PHYSIOLOGIA PLANTARUM 94: 233–240. 1995
Printed in Denmark – all rights reserved

ISSN 0031-9317

The expression of desiccation-induced damage in orthodox seeds is a function of oxygen and temperature

Olivier Leprince, Christina W. Vertucci, George A.F. Hendry and Neil M. Atherton

Leprince, O., Vertucci, C.W., Hendry, G.A.F. and Atherton, N.M. 1995. The expression of desiccation-induced damage in orthodox seeds is a function of oxygen and temperature. – Physiol. Plant. 94: 233–240.

From the premise that desiccation-induced damage is associated with a free-radical mechanism of injury, we address the hypothesis that expression of desiccation damage is dependent on metabolism. The effects of temperature and O_2 concentration on the expression of damage were studied in germinating bean (*Phaseolus vulgaris* L. cv. Pole Kentucky Wonder) axes and maize (*Zea mays* L. cv. Kelvedon Glory) radicles submitted to flash drying. Damage in desiccation-tolerant and -intolerant material was assessed by measurements of electrolyte leakage and accumulation of a stable free radical. In desiccation-tolerant material leakage rates remained low during water removal. In contrast, in desiccation-intolerant tissues, leakage profiles revealed the presence of a critical moisture content below which leakage rates increased sharply. In the desiccation-intolerant stage, a highly significant correlation was found between critical moisture contents and temperatures of drying. The concentration of the stable radical was lower if tissues were dried below 15°C and higher when tissues were dried at 30°C and above. Both leakage and build up of free radicals were highly sensitive to O_2 concentrations: damage was lower when tissues were dried in the presence of N_2, but increased several-fold when tissues were exposed to O_2 concentrations between 2 and 100%. In contrast, neither temperature nor O_2 concentrations affected electrolyte leakage in desiccation-tolerant samples. Treatment with a respiration inhibitor (KCN) prior to drying reduced the desiccation sensitivity of tissues, as noted by a reduction of the critical moisture content. We conclude that the expression of desiccation damage depends on the drying history and that factors that limit metabolism also reduce the incidence of desiccation injury.

Key words – Bean, critical moisture content, desiccation tolerance, electron paramagnetic resonance, free radicals, germination, maize, oxygen, *Phaseolus vulgaris*, temperature, *Zea mays*.

O. Leprince (corresponding author) and C.W. Vertucci, USDA-ARS, National Seed Storage Laboratory, Fort Collins, CO 80521, USA.; G.A.F. Hendry, NERC Unit of Comparative Plant Ecology, Dept of Animal and Plant Sciences and N.M. Atherton, Dept of Chemistry, The University, Sheffield S10 2TN, UK.

Introduction

In recent years, many studies of the mechanisms of desiccation tolerance in seeds have focused on protective components (e.g. carbohydrates and LEA [late embryogenesis abundant] proteins; reviewed by Crowe et al. 1992, Leprince et al. 1993, Vertucci and Farrant 1994). Correlations have been drawn between changes in concentration and types of protectants and the development or loss of desiccation tolerance in a wide variety of plants. While such correlations have been demonstrated in several systems (see Leprince et al. 1993, Vertucci and Farrant 1994), accumulating evidence also shows that these may not always hold (Blackman et al. 1992, Farrant et al. 1993, Finch-Savage and Blake 1994, Ooms et al. 1994, Still et al. 1994).

An alternative hypothesis for desiccation sensitivity maintains that destructive reactions are mediated by free radicals generated during desiccation (Hendry 1993, Leprince et al. 1990, 1993, 1994, McKersie 1991, Na-

Received 22 December, 1994; revised 17 February, 1995

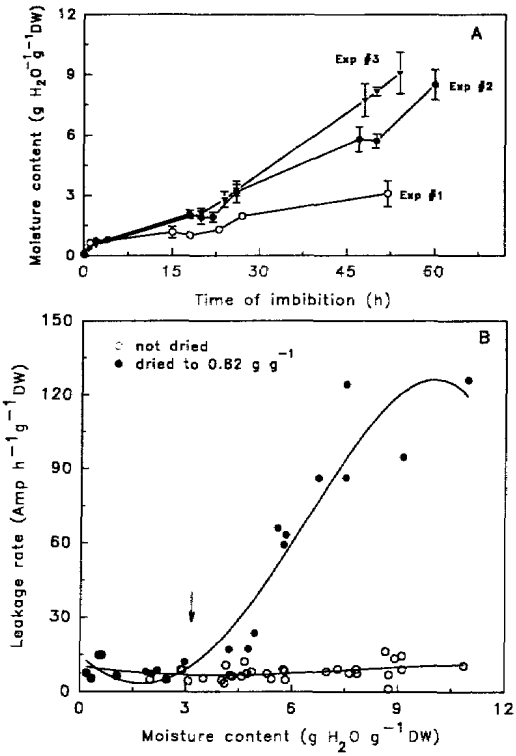

Fig. 1. A. Experimental variations of the time course of water taken up by embryonic axes of bean during imbibition of intact seeds. Data are expressed on a dry weight basis and are the means of 5–6 individual axes ± SE. B. Changes in the sensitivity of bean embryonic axes to desiccation during germination. The progress of germination is defined by the moisture content acquired by axes. Sensitivity of desiccation is expressed as the rate of electrolyte leakage from imbibed bean axes sampled before (○) and after (●) drying to 0.82 g H_2O g^{-1} dry weight. Curve describes a 4th order polynomial regression fitted to data ($r^2 = 0.955$). The arrow indicates the onset of radicle emergence.

