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# Drought and Desiccation-Tolerance and Sensitivity in Plants

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## 1. Introduction

As sessile organisms, plants encounter a plethora of stresses in their natural environment (reviewed by Janská *et al.*, 2010). They must withstand these stressors to survive. Stresses are abiotic or environmental and biotic. Environmental stressors include water, temperature, salt or salinity, light and metal ions. Water stress consists of both low – osmotic stress or water deficit: drought and drying or dehydration or desiccation (relative humidity [RH] < 100%) and high (RH = 100%) – flood and imbibition or rehydration - water stress. Salinity and water stress are closely related. Whereas high salinity or hyperosmolality is desiccating, low salinity and/or hypo-osmotic shock promotes hydration. Similarly, temperature stress comprises low – chilling/cold (0 °C > T° < 25 °C), freezing (-40 °C < T° ≤ 0 °C) and cooling (T° < original temperature [usually < -40 °]) and high – warming or thawing (T° > original temperature [usually > -196 or -80 °C]), heat (25 °C ≤ T° 50 °C) and heat shock (T° > 50 °C) – temperature stress as are light and metal ions stressors. Biotic stresses comprise micro-organisms – viruses, bacteria and fungi, insects and herbivores.

Desiccation-tolerance is not synonymous with drought tolerance (Alpert, 2005)! On one hand, desiccation-tolerance of an organism is defined as the ability of a living structure to survive drying to equilibrium with low (< 50%) RH and maintain low intracellular water concentrations (WCs). Drought tolerance (*sensu stricto*) is survival of low environmental water availability while maintaining high internal water contents (WCs), on the other. A drought-tolerant organism that is not desiccation-tolerant will die if it loses much of its water, whereas a desiccation-tolerant organism will survive under the same conditions. Thus, *desiccation-tolerance is one mechanism of drought tolerance*!

Desiccation-tolerance is generally understood to pertain to organisms that will survive dehydration to an overall WC equal to, or less than, 0.1 g (water) per g dry mass (g g$^{-1}$) (Berjak, 2006). Anhydrobiosis is commonly used synonymously with desiccation-tolerance in literature but Berjak (2006) cautions against this habit as anhydrobiosis implies complete absence of intracellular water which is not the case at WCs of around 0.1 g g$^{-1}$.

Intracellular WCs of ≤ 0.1 g g$^{-1}$ are considered to represent levels at which macromolecules can no longer be surrounded by a water monolayer, thereby precluding enzymatic activity and, thus, all metabolism (Billi and Potts, 2002). Unique water properties pertain at such low WCs. In fact, water should be viewed not only as an intracellular medium but also as a structural component of macromolecules, such as proteins, which were water to be completely removed, are likely to undergo conformational changes (Billi and Potts, 2002). In solution, proteins are held to exclude small molecules from their immediate vicinity, thus being surrounded by solute-free water (Timasheff, 1982, Parsegian, 2002).

Upon extreme dehydration, but assuming a residual layer of water remains in close association with proteins, small solutes – which must include inorganic ions – are likely to perturb the residual water, where their localised effects could contribute to macromolecule denaturation (Berjak, 2006). This phenomenon may well impose one constraint on biological material being able to survive completely anhydrous conditions, thus imposing a limitation on absolute desiccation tolerance (Berjak, 2006).

## 2. Drought tolerance

Environmental variables, especially those affecting water vailability and temperature, are the major determinants of plant growth and development (reviewed by Janská *et al.*, 2010). Drought is undoubtedly one of the prime abiotic stresses (reviewed by Ashraf, 2010). Crop yield losses due to drought stress are considrable.

Mechanisms of drought tolerance occur at four levels (reviewed by Ashraf, 2010). They include genetic fectors which underlie morphological adaptations, physiological acclimation and cellular adjustments. Morphological adaptations consists of improved root length and thickness, thick and/or waxy coverings of leaves, lower leaf size and weight, higher green leaf area, delayed leaf senescence and smaller epithelial cells. Physiological acclimation comprises higher stomatal conductance and density, slower rates of transpiration, early and reduced asynchrony between male and female flowering and maturation and better production, assimilation, accumulation and partitioning of biomass and seed yield. Cellular adjustments entail higher chlorophyll content and  particle numbers or harvest index, lower osmotic potential and mechanisms of desiccation-tolerance (see below).

## 3. Desiccation-tolerance

Desiccation-tolerence is rare but universal (Alpert, 2005)! There may be considerable commonality among the mechanisms and processes facilitating desiccation-tolerance across the spectrum of organisms that show this trait (Berjak, 2006).

