Increasing the rate of drying reduces metabolic imbalance, lipid peroxidation and critical water content in radicles of garden pea (*Pisum sativum L.*)

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ABSTRACT

Orthodox seeds become desiccation-sensitive as they undergo germination. As a result, germinating seeds serve as a model to study desiccation sensitivity in plant tissues. The effects of the rate of drying on the viability, respiratory metabolism and free radical processes were thus studied during dehydration and wet storage of radicles of *Pisum sativum*. For both drying regimes desiccation could be described by exponential and inverse modified functions. Viability, as assessed by germination capacity and tetrazolium staining, remained at 100% during rapid (< 24 h) desiccation. However, it declined sharply at *c.* 0.26 g g^{-1} dm following slow (*c.* 5 days) drying. Increasing the rate of dehydration thus lowered the critical water content for survival. Rapid desiccation was also associated with higher activities and levels of malate dehydrogenase and the oxidized form of nicotinamide adenine dinucleotide. It was also accompanied by lower hydroperoxide levels and membrane damage. In addition, the activitiy of glutathione reductase was greater during rapid drying. Ageing may have contributed to increased damage during slow dehydration, since viability declined even in wet storage after two weeks. The results presented are consistent with rapid desiccation reducing the accumulation of damage resulting from desiccation-induced aqueous-based deleterious reactions. In addition, they show that radicles are a useful model to study desiccation sensitivity in plant tissues.

Keywords: Antioxidant, desiccation, drying rate, free radical processes, lipid peroxidation, metabolism, respiration, *Pisum sativum*, seed storage.

Abbreviations: AsA, ascorbic acid; CAT, catalase; GR, glutathione reductase; MDH, malate dehydrogenase; NAD, nicotinamide adenine dinucleotide; PFK, phosphofructokinase; SOD, superoxide dismutase; TZ, tetrazolium.

INTRODUCTION

Recalcitrant seeds and vegetative tissues are desiccation-sensitive (reviewed by Berjak, 2006). As a result, recalcitrant seeds are unstorable by conventional methods for orthodox seeds (reviewed by Berjak and Pammenter, 1997a, b; 2001; 2004; 2008). In addition, they may not be produced in particular seasons. Thus conservation of their genetic resources and accessibility for study is presently limited. Radicles become desiccation-sensitive as they undergo germination. Consequently, they provide an always-accessible model to study desiccation-sensitivity in seeds. In addition, they eliminate the introduction of extraneous factors by vegetative tissues.

In addition to physical stresses that desiccation-sensitive seed tissues incur as a consequence of dehydration, they are subject 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; reviewed by 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; Walters *et al.*, 2002; Ntuli *et al.*, 2011). Of 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 the 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* radicles and orthodox *Zea mays* 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 generating the accumulation 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* radicles and orthodox *Zea mays* embryos during dehydration.

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). Roach *et*

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al. (2010) demonstrated extracellular superoxide production in response to desiccation in recalcitrant *Castanea sativa* seeds.

A number of processes associated with seed desiccation tolerance have been identified. They include, *inter alia*, accumulation of soluble sugars, the synthesis of late embryogenesis abundant (LEA) proteins and 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, 1997a; b; 2001; 2004; 2008; Pammenter and Berjak, 1999; Buitink *et al.*, 2002; Berjak, 2006). For instance, Hendry *et al.* (1992) demonstrated that dehydration of desiccation-sensitive embryonic radicles of *Quercus robur* resulted in decreases in the activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APOD) and the level of α-tocopherol. In contrast, there was an increase in the level of ascorbate (AsA) during dehydration. However, guaicol peroxidase (GPOD) 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, APOD, CAT, GR, and dehydroscorbate reductase (DHAR) and SOD, APO and CAT in radicles of *Trichilia dregeana* and Chinese wampee during dehydration (Song *et al.*, 2005; Huang *et al.*, 2009, respectively).

