cultivars/hybrids as compared to susceptible ones. The interrelationships between cell membrane stability, lipid peroxidation and related enzyme activities are discussed in the light of the present investigations.

Key words : *Cocos nucifera*, drought tolerance, enzymes, membrane, peroxidation, stability

INTRODUCTION

Coconut is one of the most important commercial crops grown in India and one of the major sources of vegetative oils in the world. It is cultivated mainly as a rainfed crop in peninsular India. An average rainfall of 150 cm is ideal for good growth and nut yield. But during summer months, the plant exhibits symptoms of atmospheric and soil droughts because of high evaporative demand. Since coconut is grown mostly on coastal sandy loam soils, this type of stress is commonly seen due to the erratic distribution of rainfall (Kasturi Bai *et al.* 1988, Rajagopal *et al.* 1988, Voleti *et al.* 1989). Under increasing atmospheric drought conditions and soil moisture deficit, coconut varieties/hybrids exhibit clear differences in stomatal resistance, epicuticular wax and other water potential components indicating their role as desirable characters under stress conditions (Rajagopal *et al.* 1990).

The amount of lipid peroxidation has also long been considered as one of the factors which indicate the severity of stress experienced by a plant (Dhindsa and Matowe 1981, Parida *et al.* 1981, Dhindsa *et al.* 1982, Choudhury and Choudhuri 1985). The membrane damage is also reflected in the solute leakage which is a measure of the cell permeability as reported by Blum and Ebercon (1981). The free radicals generated during lipid peroxidation viz. hydroxyl radical $(OH)^{\bullet}$ and single components of oxygen $(O_2)^{\bullet}$ readily react with protein and lipid biomembrane and cause cell damage (Fridovich 1978, Gardner 1979, Elstner 1982). Superoxide dismutase (SOD), catalase and peroxidase catalyse the destruction of these free radicals, thus limiting the damage due to lipid peroxidation in various annual crops, during periods of stress (Pederson and Aust 1973, Kellog and Fridovich 1975). The activities of polyphenol oxidase (PPO) and peroxidase which catalyse the oxidation reaction in various cell organelles show alterations during induced stress in coconut palms (Shivashankar, 1988) and also in plants exposed to salinity (Kalir *et al.* 1984).

The present paper deals with the extent of lipid peroxidation experienced by coconut varieties/hybrids having relative tolerance/susceptibility to drought and also the level of enzymes such as SOD, catalase, PPO and peroxidation and consequently the degree of stress.

MATERIALS AND METHODS

Eight coconut cultivars/hybrids previously screened for drought tolerance based on the water relation components (Rajagopal *et al.* 1990) viz. West Coast Tall × West Coast Tall (WCT × WCT), West Coast Tall × Chowghat Orange Dwarf (WCT × COD), WCT, Fiji, COD × WCT, COD × COD, Malayan Yellow Dwarf (MYD) and Ganga Bondam (GB) were selected for the study. The palms are grown under rainfed conditions with the normal cultural and agronomic practices and

are maintained by the Division of Crop Improvement of the Institute.

The photosynthetically active sixth leaf of the palm was taken for the estimation of lipid peroxidation and enzyme activities. All enzyme assays were performed in duplicate on three palms with two leaflets each during the months of February-March and the results are expressed as mean with s.e. of three values.

Determination of lipid peroxidation

This involved measuring the amount of lipid peroxidation in terms of absorbance values by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). One g chopped leaf sample was homogenised with 2.0 ml 0.1% trichloro acetic acid (TCA). Aliquots were taken and allowed to react with 4 ml of 20% TCA containing 0.5% TBA by boiling in a waterbath for 30 minutes and then quickly cooling in an ice bath. The absorbance at 532 nm was read and the non-specific absorption at 600 nm was substracted.

Preparation of enzyme extract

2.5 g of the chopped leaf sample was ground under ice-cold conditions using 0.1 M Na) phosphate buffer, pH 7.6 containing 0.5% β-mercaptoethanol and 5% polyvinyl pyrrolidone. The homogenate was squeezed through two layers of muslin cloth and centrifuged at 10,000 g for 20 minutes at 5°C. The enzyme was partially purified from the supernatant by ammonium sulphate precipitation (final concentration 90%) and subsequent dialysis. The clear pale yellow dialysate after centrifugation was used for all the enzyme assays.

