Full Length Research Paper

Tissue diversity in respiratory metabolism and free radical processes in embryonic axes of the white mangrove (*Avicennia marina* L.) during drying and wet storage

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Species diversity in responses to desiccation in plants is well studied and documented. However, organ and tissue variability in plant dehydration responses is not as well investigated and understood. Therefore, the responses of whole axes, hypocotyls, root primordia and plumules of white mangrove to drying and wet storage were monitored. Increasing the rate of drying lowered the critical and lethal water concentrations for survival as assessed by germination capacity and tetrazolium staining. Dehydration and hydrated storage were generally associated with decrease in activities of phosphofructokinase (PFK) and malate dehydrogenase and levels of nicotinamide adenine dinucleotide and an increase in the levels of hydroperoxides in whole axes, hypocotyls, root primordia and plumules and membrane damage in axes. Increase in the activities of superoxide dismutase and catalase and decrease in the activities of glutathione reductase and amounts of ascorbate accompanied drying and moist storage in all tissues, in general. Apart from the activity of PFK, the plumules showed the highest activities and quantities of all the enzymes and compounds among the tissues during desiccation and wet storage. It is possible that this tissue, despite its relatively small size and volume plays disproportionately an important role in the events described. Nonetheless, it is likely that physical rather than metabolic damage underlined loss of viability as it occurred at high water concentration.

Key words: Antioxidant, *Avicennia marina* desiccation, drying rate, free radical processes, lipid peroxidation, metabolism, respiration seed survival.

INTRODUCTION

Avicennia marina (Forssk.) is an Indo-Pacific tropical intertidal estuarine (mangrove) tree that produces desiccation-sensitive (recalcitrant) seeds. These seeds are shed around the autumn equinox and can be carried to the inland fringes of the mangrove by the equinoctial high spring tide. Subsequent inundation may not occur

for several months and so these seeds will die from desiccation damage. Knowledge of the response of *A. marina* seeds to desiccation contributes to an understanding of the biology of a keystone mangrove species. Furthermore, many tropical and subtropical species produce recalcitrant seeds, which have implications

in biodiversity conservation. Desiccation tolerant seeds are generally stored in the dry state and the desiccation sensitivity of recalcitrant seeds precludes their storage by conventional means. This impact negatively on attempts at long-term storage of germplasm represented by these seeds, which, in turn, has implications for long-term biodiversity conservation. It is possible to store recalcitrant seeds at their shedding water content (hydrated storage), but this is strictly a short-term measure as viability is rapidly lost (Motete et al., 1997). The studies on the response of *A. marina* to drying reported here contribute to an increased knowledge of the species, of seed recalcitrance, and the ability to conserve the germplasm of species producing these seeds

In addition to physical stresses that desiccation-sensitive seed tissues incur as a consequence of dehydration, they are subjected to metabolic damage. It has been argued that desiccation differentially affects the activities of various enzymes (Farrant et al., 1985; Leprince et al., 1993a; 1994; 1995; 1999; 2000; Côme and Corbineau, 1996; Leprince and Hoekstra, 1998; Ntuli et al., 2011) and hence results in metabolic imbalance (Finch-Savage et al., 1993; reviewed by Vertucci and Farrant, 1995; Leprince et al., 2000; Ntuli et al., 2011). Of a particular interest in this regard, is respiratory metabolism. For instance, it has been shown that phosphofructokinase (PFK) and malate dehydrogenase (MDH) were slightly and mildly affected by dehydration, respectively, while the glucose-6-phosphate dehydrogenase (G6PDH) and NADH dehydrogenase of NADH-ubiquinone (coenzyme Q) reductase (complex I) were extremely sensitive to desiccation in germinating maize (Leprince et al., 1992; 1993a; 1994). In contrast, Carpenter et al. (1987) observed that PFK was highly sensitive to *in vitro* desiccation. In this regard, it is noteworthy that the cause of the differences in sensitivity among enzymes is unknown. More recently, Song et al. (2009) reported a decline in the activity of malate dehydrogenase in recalcitrant *Antiaris toxicaria* axes and orthodox *Zea may*s embryos during dehydration.

It has been suggested that desiccation-induced disruption of the electron transport chain of mitochondrial and microsomal membranes between the ubiquinone pool and cytochrome oxidase may result in more leakage of electrons from the electron transport chain than normal, thus increasing the generation of free radical species, which are associated with lipid peroxidation (Leprince et al., 1990; 1994; 1995; Hendry et al., 1992; Hendry, 1993). More recently, Song et al. (2009) reported

a decline in the activity of cytochrome c oxidase in recalcitrant *Antiaris toxicaria* axes and orthodox *Zea may*s embryos during dehydration. Roach et al. (2010) have demonstrated extracellular superoxide production in response to desiccation in recalcitrant *Castanea sativa* seeds. However, it remains unclear whether oxidative damage is a cause or consequence of tissue damage and/or death (Hendry et al., 1992; reviewed by Leprince and Golovina, 2002).

One of the mechanisms contributing to desiccation tolerance is the nature and efficiency of free radical processing systems (reviewed by Crowe et al., 1992; Leprince et al., 1993b; Horbowicz and Obendorf, 1994; Vertucci and Farrant, 1995; Close, 1996; Berjak and Pammenter, 2008; Pammenter and Berjak, 1999; Buitink et al., 2002). For instance, Hendry et al. (1992) demonstrated that dehydration of desiccation-sensitive embryonic axes of *Quercus robur* resulted in decreases in the activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APO) and the levels of α-tocopherol. In contrast, there was an increase in the levels of ascorbate (AsA) during dehydration. However, guaicol peroxidase (GPO) was not affected by drying. No catalase (CAT) activity was detected. More recently, Song and co-workers have shown a decline in activities of SOD, APO, CAT, GR, and dehydroascorbate reductase (DHAR) and SOD, APO and CAT in axes of Chineese wampee during dehydration (Huang et al., 2009, respectively).