Several studies have shown that rapid drying of desiccation-sensitive seeds permits survival to lower water content than slow drying (Berjak *et al.*, 1984; Farrant *et al.*, 1985; 1989; Grabe, 1989; Pritchard, 1991; Pammenter *et al.*, 1991; 1998; 1999; Walters *et al.*, 2001; Wesley-Smith *et al.*, 2001; Huang *et al.*, 2009; Ntuli *et al.*, 2011). It has been proposed that such behavior depends upon rapid drying removing water sufficiently fast to reduce the accumulation of damage resulting from aqueous-based deleterious reactions (Berjak *et al.*, 1989; 1993; Pammenter *et al.*, 1991; 1998; 1999; Pritchard, 1991).

Wet storage may be equated to long-term low-intensity desiccation (e. g. Pammenter *et al.*, 1994). It is not surprising that the deleterious reactions involved during hydrated storage such as metabolic imbalance, lipid peroxidation and disruption of antioxidant systems are similar (e. g. Ntuli *et al.*, 2011).

In the present study, the effects of the rate of drying on viability, respiratory metabolism and free radical processes were monitored during dehydration and wet storage of radicles of *Pisum sativum*.

MATERIALS AND METHODS

Plant material

Seeds of garden pea (*Pisum sativum* L. var Greenfeast) were bought from Pannar, Greytown, South Africa. They were allowed to imbibe water for 6 h, surface sterilised and set out to germinate in germination trays on moist paper towel at room temperature under constant light for 72 h.

Radicles were excised from cotyledons and accumulated on moist filter paper in closed Petri dishes. Only radicles longer than 10 mm were used for subsequent experiments.

Treatments

Radicles 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]), or

(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 content determinations

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

Assessment of drying rates

Drying rates were assessed either as the rate constant (β) of an exponential decline in water content (Liang and Sun, 2000; Sun, 2002), calculated as slopes of log-transformed water contents against time, or as the constant (*b*) of the modified inverse function relating relative water content (RWC) to time (RWC = $b/(b + t)$, where RWC is the water content relative to the initial water content, and t is drying time (Pammenter *et al.*, 2002; 2003), *b* being calculated as the slope of a plot of $1/RWC$ against time.

Viabillity tests

To minimize the effects of imbibing damage, dehydrated radicles were moistened on damp filter paper in Petri dishes overnight at 20 °C before being subjected to the tetrazolium and conductivity tests.

To assess germination capacity, radicles 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. Radicles were scored as germinated when they showed greening and/or elongation and/or expansion.

Apparent radicles 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 radicles was measured using a multi-cell conductivity meter (CM100; Reid and Associates cc, Durban, SA) over 12 h. All measurements were made at 2 V whilst radicles were immersed in 1 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 radicles.

Assays of respiratory enzymes

Phosphofructokinase (PFK) activity was determined according to Leprince *et al.* (1993a). Radicles (*c*. 5 mg dry matter) were homogenized 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 $MgCl_2$, 0.6 mM ATP, 0.33 U ml^{-1} aldolase, 10 U ml^{-1} triose phosphate isomerase, 1 U ml^{-1} glycerophosphate dehydrogenase 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 homogenized 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

Radicles (*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 neutralised 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 radicles (*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) and modified as in Ntuli *et al*. (1997). Following centrifugation at 1500 *g* for 5 min, 1 ml of 0.014 M ferrous chloride ($FeCl_2$) 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 phosphate (pH 7.0), 0.25% (w/v) Triton X-100 and 1% (w/v) polyvinlypolypyrolidone, following homogenisation of radicles (*c*. 5 mg dm) to a fine powder under liquid nitrogen in a 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 H_2O_2 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 homogenization 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 neutralized 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 the reaction started with addition of 1 ml of 5 U ml^{-1} of ascorbate oxidase (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 radicles (*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 therein. 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 Tukey multiple range test was subsequently used to distinguish significantly deviating means.

