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Antioxidative Responses of Tree Species in Ayer Hitam Forest, Selangor, Peninsular Malaysia

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ABSTRAK

Tindakbalas oksidatif lapan spesies pokok, iaitu Atrocarpus elasticus, Endospermum diadenum, Vitex pinnata, Pellacalyx axillaris, Garcinia atroviridis, Gironniera nervosa, Bouea oppositifolia dan Callerya atropurpurea telah ditentukan bagi sampel yang diambil dari kedua-dua kawasan pendalaman dan tepian Hutan Simpan Ayer Hitam. Tindakbalas antioksidatif yang ditentukan termasuklah tahap penguraian oksidatif lipid pada membran sel dan juga kepekatan askorbat serta α-tokoferol, dua antioksidan yang penting. Kesemua lapan spesies menunjukkan perbezaan yang bererti pada tahap penguraian oksidatif antara kawasan pendalaman dan tepian hutan di mana secara amnya, tahap penguraian oksidatif atau peroksidaan lipid pada membran adalah lebih tinggi pada sampel yang diambil dari kawasan tepian hutan kecuali V.pinnata dan G.nervosa. Kepekatan α-tokoferol secara amnya juga didapati lebih tinggi di kawasan tepian hutan kecuali V.pinnata dan G.nervosa. Kepekatan askorbat walaubagaimanapun didapati lebih tinggi bagi semua sampel yang diambil dari kawasan tepian hutan. Ini menunjukkan bahawa terdapat tahap tegasan yang lebih tinggi dari segi tegasan oksidatif akibat gangguan yang lebih ketara di kawasan tepian hutan. C.atropurpurea mungkin merupakan satu spesies penunjuk yang baik dan sensitif dalam menentukan keadaan tegasan di dalam hutan sementara V.pinnata dan G.nervosa pula mungkin merupakan spesies pokok yang dapat mengurangkan tahap penguraian oksidatif dengan cekap dalam keadaan tegasan.

ABSTRACT

Antioxidative responses of eight tree species namely Atrocarpus elasticus, Endospermum diadenum, Vitex pinnata, Pellacalyx axillaris, Garcinia atroviridis, Gironniera nervosa, Bouea oppositifolia and Callerya atropurpurea were determined from samples collected from both the interior and fringe forest regions of Ayer Hitam Forest Reserve. These antioxidative responses measured include the extent of oxidative deterioration of cellular membrane lipids as well as the concentrations of ascorbate and α-tocopherol, two important endogeneous antioxidants. All eight species showed significant differences in the extent of oxidative deterioration between the interior and fringe forest regions where generally higher levels of membrane lipid peroxidation or oxidative deterioration were observed in samples from the fringe forest regions except for V.pinnata and G.nervosa. Concentrations of α-tocopherol were also found to be generally higher in the fringe forest regions except for V.pinnata and G.nervosa. Ascorbate concentrations were however found to be higher in all the tree species sampled from the fringe forest. This thus indicates higher levels of stress conditions with respect to oxidative stress manifested by higher levels of disturbance in the fringe forest regions. While C.atropurpurea may represent a good and sensitive indicator species in determining stress conditions in the forest, V.pinnata and G.nervosa may represent tree species that are efficient in minimising oxidative deterioration in stress conditions.

INTRODUCTION

Plants, in their natural habitats, are often subjected to various stress conditions, which may be due to abiotic factors as well as biotic factors. Abiotic factors, which include drought (Price and Hendry 1991), salinity (Fadzillah *et al.* 1997), anoxia (Crawford 1993), herbicides (Westphal *et al.* 1992), ozone (Tausz *et al.* 1994), sulfur dioxide and other gas pollutants (Bowler *et al.* 1992) as well as biotic factors such as bacterial or fungal infections (Wojtaszek 1997) often lead to decreased growth and yield of the plants affected where in severe conditions, may even lead to death. In most stress conditions, the generation of reactive oxygen species (ROS), have been implicated, where accumulation of these ROS at higher than normal levels may cause various damages at the cellular and molecular levels (Scandalios and Wright 1990). An important measure of this oxidative damage is the extent of oxidative deterioration or peroxidation of tissue and cellular membrane lipids. Through evolutionary pressures however, plants have evolved an antioxidative mechanism comprising of enzymatic as well as non-enzymatic systems. Some plants are more efficient than others in regulating a satisfactory antioxidative defense against the stress conditions, and this is often influenced in part, by their endogeneous antioxidant constituents.