Several studies have shown that rapid drying of desiccation-sensitive seeds permits survival to lower water contents than slow drying (Berjak et al., 1984; Farrant et al., 1985; Pritchard, 1991; Walters et al., 2001; Wesley-Smith et al., 2001; Huang et al., 2009; Ntuli et al., 2011). It has been proposed that such behaviour is consequent upon rapid drying removing water sufficiently fast to reduce the accumulation of damage resulting from aqueous-based deleterious reactions (Pritchard, 1991). To achieve the rates of drying to demonstrate this effect requires small pieces of tissue, and so the embryo or embryonic axis must be excised from the seed.

Although the responses of plant tissue to dehydration have been studied in a number of species, the responses of the individual seed components have been less frequently reported. This is important when studying recalcitrant seeds as different components of the seed or embryonic axes may dry at different rates and/or show differential sensitivities to dehydration.

A. marina is one of the most desiccation-sensitive

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Abbreviations: A/GPO, Ascorbate/guaicol peroxidase; **AsA,** ascorbic acid; **CAT,** catalase; **GR,** glutathione reductase; **MDH,** malate dehydrogenase; **NAD(H),** (reduced) nicotinamide adenine dinucleotide; **PFK,** phosphofructokinase; **SOD,** superoxide dismutase; **TZ,** tetrazolium.

recalcitrtant species recorded (Berjak et al., 1984) and so its response to dehydration is of particular importance. This report examines the response of respiratory metabolism and free-radical processing of whole axes, hypocotyls, root primordia and plumules of seeds of *Avicennia marina* subjected to rapid or slow drying or hydrated storage.

MATERIALS AND METHODS

Plant material

Newly-shed propagules of white mangrove (*Avicennia marina* L.) were collected from various trees in Isipingo Beach and Beachwood Nature Reserve in Durban, South Africa. Embryonic axes were excised from cotyledons and accumulated on moist filter paper in closed Petri dishes.

Treatments

Embryonic axes were partitioned into three samples. Each sample was aseptically placed in sterile containers over:

1) Activated silica gel with a fan mounted (rapid drying [close to 0% RH]),
2) A saturated solution of sodium chloride (slow dehydration [75 ± 1% RH]) or distilled water (wet storage [100% RH]). The containers were stored at 15 ±1°C.

Water concentration determinations

Water contents were determined gravimetrically after drying axes in the oven at 80°C for 48 h. Masses were determined periodically during dehydration and wet storage. The water contents are reported as means ± SE for five individual axes, expressed on a dry mass basis (g g^{-1} [dm]).

Viability tests

To minimize the effects of imbibitional damage, dehydrated embryonic axes were moistened on damp filter paper in Petri dishes overnight at 20°C before being subjected to the germination, tetrazolium and conductivity tests. To assess germination capacity, axes were cultured in Petri dishes on half MS medium (Musharige and Skoog, 1962) supplemented with 0.3 g l^{-1} sucrose, photoperiod 16 h, under sterile conditions for a period of 20 days at room temperature. Axes were scored as germinated when they showed greening and/or elongation and/or expansion.

   Apparent embryonic axis viability was determined by the tetrazolium test. Twenty moistened radicles were cut through longitudinally, soaked in 1% (w/v) 2, 3, 5- triphenyltetrazolium chloride solution for 24 h in the dark at 20°C, and scored using intensity and location of staining as criteria (International Seed Testing Association, 1996).

   Electrolyte leakage from five replicates of individual moistened embryonic axes was measured using a multi-cell conductivity meter (CM100; Reid and Associates cc, Durban) over 12 h. All measurements were made at 2 V whilst axes were immersed in 3 ml of distilled water. Leakage was recorded as the highest reading over the measurement period. The results are reported as means ±SE of the five replicate axes.

Respiratory enzymes assays

Phosphofructokinase (PFK) activity was determined according to Leprince et al. (1993a). Root primordia, hypocotyls, plumules and whole axes (*c*. 5 mg dry matter) were homogenised to a fine powder under liquid nitrogen using a pestle and mortar. Soluble proteins were extracted from the frozen powder in 5 ml of 50 mM Tris-HCl (pH 7.6) in the presence of 0.1% polyvinylpyrolidone (PVP) (Hofmann and Kopperschläger, 1982) and the homogenate centrifuged at 8 000 *g* for 5 min. An aliquot of 2 ml of the supernatant was then transferred to 1 ml of a mixture of 0.2 mM ethylenediaminetetraacetic acid (EDTA), 2 mM fructose-6-phosphate, 5 mM MgCl2, 0.6 mM ATP, 0.33 U ml^{-1} aldolase, 10 U ml^{-1} triose phosphate isomerase, 1 U ml^{-1} glycerophosphate de-hydrogenase and 0.2 mM NADH in Tris (pH 7.6) buffer. Activity was monitored by measuring the formation of fructose-1, 6-bisphosphate, as indicated by a coupled NADH-dependent reduction of dihydroxyacetone phosphate to glycerol-3-phosphate, as the change in absorbance at 340 nm over 3 min. Malate dehydrogenase (MDH) activity was monitored using the procedure of Leprince et al. (1993a). Radicles (*c*. 5 mg dry mass) were homogenised to a fine powder under liquid nitrogen using a mortar and pestle. Soluble proteins were extracted from frozen powder in 5 ml of 50 mM potassium phosphate buffer (pH 7.4) in the presence of 0.1% PVP (Siegel and Bing, 1956). The homogenate was then centrifuged at 8 000 *g* for 5 min. An aliquot of 0.1 ml of the supernatant was added to 2 ml of 0.1 M phosphate buffer (pH 7.55) and 0.1 ml of 2 mg/ml NADH. After 10 min, 0.1 ml of 0.5 M oxaloacetate in 0.1 M phosphate buffer (pH 7.0) was added. Activity was determined by observing the change in optical density of NADH at 340 nm over a 3 min period.