vari-Izzo et al. 1994, Seel et al. 1992). Several studies have found that desiccation-tolerant tissues are capable of evading damage from peroxidative reactions during drying and that loss of viability in intolerant counterparts is associated with various symptoms of free-radical injury such as lipid peroxidation, phospholipid de-esterification and accumulation of a highly stable, quinone-based radical (Hendry 1993, Hendry et al. 1992, Leprince et al. 1990, 1994, Navari-Izzo et al. 1994). The accumulation of this stable free radical has been associated with loss of desiccation tolerance in germinating maize radicles and it probably arises from desiccation-induced impairment of electron transport between complex I and the ubiquinone pool of mitochondrial membranes, leading to the transient formation of activated oxygen (Leprince et al. 1994). Based on this evidence and on a relationship between increases in respiration rates and desiccation sensitivity (Leprince et al. 1992), respiration has been suggested to play a central role in desiccation intolerance via a free-radical mechanism of injury. The present study was undertaken to evaluate the relationship between desiccation damage and metabolism. The generation of free radicals under nonstressed conditions is associated with electron transport processes such as respiration and photosynthesis (Leprince et al. 1993, 1994, Puntarulo et al. 1991 and references therein). Therefore environmental factors that influence both metabolism and free-radical processes might have an impact on the expression of damage induced by desiccation. Here, we address this hypothesis by investigating the effects of temperature, oxygen and the respiration inhibitor KCN during drying on the expression of two independent indices of damage in germinating bean and maize at both desiccation-tolerant and -intolerant stages.

Abbreviations – EPR, electron paramagnetic resonance; g g^{-1}, g H_2O g^{-1} dry weight; LEA, late embryogenesis abundant; MC, moisture content.

Materials and methods

Plant material, germination and desiccation tolerance

Bean seeds (*Phaseolus vulgaris* L. cv. Pole Kentucky Wonder) were prehydrated overnight at 100% relative humidity (RH), then rolled in paper towels and placed at 25°C for up to 56 h. Maize kernels (*Zea mays* L. cv. Kelvedon Glory) were placed on a moistened cotton-wool layer topped with filter paper and allowed to germinate at room temperature for up to 76 h. The desiccation-tolerant and -intolerant stages in maize were qualitatively determined when imbibed seeds gave 100 and 0% germination after drying and subsequent reimbibition treatment, respectively (Leprince et al. 1992). These germination percentages corresponded to 18- and 72-h-imbibed samples, respectively. In bean, it was practically impossible to characterize the development of germination in a similar way because moisture content (MC) during imbibition varied considerably among experiments (Fig. 1A). Therefore a preliminary study using electrolyte leakage measurements was undertaken to assess the desiccation-tolerant and -intolerant stages (see Results).

Drying treatments

Two drying methods were applied to bean seeds and maize kernels. Desiccation-tolerant and -intolerant embryonic axes of germinating beans were excised and dried for intervals of up to 7.5 h in flash-drying chambers under a gas flow of 15 l h^{-1} by using air compressors or gas cylinders (Pammenter et al. 1991). For germinating maize, radicles were placed in a desiccator with 250 g of activated silica gel for 24 h. This produced an MC between 0.55 and 1.22 g H_2O g^{-1} dry weight (g g^{-1}). In both treatments, dehydration rates of desiccation-tolerant or -intolerant material of both species were comparable at the onset of drying (data not shown). Moisture contents

were determined gravimetrically by weighing samples before and after drying them for 36 h at 96°C and were expressed as g of H_2O g^{-1} dry mass.

Variation in temperature during drying was achieved by placing the drying chamber or the desiccators in temperature-controlled cold rooms (4–9°C) or growth chambers (15–40°C). When air compressors were used, a constant and low RH of drying air was achieved for each temperature by connecting a cartridge of activated silica gel (40 ml bed volume) between the compressor and the drying chamber. In studies with beans, variation in O_2 concentration was achieved by drying them under dry N_2, air or a 50% O_2:N_2 mixture. In maize studies, N_2:O_2, air or a 2% O_2:98% N_2 mixture was flushed three times for 10 min through sealed desiccators equipped with sealable valves for inlet and outlet.

