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BRIEF COMMUNICATION

Water stress-induced oxidative damage and antioxidant responses in micropropagated banana plantlets

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Abstract

Oxidative injury and antioxidant responses were investigated in two banana genotypes (*Musa* AAA 'Berangan' and *Musa* AA 'Mas') subjected to 40 % PEG-induced water stress. PEG treatment resulted in oxidative injury, as expressed in increased lipid peroxidation and reduced membrane stability index, in both cultivars; however, greater oxidative injury was detected in 'Mas'. Under PEG treatment, catalase activity and glutathione reductase activity were enhanced in both cultivars, but were higher in 'Mas'. Ascorbate peroxidase activity was enhanced in 'Berangan' under water stress, but was unaffected in 'Mas'. Meanwhile, superoxide dismutase activity was inhibited in both cultivars under water stress, but higher activity was detected in 'Berangan'. Higher ascorbate peroxidase and superoxide dismutase activities were associated with greater protection against water stress-induced oxidative injury.

Additional key words: oxidative stress, ascorbate peroxidase, superoxide dismutase, glutathione reductase, catalase, lipid peroxidation.

Plants are immobile and therefore unable to escape stressful environments. In higher plants, exposure to abiotic stresses, *e.g.* water stress and high salinity, often results in oxidative injury (Smirnoff 1993, Fadzilla *et al*. 1997). Plants have evolved various antioxidative mechanisms that alleviate oxidative stress through the detoxification of reactive oxygen species (Alscher *et al*. 1997). Studies on sorghum and wheat revealed that stress-tolerant plants are usually endowed with efficient antioxidant defence systems (Jagtap and Bhargava 1995, Zhang and Kirkham 1996). Meanwhile, over-expression of genes encoding antioxidant enzymes in transgenic plants has been associated with enhanced stress tolerance (Allen *et al*. 1997).

Banana is a commercial crop in the tropical and subtropical regions of the world (Robinson 1996). As in other plants, the growth and productivity of the banana plant are adversely affected by water stress (Adam and Barakbah 1990). The role of antioxidative mechanism in the banana plant in relation to water stress, however, is largely unexplored; hence, this study was conducted. In this study, we investigated the antioxidant responses in micropropagated banana plantlets under water stress.

Micropropagated plantlets of *Musa* AAA 'Berangan' and *Musa* AA 'Mas' were prepared based on the procedure described by Vuylsteke and De Langhe (1985) and Novak *et al.* (1985) with some modifications. Sword suckers collected from Plantation Field 2, Universiti Putra Malaysia were the source of shoot tips used in culture initiation.

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Abbreviations: APX - ascorbate peroxidase; BAP - 6-benzylaminopurine, CAT - catalase; GR - glutathione reductase; IAA - indole-3-acetic acid; MDA - malondialdehyde; MSI - membrane stability index; PEG - polyethylene glycol; PPFD - photosynthetic photon flux density; SOD - superoxide dismutase.
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For the preparation of semisolid culture initiation media, Murashige and Skoog (1962, MS) basal medium was supplemented with 1 mg dm^{-3} thiamine, 100 mg dm^{-3} inositol, 30 g dm^{-3} sucrose, 10 μM 6-benzyl aminopurine (BAP) and 5 μM indole-3-acetic acid (IAA). The culture media were solidified with 5 g dm^{-3} agar and their pH was adjusted to 5.8 prior to autoclaving at 121 °C for 15 min. Semisolid and liquid multiplication media were prepared as for the culture initiation media except BAP concentration was increased to 20 μM and IAA was omitted. Semisolid rooting media were prepared as for the culture initiation media except BAP was omitted. All types of culture media were used at a volume of 50 cm^3.

Cultures on semisolid media were grown at 25 ± 2 °C with a 12-h photoperiod and a photosynthetic photon flux density (PPFD) of 65 μmol m^{-2} s^{-1}. Cultures on liquid media were placed on an orbital shaker (50 rpm) and incubated at 25 ± 2 °C with a 12-h photoperiod and PPFD of 20 μmol m^{-2} s^{-1}.

To induce water stress, each rooted banana plantlet (roots trimmed to 1 cm long) was placed in a capped test tube containing 15 cm^3 treatment medium. Treatment media were prepared as for the rooting media, supplemented with 0 % or 40 % PEG-6000, omitting agar. Test tubes were placed on an orbital shaker (50 rpm) and incubated at 25 ± 2 °C with a 12-h photoperiod and a PPFD of 20 μmol m^{-2} s^{-1}. At appropriate time intervals, the third leaf of each plantlet was used for analyses.

To determine leaf water content, leaf sample was excised from a plantlet with a sharp razor blade and weighed immediately to determine its fresh mass. For the determination of dry mass, the leaf was then placed in a pre-weighed envelope and dried to a constant mass in a drying oven at 70 °C. Leaf water content (LWC) was expressed as percentage of fresh mass (% of f.m.).