Oxidised nicotinamide adenine dinucleotide (NAD) assay

Root primordia, hypocotyls, plumules and whole axes (*c*. 5 mg dry mass) were homogenized in 5ml of 0.2 M HCl following homogenization to a fine powder under liquid nitrogen in the mortar with a pestle, heated in a boiling water bath for 5 min, cooled in an ice bath and centrifuged at 14 000 *g* for 10 min (Zhao et al., 1987). An aliquot of 0.5 ml of the supernatant was then transferred to 1.0 M Bicine-NaOH buffer (pH 8.0) and neutralized with 0.2 M NaOH in the dark (Matsumura and Miyachi, 1980). Following the addition of 0.1 ml each of 40 mM EDTA, 4.2 mM 3-(4,5 dimethyl-thiazoyl-2)-2,5-diphenyltetrazolium bromide (MTT), 16.6 mM phenol ethosulfate and 5.0 M ethanol, 0.1 ml of 500 U ml^{-1} alcohol dehydrogenase was added after 5 min at 37°C. The level of NAD was determined by measuring the rate of reduction of MTT as absorbance at 570 nm after 30 min.

Lipid peroxidation assessment

Lipids were extracted from radicals (*c*. 5 mg dry mass) that had been homogenized under liquid nitrogen in a mortar with a pestle, in 5 ml of dichloromethane/methanol (2:1 v/v) containing butylated hydroxytoluene (50 mg l^{-1}) according to Hailstones and Smith (1988). Following centrifugation at 1500 *g* for 5 min, 1 ml of 0.014 M ferrous chloride (FeCl2) was added to 2 ml of the lipid extract in dichloromethane/methanol and shaken. Twenty µL of 30% potassium thiocyanate (KSCN) were then added. Hydroperoxide levels were estimated by the oxidation of Fe^{2+} as the absorbance recorded at 505 nm.

Antioxidant assays

Superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) were extracted in 10 ml of 50 mM potassium
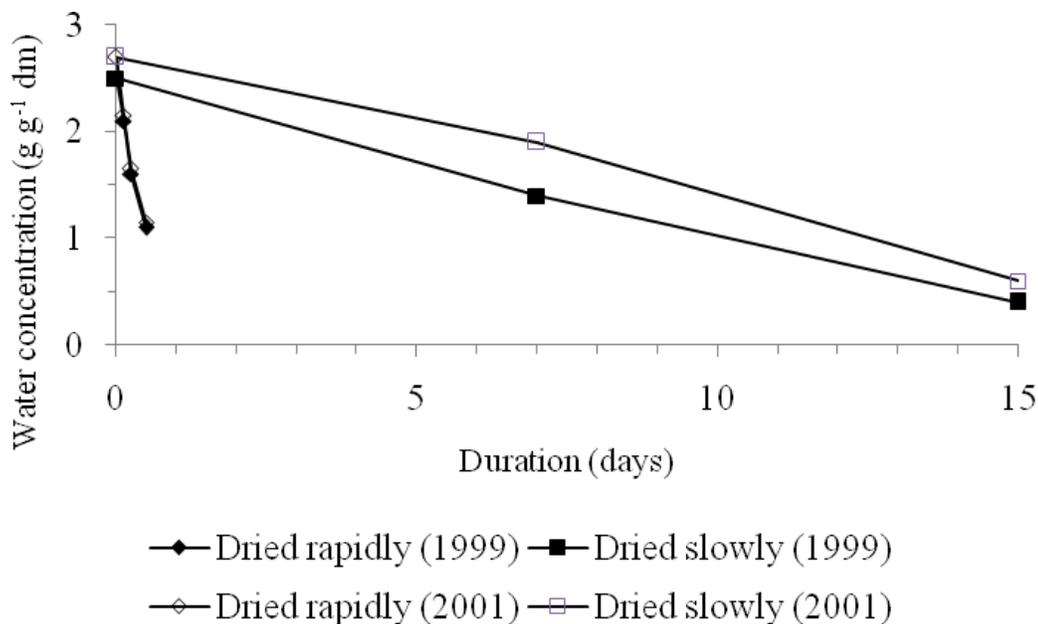
Figure 1. Water concentrations of embryonic axes of *Avicennia marina* during desiccation. (Error bars represent means ± SE, n = 5, all smaller than symbols).

phosphate (pH 7.0), 0.25% (w/v) Triton X-100 and 1% (w/v) poly-vinylpolypyrolidone, following homogenisation of Root primordia, hypocotyls, plumules and whole axes (*c.* 5 mg dm) to a fine powder under liquid nitrogen in the mortar with a pestle, following the procedures of Mishra et al. (1993; 1995). The homogenate was then centrifuged at 8 000 g for 15 min. For SOD, the reaction was performed with an aliquot of 2 ml of the supernatant and 1 ml of 50 mM potassium phosphate (pH 7.8), 0.1 mM EDTA containing 18 µM cytochrome *c* and 0.1 mM xanthine and the reaction started with addition of 0.02 ml of 1 U ml-1 of xanthine oxidase (McCord and Fridovich, 1969; Schoner and Krause, 1990). Activity was monitored by measuring the rate of reduction of cytochrome c as the change in absorbance at 550 nm.