RESULTS

Desiccation kinetics

Radicles of *P. sativum* seeds were at a water content of *c*. 2.0 g H_2O /g dry matter (g g^{-1} dm) after imbibition (data not shown). Radicles of different lengths showed different drying curves (data not shown). Radicles reached a water content of *c*. 4.5 g g^{-1} dm following germination subsequent to imbibition (data not shown). The rate of water loss could be described by exponential and modified inverse functions for both drying regimes.

Radicles reached a water content of c. 0.19 g g^{-1} dm in 24 h (β [rate constant of water loss/drying rate {Liang and Sun, 2000; Sun, 2002}] = 1.4, r^2 = 0.75 and p = 0.01; b = 1.05 h, r^2 = 0.99 and p < 0.01) following rapid drying (Fig. 1). In contrast, slow dehydration achieved a final water content of c. 0.22 g g^1 dm in 7 days (β = 0.19, r^2 = 0.58 and p = 0.24; b = 8.25 h, r^2 = 0.83 and p = 0.09). Thus, rapid dehydration facilitated desiccation approximately seven times more rapidly than slow dying. Conversely, the water content of the radicles remained constant at c. 4.5 g g^{-1} dm over four weeks (β = 0, r^2 = 0.09 and p = 0.70) during wet storage (data not shown).

Viability

Viability, as assessed by germination capacity and tetrazolium staining, of fresh (undried) *P. sativum* radicles was 100%. It remained at that level throughout rapid drying (Fig. 2A). However, there was a precipitous decline in viability at c. 0.26 g g^{-1} dm following slow desiccation. Similarly, there was a gradual decrease in viability after two weeks of hydrated storage (Fig. 2B).

Electrolyte leakage

Untreated radicles of *P. sativum* recorded a mean electrolyte leakage of c. 47 mS cm^{-1} g^{-1} dm after 12 h. Subsequently there was a progressive increase in electrolyte leakage with drying and wet storage (Figs 2C and D). The highest conductivity readings for radicles that were dried rapidly and slowly were c. 68.5 and c. 122 mS cm^{-1} g^{-1} dm, respectively. Radicles dried slowly registered more leakage than those dried rapidly at a corresponding water content.

Respiratory metabolism

There was a c. 26% decrease and a 50% increase in the activity of PFK before the onset of viability loss with rapid and slow desiccation (at c. 0.19 and 0.26 g g^{-1} dm), respectively (Fig
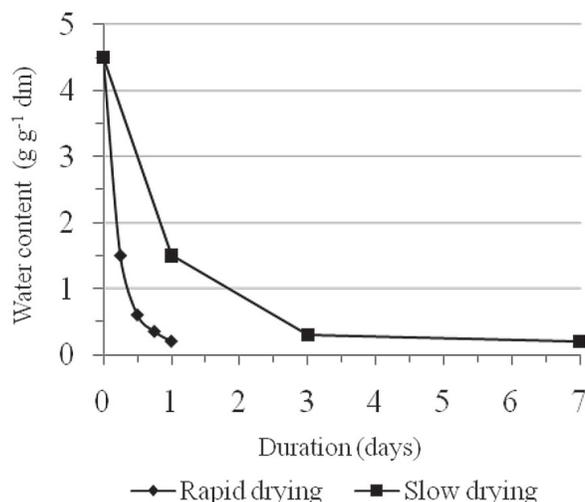

Figure 1. Water contents of embryonic radicles of *Pisum sativum* during slow and rapid desiccation. (Error bars represent means ± SE, n = 5, all smaller than symbols).

3A). During viability loss there was a c. 41% decrease in PFK activity upon slow drying. There was no significant change in the activity of PFK prior to the onset of or during viability loss with hydrated storage (Fig. 3B).

A c. 42% and a c. 75% reduction in the activity of MDH were observed before the onset of viability loss upon rapid and slow dehydration, respectively (Fig. 3C). A c. 54% decline in the activity of MDH preceded the onset of viability loss during wet storage (Fig. 3D). A further c. 21% decrease in MDH activity accompanied loss of viability during wet storage.

