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Response of Chinese Wampee Axes and Maize Embryos to Dehydration at Different Rates

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Abstract

Survival of wampee (*Clausena lansium* Skeels) axes and maize (*Zea mays* L.) embryos decreased with rapid and slow dehydration. Damage of wampee axes by rapid dehydration was much less than by slow dehydration, and that was contrary to maize embryos. The malondialdehyde contents of wampee axes and maize embryos rapidly increased with dehydration, those of wampee axes were lower during rapid dehydration than during slow dehydration, and those of maize embryos were higher during rapid dehydration than during slow dehydration. Activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) of wampee axes markedly increased during the early phase of dehydration, and then rapidly decreased, and those of rapidly dehydrated axes were higher than those of slow dehydrated axes when they were dehydrated to low water contents. Activities of SOD and APX of maize embryos notable decreased with dehydration. There were higher SOD activities and lower APX activities of slowly dehydrated maize embryos compared with rapidly dehydrated maize embryos. CAT activities of maize embryos markedly increased during the early phase of dehydration, and then decreased, and those of slowly dehydrated embryos were higher than those of rapidly dehydrated embryos during the late phase of dehydration.

Key words: *Clausena lansium* axis; desiccation-sensitivity; desiccation-tolerance; malondialdehyde; orthodox seed; reactive oxygen species scavenging enzyme; recalcitrant seed; *Zea mays* embryo.

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Recalcitrant seeds undergo no maturation drying, have high water content when they are shed, and remain metabolically active throughout development. Such seeds are highly susceptible to desiccation, and for most of these seeds, to chilling injury (Pammenter and Berjak 1999; Song et al. 2003). Sufficient evidence has now accumulated to show that the response to dehydration depends not only on the inherent characteristics of the species, but also on the developmental status of the seeds and the conditions under which they are dried, particularly the rate of dehydration (Pammenter and Berjak 1999). It has been found that the more rapidly dehydration can be achieved, the lower the water content to which the seeds or axes can be dried before viability is lost. This is particularly marked when excised axes are dried (Pammenter et al. 1991, 2000; Berjak et al. 1993). However, there is a contrary report about the effect of drying rate on survival of axes excised from recalcitrant *Quercus robur* seeds (Finch-Savage 1992).

It has been suggested that a number of processes or mechanisms confer, or contribute to, desiccation tolerance of seeds (Pammenter and Berjak 1999; Berjak and Pammenter 2004). Among these mechanisms, the presence and efficient operation of antioxidant systems is considered as one of the major factors associated with desiccation tolerance of orthodox recalcitrant seeds (Pammenter and Berjak 1999; Berjak and Pammenter 2004). Recalcitrant seeds (or embryonic axes) also appear to possess antioxidant mechanisms (Hendry et al. 1992; Finch-Savage et al. 1994). However, these protective mechanisms might become impaired under water stress (Smith and Berjak

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1995; Walters et al. 2002) and most certainly are ineffective in protecting recalcitrant seeds against desiccation damage.

Chinese wampee is a well known subtropical fruit, the seeds of which are sensitive to desiccation and chilling, and so are typically recalcitrant (Song and Fu 1997). It has been shown that desiccation sensitivity of Chinese wampee seeds is associated with lipid peroxidation (Song and Fu 1997). What are the responses of Chinese wampee axes to dehydration at different rates? Are such responses implicated in reactive oxygen species (ROS) scavenging enzymes? And are there some effects of dehydration at different rates on desiccation tolerance of orthodox embryos? Recalcitrant Chinese wampee axes and orthodox maize embryos were used as materials and the effects of dehydration at different rates on desiccation tolerance, changes in contents of malondialdehyde (MDA, thiobarbituric acid-reactive products) and in activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), were studied.

Results

Changes in water content and survival

The water content of fresh Chinese wampee axes at the phase of physiological maturation was 0.82 g/g. The decrease in water content of Chinese wampee axes caused by rapid dehydration (P value $\leq$ 0.001) was much faster than by slow dehydration (P value $\leq$ 0.001); the time at which their water content decreased by 50% by rapid and slow dehydration was 0.3 h and 40 h, respectively (Figure 1A). The changes in water contents by slow dehydration did not occur after the water content of Chinese wampee axes were dehydrated to 0.24 from 0.82 g/g over a saturated solution of sodium chloride (Figure 1A).

Survival of Chinese wampee axes decreased with both rapid and slow dehydration (Figure 1B) (P value $\leq$ 0.001). Damage to rapidly dehydrated axes, as measured by axis survival and dry weight of seedlings produced by surviving axes, occurred at lower water contents than that of slowly dehydrated axes (Figure 1B,C). For example, survival of rapidly dehydrated Chinese wampee axes remained at 100% during dehydration from 0.82 to 0.13 g/g and that of slowly dehydrated axes was only 52.4% during dehydration from 0.82 g/g to 0.24 g/g. The W_{50}s (the water content at which 50% of axes had been killed by dehydration) of axes caused by rapid and slow dehydration were 0.09 and 0.24 g/g, respectively (Figure 1B).

The water content of freshly collected immature maize embryos was 2.53 g/g, and rapid dehydration decreased this by 50% in about 0.5 h (P value $\leq$ 0.001) (Figure 2A), whereas the water content of those dehydrated by slow dehydration decreased by 50% in about 13 h (P value $\leq$ 0.001) (Figure 2A). When dehydrated slowly, water loss from maize embryos was still rapid until 24 h, after which water loss slowed.