CAT was assayed in an aliquot of 2 ml of the supernatant added to 1 ml of 50 mM potassium phosphate containing 11 mM H_2O_2 (pH 7.0). Activity was determined as the decomposition of H2O2 by the decrease in absorbance at 240 nm (Aebi, 1983). GR was assayed in an aliquot of 2 ml of the supernatant added to 1 ml of 25 mM Tris- HCl containing 0.5 mM oxidised glutathione (GSSG) and 0.12 mM NADPH (pH 7.8) (Foyer and Halliwell, 1976). Activity was recorded by measuring the oxidation of NADPH as the decrease in absorbance at 340 nm.

AsA was extracted in 4 ml of 2.5 mM perchloric acid ($HClO_4$) following homogenisation of radicles (*c.* 5 mg dm) in liquid nitrogen in the mortar with a pestle according to Foyer *et al.* (1983). The homogenate was then centrifuged at 8 000 g for 5 min and neutral-lised to pH 5.6 with 1.25 M K_2CO_3. One ml of the extract was transferred to 1 ml of 0.1 M sodium phosphate buffer (pH 5.6) and reaction started with addition of 1 ml of 5 U ml^{-1} of ascorbate oxi-dase (Hewitt and Dickes, 1961). The level of AsA was estimated by measuring its oxidation as the decrease in absorbance at 265 nm.
For all the biochemical assays, the extractions were performed on five embryonic axes (*c.* 5 mg dm) in at least three replicates at 4°C. All the assays were carried out at 25°C. All chemicals were bought from Sigma and the units are as defined thereby. Results are reported as means   SE of the replicate extractions and % change of the initial value.

Dry mass estimations

For the conductivity tests and all biochemical assays, dry mass was calculated from wet mass and water contents expressed on a dry mass basis. Wet mass was determined before the tests or assays were conducted.

Statistical analysis

Data were subjected to one-way ANOVA test. Where significant effects were found to occur, the Turkey multiple range test was subsequently used to distinguish among significantly deviating means.

RESULTS

Desiccation kinetics

Both slow and rapid drying showed almost linear dehy-dration. Whole axes reached a water concentration of 1.1 g g^{-1} dm in 12 h during rapid drying while a final water content of 0.29 g g^{-1} dm was attained in 15 days upon slow dehydration (Figure 1). As a result, rapid desicca-tion facilitated drying around 20 times more rapidly than slow dehydration, water contents remained constant during wet storage (data not shown).

Survival characteristics

Newly-shed axes registered 100% germination and tetra-zolium staining for both harvests (Figures 2A and C). A 40% decrease in germination capacity occurred at *c.* 2.0 and 2.4 g g^{-1} dm during fast and slow drying of axes from

seeds harvested in 1999, respectively. A similar decline in germination capacity was observed at *c.* 2.0 and 2.6 g g^{-1} dm following rapid and slow dehydration of axes from the 2001 harvest, respectively. Total loss of the ability to germinate occurred at *c.* 0.3 and 0.7 g g^{-1} dm upon slow desiccation. Tetrazolium staining remained at 100% for both harvests during fast dehydration. Conversely, an abrupt decline occurred on slow drying at *c.* 1.4 and 2.0 g g^{-1} dm for 1999 and 2001 harvests, respectively. Germination capacity and tetrazolium staining decreased sharply after one week of wet storage for both harvests (Figures 2B and D). Loss of ability to germinate was complete after two weeks of hydrated storage.

Electrolyte leakage

Axes of *A. marina* recorded a mean electrolyte conductivity of *c.* 2.9 mS cm^{-1} g^{-1} dm after 12 h of leakage. Subsequently, there was gradual increase in electrolyte leakage with drying and wet storage (Figures 2E and F). The highest conductivity readings for axes dried rapidly and slowly were *c.* 4.5 and *c.* 15.9 mS cm^{-1} g^{-1} dm for the 1999 and 2001 harvests, respectively. Axes dried slowly recorded more leakage than those dried rapidly at a corresponding water content.

Respiratory metabolism

Phosphofructukinase (PFK)

A significant *c.* 70% decrease in the activity of PFK in whole axes accompanied the 40% decline in germination capacity during fast drying (Figures 3A and Table 1). Similarly, a significant *c.* 80% reduction in PFK activity in axes was associated with germination loss upon slow dehydration. There was no significant change in the activity of PFK in axes before the onset of germination loss during wet (Figure 3B).

The activity of PFK in hypocotyls was significantly diminished by *c.* 83% during germination loss upon rapid desiccation (Figure 3A). There were no significant changes in the PFK activity in hypocotyls during slow drying and hydrated storage. There were no significant changes in the activities of PFK in root primordial and plumules during fast and slow drying and moist storage.

Malate dehydrogenase (MDH)

A significant *c.* 40% decrease in the activity of MDH in axes occurred during loss of germination following slow drying (Figure 3C). Similarly a significant *c.* 85% decline preceded the onset of germination loss during wet storage (Figure 3D). MDH activity in hypocotyls, root primordia and plumules did not change significantly upon dehydration and hydrated storage.