The levels of NAD were enhanced by c. 87% before the onset of loss of viability upon rapid dehydration (Fig. 3E). A c. 30% reduction in NAD levels preceded the onset of loss of viability as slow desiccation proceeded. A c. 102% increase in the levels of NAD accompanied loss of viability during slow drying. Loss of viability was preceded by a c. 43% decline in the levels of NAD during wet storage (Fig. 3F). No significant change in the level of NAD accompanied the period of loss of viability during hydrated storage.

Hydroperoxide levels

An initial c. 52% and a subsequent c. 64% decrease in level of hydroperoxides preceded the onset of loss of viability during rapid dehydration (Fig. 3G). No significant change in the level of hydroperoxides accompanied loss of viability during slow desiccation. The onset of loss of viability was preceded by a c. 72% increase in the hydroperoxide levels during wet storage (Fig. 3H). No significant change in the level of hydroperoxides accompanied the period of loss of viability during hydrated storage.

Antioxidant levels

There was an initial c. 46% decrease and a subsequent c. 38% increase in the activity of SOD before the onset of loss of viability during rapid dehydration (Fig. 4A). In contrast, there was a c. 120% stimulation in SOD activity before the onset of viability loss following slow drying. During loss of viability there was a c. 88% decrease in SOD activity with slow dehydration. There was a c. 142% enhancement in the activity of SOD prior to the onset of viability loss during wet storage (Fig. 4B). Viability loss was not accompanied by significant change in SOD activity during hydrated storage. There were no significant changes in CAT activity during drying and wet storage (Fig. 4C and D).

The activity of GR initially increased by c. 93% and was then subsequently stimulated by c. 151% before the onset of viability loss during rapid drying (Fig. 4E). In contrast, GR activity was initially diminished by c. 68% and subsequently increased by c. 113% prior to the onset of viability loss upon slow dehydration. Loss of viability did not produce significant change in GR activity upon slow desiccation. The onset of loss of viability was preceded by a c. 248% stimulation in GR activity during wet storage (Fig. 4F). However, no further significant change in GR activity accompanied the period of viability loss with hydrated storage.

There was an initial c. 51% decrease and a subsequent c. 51% increase in the levels of AsA before the onset of viability loss during rapid drying (Fig. 4G). Similarly, an initial c. 30% decrease and a c. 28% increase in AsA levels preceded the onset of and accompanied loss of viability upon slow dehydration,

respectively. During wet storage, levels of AsA did not change significantly during the experimental period (Fig 4H).

DISCUSSION

Water loss during desiccation of radicles of *P. sativum* can be described by exponential and modified inverse functions (Ntuli and Pammenter, 2009). In this regard, it should be remembered that the difference in water potentials between the cell and the surrounding air, which drives water loss, is exponentially related to the relative humidity of the surrounding air through the water vapour pressure (Wexler, 1997).

In contrast, the water content of radicles of *P. sativum* remained constant during wet storage as the surrounding air is fully hydrated and there is no direct contact between the radicles and the water. Hence, water loss from the seed to the surrounding air and water uptake by the radicles are in equilibrium.

In agreement with studies on other species (Berjak *et al.*, 1984; Farrant *et al.*, 1985; 1989; Grabe, 1989; Pritchard, 1991; Pammenter *et al.*, 1998; Ntuli *et al.*, 2011), germination and tetrazolium tests revealed that rapid dehydration of radicles of hydrated pea seeds markedly decreased the water content at which abrupt viability loss occurred in comparison with slow desiccation.

Loss of viability occurred at low ($\leq$ 0.5 g g^{-1} dm) water contents during slow dehydration of radicles of *P. sativum*. This observation indicates that desiccation damage (*sensu stricto*) underlies loss of viability during slow desiccation in radicles of *P. sativum*. In wet storage, viability also began to decline after two weeks, suggesting that ageing may have also contributed to viability loss during slow drying.