Electrolyte leakage assays

All measurements of electrolyte leakage were performed with an ASAC-1000 multiwell conductivity meter (Neogen Food Tech Corp., Ann Arbor, MI, USA). Control and dried samples were slowly prehydrated for 20 min on damp filter paper to avoid imbibitional damage, then soaked individually in 2 ml of distilled water. Conductivity measurements were taken from individual axes every 5 min for 1 h. Rates of solute leakage were determined for 5–6 axes for each of the various drying times. Rates of leakage were computed by a linear regression of conductivity values over time ($r^2 = 0.90$–0.98). To determine a desiccation-tolerant and -intolerant stage in germinating bean, embryonic axes were excised from seeds imbibed up to 56 h then dried to a single MC of 0.82 g g^{-1}. The duration of drying was standardized accordingly since the samples exhibited a range of MC values prior to drying. An MC of 0.82 g g^{-1} constituted a compromise value enabling fully hydrated tissues to be dried within the time scale of a flash drying procedure (i.e. 7 h). Once desiccation-tolerant and -intolerant stages were established, the effects of environmental factors on these stages were studied in embryonic axes exposed to six to eight drying times. The relation between leakage rates and MC can be described by two separate linear regression equations of different slopes. The water content at the regression intercept corresponds to a breakpoint in the relation between MC and leakage rate and is defined as the critical MC. The objective criteria and method used to set the two regressions in the data point population were those of Berjak et al. (1993). In addition, prior to fitting the linear regressions, polynomial regressions were fitted through our data in order to bring out the trends of the relation between leakage rates and MC. The effect of inhibiting respiration on the expression of desiccation damage was measured by incubating bean seeds at the desiccation-intolerant stage for 30 min in 0.5 m*M* KCN in 4 m*M* 2-(N-morpholino)ethanesulfonic acid (MES)-KOH buffer, pH 6.6, or in the buffer alone (control), then 5 min in distilled water twice. Embryonic axes were then excised and dried for different intervals as described above. Measurements of electrolyte leakage rates in maize were taken following the same procedure described above but were limited to samples that were dried overnight in desiccators with silica gel.

Electron paramagnetic resonance

The influence of atmospheric composition and temperature on free-radical-induced injury was also assessed by electron paramagnetic resonance (EPR) by using maize radicles, since the EPR response to desiccation is well characterized for this species (Leprince et al. 1990, 1994). Preliminary EPR experiments on dried bean axes showed that the actual signal was too weak to be significantly manipulated (data not shown). Following settings described in Atherton et al. (1993) and Leprince et al. (1994), EPR spectra of samples containing 20–30 dried radicles were recorded on a Brucker ER 200D spectrometer (Bruker Spectrospin, Coventry, UK) at room temperature. We concentrated on the signal for a stable, quinone-based radical having a g value of 2.0050. This free radical has been ubiquitously associated in seeds and other tissues with loss of viability (Atherton et al. 1993, Hendry et al. 1992, 1994, Leprince et al. 1994). The concentration of radicals was estimated by the height of the second derivative amplitude corrected for instrument gain and dry mass. Three replicate samples per treatment were each scanned twice and the amplitude heights were averaged.

Results

Defining desiccation-tolerant and -intolerant stages in germinating beans

Desiccation-tolerant and -intolerant stages in germinating bean axes were determined by electrolyte leakage assays whose reliability as an index of desiccation tolerance has been previously demonstrated (Berjak et al. 1992, Pammenter et al. 1991, Sun and Leopold 1993, Vertucci et al. 1993). Leakage rates are given as a function of the initial MC measured prior to drying (Fig. 1B). In control, non dried samples, the leakage rates were constant as imbibition progressed. When samples were dried to 0.82 g g^{-1}, the extent of electrolyte leakage was dependent on the MC to which embryos were initially hydrated. In samples hydrated to less than 3 g g^{-1} prior to drying, leakage rates following drying were not significantly different from control levels. In samples hydrated to 3 g g^{-1} or greater, the extent to which electrolytes leaked from dried tissues increased as the MC to which embryos were initially hydrated increased. An MC of 3 g g^{-1} was also coincident with the onset of radicle emergence. Based on these data, desiccation-tolerant material was defined as nongerminated axes (4–6 mm in length) with an MC between 2.2 g g^{-1} and 3 g g^{-1} and desiccation-intolerant material was defined as germinated axes (12–14 mm in length) with an

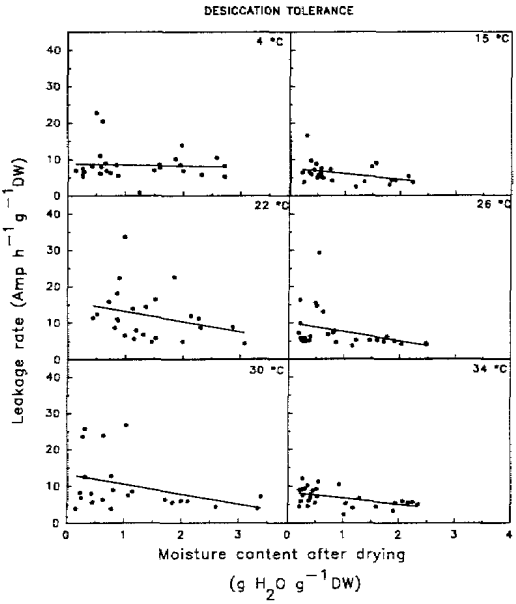

Fig. 2. Relation between moisture contents to which germinating bean axes excised at the desiccation-tolerant stage were dried at different temperatures and leakage rates after drying.

