UNIVERSITI PUTRA MALAYSIA

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By

CHAI TSUN THAI

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the Degree of Master of Science

ANTIOXIDATIVE RESPONSES IN BANANA (*MUSA* sp.) CULTIVARS UNDER WATER STRESS

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CHAI TSUN THAI

May 2003

Chairperson : **Associate Professor Dr. Nor'Aini Mohd. Fadzillah**

Faculty : **Science and Environmental Studies**

Responses of the enzymatic and non-enzymatic antioxidant defence systems as well as oxidative injury in banana plants (*Musa* sp.) subjected to polyethylene glycol (PEG)-induced water stress were investigated. Micropropagated plantlets of two banana cultivars, 'Berangan' and 'Mas' were subjected to 0%, 10%, 20% and 40% PEG 6000 treatments *in vitro* for a duration of 14 days.

Progressive decline in the leaf water content of both cultivars with time and increased concentrations of PEG used, indicates that water stress was successfully imposed. Based on the enhanced levels of lipid peroxidation and membrane permeability, this study shows that water stress resulted in oxidative injury in both cultivars. Relatively lower levels of membrane leakage and malondialdehyde concentrations in 'Berangan'

indicate that it was more tolerant than 'Mas' to water stress-induced oxidative damage.

Generally, activities of catalase and glutathione reductase were enhanced, while activities of superoxide dismutase were inhibited to different extent in both cultivars under water stress. Ascorbate peroxidase activity, meanwhile, was enhanced in 'Berangan' plantlets but generally unaffected in 'Mas' under water stress. Tissue concentrations of ascorbate, reduced glutathione and tocopherols were generally increased in both cultivars under water stress. Overall, activities of ascorbate peroxidase and superoxide dismutase as well as the tissue concentrations of ascorbate and reduced glutathione were higher in 'Berangan' compared to 'Mas'. On the other hand, activities of catalase and glutathione reductase as well as the tissue concentrations of tocopherols were higher in 'Mas' compared to 'Berangan'.

These data indicate that the capacity for detoxifying hydrogen peroxide and superoxide radicals (which involves ascorbate peroxidase and superoxide dismutase) before they could lead to oxidative damage, may be more crucial than the capacity to terminate lipid peroxidation (which involves tocopherols) in conferring protection against water deficit-induced oxidative stress in banana plants.

Abstrak tesis ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat untuk mendapat ijazah Master Sains

TINDAKBALAS ANTIOKSIDATIF TERHADAP KEPAYAHAN (TEGASAN) AIR PADA KULTIVAR PISANG (*MUSA* sp.)

Oleh

CHAI TSUN THAI

Mei 2003

Pengerusi : **Profesor Madya Dr. Nor'Aini Mohd. Fadzillah**

Fakulti : **Sains and Pengajian Alam Sekitar**

Kajian ke atas tindakbalas sistem pertahanan antioksidan berenzim dan bukan-enzim serta kecederaan oksidatif telah dijalankan pada tumbuhan pisang (*Musa* sp.) yang mengalami tegasan air ekoran rawatan polietilena glikol (PEG). Anak pokok dua kultivar pisang, iaitu 'Berangan' dan 'Mas' yang disediakan melalui teknik mikropropagasi telah diberi rawatan PEG 6000 pada kepekatan 0%, 10%, 20% dan 40% selama 14 hari.

Penurunan kandungan air dalam tisu daun yang sejajar dengan peningkatan tempoh rawatan dan kepekatan PEG menunjukkan bahawa dua kultivar pisang tersebut telah mengalami tegasan air dalam kajian ini. Berdasarkan peningkatan tahap pengoksidaan lipid dan kebocoran membran, kajian ini menunjukkan bahawa kecederaan oksidatif telah berlaku pada kedua-dua kultivar tersebut. Namun, tahap kebocoran membran dan malondialdehida

yang lebih rendah dalam anak pokok 'Berangan' menunjukkan bahawa 'Berangan' lebih toleran terhadap kecederaan oksidatif yang diaruh oleh tegasan air berbanding dengan 'Mas'.