Nicotinamide adenine dinucleotide (NAD)

No significant changes were observed in the levels of NAD in axes during fast drying and wet storage (Figures 3E and F). However, a significant *c.* 80% decrease in NAD level occurred following slow dehydration (F = 427.40 and p = 0.04).

There were no significant changes in the NAD level in hypocotyls upon desiccation and hydrated storage. A significant *c.* 90% decline in the level of NAD in root primordia accompanied germination loss during slow drying. The onset of germination loss was preceded by a *c.* 80% reduction in NAD level upon hydrated storage. No significant changes occurred in the level of NAD in plumules following fast dehydration and moist storage. Nonetheless, germination loss was associated with a *c.* 90% decrease in NAD level during slow desiccation.

Hydroperoxide levels

No significant changes in the levels of hydroperoxides in axes, root primordia and plumules were associated with drying and wet storage (Figures 3G and H). However, the hydroperoxide levels in hypocotyls increased significantly by *c.* 440% and 145% during fast and slow dehydration.

Antioxidant levels

Superoxide dismutase (SOD)

Significant *c.* 540 and 200% increases in the activity of SOD in axes occurred during fast drying and wet storage (Figures 4A and B). No significant changes in SOD activity in hypocotyls, root primordia and plumules were observed following dehydration and hydrated storage.

Catalase (CAT)

The activities of CAT did not significantly change in axes, hypocotyls and plumules during drying (Figures 4C and D). In contrast, a significant *c.* 360% in CAT activity in root primordia preceded the onset of germination loss following wet storage.

Glutathione reductase (GR)

The activity of GR in axes significantly decreased by *c.* 90% upon desiccation (Figure 4E). Similarly, the onset of the loss of germination was preceded by a *c.* 95% decline in GR activity during wet storage. Complete loss of germination was associated with a significant *c.* 40% reduction in the activity of GR in hypocotyls following slow dehydration. No significant changes in GR activity occurred in root primordia during drying and hydrated storage. A significant *c.* 90% decrease in the activity of GR in plumules accompanied loss of germination upon slow desiccation.
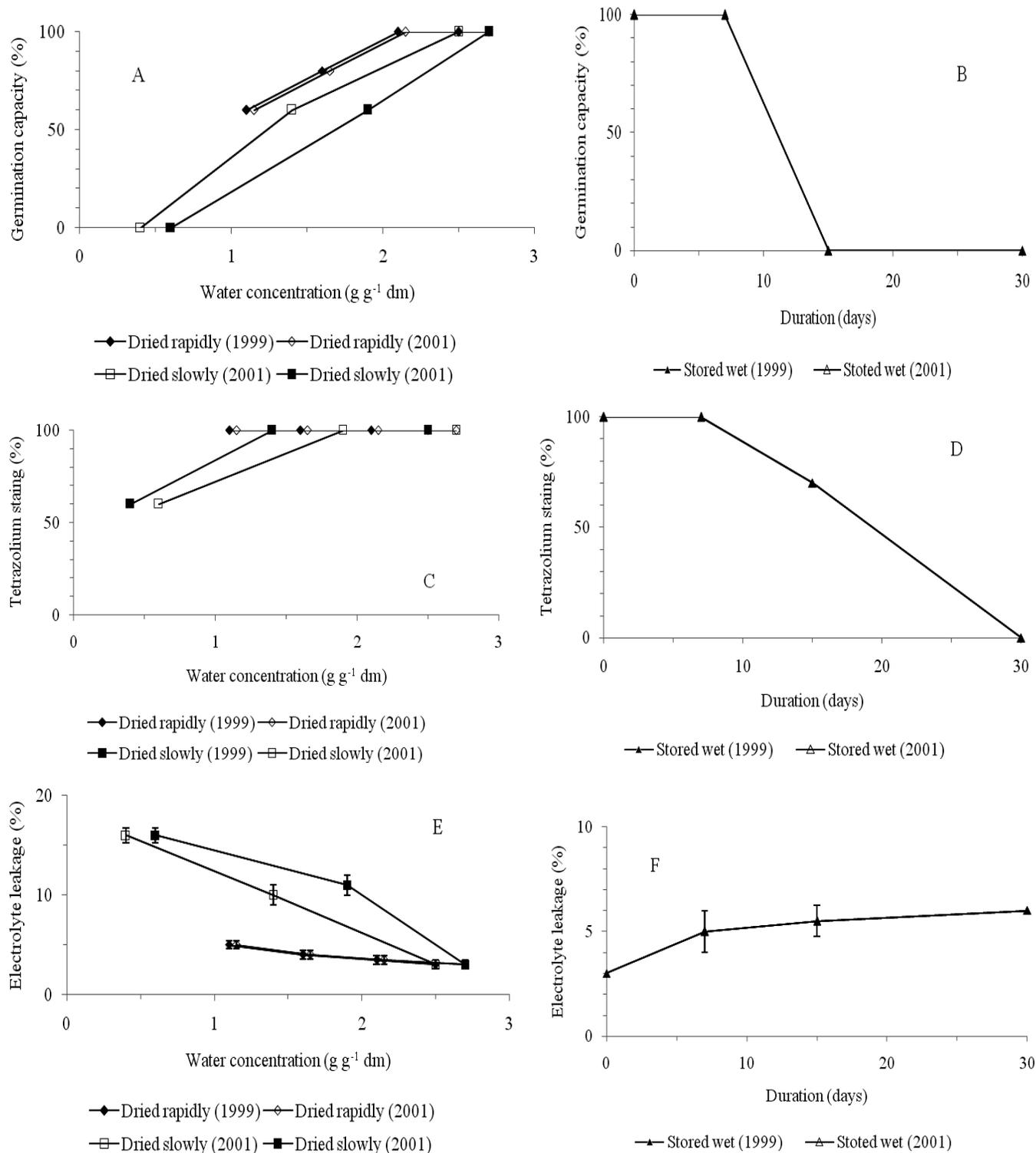
Figure 2. Germination capacity, tetrazolium staining and electrolyte leakage of *A. marina* axes following dehydration (A, C and E) or wet storage (B, D and F) in 1999 (open symbols) and 2001 (closed symbols). (Error bars represent means ± SE, n = 10, some smaller than symbols).