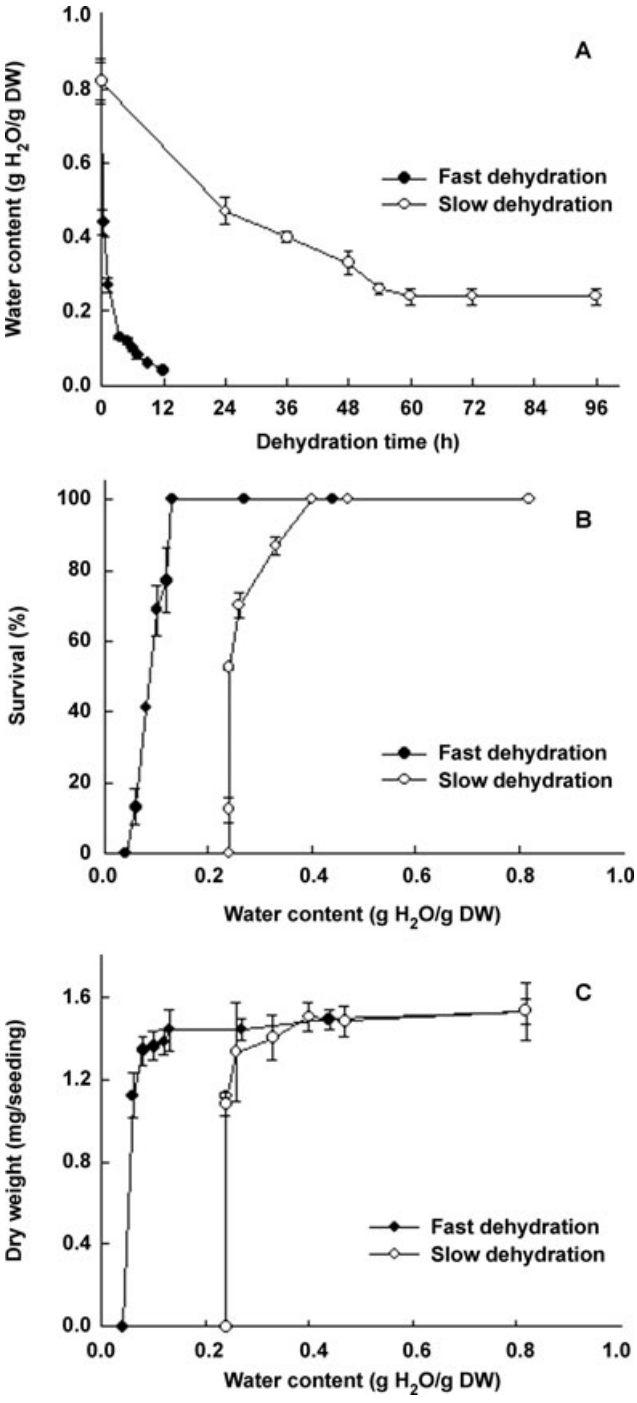

Figure 1. Changes in water content **(A)**, survival **(B)** and dry weight of seedlings produced by surviving axes **(C)** during rapid and slow dehydration of Chinese wampee axes.

After excising from seeds, axes were dehydrated for an indicated time and to an indicated water content at 15°C, and then germinated at 30°C for 6 d. All values are means ± *SD* of three replicates of 20 axes each.