Secara umum, dalam kedua-dua kultivar tersebut, aktiviti katalase dan glutathion reduktase telah meningkat, manakala aktiviti superoksida dismutase telah menurun lantaran tegasan air. Aktiviti askorbat peroksidase meningkat di dalam anak pokok 'Berangan' ekoran tegasan air, tetapi secara keseluruhan tidak dipengaruhi dalam anak pokok 'Mas'. Secara keseluruhan, kepekatan askorbat, glutathion bentuk terturun dan tokoferol meningkat dalam kedua-dua kultivar ekoran tegasan air. Di dalam anak pokok 'Berangan', paras aktiviti askorbat peroksidase dan superoksida dismutase serta kepekatan askorbat dan glutathion bentuk terturun adalah lebih tinggi berbanding dengan 'Mas'. Sebaliknya, paras aktiviti katalase dan glutathion reduktase serta kepekatan tokoferol adalah lebih tinggi di dalam anak pokok 'Mas' berbanding dengan 'Berangan'.

Keputusan kajian ini menunjukkan bahawa keupayaan untuk melupuskan hidrogen peroksida dan radikal superoksida (melibatkan askorbat peroksidase dan superoksida dismutase) adalah lebih mustahak daripada keupayaan untuk menamatkan tindakbalas pengoksidaan lipid (melibatkan tokoferol) dalam mempertahankan anak pokok pisang daripada mengalami kecederaan oksidatif di bawah aruhan tegasan air.

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I certify that an Examination Committee met on 12th May 2003 to conduct the final examination of Chai Tsun Thai on his Master of Science thesis entitled "Antioxidative Responses in Banana (*Musa* sp.) Cultivars Under Water Stress" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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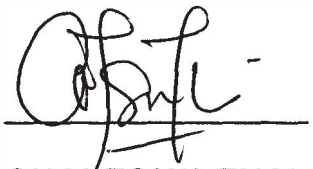
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



CHAI TSUN THAI

Date: 20/5/2003

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LIST OF ABBREVIATIONS

Δ A/min	Change of absorbance per minute
$^1O_2$	Singlet oxygen
Abs	Absorbance
APX	Ascorbate peroxidase
BAP	Benzyl aminopurine
CAT	Catalase
cDNA	Complementary DNA
Chl	Chlorophyll
CO_2	Carbon dioxide
Cu^+	Copper (I)
Cu^{2+}	Copper (II)
Cu/Zn SOD	Copper/Zinc superoxide dismutase
DHAR	Dehydroascorbate reductase
DNA	Deoxyribonucleic acid
DW	Dry weight
EDTA	Ethylenediamine tetraacetic acid
Fd	Ferredoxin
Fd_{red}	Reduced ferredoxin
Fe^{2+}	Iron (II)
Fe^{3+}	Iron (III)
FeSOD	Iron superoxide dismutase
FW	Fresh weight
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidised glutathione
h	Hour
H_2O	Water
H_2O_2	Hydrogen peroxide
HNE	4-hydroxy-2-nonenal
IAA	Indole-3-acetic acid
MDA	Malondialdehyde
$MDA^{\bullet}$	Monodehydroarscorbate radical
MDHAR	Monodehydroascorbate reductase
Min	Minute
MnSOD	Manganese superoxide dismutase
mRNA	Messenger Ribonucleic acid
MSI	Membrane stability index
NaCl	Sodium chloride
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidised form)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NBT	Nitro blue tetrazolium
O_2	Oxygen
$O_2^{\bullet -}$	Superoxide anion radical
OH^-	Hydroxyl ion

$OH^{\bullet}$	Hydroxyl radical
PEG	Polyethylene glycol
PPFD	Photosynthetic photon flux density
PSI	Photosystem I
PSII	Photosystem II
PWD	Plant water deficit
$R^{\bullet}$	Carbon-centred radical
$RO^{\bullet}$	Alkoxyl radical
$ROO^{\bullet}$	Peroxyl radical
ROOH	Lipid hydroperoxide
ROS	Reactive oxygen species
SOD	Superoxide dismutase
tAPX	Thylakoid-bound ascorbate peroxidase
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
tSOD	Thylakoid-bound superoxide dismutase
WC	Water content

CHAPTER I

INTRODUCTION

Oxidative stress is exerted when the antioxidant capacity of plants is overwhelmed by the enhanced production of reactive oxygen species (Zhang and Kirkham, 1996a). Oxidative stress is implicated in plants subjected to stressful environmental conditions, one of which being water stress. Studies on various crop species show that drought-tolerant plants are usually endowed with efficient antioxidant defence system (Jagtap and Bhargava, 1995; Sairam *et al.*, 1998). Meanwhile, field trials of a transgenic alfalfa overexpressing superoxide dismutase showed that oxidative tolerance is related to enhanced growth and survival in the field (McKersie *et al.*, 1996).