### 3.1 Occurrence

Desiccation-tolerance occurs in organisms or life stages of species or taxa of higher – 'resurrection plants' and 'orthodox seeds' – and lower – mosses – plants, animals – nematodes and bdelloid rotifers and microorganisms - terrestrial micro-algae, lichens: symbionts of fungi (mycobiont) and algae or cyanobacteria (photobiont), bacteria and yeast (Berjak, 2006).

'Resurrection plants', so far known to be constituted of 330 species, have been described from nine pteridophyte and ten angiosperm families (Proctor and Pence, 2002). Their

vegetative tissues are characterised by desiccation-tolerance. The plants concerned are able to remain viable despite considerable dehydration, resuming metabolic activity when water becomes available. Desiccation-tolerance in angiospermous resurrection plants, like orthodox seeds, is based on a spectrum of mechanisms that accompany drying (Illing *et al.*, 2005). Developing 'orthodox seeds' acquire the ability to tolerate desiccation relatively early, preceding the final developmental phase of maturation drying on the parent plant by some time (Bewley and Black, 1994; Vertucci and Farrant, 1995 ; Kermode and Finch-Savage, 2002).

Although desiccation-tolerance appears to be far more restricted across the spectrum of animals than plants (Alpert, 2005), the phenomenon has been documented for nematodes (Solomon *et al.*, 2000 ; Browne *et al.*, 2002) and bdelloid rotifers (Laprinski and Turnacliffe, 2003 ; Caprioli *et al.*, 2004), for example, and, classically, for the encysted embryos of the brine shrimp, *Artemia* spp. (Clegg, 1986, 2005).

Lichens represent a symbiosis between a fungus – the mycobiont and a green alga or cyanobacterium - the photobiont. The remarkable outcome of the symbiosis is that neither of the partners remains constrained to the cryptic habitats that would be obligatory for either one alone (Kranner *et al.*, 2005).

Prokaryotes in soils, which might periodically become very dry, need to be able to protect against the consequences of dehydration (Billi and Potts, 2002). In addition, survival of bacteria in the dry state is  important in health issues (Berjak, 2006).

### 3.2 Mechanisms

Bewley (1979) pioneered the idea that desiccation-tolerance may be protoplasmic in a landmark article! The trait of desiccation-tolerance is an outcome of the interaction of a spectrum of phenomena and properties (Pammenter and Berjak, 1999) described as intrinsic cell characteristics (Walters *et al.*, 2005a). However, the degree of expression of these characteristics and, indeed, whether all or some are present underlie the differences in responses to drying between desiccation-tolerant and variously desiccation-sensitive organisms (Pammenter and Berjak, 1999). The modes and interaction of the operation of the protective mechanisms and processes of desiccation-tolerance remain largely conjectural (Berjak *et al.*, 2007; Berjak and Pammenter, 2008). Berjak (2006) stresses the point that desiccation-tolerance involves not only the facility to survive extreme water loss, but also the ability to survive for prolonged periods in the dehydrated state.

### 3.2.1 Intracellular physical characteristics

The first set of major components of the suite of mechanisms of desiccation-tolerance involves intracellular physical features (Pammenter and Berjak, 1999). It was first demonstarted by Berjak and co-workers (Berjak *et* al., 1984, 1989). They include minimization of vacuolation, protection of (the integrity of) DNA and orderly dismantling of cytoskeletal elements.

Both 'orthodox and recalcitrant seeds' deal with the problem of volume reduction by the accumulation of space-filling insoluble reserves (Berjak and Pammenter, 2008). There is the notable exception of 'highly-recalcitrant propagules' of *Avicennia marina*.

Boubriak *et al.* (2000) found DNA to be severely damaged after slight drying in embryos of *A. marina*. This event is followed by the inability for its repair after loss of 22 % of the water present at shedding. More recently, studies have shown putative role of helicases in plant abiotic stress tolerance (Owttrim, 2006; Vashisht and Tuteja, 2006).

With respect to the cytoskeleton, Faria *et al.* (2005) used an α-tubulin antibody in an immunocytochemical (ICC) assay to show that only disassociated tubulin granules were present in the radicle cells in the dry state of 'orthodox seeds' of *Medicago truncutula*. In contrast, well-established and extensive cortical arrays of microtubules were present in germinating *M. truncutula* and 'recalcitrant' *Inga vera* seeds (Faria *et al.*, 2005 and 2004, respectively). Microtubules disassociated on drying, giving rise to tubulin granules which disappeared following further dehydration. The damaged cells appeared to have lost the capacity for microtubule reconstitution upon rehydration. These observations support earlier findings indicating failure of the reconstitution of microfilaments and, hence, a complete cytoskeleton following desiccation in embryonic axes of *Quercus robur* (Mycock *et al.*, 2000) and *Trichilia dregeana* (Gumede *et al.*, 2003).