Ascorbic acid (AsA)

A significant *c.* 80% decrease in the levels of AsA in axes

occurred during loss of germination during slow drying (Figure 4G). No significant changes were observed in the levels of AsA in hypocotyls, root primordia and plumules
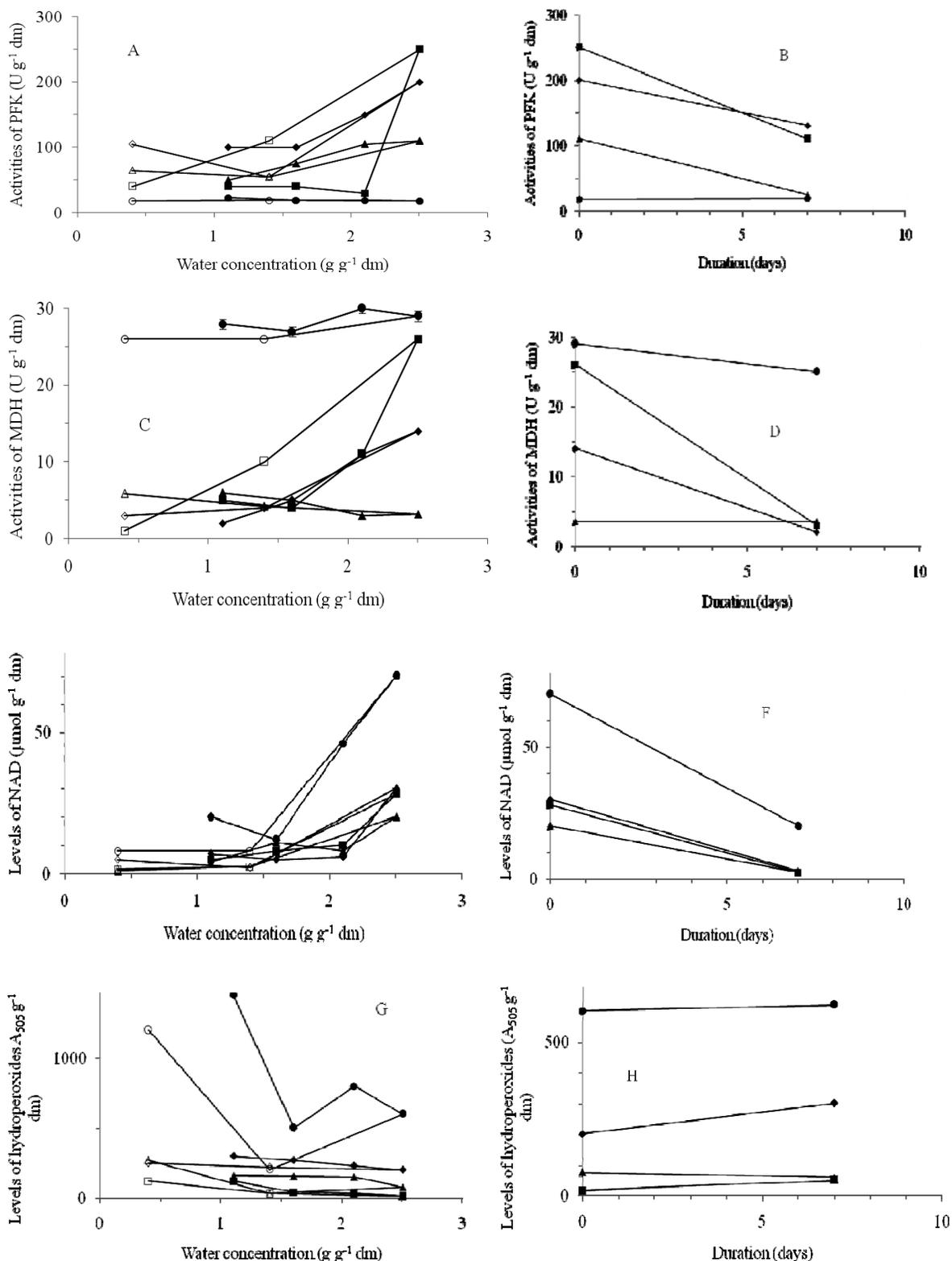
Figure 3. Activities of phosphofructokinase and malate dehydraogenase and levels of nicotinamide adenine dinucleotide and hydroperoxides in whole axes (diamonds), hypocotyls (squares), root primordia (triangles) and hypocotyls (circles) of *A. marina* during rapid (closed symbols) and slow (open symbols) drying (A, C, E and G) or hydrated storage (B, D, F and H). (1 U of PFK will convert 1 μmol of fructose-6-phosphate and ATP to fructose-1,6-bisphosphate and ADP per minute at pH 8.0 at 30 °C and 1 U of MDH will convert 1 μmol of oxaloacetate and NADH to malate and NAD per minute at pH 7.5 at 25°C).