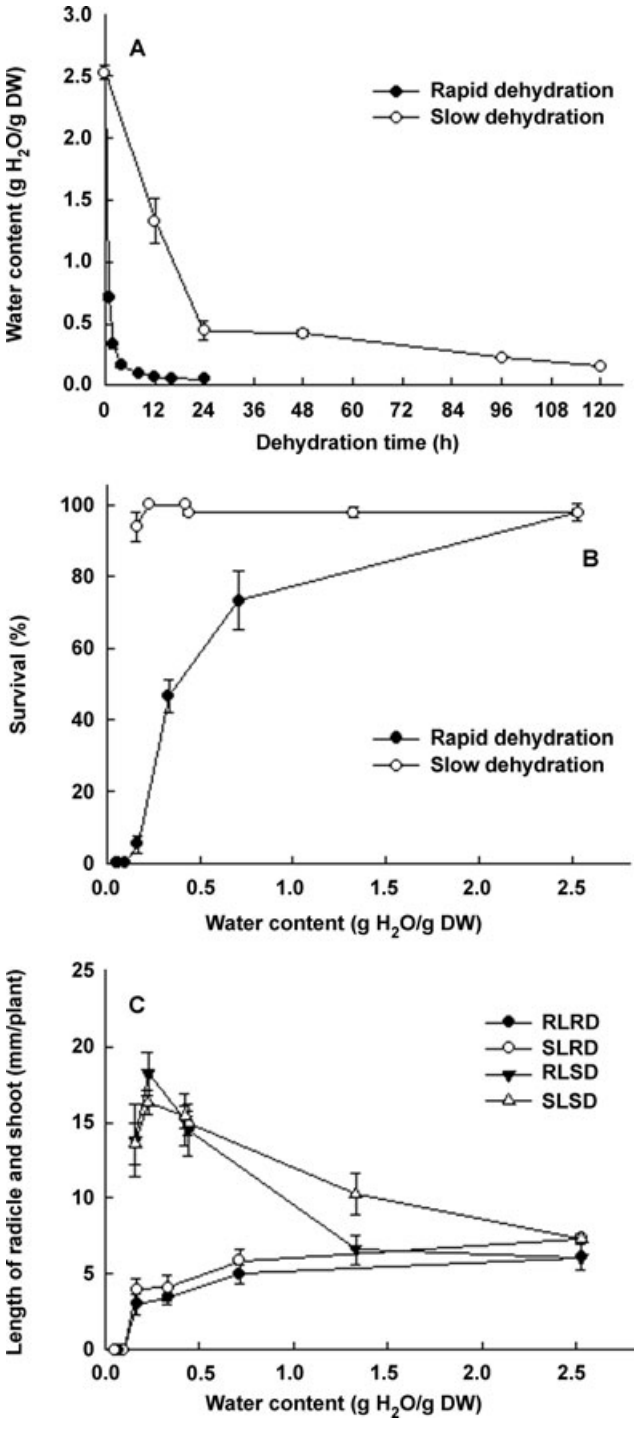

Figure 2. Changes in water content **(A)**, survival **(B)** and length of radicle and shoot produced by surviving embryos **(C)** during rapid and slow dehydration of maize embryos.

After excising from seeds, embryos were dehydrated for an indicated time and to an indicated water content at 15°C, and then germinated at 25°C for 5 d. All values are means ± *SD* of three replicates of 20 axes each. RLRD, radicle length after rapid dehydration; RLSD, radicle length after slow dehydration; SLRD, shoot length after rapid dehydration; SLSD, shoot length after slow dehydration.

In contrast to Chinese wampee axes, damage to rapidly dehydrated maize embryos (*P* value ≤ 0.001), as measured by embryo survival and length of radicles and shoots produced by embryos that survived, was much greater than that of slowly dehydrated embryos (Figure 2B,C) (*P* value ≤ 0.015). For example, the survival of maize embryos dehydrated slowly remained at 94% during dehydration from 2.53 to 0.16 g/g, but for embryos dehydrated rapidly it was only 5.3% after dehydration from 2.53 to 0.17 g/g. The W_{50}s of embryos dehydrated rapidly was 0.39 g/g, but could not be observed in embryos that underwent slow dehydration (Figure 2B).

Changes in MDA contents

The MDA contents of Chinese wampee axes increased with both rapid and slow dehydration (*P* value ≤ 0.001), but a rapid increase in MDA contents occurred at a higher water content during slow dehydration (approximately 0.26 g/g) than during rapid dehydration (approximately 0.12 g/g) (Figure 3A).

Malondialdehyde contents of immature maize embryos increased throughout slow dehydration, whereas with rapid dehydration the initial increase was followed by a decline at low water content (*P* value ≤ 0.001) (Figure 3B). The MDA contents produced during rapid dehydration were, however, higher than those produced during slow dehydration (Figure 3B).

Effects of dehydration at different rates on activities of SOD, APX and CAT

Changes in activities of SOD, APX and CAT were monitored during dehydration of Chinese wampee axes and maize embryos. Activities of SOD, APX and CAT of Chinese wampee axes markedly increased during the early phase of rapid and slow dehydration, and then rapidly decreased (*P* value ≤ 0.001) (Figure 4). However, the peak values of SOD, APX and CAT activities occurred at different water contents during rapid and slow dehydration. Activities of SOD, APX and CAT of rapidly dehydrated axes were higher than those of slowly dehydrated axes when these axes were dehydrated to lower water content (Figure 4).

Activities of SOD and APX of immature maize embryos decreased with both rapid and slow dehydration (*P* value ≤ 0.001) (Figure 5). However, during dehydration, SOD activities of slowly dehydrated maize embryos were higher than those of rapidly dehydrated embryos, and APX activities of rapidly dehydrated maize embryos were higher than those of slowly dehydrated embryos (Figure 5). CAT activities of maize embryos markedly increased during the early phase of both rapid and

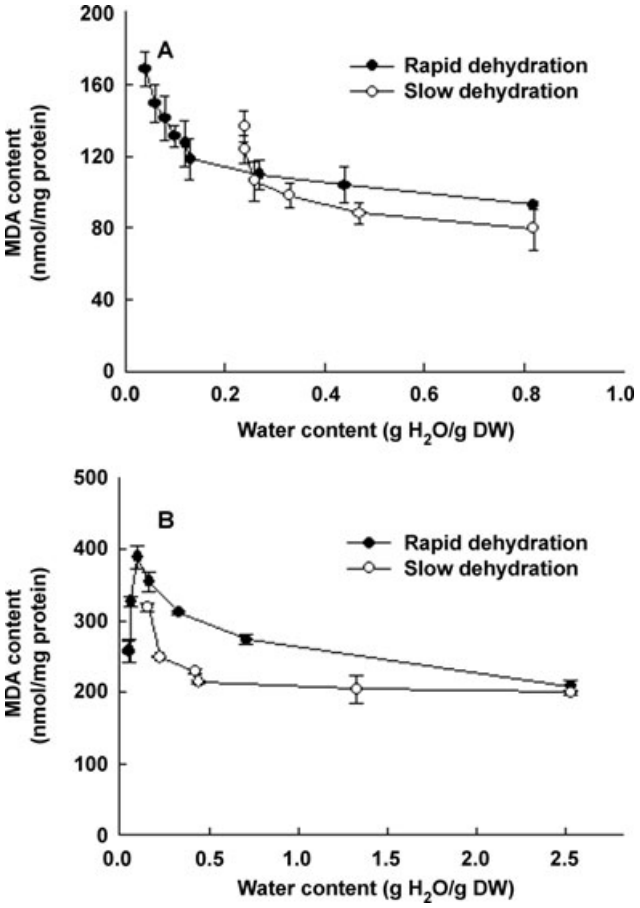

Figure 3. Effects of dehydration at different rates on malondialdehyde (MDA) contents during rapid and slow dehydration of Chinese wampee axes **(A)** and maize embryos **(B)**.