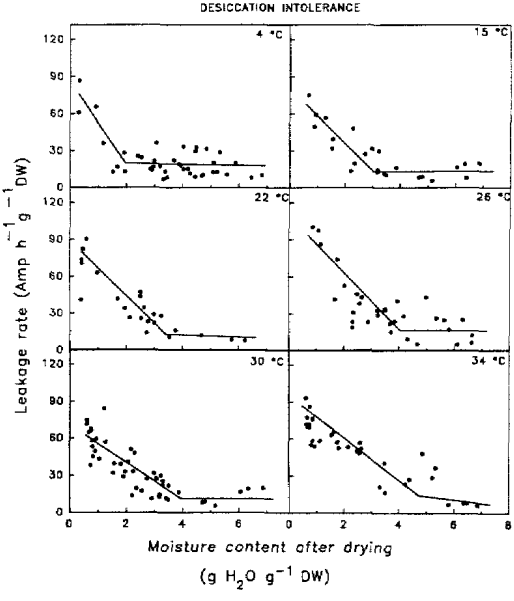

Fig. 3. Relation between moisture contents to which axes excised at the desiccation-intolerant stage were dried at different temperatures and leakage rates after drying. The intersection of the 2 regression lines corresponds to the critical moisture content. The r^2 for the regression fitting the data in the lower moisture content range is 0.895 at 4, 0.805 at 15, 0.835 at 22, 0.669 at 26, 0.831 at 30 and 0.703 at 34°C, respectively. Note the difference in the ordinates of Figs 2 and 3.

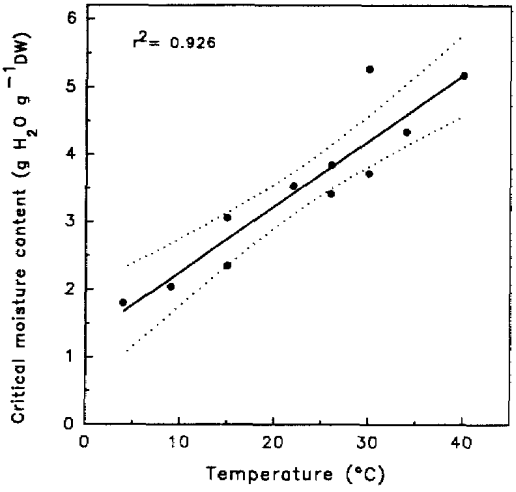

Fig. 4. Relation given by linear regression between temperature of drying and critical moisture contents of desiccation-intolerant bean axes. Regression coefficient and 95% confidence intervals (dotted lines) were calculated using SigmaPlot.

MC ranging from 5 to 7 g g^{-1}. The percentages of germination following drying to 0.82 g g^{-1} were 100 and 0% in desiccation-tolerant and intolerant axes, respectively.

Effect of temperatures on desiccation-induced injury

The temperature during drying had different effects on electrolyte leakage measurements, depending on whether bean axes were tolerant (Fig. 2) or intolerant (Fig. 3) of drying. In desiccation-tolerant samples, leakages rates were not greatly influenced by decreasing MC at any temperature (Fig. 2). In desiccation-intolerant samples, the relationship between leakage rates and MC after drying revealed a breakpoint corresponding to the critical MC (Fig. 3). At MC values less than this critical value,

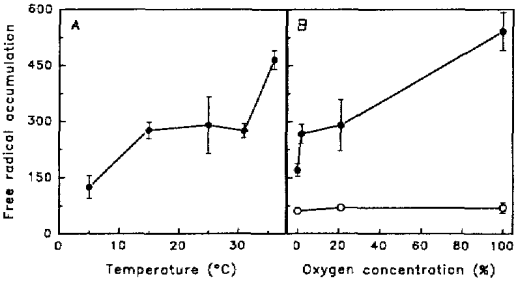

Fig. 5. Effect of drying temperatures (A) and exposure to different oxygen concentrations during drying (B) on the accumulation of a quinone-based free radical in maize radicles dehydrated to about 0.6 g H_2O g^{-1} dry weight at the tolerant (○) and intolerant (●) stages. Free-radical accumulation is expressed as the amplitudes of the EPR second derivative signal corrected for the dry mass (Leprine et al. 1994). Data are the means of triplicates of 20–30 excised radicles ±SE.