Table 1. Statistical analysis of results.

Test	F -value	p-value
Phosphofructokinase		
1	F = 1.585.70	p = 0.02
2	F = 113.32	p = 0.01
Malate dehydrogenase		
1	F = 288.00	p < 0.01
2	F = 288.00	p < 0.01
Nicotinamide adenine dinucleotide 1	F = 791.50	p < 0.01
Hydroperoxide levels		
1	F = 80.50	p < 0.01
2	F = 120.20	p < 0.01
Superoxide dismutase		
1	F = 1 789.80	P = 0.02
2	F = 288.00	p = 002
Catalase 1	F = 37.00	p = 0.03
Glutathione reductase 1	F = 33.74	p < 0.01
2	F = 61.20	p < 0.01
3	F = 63.10	p = 0.02
4	F = 27.10	p = 0.01
5	F = 25.70	p = 0.01
Ascorbic acid 1	F = 29.00	p = 0.03

during drying and wet storage (Figures 4G and H).

DISCUSSION

The water content of axes of *A. marina* remained constant during wet storage as the surrounding air is fully hydrated and there is no direct contact between the axes and the water. Hence, water loss from the seed to the surrounding air and water uptake by the axes are in equilibrium. In agreement with studies on other species (Berjak et al., 1984; Farrant et al., 1985; Pritchard, 1991; Ntuli et al., 2011), germination and tetrazolium tests revealed that rapid drying of axes of white mangrove seeds markedly decreased the water content at which abrupt loss of viability occurred in comparison with slow dehydration.

Loss of viability occurred at high (0.8 g g^{-1} dm) water contents during desiccation of axes of *A. marina*. This observation indicates that physical damage underlay loss of viability during desiccation in *A. marina* axes. In wet storage, survival began to decline after a week suggesting that ageing may have contributed to viability loss du-

during slow drying.

The relationship between electrolyte leakage and water concentration during drying did not show the typical pattern in which there is constant leakage to a critical water content, at which point a sudden increase is observed. Rather, there was a gradual increase in the degree of leakage as dehydration proceeded and Ntuli et al. (2011) showed a similar pattern during drying of whole seeds of *Ekebergia capensis* and excised axes of *Quercus robur*, respectively. Nevertheless, less electrolyte leakage was observed during rapid than slow dehydration. It is suggested that less membrane damage occurred during rapid desiccation because of the limited period of exposure to stress compared with slow drying.

The activities of PFK and MDH decreased upon drying and wet storage of axes, hypocotyls, root primordia and plumules of *A. marina*. This observation is in agreement with previous findings where PFK and MDH activities were reported to be adversely affected by desiccation (Leprince et al., 1993a; Côme and Corbineau, 1996; Song et al., 2009; Ntuli et al., 2011).

The levels of NAD also declined during dehydration and hydrated storage in all four tissues. This reduction is
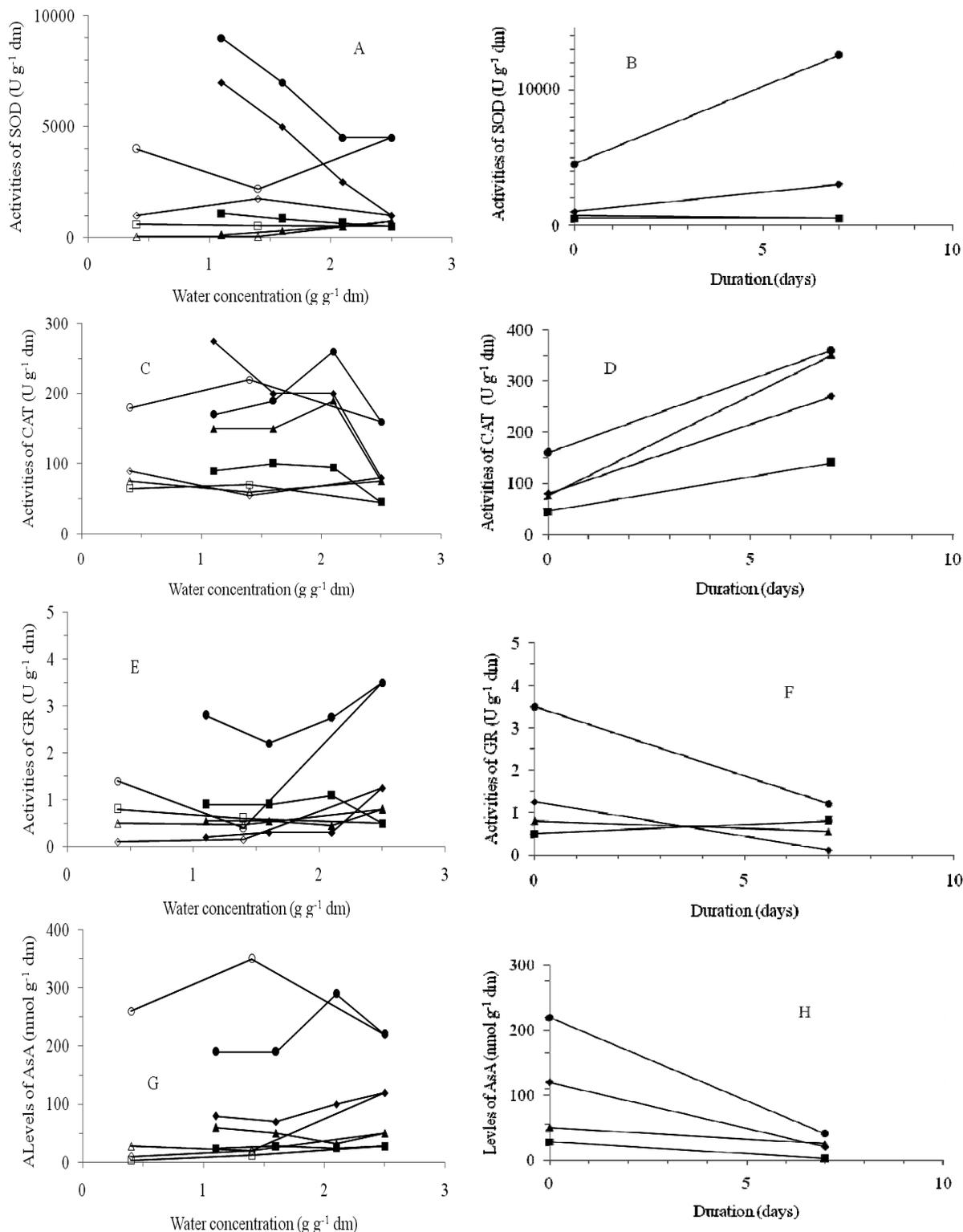
Figure 4. Activities of superoxide dismutase, catalase and glutathione reductase and levels of ascorbate in whole axes (diamonds), hypocotyls (squares), root primordia (triangles) and hypocotyls (circles) of *A. marina* during rapid (closed symbols) and slow (open symbols) desiccation (A, C, E and G) or moist storage (B, D, F and H). (1 U of SOD will inhibit the rate of reduction of cytochrome *c* by 50% in a coupled system with xanthine and xanthine oxidase at pH 7.8 at 25 °C, 1 U of CAT will decompose 1 μmol of hydrogen peroxide (H_2O_2) per minute at pH 7.0 at 25 °C, while the concentration of H_2O_2 falls from 10.3 to 9.2 mM and 1 U of GR will reduce 1 μmol of oxidized glutathione per minute at pH 7.6 at 25 °C).