### 3.2.2 Intracellular de-differentiation and 'metabolic repression or switch-off or shut-down'

De-differentiation and 'metabolic switch-off or shut-down' constitute the second set of characteristics of the acquisition of desiccation-tolerance in 'orthodox seeds' (Pammenter and Berjak, 1999). Comparisons with developing 'recalcitrant seeds' indicate that these phenomena do not occur, although the metabolic rate may be at its lowest at, or shortly before, the seeds are shed (Farrant *et al.*, 1997).

Rogerson and Matthews (1977) observed a sharp decline in the levels of sugars, which preceded a fall in the respiratory rate, prior to the acquisition of desiccation-tolerance in developing seeds of *Pisum sativum*. Those authors suggested that such an event facilitated desiccation-tolerance in these tissues by, presumably, obviating metabolic damage.

Furthermore, Brunori (1967) showed that cell cycling was arrested at G1 phase during maturation drying in 'orthodox seeds'. The first round of S-phase replication occurred during G2 phase following imbibition. Desiccation-tolerance is lost as soon as cells enter G2M during which mitosis takes place (Sen and Osborne, 1974).

There is only a transient cessation of DNA replication at shedding, with re-entry into the S-phase soon thereafter, in embryos of *A. marina* (Boubriak *et al.*, 2000). In addition, the 4C DNA content was found to be relatively low and constant in both shoot and root apices in *I. vera* embryos from six weeks after flowering to shedding (Faria *et al.*, 2004). In contrast to the findings for *A. marina*, it did not change significantly after 13 h of imbibition of mature seeds. Reviewing previously published information for a range of 'recalcitrant-seeds species', Faria *et al.* (2004) concluded that the majority of cells appeared to be arrested in the G1 phase of the cell cycle, thus the more vulnerable 4C phase would be avoided when the seeds are shed and at the greatest risk of drying.

### 3.2.3 Sugars and oligosaccharides

It seems invariable that sucrose and certain raffinose series oligosaccharides or galactosyl cyclitols accumulate in 'orthodox seeds' during maturation drying as demonstrated by the

pioneering studies of Leopold and co-workers and others (e. g. Blackman *et al*., 1995; Steadman *et* al., 1996; Black *et al*., 1999; reviewed by Leprince *et al*., 1993; Horowicz and Obedendorf, 1994; Obendorf, 1997). In addition, high sucrose concentrations are also common to desiccated 'resurrection plant' tissues (reviewed by Berjak *et al*., 1997).

Leopold and co-workers contend that the role of sucrose is dynamic in hindering the close approach of membranes to one another, and hence preventing their lateral proximity (Bryant *et al*., 2001, Koster and Bryant, 2005; Halperin and Koster, 2006). In this regard, it is noteworthy that membrane lateral proximity promotes phase transitions of some phospholipids and even the demixing of membrane components which is accompanied by the exclusion of integral proteins (e. g. Ntuli *et al*., 1997).

It appears that sucrose with raffinose or stachyose accumulates in the axes and cotyledons of developing 'recalcitrant seeds' (Berjak and Pammenter, 2008). 'Highly recalcitrant' embryos of *A. marina* were found to accumulate substantial amounts of sucrose and stachyose (Farrant *et al*., 1993b). In addition, sucrose accumulation accompanied dehydration in the less 'recalcitrant' *Camellia sinensis* embryonic axes (Berjak *et al*., 1989). Similarly, embryos of *Qurcus robur*, which are more desiccation-tolerant than those of the latter counterpart, accumulate sucrose and raffinose concomitant with the later stage of reserve accumulation (Finch-Savage *et al*., 1993; Finch-Savage and Blake, 1994). Furthermore, *Quercus alba* embryos have a high sucrose content (Connor and Sowa, 2003).