For the determination of membrane stability index (MSI), leaf cuts (1 × 0.5 cm) were used. Electrolyte leakage determination was carried out as described by Kraus and Fletcher (1994). Leaf membrane stability index (MSI) was calculated as described by Sairam *et al.* (1997/98).

The concentration of malondialdehyde (MDA), a product of lipid peroxidation, was determined by the thiobarbituric acid (TBA) test described by Shaw (1995).

Catalase (CAT) activity was assayed according to Fadzilla *et al.* (1997). Ascorbate peroxidase (APX) activity was assayed according to Nakano and Asada (1981). The reaction mixture (3 cm^3) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 1 mM hydrogen peroxide and 0.2 cm^3 of enzyme extract. Superoxide dismutase (SOD) activity was assayed according to Beauchamp and Fridovich (1971). The reaction mixture (1 cm^3) consisted of 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM nitro blue tetrazolium (NBT), 0.05 mM xanthine, 0.025 unit of xanthine oxidase and 0.05 cm^3 of enzyme extract. The enzyme extract was prepared as described by Dhindsa *et al.* (1981). One unit of SOD activity is equivalent to 50 % decline in the control rate of NBT reduction. Glutathione reductase (GR) activity was assayed as described by Hodges *et al.* (1997). The reaction mixture (3 cm^3) contained 50 mM potassium phosphate buffer (pH 7.8), 3 mM EDTA, 0.15 mM NADPH, 0.2 mM oxidised glutathione and 0.3 cm^3 of enzyme extract. Protein concentration was determined according to Bradford (1976).

Results were presented as means ± standard errors based on four replications. The significance of differences between mean values were evaluated by the Student's *t*-test. Differences were considered significant at $P < 0.05$.

Leaf water content (LWC) in 40 % PEG-treated 'Berangan' and 'Mas' plantlets declined significantly compared to their respective controls (Table 1). Nevertheless, the two cultivars showed no significant differences in LWC on day 7 and day 14 of 40 % PEG

Table 1. Effect of PEG treatment on the leaf water content [% of f.m.], membrane stability index [%], and malondialdehyde (MDA) concentration [nmol g^{-1}(f.m.)] of *Musa* AAA 'Berangan' and *Musa* AA 'Mas' plantlets.

Parameter	Time [d]	'Berangan' control	PEG	'Mas' control	PEG
Leaf water content	0	92.86 ± 0.12	92.86 ± 0.12	92.13 ± 0.33	92.13 ± 0.33
	7	92.70 ± 0.04	81.14 ± 0.99	91.32 ± 0.27	79.74 ± 0.63
	14	92.98 ± 0.03	73.09 ± 1.08	91.92 ± 0.35	75.03 ± 0.86
Membrane stability index	0	85.32 ± 0.23	85.32 ± 0.23	85.15 ± 0.34	85.15 ± 0.34
	7	85.74 ± 0.69	81.12 ± 1.58	87.43 ± 0.98	76.73 ± 2.52
	14	86.53 ± 1.23	61.43 ± 0.84	81.43 ± 0.33	37.37 ± 2.06
MDA content	0	13.75 ± 0.88	13.75 ± 0.88	20.14 ± 1.20	20.14 ± 1.20
	7	12.82 ± 0.78	43.17 ± 2.58	26.46 ± 2.73	73.48 ± 6.75
	14	13.44 ± 1.21	54.59 ± 1.67	20.27 ± 1.34	82.91 ± 5.48

Table 2. Effect of PEG treatment on the activities of catalase (CAT) [μmol(H_2O_2 consumed) s^{-1} mg^{-1}(protein)], ascorbate peroxidase (APX) [nmol(ascorbate consumed) s^{-1} mg^{-1}(protein)], superoxide dismutase (SOD) [U mg^{-1}(protein)], and glutathione reductase (GR) [nmol(NADPH) s^{-1} mg^{-1}(protein)] in *Musa* AAA 'Berangan' and *Musa* AA 'Mas' plantlets.