Determination of enzyme activities

SOD activity was determined by measuring its ability to initiate photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971). Catalase was assayed titrimetrically using $KMnO_4$ based on the method of Kar and Mishra (1976). The activity was expressed in units where one unit is defined as the amount of enzyme which consumes 1 mole of H_2O_2/minute under the conditions of the assay. Activities of peroxidase and PPO were determined by the method of Kar and Mishra (1976). The absorbance of the reaction product was read at 420 nm. The enzyme activity was expressed as changes in absorbance (ΔA/min/mg protein). An aliquot of the enzyme extract was used to determine its protein content by the method of Lowry *et al.* (1951).

RESULTS

The drought-induced changes in the levels of lipid peroxidation are shown in Table 1. It is seen that there is a marked variation in the peroxide levels in the various cultivars/hybrids. MYD and GB recorded significantly higher peroxidation than the rest of the genotypes. The rate of peroxide formation ranged from 0.0763 (WCT × WCT) to 0.569 (MYD and GB).

Table 1. Lipid peroxidation in coconut cultivars (expressed in terms of absorbance values)

Cultivar	*Peroxidation*
Drought tolerant	
WCT × WCT	0.076
WCT × COD	0.186
WCT	0.183
Fiji	0.219
Drought susceptible	
COD × COD	0.396
COD × WCT	0.459
MYD	0.569
GB	0.569
S.E./Plot	0.254
Gen. Mean	0.3322
CV%	8.658
CD	0.0440

Table 2 shows the specific activities of SOD, catalase, peroxidase and PPO in the different cultivars/hybrids. SOD and catalase activities showed more or less a gradual decline in the genotypes with increasing level of susceptibility to stress. The decline corresponded with an increase in the levels of lipid peroxidation and the degree of stress, as shown earlier.

In contrast, the drought susceptible cultivars, especially MYD and GB showed significant increase in the activity of PPO and decrease in peroxidase activity. These were found to be directly correlated with the peroxide levels.

Fig. 1 illustrates the correlations between lipid peroxidation and enzyme activities. SOD, catalase and peroxidase exhibit a negative correlation with peroxidation levels while PPO shows a positive correlation. Based on these characters, the cultivars/hybrids were grouped by means of average link method of hierarchical clustering and the results are summarised in Fig. 2.

Table 2. Activities of SOD, catalase, peroxidase and polyphenol oxidase in coconut cultivars (expressed as enzyme units mg^{-1} protein $min.^{-1}$)

Cultivar/Hybrid	SOD	Catalase ($\times 10^{-3}$)	Peroxidase ($\times 10^{-3}$)	Polyphenol oxidase ($\times 10^{-3}$)
WCT × WCT	5.19	5.04	59.24	30.37
WCT × COD	4.15	3.73	59.1	33.87
WCT	3.68	4.60	60.4	38.26
Fiji	2.59	2.68	54.9	46.80
COD × COD	2.30	2.52	38.7	56.24
COD × WCT	1.75	1.49	32.1	61.54
MYD	1.58	1.33	41.95	93.31
GB	1.43	1.71	35.9	73.26
SE/plot	0.54	0.49	8.29	4.83
Gen. Mean	2.83	2.89	48.73	54.20
CV (%)	18.26	17.13	17.00	8.21
CD	0.93	0.8561	14.34	8.36

DISCUSSION

Lipid peroxidation affects normal cell functions causing damage to the cell constituents and consequently increased cell permeability and leakage, which is seen during varying stress conditions in different crops including coconut (Dhindsa and Bewley 1977, Dhindsa and Matowe 1981, Dhindsa *et al.* 1981, Kurup 1989). The present investigations on coconut cultivars/hybrids possessing different degrees of drought tolerance also support the above findings. Thus WCT × WCT, WCT × COD and WCT, which are established as drought tolerant (Rajagopal *et al.* 1990), exhibited lower levels of lipid peroxidation as compared to drought susceptible ones viz. MYD and GB. Thus there exists a direct correlation between peroxide levels and stress induced damages in coconut leaf tissue.