The Ayer Hitam Forest Reserve, flanked by the Puchong Damansara Highway represents a forest ecosystem which can be divided into a more disturbed fringe forest region and a relatively less disturbed interior forest region. Disturbances arising mainly from human activities near the fringe forest which include atmospheric gas pollution may give rise to conditions of oxidative stress which can be measured from the concentration of malondialdehyde (MDA), representing the extent of oxidative deterioration of cellular membrane lipids. Indications of oxidative deterioration as well as status of endogeneous antioxidant, namely ascorbate and α-tocopherol, not only provide information on the level of stress and defense capacity of the plants, but may also provide information on the sensitivity of the plant species in detecting stress conditions. In this study, eight tree species were selected from both the interior and fringe forest regions of Ayer Hitam to determine differences between these two regions in terms of antioxidative responses manifested by different levels of disturbances to these regions. Concentrations of MDA and two important endogeneous antioxidants, namely ascorbate and α-tocopherol, were determined in all the samples collected.

MATERIALS AND METHODS

Plants Samples:

Leaves of eight forest tree species namely *Atrocarpus elasticus* (terap), *Endospermum diadenum* (sesenduk), *Vitex pinnata* (leban), *Pellacalyx axillaris* (membuluh), *Garcinia atroviridis* (kandis), *Gironniera nervosa* (hampas tebu), *Bouea oppositifolia* (kundang), and *Callerya atropurpurea* (tulang daing) were collected from the fringe and interior regions of the Ayer Hitam Forest, Selangor. Leaf tissue of each plant collected was placed in sealed polythene bags, kept in crushed ice and quickly transported to the laboratory for immediate analyses.

Determination of Lipid Peroxidation :

The level of lipid peroxidation in the leaf tissue, measured from concentration of MDA was determined by the thiobarbituric acid (TBA) reaction based on the method by Heath and Packer (1968) with slight modifications by Shaw (1995). Fresh samples (approximately 0.2 g) was homogenized in 1.5 ml 0.1 % (w/v) trichloroacetic acid and clean sand in a prechilled mortar and pestle at 0-4 °C. The homogenate was centrifuged at 10, 000 xg (Universal 16R) for 5 minutes. 0.75 ml of the supernatant obtained was added into 2.25 ml of TBA reagent and the mixture was heated at 95 °C for 30 minutes and quickly cooled in an ice bath for 15 minutes. After centrifuging at 10, 000 xg for 10 minutes, the absorbance of the supernatant obtained was measured at 532 nm with the value of non-specific absorption at 600 nm subtracted from the absorbance values. The concentrations of MDA were calculated using its extinction coefficient of 155 $mM^{-1}cm^{-1}$ and expressed as nmol MDA/g fresh weight of sample. A total of five replicates were used for each plant species from each of the two (interior and fringe forest) locations.

Determination of α-tocopherol :

α-tocopherol was extracted from the leaves tissue based on the method by Hodges *et al.* (1996). Under dim light and over ice, 0.15g of fresh

sample was ground up with 1.5 ml acetone and clean sand in a mortar and pestle at 0-4°C. The mixture was extracted with 0.5 ml hexane followed by vortexing for about 30 seconds. The mixture was then centrifuged at 1000xg for 10 minutes. After the centrifugation, the top layer was removed and the hexane extraction was repeated twice. The assay mixture was prepared as described by Kanno and Yamauchi (1997). 0.5 ml of the hexane-extract was added into 0.4 ml 0.1% (w/v) PDT, (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine, prepared in ethanol) and 0.4 ml 0.1% (w/v) ferric chloride (prepared in ethanol). The volume was made up to 3.0 ml with absolute ethanol and the mixture was gently swirled and left for 4 minutes for colour development. Following this, 0.2 ml of 0.2 M orthophosphoric acid was added to the mixture and allowed to stand for 30 minutes at room temperature before absorbance of the mixture was measured at 554 nm. The blank was prepared in the same manner except that the absolute ethanol was used instead of the hexane-extract. A standard curve was prepared using α-tocopherol (Sigma, type V) at various concentrations (0-1.4 μg/ml). 0.5 ml of α-tocopherol was added into the solution as described above and amount of α-tocopherol in the leaf sample was calculated based on the standard curve. A total of five replicates were used for each plant species from each of the two (interior and fringe forest) locations.

Determination of Ascorbate:

Ascorbate was extracted from the leaf tissue based on the method of John and Hughes (1983). 0.15 g of fresh sample was ground with pre-chilled mortar and pestle in 2.0 ml of 6% orthophosphoric acid in ice-cold conditions. The ground samples were then centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant obtained was obtained carefully titrated with DCPIP until a pink colouration was obtained. The volume of DCPIP used was compared against a standard curve to determine the amount of ascorbic acid in the samples. A total of five replicates were used from each of the two (interior and fringe forest) locations.