After excising from seeds, Chinese wampee axes and maize embryos were dehydrated to an indicated water content, and then MDA contents were immediately measured. All values are means ± *SD* of four replicates.

slow dehydration, and then decreased, and CAT activities of slowly dehydrated embryos were higher than those of rapidly dehydrated embryos during the late phase of dehydration (Figure 5).

Discussion

Survival of Chinese wampee axes decreased with both rapid and slow dehydration (Figure 1B), showing that Chinese wampee axes were sensitive to dehydration. These results are similar to those of Song and Fu (1997), who reported that Chinese wampee seeds were desiccation-sensitive and were typically recalcitrant seeds. Dehydration at different rates markedly influenced the desiccation response of Chinese

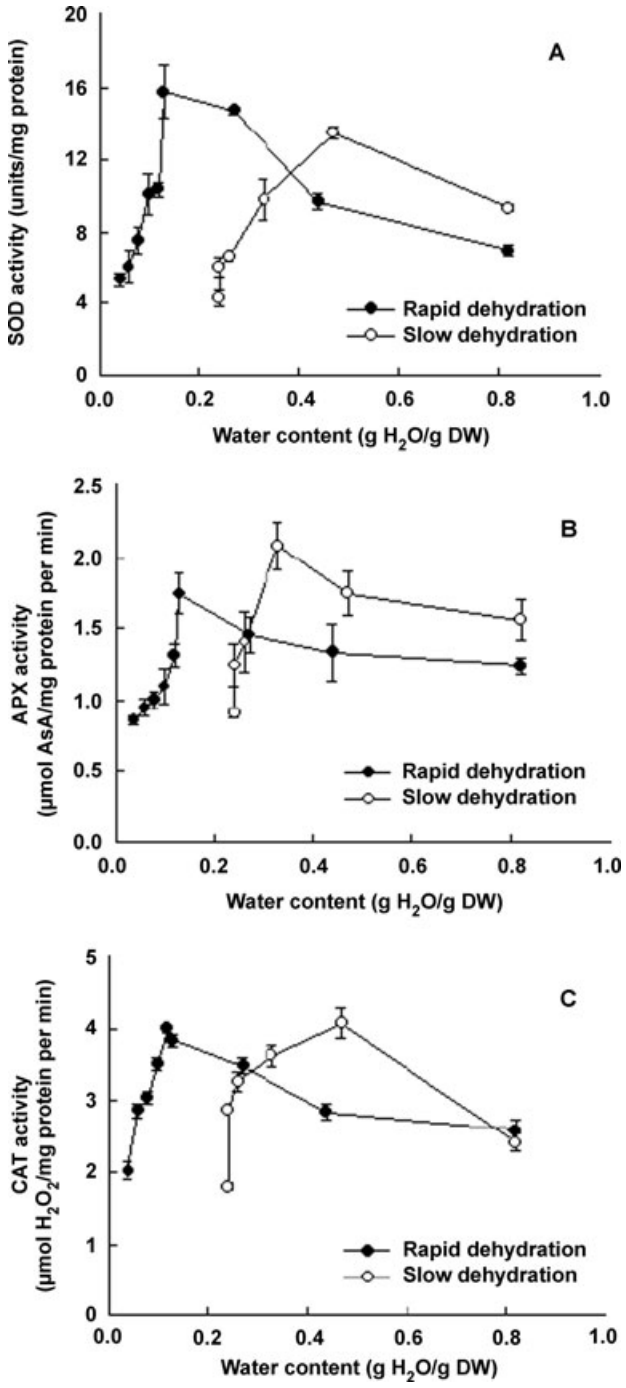

Figure 4. Effects of dehydration at different rates on activities of superoxide dismutase (SOD) **(A)**, ascorbate peroxidase (APX) **(B)**, and catalase (CAT) **(C)** of Chinese wampee axes.

After excising from seeds, axes were dehydrated to an indicated water content, and such enzyme activities were assayed as described in Materials and Methods. All values are means ± *SD* of five replicates.