The uncontrolled lipid peroxidation as observed here, is well supported by lower activities of SOD and catalase. SOD plays an important role in scavenging toxic intermediates of incomplete oxidation in tissues. A decrease in the activity of the enzyme can result in the formation of superoxide radical $(O_2)^{\bullet}$ and H_2O_2 which in turn can form the hydroxyl radical $(OH)^{\bullet}$. These free radicals can participate in a number of toxic reactions, thus damaging the cell membrane integrity (Elstner, 1982). The fact that SOD activity was present at higher levels in the drought tolerant varieties suggests that detoxification is affected with less cell damage.

The decline in the activity of catalase in drought susceptible varieties favours further accumulation of H_2O_2, and subsequent free radical production and cell injury. There are several reports on the increase in H_2O_2 (Brennen and Frenkel 1977, Mondal and Choudhuri 1981, Ferguson *et al.* 1983) coupled with the decrease in the activity of SOD and catalase (Patra *et al.* 1979, Dhindsa *et al.* 1981, Parida *et al.* 1981) during senescence and during fruit ripening.

The increased levels of hydroperoxides associated with drought susceptibility can well be accounted for by the higher activity of PPO and lower activity of peroxidase in leaf tissue.

Polyphenol oxidase (PPO) activity was also found to be higher during stress in the susceptible varieties studied. Even though it is a plastid enzyme and hence has limited access to phenols present in the vacuoles, at times of stress, PPO is released to the cytoplasm following cell injury (Mayer and Harel, 1979, Vaughn and Duke 1984). This is evinced by higher levels of the enzyme during stress in susceptible ones and is directly correlated with the membrane peroxide levels. Thus, in coconut, drought susceptible cultivars/hybrids, due to their higher rate of cell injury, exhibited higher PPO activity. Higher PPO activity was also observed in certain talls and hybrids under water stress (Shivashankar 1988).

The above findings are summarised in Figs. 1 and 2. It is evident from Fig. 1 that the activities of SOD, cata-