In the present study, the relationship between electrolyte leakage and water content 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 (e. g. Pammenter *et al.*, 1991). Rather, there was a gradual increase in the degree of leakage as drying proceeded. Pammenter *et al.* (1998) and Ntuli *et al.* (2011) showed a similar pattern during drying of whole seeds of *Ekebergia capensis* and excised radicles of *Quercus robur*. Nevertheless, less electrolyte leakage was observed during rapid than slow drying. It is suggested that less membrane damage occurred during rapid dehydration because of the limited period of exposure to stress compared with slow desiccation.

The activities of PFK and MDH were reported to be adversely affected by desiccation (Leprince *et al.*, 1993a; reviewed by Côme and Corbineau, 1996; Song *et al.*, 2009; Ntuli *et al.*, 2011). PFK activity decreased upon rapid drying but was stimulated during slow dehydration. This apparent anomaly is attributed to the minimally desiccation-sensitive
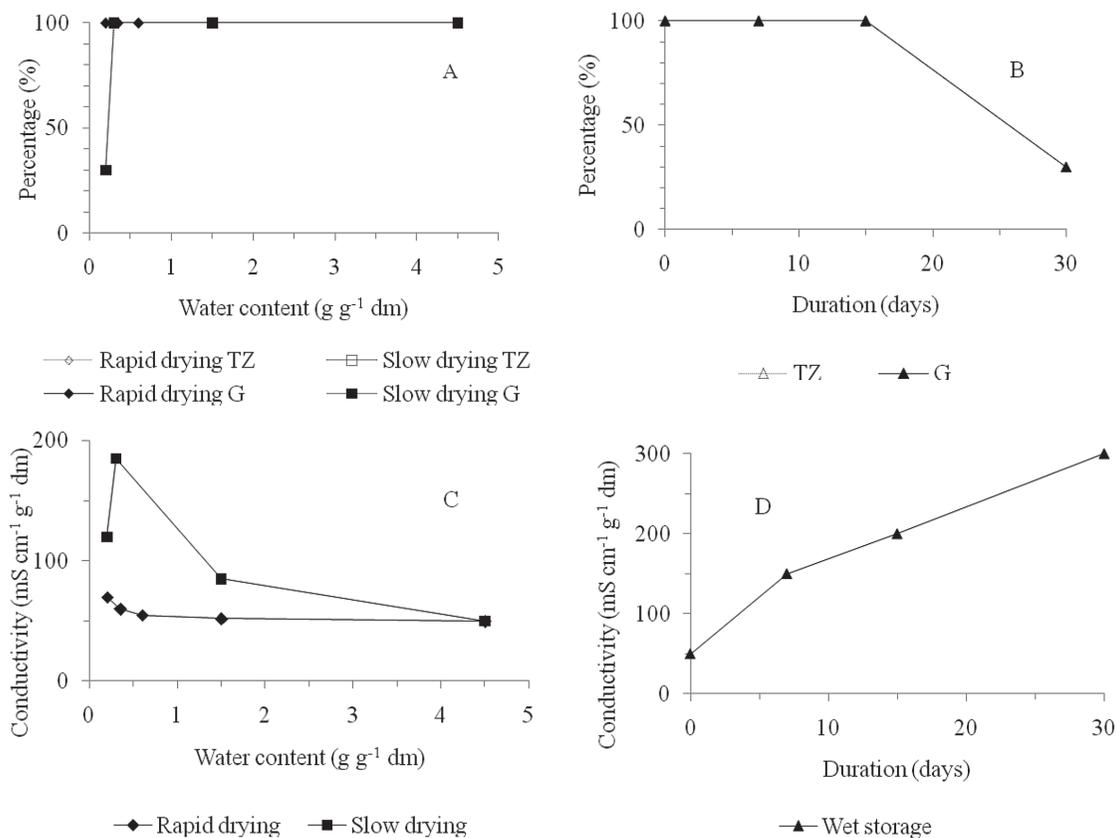