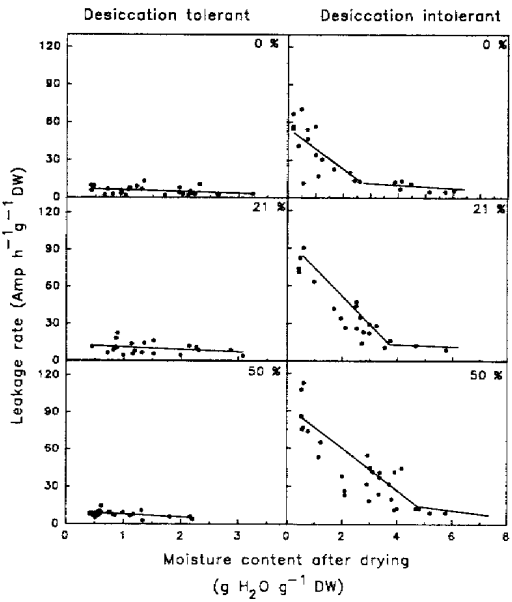

Fig. 6. Effect of O_2 concentration on the relation between moisture content and leakage rates of dried germinating bean axes at the desiccation-tolerant and -intolerant stages. The r^2 of the linear regression fitting the data in the lower moisture content range in desiccation-intolerant samples is 0.727 for 0%, 0.872 for 21% and 0.739 for 100% O_2.

leakage rates markedly increased as a function of decreasing moisture contents. The critical MC increased with increasing temperature from 1.8 g g^{-1} at 4°C to 4.8 g g^{-1} at 34°C. The relationship between temperature and critical MC showed a highly significant positive correlation ($r^2 = 0.926$, Fig. 4).

Extremes of temperatures during drying also influenced the accumulation of the stable free radical in intolerant maize radicles dried to about 0.6 g g^{-1} (Fig. 5A). Since the electron paramagnetic resonance (EPR) spectral characteristics and lineshape of the signal conformed to those previously published (Atherton et al. 1993, Hendry et al. 1992, 1994, Leprince et al. 1990, 1994), the spectra of the stable free radical are not reproduced here. No significant differences in signal amplitudes were observed in intolerant maize dried between 15 and 30°C. However drying treatments at 5 and 36°C induced, respectively, a 2-fold decrease and a 2-fold increase in radical concentrations, indicating that the free-radical accumulation is temperature dependent.

Effect of O_2 concentration on desiccation-induced injury

The effect of O_2 concentration on the accumulation of the stable free radical and on leakage was investigated by using different gas compositions during drying. In the EPR studies, no differences in the signal lineshape were found with the different O_2 treatments (data not shown) and no marked effect on signal amplitude was observed in desiccation-tolerant material (Fig. 5B). When desiccation-intolerant radicles were exposed to increasing concentrations of O_2, the radical concentration increased concomittantly. The absence of O_2 during the drying of intolerant material did not reduce the signal to levels similar to that of tolerant tissues.

Contrasting effects of O_2 concentration on leakage rates were also found in desiccation-tolerant and -intolerant bean (Fig. 6) and maize (Tab. 1) samples. Desiccation-tolerant bean axes could be dried at room temperature down to 0.5 g g^{-1} in the presence of up to 50% O_2 with no effect on the leakage rates. In contrast, the O_2 concentration markedly affected both critical MC and leakage rates of desiccation-intolerant material. The critical MC increased from 2.5 in the absence of O_2 to 4.44 g g^{-1} in the presence of 50% O_2. Similarly the rates of leakage from axes dried to about 0.5 g g^{-1}, increased from 64 ± 5 to 91 ± 8 Amp g^{-1} dry weight h^{-1} after exposure to 0 and 50% O_2, respectively (Fig. 6). When desiccators were used instead of a gas stream as an alternative flash drying method, a somewhat similar interaction between O_2 concentration and leakage occurred in dried maize radicles (Tab. 1).

Effect of a respiration inhibitor on critical moisture content

Previously we showed that the desiccation-induced accumulation of free-radicals in maize can be reduced by inhibitors of respiration (Leprince et al. 1994). Here we investigated whether a similar effect on leakage rates and critical MC could be found with desiccation-intolerant bean axes. When axes were exposed to KCN prior to drying, leakage rates were reduced and the critical MC decreased from 2.5 g g^{-1} in control tissues to 1.8 g g^{-1} in

Tab. 1. Effect of different atmospheric compositions during drying on leakages rates (expressed as Amp g^{-1} dry weight h^{-1}) of excised maize radicles. Material was collected at the desiccation-tolerant and -intolerant stages. The average moisture contents to which desiccation-tolerant and -intolerant samples were dried are expressed as g H_2O g^{-1} dry weight. Data are the means of 4–6 individual samples ± SE.