RESULTS AND DISCUSSION

Oxidative deterioration of all the eight species sampled, as indicated by concentrations of MDA in their leaf tissues, were found to be significantly different between the interior forest and fringe forest regions (*Fig.1*). The MDA concentration was generally found to be higher in the leaf tissues sampled from the fringe forest which was relatively more disturbed compared to samples taken from the relatively undisturbed interior forest except for *Vitex pinnata* (Leban) and *Gironniera nervosa* (Hampas tebu) which had higher MDA concentration in the interior forest region . Higher levels of oxidative deterioration in the samples taken from the fringe forest indicate that the plants were subjected to higher levels of stress conditions which may partly be attributed by greater exposure to atmospheric pollution and inferior soil conditions (Bowler *et al.* 1992). The highest degree of difference in MDA concentrations between the interior and fringe forest with reference to the ratio of the fringe forest region MDA concentrations to interior forest region MDA concentrations was exhibited by *C. atropurpurea* followed by *B. oppositifolia, G . atroviridis, P. axillaris , A. elasticus, E. diadenum, V. pinnata* and *G. nervosa.*

Concentration of α-tocopherol (*Fig. 2*), a lipid soluble antioxidant was also found to be generally higher in the fringe forest regions compared to the interior forest region except for *V. pinnata* and *G. nervosa* where significant differences were found in *E. diadenum , V. pinnata, C. atropurpurea.* The apparent ability of *V. pinnata* and *G. nervosa* to minimize oxidative deterioration in stress conditions as indicated by the lower levels of MDA concentrations in the fringe forest region may be attributed to their efficient ability in modulating and regulating the endogeneous α-tocopherol to impede the chain reactions of oxidative deterioration. This may thus explain the lower levels of α-tocopherol in both *V. pinnata* and *G. nervosa* sampled from the fringe forest regions. Concentration of ascorbate (*Fig.3*), a water-soluble antioxidant on the other hand, was found to be higher for all species sampled from the fringe forest region compared to samples from the interior region where significant differences between these two forest regions were shown by *A. elasticus* (terap), *E. diadenum* (sesenduk), *V. pinnata* (leban), *B. oppositifolia* (kundang) and *C. atropurpurea* (tulang daing). Ascorbate, a reductant in the Halliwell-Asada pathway, may be more directly regulated by the environmental conditions compared to α-tocopherol and thus more sensitive with respect to the antioxidative response shown by all the species samples.

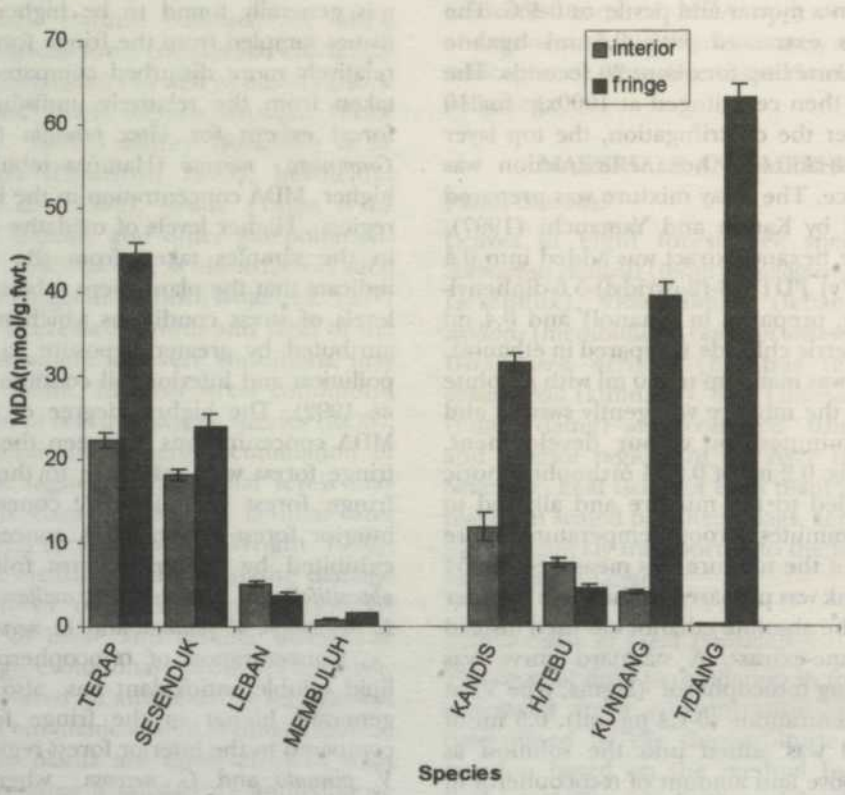

Fig. 1. Malondialdehyde concentration of eight tree species from the interior and fringe forest regions of Ayer Hitam Forest Reserve. Data are means ± se (n=5 replicates)