Figure 2. Viability, as assessed by tetrazolium staining (open symbols and dotted lines), germination capacity (closed symbols and solid lines) and maximum electrolyte leakage of *P. sativum* radicles following slow and rapid dehydration (A and C) or hydrated storage (B and D). (Error bars represent means ± SE, n = 10, all smaller than symbols). The TZ test gave exactly the same results as germination assay so the TZ data are not visible on these figs.
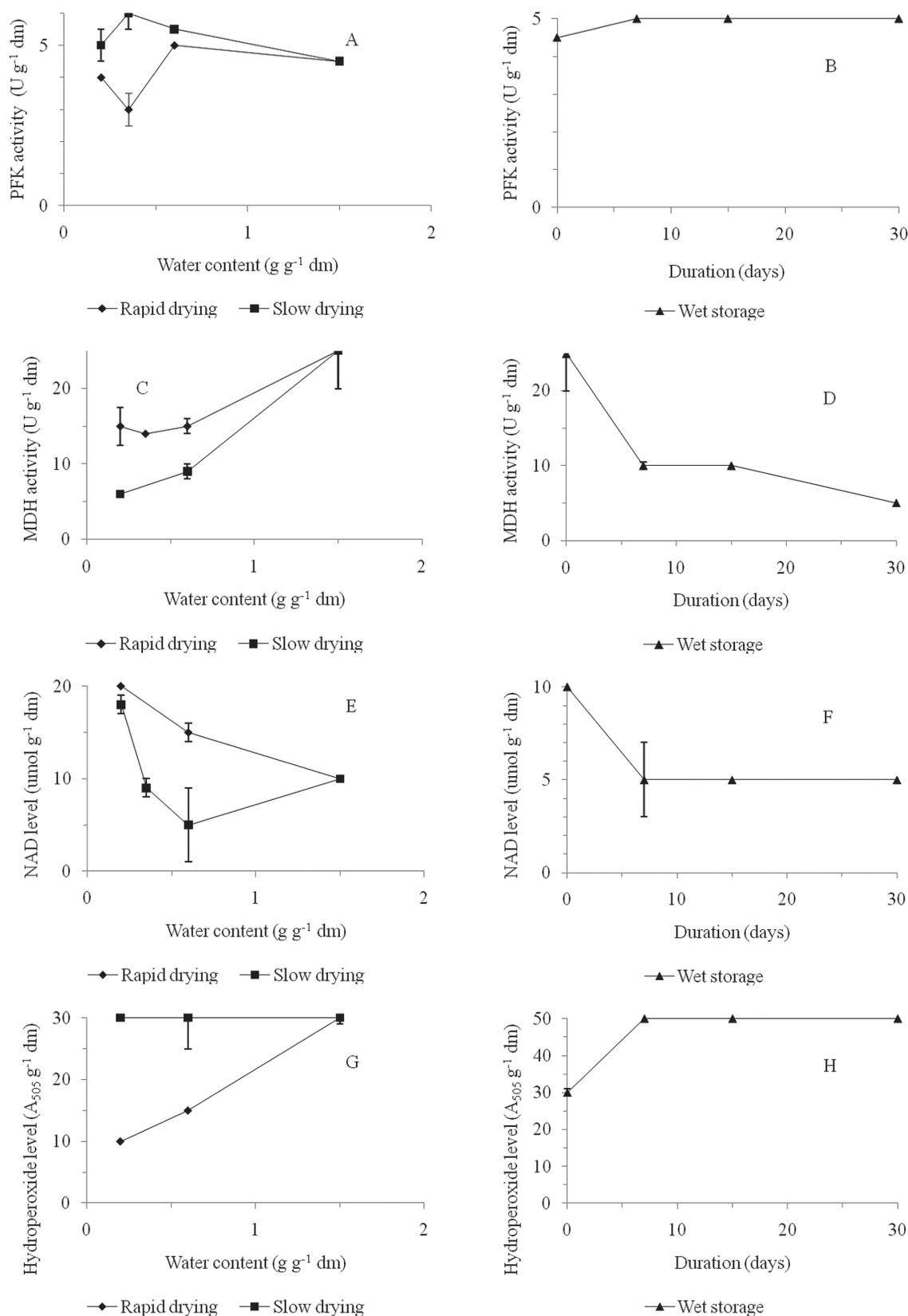
Figure 3. Activities of phosphofructokinase and malate dehydraogenase and levels of NAD and hydroperoxides in radicles of *P. sativum* during dehydration at different rates (A, C, E and G) or wet storage (B, D, F and H). (1 U of PFK will convert 1 μmol of fructose-6-phosphate and ATP to fructose-1,6-bisphosphate 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. Error bars represent means ± SE, n = 3, some smaller than symbols).
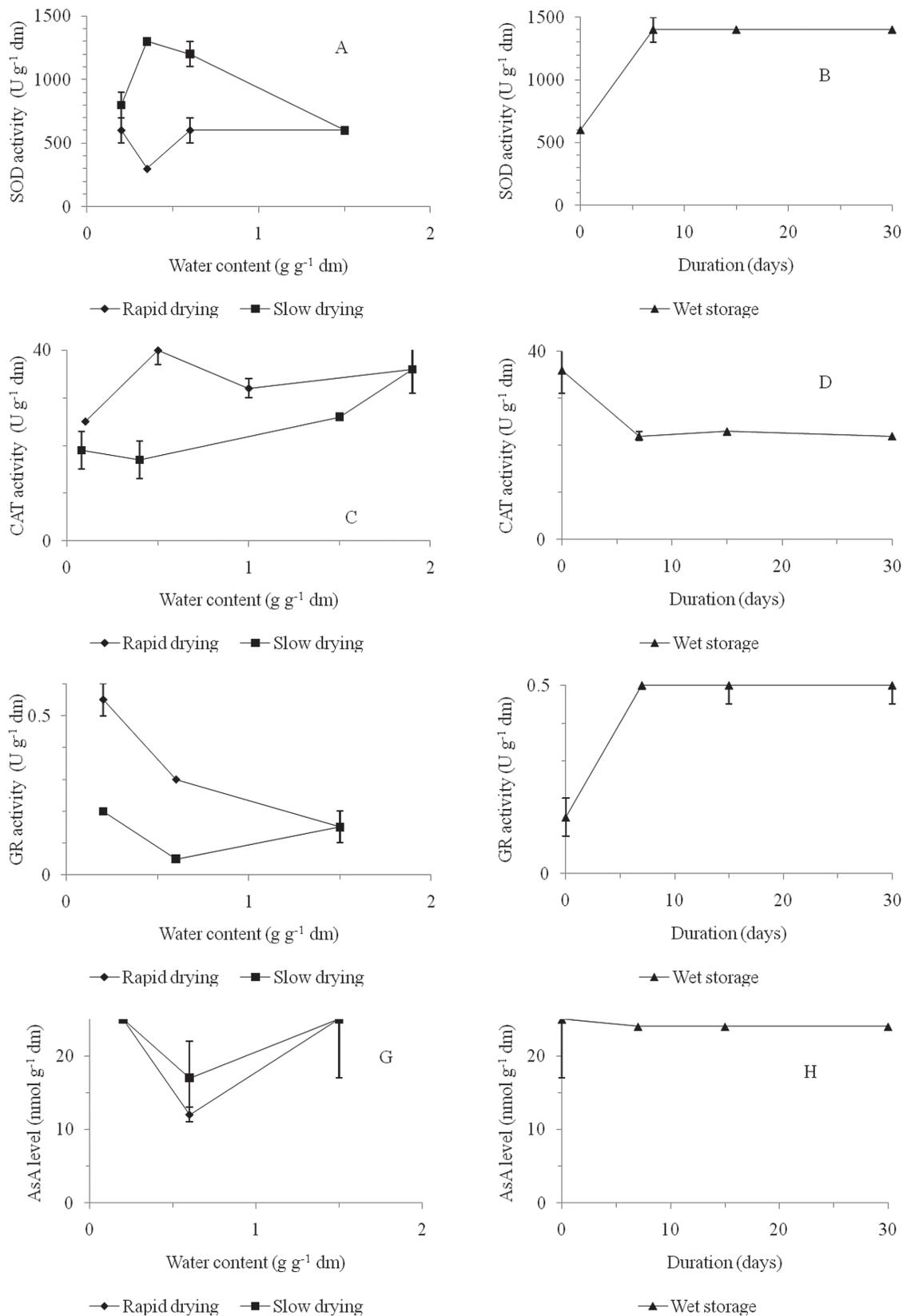
Figure 4. Activities of superoxide dismutase, catalase and glutathione reductase and levels of ascorbate in radicles of *P. sativum* during desiccation at different rates (A) or wet storage (B). (1 U of SOD will inhibit the rate of reduction of cytochrome *c* by 50% in a coupled system with xanthine 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. Error bars represent means ± SE, n = 3, some smaller than symbols).