Germination stages	Moisture content after drying	Gas composition		
		100% N_2	Air	50% O_2; 50% N_2
Tolerant	0.61 ± 0.03	49.5 ± 7.1	55.8 ± 4.9	36.3 ± 12.1
Intolerant	1.02 ± 0.05	160 ± 13	186 ± 9	223 ± 13

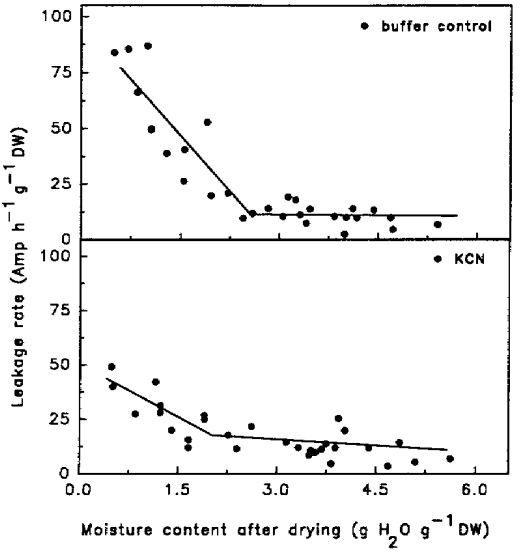

Fig. 7. Effect of incubation in 0.5 m*M* KCN (in MES buffer, pH 6.6) prior to drying on leakage rates of desiccation-intolerant bean axes dried to different moisture contents. Controls correspond to material preincubated in MES buffer alone.

treated tissues (Fig. 7). In a replicate experiment with slightly older axes, the critical MC values were 4.1 g g^{-1} and 3.55 g g^{-1} for control and treated samples, respectively.

Discussion

In the present study, we investigated the effects of two environmental factors on the expression of desiccation sensitivity in bean and maize, two species having a comparable desiccation tolerance pattern during germination (Fig. 1B, Tab. 1). The accumulation or build up of a stable free radical during drying is considered to be an end product of degradative free-radical processes induced during drying (Atherton et al. 1993, Hendry et al. 1994). Furthermore, its correlation with desiccation sensitivity has been demonstrated in various plant systems by using different conditions of drying (seeds, Hendry et al. 1992, Leprince et al. 1990; sub-cellular extracts, Leprince et al. 1994; mosses, Seel et al. 1992; senescent leaves, Atherton et al. 1993). The accumulation of this stable radical appears to be dependent on extremes of temperature and O_2 concentrations. We therefore suggest that the measurement of its concentration in dried tissues can be used as a qualitative measurement of desiccation sensitivity. Sensitivity to desiccation in orthodox and recalcitrant seed tissues can also be quantitatively expressed in terms of a critical MC determined by a leakage assay (Berjak et al. 1993, Pammenter et al. 1991, 1993, Sun and Leopold, 1993, Vertucci et al. 1993). Under our experimental conditions, desiccation-intolerant bean axes exhibited critical MC ranging from 2 to 5 times higher (Figs 3 and 4) than values reported for either developing orthodox or recalcitrant seeds (Pammenter et al. 1991, 1993, Vertucci and Farrant 1994), indicating that our material was considerably more sensitive to desiccation than those reported in the literature (Pammenter et al. 1993, Vertucci and Farrant 1994).

There are several reasons for the existence of a critical MC that is associated with loss of viability during drying. It has been suggested that the loss of "structural" water is associated with desiccation damage and the expression of a critical MC (Finch-Savage 1992, Pammenter et al. 1991). However, at the critical MC reported here, water behaves as a dilute solution (Vertucci and Farrant 1994), and thus it is unlikely that desiccation damage in our system is related to a loss of "structural" water. Leakage of cellular constituents is generally attributed to changes in the membrane semi-permeability. The origin of such changes has been ascribed to lipid phase separation or transitions (Crowe et al. 1992) and/or alterations in their composition due to free-radical degradative reactions (McKersie 1991, McKersie et al. 1989, Senaratna et al. 1987). We do not believe that the increases in electrolyte leakage from axes dried below the critical MC resulted from a direct effect of desiccation on membrane phase behavior. The critical MC values that we observed here were higher than expected for this mechanism to be operative. In addition, we observed that low temperature lessened the extent of desiccation damage (Fig. 3) which is the opposite of what would be predicted from the membrane phase transition hypothesis.

Several lines of evidence, reviewed by McKersie (1991) and Leprince et al. (1993), suggest that membrane alterations result from highly reactive free radicals in the form of activated oxygen produced during desiccation. Extensive leakage from various plant cells and liposomes has been correlated with the appearance of large amounts of peroxidation products (Crowe et al. 1989, McKersie et al. 1989, van Bilsen and Hoekstra 1993). Based on these observations, we suggest that the critical MC is linked to membrane damage during drying via a free-radical mechanism of injury. Similarities in the responses of the critical MC and build-up of stable free radicals to temperature (Figs 3 and 5A) and O_2 concentrations (Figs 5A and 6, Tab. 1) support this hypothesis. Although measurements of markers of oxidative damage (i.e. leakage rates and build up of free radicals) can be used to assess desiccation sensitivity, the mechanisms that initiate the free-radical generation and that lead to their expression following drying are unknown. The sensitivity of both symptoms of oxidative injury to O_2 supports the hypothesis that the transient formation of activated oxygen is directly implicated in the free-radical-induced desiccation sensitivity (Leprince et al. 1990, 1994, McKersie 1991). The influence of O_2 on desiccation-induced damage is likely to be complex. The lack of O_2 supply during drying may decrease respiration rates and provide less substrate for the peroxidative reactions to ravage cellular membranes.