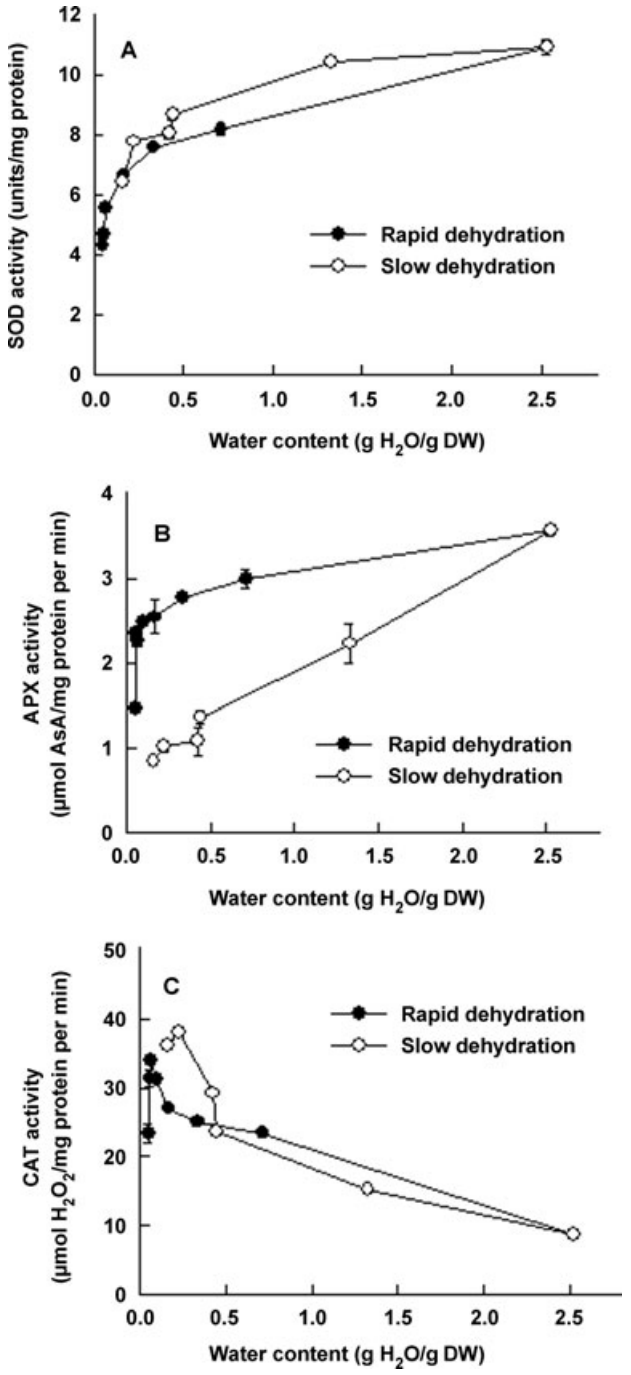

Figure 5. Effects of dehydration at different rates on activities of superoxide dismutase (SOD) **(A)**, ascorbate peroxidase (APX) **(B)**, and catalase (CAT) **(C)** of maize embryos.

After excising from seeds, embryos were dehydrated to an indicated water content, and such enzyme activities were assayed as described in Materials and Methods. All values are means ± *SD* of five replicates.

wampee axes; the more rapidly axes were dehydrated, the lower was the water content to which the axes could be dried before viability was lost (Figure 1B). These results are in accordance with those of Berjak et al. (1990) for *Landolphia kirkii* seeds, Pammenter et al. (2000) for excised axes of *Trichilia dregeana*, *Castanospermum australe* and *Camellia sinensis*. It is noted that rapid dehydration does not truly confer the property of desiccation tolerance on Chinese wampee axes, because such axes will not survive for longer than a few days at 25 °C and at the same water content at which viability was not lost after rapid dehydration (data not shown).

In addition, survival of Chinese wampee axes decreased by 47.6% during dehydration from 0.82 g/g to 0.24 g/g by slow dehydration. Although water contents of axes have no change during slow dehydration from 60 h to 96 h of dehydration, their survival decreased to zero from 52% (Figure 1B). Thus the decrease in survival was not only a direct response to dehydration, but also involved the time over which axes were dried. From this perspective, desiccation damage is a time-dependent process – an ageing phenomenon (Walters et al. 2002). Therefore, dehydration time cannot be ignored in the dehydration experiments, and it is very difficult to determine a 'critical water content' for viability loss of axes (or seeds) (Pammenter and Berjak 1999).

In contrast to Chinese wampee axes, damage to immature maize embryos caused by rapid dehydration was much greater than by slow dehydration (Figure 2B,C), with the W_{50}s of maize embryos being 0.39 g/g for rapid dehydration, and not being observed for slow dehydration (Figure 2B) in this study. It has been reported that whole seeds of several legumes (Adams et al. 1983; Ellis et al. 1987) and *Ricinus communis* (Kermode and Bewley 1985) are unable to withstand rapidly imposed drying at early stages of development (prior to maturation drying) and exhibit no germinability upon subsequent rehydration. This contrasts with seeds at the same stage of development dried slowly over saturated salt solutions, where full germinability is evident. Kermode and Finch-Savage (2002) suggested that for orthodox seeds, gradual water loss may allow protective changes to occur and hence increase the resistance of the seed to disruption by dehydration, and rapid drying presumably would not allow such protective changes to take place and may cause considerable disruption to cellular membranes and internal structures.

Lipid peroxidation has considerable potential to damage membranes and may be a principal cause of deterioration in orthodox seeds (Smith and Berjak 1995). Viability loss during desiccation of the recalcitrant seeds of *Q. robur* (Hendry et al. 1992; Finch-Savage et al. 1994), *Castanea sativa* and *Aesculus hippocastanum* (Finch-Savage et al. 1994), *Litch chinensis* (Song and Fu 1999), *Clausena lansium* (Song and Fu 1997), *Trichilia dregeana* (Song et al. 2004) was accompanied by increased lipid peroxidation. The MDA contents of Chinese wampee axes and maize embryos notable increased with rapid