attributed to the impairment of mitochondrial electron transport chain at the NADH dehydrogenases of NADH-coenzyme Q reductase (complex I) and NADH-cytochrome *c* reductase (complex IV) and the alternative oxidase (Leprince et al., 1993a; 1994; 1995; 2000; Côme and Corbineau, 1996; Leprince and Hoekstra, 1998; Song et al., 2009).

There was an increase in hydroperoxide levels during desiccation and moist storage in all tissues. This event is in concurrence with those in previous studies (for example, Leprince et al., 1990; Song et al., 2004; Huang et al., 2009; Ntuli et al., 2011).

It appears that the patterns of physiological and biochemical response of the free radical processing systems in desiccation-sensitive seeds to oxidative stress differ among both tissues and species, as in germinating orthodox seeds. For instance, the defence against oxidative attack on axial tissue of *Quercus robur* was largely dependent on antioxidants whereas it was predominantly enzymic in cotyledons (Hendry et al., 1992). Moreover, the activities of SOD and GR in axes decreased during desiccation (Hendry et al., 1992). In contrast, SOD and GR activity increased in the cotyledons upon drying. Furthermore, there was a decrease in the levels of α-tocopherol in axes during dehydration compared with an increase in the cotyledons (Hendry et al., 1992). However, the activity of SOD increased significantly during drying in *Shorea robusta* seeds (Chaitanya and Naithani, 1994) but there was a rapid decrease in activities of SOD and peroxidases in *Theobroma cacoa* axes corresponding to loss of viability (Li and Sun, 1999). Additionally, an increase in both the amount of tocopherol and activity of SOD was observed in the plumule of axes of *Avicennia marina* following dehydration (Greggains et al., 2001).

The activities of SOD and CAT increased during desiccation and wet storage of *A. marina* axes, hypocotyls, root primordia and plumules in the present study. In contrast, the GR activities and levels of AsA decreased upon drying and hydrated storage.

In summary, respiratory enzymes, PFK, MDH and the NADH dehydrogenases of the electron transport chain of axes of *A. marina* showed sensitivity to desiccation and wet storage. These data are consistent with the view that these events led to metabolic imbalance which, in turn, resulted in more leakage of electrons than normal from the mitochondrial electron transport chain. The increased free radical activity caused the enhanced formation of hydroperoxides during lipid peroxidation. In addition, desiccation and wet storage impaired the efficiency of the free radical processing systems. This situation is less prevalent during rapid drying than slow dehydration and hydrated storage.

The results of the present study support the hypothesis that rapid desiccation lowers the critical and lethal water concentrations for the survival of desiccation-sensitive seeds. Furthermore, they show that such a phenomenon arises as a consequence of lesser adverse effects upon

metabolism and free radical processing systems when drying is more rapid. However, viability loss in axes of *A. marina* is underlain by physical rather than metabolic damage as indicated by the high water contents at which it occurred.

In conclusion, the activities and levels of all the enzymes and compounds studied, with the exception of PFK, were the highest in the plumules. However, the responses were remarkably qualitatively similar in all tissues. It is possible that the plumule, despite its relatively small volume and mass, plays a disproportionately important role in the loss of viability in axes of *A. marina*.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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