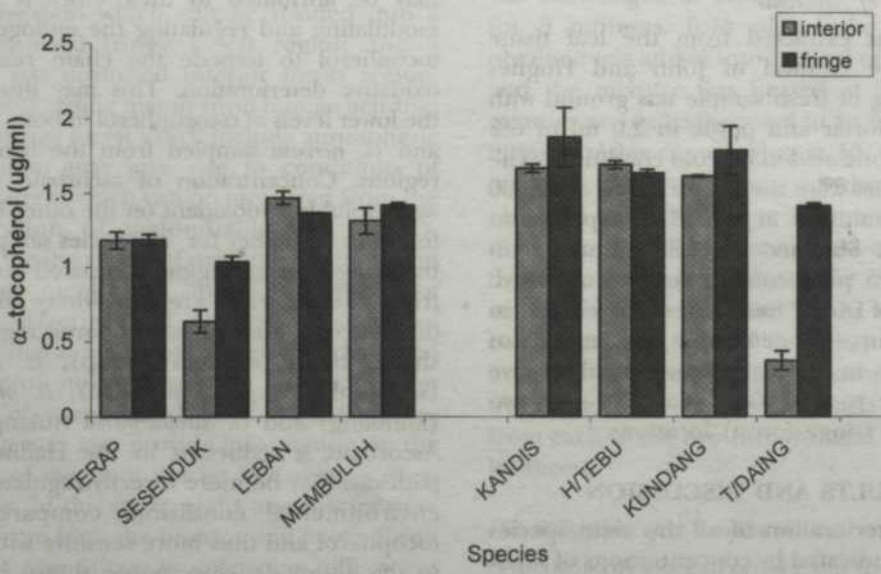

Fig. 2. α-tocopherol concentration of eight tree species from the interior and fringe forest regions of Ayer Hitam Forest Reserve. Data are means ± se (n=5 replicates)

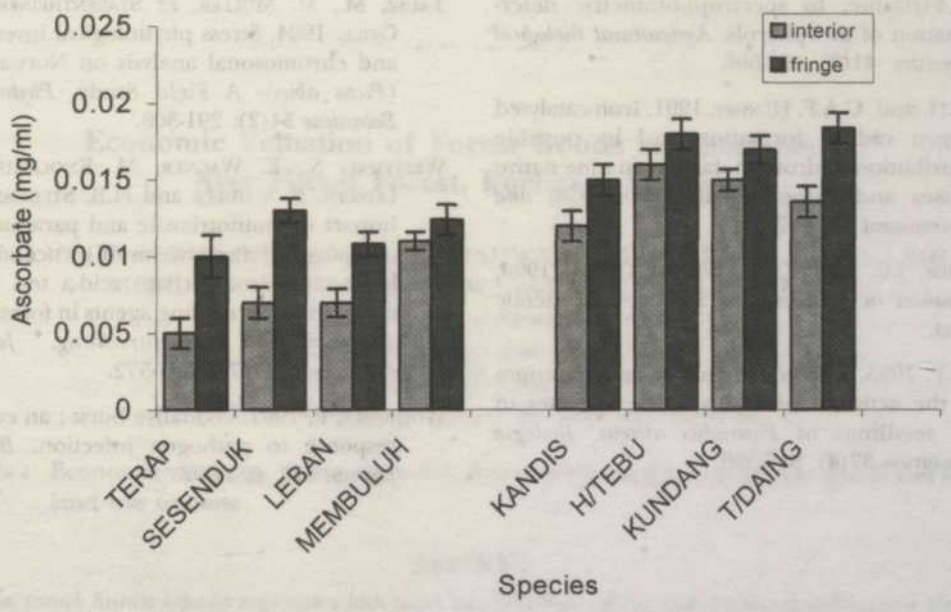

Fig. 3. Ascorbate concentration of eight tree species from the interior and fringe forest regions of Ayer Hitam Forest Reserve. Data are means ± se (n=5 replicates).

CONCLUSION

In agreement with a previous study (Fadzillah and Faridah Hanum 1999), oxidative deterioration has been shown to be a significant antioxidative response and may be a suitable indicator of forest disturbance. In addition, *C. atropurpurea* may be a good and sensitive indicator species in determining stress conditions in the forest regions while *V. pinnata* and *G. nervosa* represent tree species that are efficient in modulating their endogeneous α-tocopherol content to minimize oxidative deterioration in stress conditions.

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