and slow dehydration (Figure 3). Correlation analysis indicated that decrease in desiccation tolerance of Chinese wampee axes was closely related with increase in MDA contents during rapid ($r = -0.943$) and slow ($r = -0.972$) dehydration. It was noted that for Chinese wampee axes, a rapid increase in MDA contents occurred at a higher water content during slow dehydration than during rapid dehydration (Figure 3A). For maize embryos, the MDA contents produced during rapid dehydration were higher than those produced during slow dehydration (Figure 3B), the decrease in desiccation tolerance was related to increases in MDA contents during rapid ($r = -0.581$) and slow ($r = -0.659$) dehydration. At intermediate water contents, uncontrolled oxidative reactions that are dependent upon metabolism can occur, leading to desiccation damage (Leprince et al. 1996; Walters et al. 2001). At lower hydration levels, the remaining water is non-freezable and is associated with macromolecular structures. Its removal can lead to conformational changes that may be irreversible and damaging. Thus, at least two types of damage that can occur on drying recalcitrant seeds are envisaged: strict desiccation damage that occurs when sufficient water is removed to lead to damage to macromolecular structures, and aqueous-based oxidative damage that occurs at intermediate water contents and is a consequence of unregulated metabolism (Pammenter et al. 2000; Walters et al. 2001). For Chinese wampee axes, MDA contents during slow dehydration were higher than those during rapid dehydration (Figure 3A), supporting the suggestion that rapidly dried axes can survive to lower water contents because the tissue spends insufficient time at intermediate water contents for damage consequent upon the deleterious aqueous-based reactions to accumulate (Pammenter et al. 1991). In contrast with Chinese wampee axes, MDA contents of maize embryos during the early phase of rapid dehydration were higher than those during slow dehydration (Figure 3B), showing that gradual water loss may allow protective changes to occur and hence improve desiccation tolerance. During the late phase of rapid dehydration of maize embryos, the reason why MDA contents decreased might be owing to damage of cell structure, so that they leak out from the embryos because of volatility of MDA.

$^{\cdot}O_2^-$ and H_2O_2 are synthesized at high rates in the cells, even under normal conditions. The chief toxicity of $^{\cdot}O_2^-$ and H_2O_2 is thought to reside in their ability to initiate cascade reactions that result in the production of the hydroxyl radical ($^{\cdot}OH$) and other destructive species such as lipid peroxides (Noctor and Foyer 1998). Efficient destruction of $^{\cdot}O_2^-$ and H_2O_2 requires the action of several ROS scavenging enzymes acting in synchrony. Enzymatic ROS scavenging mechanisms in plants include SOD, APX and CAT. SOD acts as the first line of defence against ROS, dismutating superoxide to H_2O_2. APX and CAT subsequently detoxify H_2O_2 (Møller 2001; Apel and Hirt 2004). Changes in activities of SOD, APX and CAT during rapid and slow dehydration of Chinese wampee axes (Figure 4) and maize embryos (Figure 5) showed that activities of these enzymes of Chinese wampee axes can be induced by slight dehydration, and that decrease in survival of Chinese wampee axes and maize embryos was accompanied by decreased activities of SOD, APX and CAT. Activities of SOD, APX and CAT of Chinese wampee axes dehydrated rapidly were higher than those of slowly dehydrated axes when these axes were dehydrated to a lower water content compared with those that were slowly dehydrated (Figure 4). Dehydration at different rates remarkably influenced changes in the activities of SOD, APX and CAT of immature maize embryos (Figure 5), and such effects were very different from recalcitrant Chinese wampee axes. Apel and Hirt (2004) considered that the balance of SOD, APX, and CAT activities are crucial for suppressing toxic ROS levels in cells. In this regard, Li and Sun (1999) found that the activities of SOD, APX and peroxidase significantly decreased with dehydration of immature and mature *Theobroma* cacao axes.

There are a number of processes or mechanisms associated with desiccation tolerance of seeds (Pammenter and Berjak 1999; Berjak and Pammenter 2004; Berjak 2006). The parallel processes involving late embryogenic abundant (LEA) or LEA-like proteins, accumulation of sugars, aspects of ROS and non-enzymatic and enzymatic antioxidants have been the recent focus of attention (Berjak 2006). Whether LEA-like proteins and accumulation of sugars are implicated in dehydration at different rates remains unknown.

Materials and Methods

Plant material

Fruits of Chinese wampee (*Clausena lansium* (Lour.) Skeels cv. Jixin) were collected at physiological maturity from trees growing in Guangzhou Institute of Pomology, Jiufu, Guangzhou. After removal from the fruits, the seeds were cleaned in water, then surface-sterilized in a solution of 1% sodium hypochlorite, and rinsed three times in sterilized water. Axes were excised from seeds and dehydrated according to the methods of Huang et al. (2000). When axes are excised, a small amount of cotyledon has to leave attaching to the axis in order to protect the shoot apical meristem from mechanical damage.

Immature maize (*Zea mays* L.) seeds were manually collected at 18 d after pollination (DAP) from plants growing in the Xishuangbanna Tropic Botanic Garden in July 2005. After excision from seeds, embryos were dehydrated.

Dehydration of axes and embryos

Rapid dehydration of Chinese wampee axes and maize embryos was achieved by placing axes and embryos over activated silica gel for different times within a closed desiccator. Slow dehydration of axes and embryos was achieved by placing them over a saturated solution of sodium chloride (75% RH)

for different times within a closed container. The temperature when seeds were dehydrated was 15 ± 1 °C.

Water content determinations

Water contents of Chinese wampee axes and maize embryos were determined gravimetrically (after drying at 80 °C for 48 h). Five axes and embryos were sampled each time for these determinations. Water contents are expressed on a dry mass basis (g H_2O/g DW, g/g).