Parameter	Time [d]	'Berangan' control	PEG	'Mas' control	PEG
CAT	0	2.49 ± 0.20	2.49 ± 0.20	2.49 ± 0.22	2.49 ± 0.22
	7	2.11 ± 0.14	3.11 ± 0.18	2.47 ± 0.07	4.18 ± 0.46
	14	2.23 ± 0.10	2.85 ± 0.08	2.33 ± 0.06	3.50 ± 0.12
APX	0	34.60 ± 2.96	34.60 ± 2.96	39.57 ± 2.85	39.57 ± 2.85
	7	45.08 ± 0.42	71.13 ± 6.02	40.98 ± 5.26	38.52 ± 2.75
	14	46.28 ± 2.41	67.64 ± 2.39	43.73 ± 2.34	44.91 ± 0.86
SOD	0	245.52 ± 18.68	245.52 ± 18.68	257.99 ± 36.68	257.99 ± 36.68
	7	414.89 ± 13.91	223.30 ± 14.26	250.21 ± 12.59	131.86 ± 4.21
	14	381.69 ± 22.01	216.70 ± 8.67	200.50 ± 8.23	167.70 ± 12.17
GR	0	0.70 ± 0.02	0.70 ± 0.02	0.90 ± 0.08	0.90 ± 0.08
	7	0.68 ± 0.01	1.19 ± 0.02	1.27 ± 0.10	1.76 ± 0.16
	14	0.65 ± 0.04	1.01 ± 0.08	1.26 ± 0.05	1.93 ± 0.23

treatment. Reduction in LWC is characteristic for water stress (Zhang and Kirkham 1996). Therefore, the two cultivars were water-stressed when subjected to 40 % PEG treatment.

For PEG-treated 'Berangan' plantlets, membrane stability index (MSI) only declined significantly on day 14 (Table 1). For 'Mas' plantlets, PEG treatment resulted in reduced MSI on day 7 and day 14. Under PEG treatment, MSI of 'Berangan' was higher compared to 'Mas'. Malondialdehyde (MDA) concentration in both 'Berangan' and 'Mas' was increased on day 7 and day 14 when subjected to PEG treatment (Table 1), however, it was higher in 'Mas' compared to 'Berangan'. Enhanced oxidative injury, indicated by increased membrane permeability and MDA content, is common in plant cells subjected to water stress (Sairam *et al*. 1997/98, Zhang and Kirkham 1996). Data obtained thus indicate water stress-induced oxidative injury in the PEG-treated banana cultivars. Lower MDA content and higher MSI in 'Berangan' compared to 'Mas' indicated that 'Berangan' may be better protected against cellular membrane injury under water stress, a characteristic of drought-tolerant plants. Drought-tolerant sorghum and wheat genotypes were better protected against cellular membrane damage when subjected to water stress (Jagtap and Bhargava 1995, Sairam *et al*. 1997/98).

Catalase (CAT) catalyses the dismutation of hydrogen peroxide into water and oxygen (McKersie and Leshem 1994). When subjected to PEG treatment, CAT activities in both banana cultivars were increased compared to control plantlets (Table 2). Under PEG-induced water stress, CAT activity in 'Mas' was higher compared to 'Berangan'. Therefore, it is suspected that CAT may not be directly responsible for the better protection against oxidative injury in 'Berangan'.

Ascorbate peroxidase (APX) is a hydrogen peroxide-scavenging enzyme (McKersie and Leshem 1994). APX activity in 40 % PEG-treated 'Berangan' plantlets was markedly increased compared to control plantlets (Table 2). APX activity in 'Mas', on the other hand, was not significantly affected by PEG treatment. Overall, when subjected to water stress, APX activity in 'Berangan' was higher compared to 'Mas'. Therefore, APX activity may be a more crucial antioxidant defence than CAT in water-stressed banana plants.

Superoxide dismutase (SOD) catalyses the dismutation of superoxide radicals to hydrogen peroxide and water (McKersie and Leshem 1994). PEG-induced water stress inhibited SOD activity in both cultivars (Table 2), however, higher SOD activity was detected in 'Berangan'. Higher SOD activity was therefore associated with better protection against water stress-induced oxidative injury in this banana cultivar. Comparable to our finding, decreased SOD activity was detected in water-stressed jute plants, which also showed an association of lower SOD activity with greater degree of oxidative damage (Chowdhury and Choudhuri 1985).

Glutathione reductase (GR) catalyses the reduction of oxidised glutathione (GSSG) to reduced glutathione (GSH), an important endogenous antioxidant (McKersie and Leshem 1994). GR activity was enhanced in both cultivars in response to PEG treatment (Table 2); however, higher GR activity was detected in 'Mas'. Activity of APX, which catalyses the first step of the ascorbate-glutathione cycle, was generally not affected in water-stressed 'Mas' plantlets. Lack of concerted action between GR and APX may account for the higher level of oxidative injury in 'Mas' despite its higher GR activity compared to 'Berangan'. Noctor and Foyer (1998) pointed out that efficient destruction of reactive oxygen species requires the actions of several antioxidant enzymes acting in synchrony.

In summary, this study demonstrated that oxidative stress tolerance differs between banana cultivars. Compared to 'Mas', 'Berangan' was more tolerant to water stress-induced oxidative damage as evidenced by its lower MDA content and higher MSI. Enzymatic antioxidants responded differently in banana cultivars under water stress. Nevertheless, higher APX and SOD activities in 'Berangan' were associated with its better protection against water stress-induced oxidative damage.

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