In Malaysia, unpredictable short periods of dry season are possible in banana-cultivated areas (Zakaria *et al.*, 2000), which may adversely affect growth and productivity (Bohnert and Jensen, 1996) of the plant. According to Bajaj *et al.* (1999), antioxidant gene transfer is one of the main strategies for engineering drought tolerance in plants. However, before such technology could be applied to banana plants, knowledge of the responses of antioxidant defence systems of banana under water stress is fundamental.

Despite various efforts of banana improvement in the country (Siti Hawa, 2000), the antioxidant defence and oxidative stress responses of banana are areas largely unexplored. Worldwide, it is the same scenario. Considering

the commercial value of banana crop and the potential benefit that enhancing antioxidant defence in crop plants could offer, it therefore seems reasonable and appropriate to investigate the antioxidant responses of local banana cultivars.

In this study, I have examined the antioxidant defences and oxidative injury of two local banana cultivars, which are *Musa* AAA 'Berangan' and *Musa* AA 'Mas' under PEG-induced water stress. Banana is an important fruit crop in Malaysia, a centre of origin and diversity for bananas (*Musa acuminata*) (Robinson, 1996). The availability of a wide selection of banana cultivars locally eliminates the difficulty of obtaining plant material for research. One of the reasons 'Berangan' and 'Mas' were chosen for this study is their commercial value where local and export market demands are concerned. In addition, based on the levels of leaf proline accumulation, a previous study has shown that 'Berangan' was more water stress-tolerant compared to 'Mas' (Zulkifli, 1997). Nevertheless, the information regarding the antioxidant defence of 'Berangan' and 'Mas' is scarce. The use of two different banana cultivars of different genomic compositions (i.e. AAA and AA) in this study thus allows intercultivar comparisons of antioxidant defences in banana plants under PEG-induced water stress. Micropropagated banana plantlets were used in this study as they could be rapidly prepared and were uniform in shape and size, and can be maintained in axenic conditions. In this study, the degree of membrane permeability and lipid peroxidation were examined to compare the relative tolerance of the two cultivars under water deficit-induced oxidative stress. In addition, enzymatic antioxidants (superoxide

dismutase, catalase, ascorbate peroxidase and glutathione reductase) and non-enzymatic antioxidants (ascorbate, reduced glutathione and tocopherols) were determined to study their roles in the protection of banana leaf cells against water deficit-induced oxidative stress. It is hoped that information obtained in this study could be useful for banana crop improvement concerning the development of stress-tolerant banana cultivars.

Objectives

A. Comparison of two banana cultivars for oxidative injury under water deficit stress.

B. To elucidate the antioxidative mechanism in banana plants under water deficit stress.

C. Characterization of enzymatic and non-enzymatic antioxidative defence in two banana cultivars under water deficit stress.

CHAPTER II

LITERATURE REVIEW

Research Plant: Banana

General

Banana (*Musa* sp., Family Musaceae) is one of the most important fruit crops in the world. It is native to Southeast Asia and has been cultivated here for millennia (Piper, 1989; Valmayor *et al.*, 1990). Malaysia is the main centre of origin for *Musa acuminata* types, e.g. *Musa* AA 'Mas' (Robinson, 1996). In Malaysia, a national collection of cultivars is maintained at the Malaysian Agricultural Research and Development Institute (MARDI), Serdang. From Southeast Asia, cultivation of bananas has been expanded throughout the tropics and into the subtropics of Asia, America, Africa and Australia. Southeast Asia is not only the centre of origin, but also the centre of diversity for bananas (Espino *et al.*, 1991; Valmayor *et al.*, 1990).

There are three important species of *Musa* in present-day agriculture, namely *Musa textalis* (abaca, grown for its fibre), *Musa acuminata* and *Musa balbisiana*. The last two species are important to food production (Dale, 1990).

Currently edible banana cultivars are the intraspecific hybrids within *M. acuminata* and within *M. balbisiana* and also interspecific hybrids between