The term, W_{50}, was used to describe the water content at which 50% of axes and embryos had been killed by dehydration.

Survival assessment of Chinese wampee axes and maize embryos

For Chinese wampee axes, batches of 20 axes were germinated on moist filter paper in closed Petri dishes in the dark at 30 °C for 6 d; for maize embryos, batches of 20 embryos were also germinated on filter paper at 25 °C for 5 d. Axes and embryos were counted as surviving after dehydration and subsequent germination when they appeared to increase markedly in length and volume and became light green, and the axes and embryos that showed no increase in length and volume and appeared dark brown were counted as dead.

Determination of MDA contents

Malondialdehyde contents of Chinese wampee axes and maize embryos were determined as originally described by Heath and Packer (1986) but as modified by Hendry et al. (1993). The MDA contents were quantified from the second derivative spectrum against standards prepared from 1,1,3,3-tetraethoxypropane. The MDA contents were expressed as nmol/mg protein.

Assay of SOD

Forty Chinese wampee axes or maize embryos were homogenized to a fine powder with a mortar and pestle in liquid nitrogen. Subsequently, soluble proteins were extracted by grinding the powder in an extraction mixture composed of 50 mmol/L phosphate buffer (pH 7.0), 1.0 mmol/L ethylenediaminetetraacetic acid (EDTA), 0.05% (v/v) Triton X-100, 2% (w/v) polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 16 000 *g* for 15 min, after which the supernatant was transferred to a new tube and kept at −20 °C. The extraction procedures were carried out at 4 °C.

Superoxide dismutase (EC 1.15.1.1) activity assay was based on the method of Beauchamp and Fridovich (1971), which measures inhibition of the photochemical reduction of nitroblue tetrozolium (NBT) at 560 nm. The procedure was modified as follows: the reaction mixture of 1 mL contained 50 mmol/L phosphate buffer (pH 7.8), 0.1 mmol/L EDTA, 13 mmol/L methionine, 75 μmol/L NBT, 16.7 μmol/L riboflavin and enzyme extract (approximately 20 μg protein). Riboflavin was added last and the reaction was initiated by placing the reaction mixture under two 9-W fluorescent lamps. The reaction was terminated after 5 min by removal of the light. An illuminated blank without protein gave the maximum reduction of NBT, and therefore the maximum absorbance at 560 nm. SOD activity was presented as the absorbance of sample at 560 nm divided by the absorbance of the blank, giving the percentage of inhibition. In this assay, one unit of SOD was defined as the amount required to inhibit the photoreduction of NBT by 50%. The specific activity of SOD was expressed as unit/mg protein.

Assays of APX and CAT

Forty wampee axes or maize embryos were homogenized in liquid nitrogen to fine powder in a mortar. The powder was ground in 5 mL of 50 mmol/L Tris-HCl (pH 7.0), containing 20% (v/v) glycerol, 1 mmol/L ascorbic acid (AsA), 1 mmol/L dithiothreitol, 1 mmol/L EDTA, 1 mmol/L reduced glutathione (GSH), 5 mmol/L $MgCl_2$ and 1% (w/v) PVPP. After differential centrifugation (at 12 000 *g* for 6 min and at 26 900 *g* for 16 min, respectively), the supernatants were obtained as crude enzyme extraction of APX and CAT, and stored at −20 °C for later determinations of enzyme activities.

Ascorbate peroxidase (EC 1.11.1.7) activity was assayed by the decrease in absorbance of AsA at 290 nm (2.8/mM per cm) due to AsA oxidation using the method of Nakano and Asada (1981). The reaction mixture contained 50 mmol/L potassium phosphate (pH 7.0), 1 mmol/L AsA and the enzyme source (ca 15 μg protein) in a final volume of 1 ml at 25 °C.

Catalase (EC 1.11.1.6) activity was determined by directly measuring the decomposition of H_2O_2 at 240 nm (0.04/mM per cm), as described by Aebi (1983). The assay was conducted in 50 mmol/L potassium phosphate (pH 7.0), containing 10 mmol/L H_2O_2 and the enzyme source (approximately 15 μg protein) made up to a final volume of 1 mL at 25 °C.

Protein assay

Protein concentrations were determined according to Bradford (1976) with bovine serum albumin as the standard.

Statistical analysis

The data of changes in water content, survival, MDA contents and SOD, APX and CAT activities during both rapid and slow dehydration of Chinese wampee axes and immature maize embryos were analyzed using the one-way ANOVA model from the SPSS 11.0 package for Windows (SPSS Inc. Chicago, IL, USA).

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References

Adams CA, Fjerstad MC, Rinne RW (1983). Characteristics of soybean seed maturation: necessity for slow dehydration. *Crop Sci.* **23**, 265–267.

Aebi HE (1983). Catalase. In: Bergmeyer HU, ed. *Methods of Enzymatic Analyses*, Vol. 3. Verlag Chemie, Weinheim. pp. 273–282.

Apel K, Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* **55**, 373–399.

Beauchamp C, Fridovich I (1971). Superoxide dismutase improved assays and an assay applicable to acrylamide gel. *Anal. Biochem.* **44**, 276–287.

Berjak P (2006). Unifying perspectives of some mechanisms basic to desiccation tolerance across life forms. *Seed Sci. Res.* **16**, 1–15.

Berjak P, Farrant JM, Mycock DJ, Pammenter NW (1990). Rcalcitrant (homoiohydrous) seeds: the enigma of their desiccation sensitivity. *Seed Sci. Technol.* **18**, 297–310.