A role for metabolism in the expression of desiccation damage has been suggested in other studies based on ultrastructural observations (Berjak et al. 1993). We previously showed that the source of the stable free radical studied here lies, at least in part, in the mitochondria, and its generation during drying probably arises from the impairment of electron transport within the respiratory electron transport chains (Leprince et al. 1994). In addition, we observed that the rate of respiration is associated with increased peroxidative injury and loss of desiccation tolerance (Leprince et al. 1992, 1994). Given that metabolic rates are dependent on temperature, the highly significant correlation between temperature and critical MC (Fig. 4) strongly supports our previous observations. Moreover, both the build up of the stable radical (Leprince et al. 1994) and the leakage rates (Fig. 7) were significantly reduced when the cytochrome pathway was inhibited by 0.5 m*M* KCN prior to desiccation. Therefore, we conclude that a high and unabated metabolic activity during drying influences the susceptibility to desiccation damage as previously suggested (Hendry et al. 1994, Leprince et al 1992, 1994). It follows logically that conditions that lessen the generation of free radicals as a result of metabolism will also reduce the incidence of desiccation damage.

Factors known to influence the critical MC in both developing recalcitrant and orthodox seeds are drying rates and developmental status (Berjak et al. 1993, Pammenter 1991, Sun and Leopold 1993, Vertucci et al. 1993). Additional factors have been identified in this study, namely temperature and O_2 concentrations. This indicates that the expression of desiccation sensitivity in seeds is a multifactorial feature in which environmental conditions and metabolism play a distinct role. The presence of these interacting factors might also explain the lack of significant correlations between levels of desiccation tolerance and protecting components (Blackman et al. 1992, Farrant et al. 1993, Ooms et al. 1994, Still et al. 1994). In this respect, Crowe et al. (1989) reported that dried liposomes which were prepared from phospholipids mixed with free fatty acids, a product of free-radical attack on membranes, could not be protected by sugars, regardless of their concentration. It follows that free-radical processes that occur in highly desiccation-sensitive tissues during the first stages of desiccation could jeopardize the effects of protective substances at lower MC.

We conclude that the expression of desiccation damage depends on the conditions of drying and the metabolic status. Free radical reactions occur as a consequence of metabolism. Environmental factors that limit rates of metabolism also reduce the incidence of desiccation damage. Tissues that are highly sensitive to desiccation can be characterized by their inability to switch off their metabolism or by incomplete free-radical scavenging systems and consequently by a prolonged exposure to free radicals.

Acknowledgment – G. A. F. H. acknowledges the Natural Environment Research Council for support.

References

Atherton, N. M., Hendry, G. A. F., Mobius, K., Rohrer, M. & Torring, J. 1993. On the identity of a free radical ubiquitously associated with senescence in plants. – Free Radic Res. Commun. 19: 297–301.

Berjak, P., Pammenter, N. W. & Vertucci, C. W. 1992. Homoiohydrous (recalcitrant) seeds: developmental status, desiccation sensitivity and the state of water in axes of *Landolphia kirkii* Dyer. – Planta 186: 249–261.

– , Vertucci, C. W. & Pammenter, N. M. 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.

Blackman, S. A., Wettlaufer, S. H., Obendorf, R. L. & Leopold, A. C. 1992. Maturation proteins associated with desiccation tolerance in soybean. – Plant Physiol. 96: 868–874.

Crowe, J. H., McKersie, B. D. & Crowe, L. M. 1989. Effects of free fatty acids and transition temperature on the stability of dry liposomes. – Biochim. Biophys. Acta 979: 7–10.

– , Hoekstra, F. A. & Crowe, L. M. 1992. Anhydrobiosis. – Annu. Rev. Physiol. 54: 579–599.

Farrant, J. M., Pammenter, N. W. & Berjak, P. 1993. Seed development in relation to desiccation tolerance: a comparison between desiccation-sensitive (recalcitrant) seeds of *Avicennia marina* and desiccation-tolerant types. – Seed Sci. Res. 3: 1–13.

Finch-Savage, W. E. 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.

– & Blake, P. S. 1994. Indeterminate development in desiccation-sensitive seeds of *Quercus robur* L. – Seed Sci. Res. 4: 127–133.

Hendry, G. A. F. 1993. Oxygen, free-radical processes and seed longevity. – Seed Sci. Res. 3: 141–153.