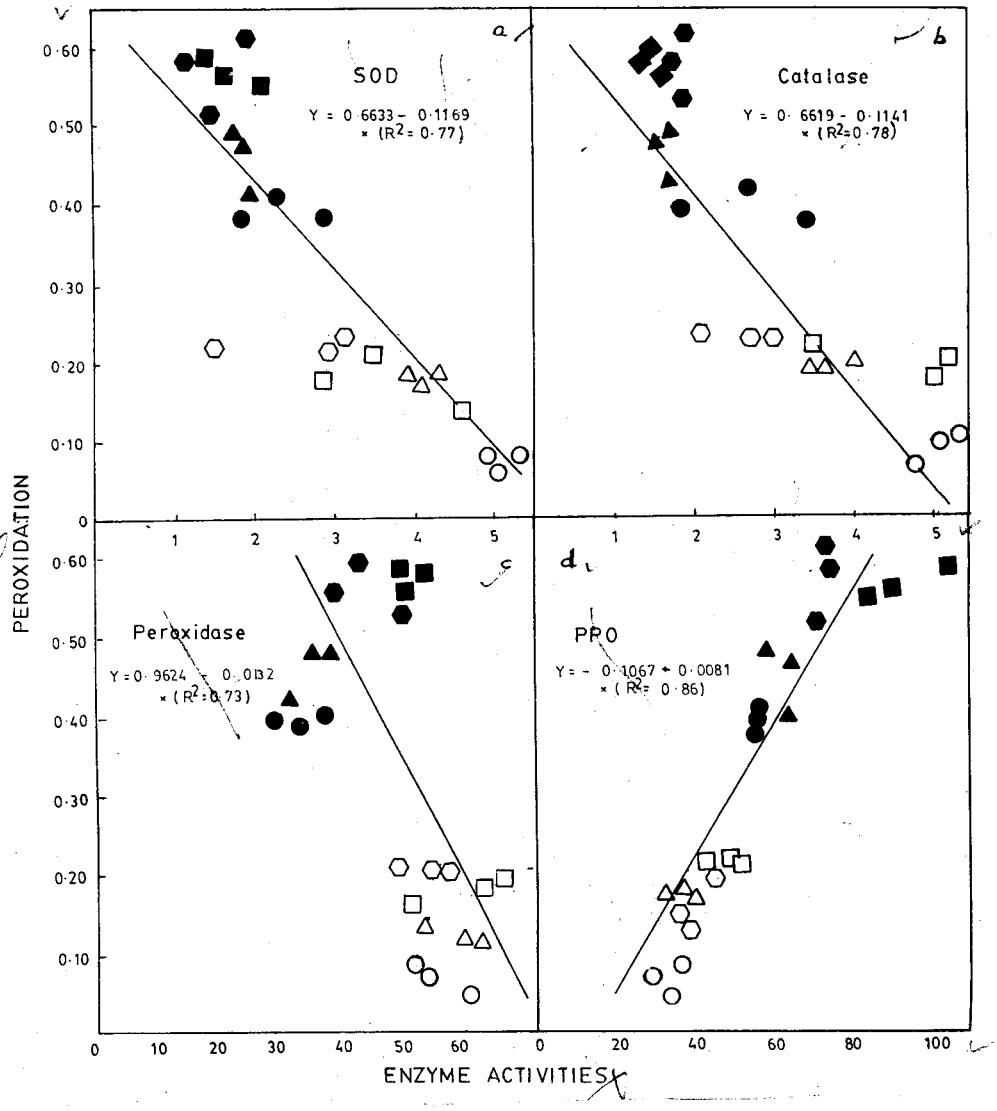

Fig. 1. Scatter diagram for peroxidation levels and enzyme activities.

	Tolerant	*Susceptible*
a - Superoxide dismutase	○ - T × T	● - D × D
b - Catalase	△ - T × D	▲ - D × T
c - Peroxidase	□ - WCT	■ - MYD
d - Polyphenol oxidase	⬡ - Fiji	⬢ - GB

lase and peroxidase have a negative correlation with the tissue peroxidation levels, while PPO exerts a positive correlation. The dendrogram shown in Fig. 2 is also a clear indication of the grouping of drought tolerant and susceptible varieties/hybrids, based on enzyme levels and peroxidation. This is in conformity with the earlier grouping based on water relation components (Rajagopal *et al* 1988). These studies on lipid peroxidation demonstrate that there is a direct relationship between drought tolerance of a plant and its capacity to control the levels of lipid peroxidation and related enzyme activities. But much remains to be unveiled, particularly with regard to the cell membrane integrity during stress and the actual mechanism underlying lipid peroxidation. Probably the protective role of specific enzymes to limit cell damage during stress, as is shown in the present investigation, may be taken as one of the important facets of drought tolerance in plants.

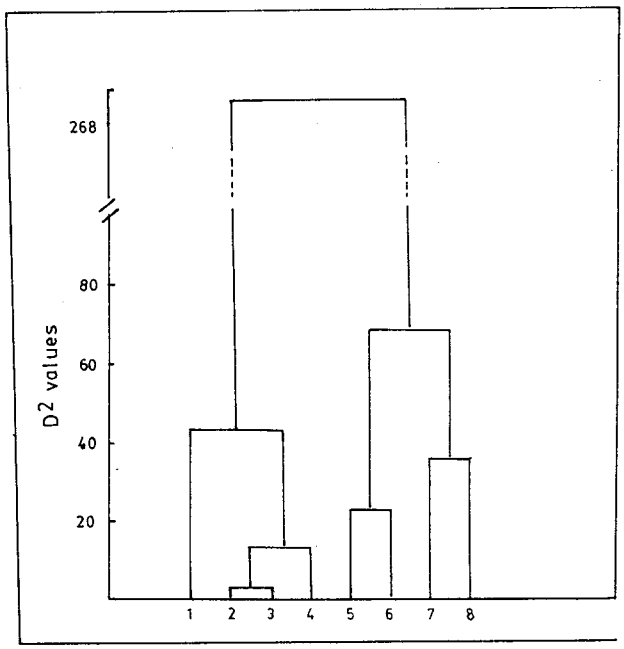

Fig. 2. Dendrogram based on average link method of clustering of coconut cultivars. 1 - WCT × WCT; 2 - WCT × COD; 3 - WCT; 4 - Fiji; 5 - COD × COD; 6 - COD × WCT; 7 - MYD; 8 - GB.

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