Berjak P, Pammenter NW (2004). Biotechnological aspects of non-orthodox seed: an African perspective. *S. Afr. J. Bot.* **70**, 102–108.

Berjak P, Vertucci CW, Pammenter NW (1993). Effects of developmental status and dehydration rate on characteristics of water and desiccation-sensitivity in recalcitrant seeds of *Camellia sinensis*. *Seed Sci. Res.* **3**, 155–166.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

Ellis RH, Hong TD, Roberts EH (1987). The development of desiccation-tolerance and maximum seed quality during seed maturation in six grain legumes. *Ann. Bot.* **59**, 23–29.

Finch-Savage WE (1992). Embryo water status and survival in the recalcitrant species *Quercus robur* L.: evidence for a critical moisture content. *J. Exp. Bot*. **43**, 663–669.

Finch-Savage WE, Hendry GAF, Atherton NM (1994). Free radical activity and loss of viability during drying of desiccation-sensitive tree seeds. *Proc. Roy. Soc. Edinb*. **102B**, 257–260.

Heath RL, Packer L (1986). Photo-peroxidation in isolated chloroplasts. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **125**, 189–198.

Hendry GAF, Finch-Savage WE, Thorpe PC, Atherton NM, Buckland SM, Nillsson KA et al. (1992). Free radical processes and loss of viability during desiccation in the recalcitrant species *Quercus rober* L. *New Phytol.* **122**, 273–279.

Hendry GAF, Thorpe PC, Merzlyak MN (1993). Stress indicators: lipid peroxidation. In: Hendry GAF, Grime, JP, eds. *Methods in Comparative Plant Ecology*. Chapman & Hall, London. pp. 154–156.

Huang XM, Fu JR, Song SQ (2000). The desiccation-sensitivity of wampee [*Clausena lansium* (Lour.) Skeels] axes in relation to seedling formation. *Acta Sci. Nat. Univ. Sunyatseni* **39**, 87–90 (in Chinese with an English abstract).

Kermode AR, Bewley JD (1985). The role of maturation drying in the transition from seed development to germination. I. Acquisition of desiccation-tolerance and germinability during development of *Ricinus communis* L. seeds. *J. Exp. Bot.* **36**, 1906–1915.

Kermode AR, Finch-Savage BE (2002). Desiccation sensitivity in orthodox and recalcitrant seeds in relation to development. In: Black M, Prichard, HW, eds. *Desiccation and Survival in Plant: Drying without Dying*. CAB International, Oxon. pp. 149–184.

Leprince O, Hendry GAF, Atherton NM, Vertucci-Walters C (1996). Free radicals and metabolism associated with acquisition and loss of desiccation tolerance in developing seeds. *Biochem. Soc. Trans.* **24**, 451–455.

Li C, Sun WQ (1999). Desiccation sensitivity and activities of free radical-scavenging enzymes in recalcitrant *Theobroma cacao* seeds. *Seed Sci. Res.* **9**, 209–217.

Møller IM (2001). Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52**, 561–591.

Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **22**, 867–880.

Noctor G, Foyer CH (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**, 249–279.

Pammenter NW, Berjak P (1999). A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Sci. Res.* **9**, 3–37.

Pammenter NW, Berjak P, Walters C (2000). The effects of drying rate on recalcitrant seeds: 'lethal water content', causes of damage, and quantification of recalcitrance. In: Black M, Bradford KJ, Vázquez-Ramos J, eds. *Seed Biology: Advances and Applications*. CABI Publishing, Oxon. pp. 215–221.

Pammenter NW, Vertucci CW, Berjak P (1991). Homeohydrous (recalcitrant) seeds: dehydration, the state of water and viability characteristics in *Landolphia kirkii*. *Plant Physiol.* **96**, 1093–1098.

Smith MT, Berjak P (1995). Deteriorative changes associated with the loss of viability of stored desiccation-tolerant and -sensitive seeds. In: Kigel J, Galili G, eds. *Seed Development and Germination*. Marcel Dekker Inc, New York. pp. 701–704.

Song SQ, Berjak P, Pammenter N (2004). Desiccation sensitivity of *Trichilia dregeana* axes and antioxidant role of ascorbic acid. *Acta Bot. Sin*. **46**, 803–810.

Song SQ, Berjak P, Pammenter N, Ntuli TM, Fu JR (2003). Seed recalcitrance: a current assessment. *Acta Bot. Sin.* **45**, 638–643.

Song SQ, Fu JR (1997). Desiccation sensitivity and lipid peroxidation in Chinese wampee (*Clausena lansium* (Lour.) Skeels) seeds. *Acta Phytophysiol. Sin.* **23**, 163–168 (in Chinese with an English abstract).

Song SQ, Fu JR (1999). Desiccation sensitivity and peroxidation of membrane lipids in lychee seeds. *Trop. Sci.* **39**, 102–106.

SPSS (2006). *SPSS 11.0 for Windows*. SPSS Inc., Chicago, IL, USA.

Walters C, Farrant JM, Pammenter NW, Berjak P (2002). Desiccation stress and damage. In: Black M, Pritchard HW, eds. *Desiccation and Survival in Plants: Drying without Dying*. CABI Publishing, Oxon. pp. 263–291.

Walters C, Pammenter NW, Berjak P, Crane J (2001). Desiccation damage, accelerated aging and metabolism in desiccation tolerant and sensitive seeds. *Seed Sci. Res.* **11**, 135–148.

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