nature of radicles which have just begun germination, which allows the accumulation of the enzyme with passage of time and the relative tolerance of the enzyme to water stress. During wet storage there was no significant change in the activity of PFK. Expectedly, a decline in MDH activity occurred during desiccation and wet storage.

Following drying of germinating maize seeds, impairment of mitochondrial electron transport chain was marked in the NADH dehydrogenase 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; reviewed by Côme and Corbineau, 1996; Leprince and Hoekstra, 1998; Song *et al.*, 2009). In the present investigation, the level of NAD was surprisingly enhanced during drying. In contrast, it declined upon hydrated storage. The increase in NAD levels during dehydration is attributed to the minimally desiccation-sensitive nature of pea seeds which have just entered germination. It is also possible that the increase in NAD levels during the period of viability loss upon slow drying is a result of spontaneous NADH oxidation in dead tissue. It is suggested that the decrease in NAD levels during wet storage is a result of the impairment of the NADH dehydrogenases of the electron transport chain complexes I and IV as shown in the aforementioned studies.

An increase in the level of hydroperoxides was recorded in germinating seeds of maize and excised radicles of forest mahogany, Chinese wampee and English oak during desiccation (Leprince *et al.*, 1990 and Song *et* al., 2005; Huang *et al.*, 2009; Ntuli *et al.*, 2011, respectively). Surprisingly, there was a decrease in hydroperoxide levels during rapid dehydration in the present study. However, slow drying and hydrated storage resulted in no significant change and an increase in levels of hydroperoxides respectively.

Like germinating orthodox seeds, 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. For instance, the defense against oxidative attack on axial tissue of *Quercus robur* was largely dependent on antioxidants whereas it was predominantly enzymatic in cotyledons (Hendry *et al.*, 1992). Moreover, the activities of SOD and GR in radicles 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 radicles during dehydration compared to 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* radicles 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 radicles of *Avicennia marina* following dehydration (Greggains *et al.*, 2001).

The activity of SOD did not change significantly during rapid desiccation in the present study. In contrast, it increased upon slow drying and hydrated storage. Similarly, CAT activity did not change significantly during dehydration and wet storage. The activity of GR increased upon desiccation and hydrated storage. No significant changes in the levels of AsA accompanied viability loss during drying and wet storage. It is suggested that the increase in GR activity during rapid drying led to the decrease in hydroperoxide levels.

Nonetheless the increases in the activities of SOD and GR during slow drying or wet storage could not bring down the levels of hydroperoxides, which stayed constant or increased, respectively.

In summary, the respiratory enzyme MDH of radicles of *P. sativum* showed sensitivity to desiccation and wet storage. Dehydration surprisingly enhanced the activities of PFK and the NADH dehydrogenases of the electron transport chain complexes I and IV. 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, hence there were lower critical water contents with rapid desiccation.

In conclusion, the results of the present study support the hypothesis that rapid dehydration lowers the critical water content 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. In addition, they demonstrate that radicles are a readily-available useful model to study desiccation-sensitivity in plants, especially seed tissues. However, caution is advised in transference of observations based on model systems – germinating and detached radicles – and other systems – recalcitrant seed and vegetative tissues and attached radicles

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