– , Finch-Savage, W. E., Thorpe, P. C., Atherton, N. M., Buckland, S. M., Nilsson, K. A. & Seel, W. A. 1992. Free-radical processes and loss of seed viability during drying in the recalcitrant species *Quercus robur* L. – New Phytol. 122: 273–279.

– , Atherton, N. M., Seel, W. E. & Leprince, O. 1994. The occurrence of a stable quinone radical accumulating in vivo during natural and induced senescence in a range of plants. – Proc. R. Soc. Edinburgh 102B: 501–503.

Leprince, O., Deltour, R., Thorpe, P. C., Atherton, N. M. & Hendry, G. A. F. 1990. The role of free radicals and radical processing systems in loss of desiccation tolerance in germinating maize (*Zea mays* L.). – New Phytol. 116: 573–580.

– , van der Werf, A., Deltour, R. & Lambers, H. 1992. Respiratory pathways in germinating maize radicles correlated with desiccation tolerance and soluble sugars. – Physiol. Plant. 85: 581–588.

– , Hendry, G. A. F. & McKersie, B. D. 1993. The mechanisms of desiccation tolerance in developing seeds. – Seed Sci. Res. 3: 231–246.

– , Atherton, N. M., Deltour, R. & Hendry, G. A. F. 1994. The involvement of respiration in free-radical processes during loss of desiccation tolerance in germinating *Zea mays* L. An electron paramagnetic resonance study. – Plant Physiol. 104: 1333–1339.

McKersie, B. D. 1991. The role of oxygen free radicals in mediating freezing and desiccation stress in plants. – *In* Active Oxygen/Oxidative Stress and Plant Metabolism (E. J. Pell and K. Steffen, eds), pp. 107–118. American Society of Plant Physiologists, Rockville, MD. ISBN 0-943088-22-5.

– , Crowe, J. H. & Crowe, L. M. 1989. Free fatty acid effects on leakage, phase properties and fusion of fully hydrated model membranes. – Biochim. Biophys. Acta 982: 156–160.

Navari-Izzo, F., Pinzino, C., Quatarcci, M. F. & Sgherri, C. L. M.

1994. Intracellular membranes: kinetics of superoxide production and changes in thylakoids of resurrection plants upon dehydration and rehydration. – Proc. R. Soc. Edinburgh 102B: 187–192.

Ooms, J.J.J., Wilmer, J.A. & Karssen, C.M. 1994. Carbohydrates are not the sole factor determining desiccation tolerance in seeds of *Arabidopsis thaliana*. – Physiol. Plant. 90: 431–436.

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

– , Vertucci, C.W. & Berjak, P. 1993. Responses to dehydration in relation ton non-freezable water in desiccation-sensitive and -tolerant seeds. – *In* Basic and Applied Aspects of Seed Biology, Fourth International Workshop on Seeds (D. Côme and F. Corbineau, eds), pp. 867–872. ASFIS, Paris. ISBN 2-9507351-4-2.

Puntarulo, S., Galleano, M., Sanchez, R.A. & Boveris, A. 1991. Superoxide anion and hydrogen peroxide metabolism in soybean embryonic axes during germination. – Biochim. Biophys. Acta 1074: 277–283.

Seel, W.E., Hendry, G.A.F. & Lee, J.A. 1992. Effects of desiccation on some activated oxygen processing enzymes and anti-oxidants in mosses. – J. Exp. Bot. 43: 1031–1037.

Senaratna, T., McKersie, B.D. & Borochov, A. 1987. Desiccation and free-radical mediated changes in plant membranes. – J. Exp. Bot. 38: 2005–2014.

Still, D.W., Kovach, D.A. & Bradford, K.J. 1994. Development of desiccation tolerance during embryogenesis in rice (*Oryza sativa*) and wild rice (*Zizania palustris*). Dehydrin expression, abscisic acid content and sucrose accumulation. – Plant Physiol. 104: 431–438.

Sun, W.Q. & Leopold, A.C. 1993. Acquisition of desiccation tolerance in soybeans. – Physiol. Plant. 87: 403–409.

van Bilsen, D.G.J.L. & Hoekstra, F.L. 1993. Decreased membrane integrity in aging *Typha latifolia* L. pollen. Accumulation of lysolipids and free fatty acids. – Plant Physiol. 101: 675–682.

Vertucci, C.W. & Farrant, J.M. 1994. Acquisition and loss of desiccation tolerance. – *In* Seed Development and Germination (J. Kigel and G. Galeli, eds), pp. 237–271. Marcel Dekker, New York, NY. ISBN 0-8247-9229-7.

– , Farrant, J.M. & Crane, J. 1993. The status of and requirement for water in developing bean seeds. – *In* Plant Response to Cellular Dehydration during Environmental Stress (T.J. Close and E.A. Bray, eds), pp. 259–260. American Society of Plant Physiologists, Rockville, MD. ISBN 0-943088-26-7.

Edited by T. M. Murphy