From a wide-ranging survey of sucrose accumulation among both 'orthodox and recalcitrant seeds', it seems that a variety of 'recalcitrant seeds' accumulate substantial quantities of sucrose relative to oligosaccharide (Steadman *et al*., 1996). However, Berjak and Pammenter (2008) argue that sucrose cannot play a part in protecting against desiccation damage as conjectured for 'orthodox seeds' as upon drying in the natural environment, 'recalcitrant seeds' would have already lost viability at WCs well in excess of those at which any benefits could be derived by the contribution of this disaccharide to the 'intracellular glass(y state)' (see below) or in counteracting lateral contact between membranes, as discussed above. It is probable that hydrolysis of sucrose affords a readily available respiratory substrate required to sustain ongoing development which grades imperceptibly into germination, followed by seedling establishment under favourable conditions in 'recalcitrant seeds' (Berjak and Pammenter, 2008).

### 3.2.4 Late embryogenesis-abundant/accumulating, (small) heat-shock proteins, oleosins and aquaporins

Galau and co-workers were the first advocates of the role of late embryogenesis-abundant/accumulating (LEA) proteins in desiccation-tolerance (Galau *et al*., 1986, 1987; Galau and Hughes, 1987). LEA proteins, together with sucrose, have been the focus of much recent attention in the context of the acquisition and retention of desiccation-tolerance in 'orthodox seeds' (reviewed by Buitink *et al*., 2002; Kermode and Finch-Savage, 2002; Berjak, 2006; Berjak *et al*., 2007; Berjak and Pammenter, 2008).

Six groups of LEA proteins have been identified on the basis of particular peptide motifs (Cumming, 1999). These proteins generally lack cysteine residues, are composed predominantly of charged and uncharged polar amino acid and, with the exception of Group 5 LEA proteins, are highly hydrophilic and heat stable.

Buitink *et al.* (2006) have demonstrated that 18 genes coding for LEA and two heat-shock proteins (HSPs) were upregulated and identified as being common to the acquisition of desiccation-tolerance in *M. truncutula* seeds. The same situation prevailed during its experimental re-imposition in the seedlings.

It has been suggested that LEA proteins of some groups could provide a protective hydration shell around intracellular structures and macromolecules while others have been hypothesized to sequester ions during dehydration and in the desiccated state (Berjak and Pammenter, 2008). This action has been attributed to their hydrophilicity.

It has also been proposed that the lysine-rich K segment of Group 2 LEA proteins, dehydrins, might stabilize hydrophobic domains of other proteins which could become exposed as dehydration proceeds (Close, 1997). Such activity is ascribed to the propensity of dehydrins to form α-helices (Close, 1996). Such interactions could counteract inappropriate intermolecular hydrophobic associations (Cuming, 1999). A similar function has been suggested for small HSPs (sHSPs) (reveiwed by Buitink *et al.*, 2002; Berjak *et al.*, 2007).

What is especially significant in terms of desiccation-tolerance is that dehydration, particularly in the presence of sucrose, induces at least some LEA proteins to assume α-helical conformation (Wolkers *et al.*, 2001). Such conformational change is suggested to be the basis of the formation and maintenance of the intracellular 'glass(y state)' (Berjak, 2006, Berjak *et al.*, 2007, Berjak and Pammenter, 2008). An additional feature linking LEA proteins to desiccation-tolerance is their concomitant appearance with abscisic acid (ABA) regulation of *lea* gene transcription (reviewed by Bray, 1993; Kermode, 1990, 1995; Cuming, 1999; Kermode and Finch-Savage, 2002; Berjak, 2007).

Berjak and Pammenter contend that although the evidence for LEA protein involvement in desiccation-tolerance is 'correlative and circumstantial rather than by direct experimental demonstration' (Cumming, 1999), it is compelling and, indeed, convincing! The appearance of LEA proteins is associated with 'orthodox seed' maturation, as it is with the imposition of a variety of stresses causing water deficits in plant cells (Cumming, 1999).

The situation regarding the occurrence of LEA proteins in 'recalcitrant seeds' is equivocal (Berjak and Pammenter, 2008). They occur in a range of species from different habitats while apparently being absent from others. Group 2 LEA proteins, dehydrins, have been identified in 'recalcitrant seeds' of some temperate trees (Finch-Savage *et al.*, 1994; Gee *et al.*, 1994), other temperate species and some of (sub-)tropical provenance (Farrant *et al.*, 1996) and in grasses typified by *Portersia coarctata*, *Zizania* spp. and *Spartina anglica* (Gee *et al.*, 1994). However, no dehydrin-type LEA proteins could be found in seeds of ten tropical wetland species (Farrant *et al.*, 1996).

Berjak and Pammenter (2008) argue that it is difficult to envisage a functional role for LEA proteins in 'recalcitrant seeds' based on the conjecture about functionality of such proteins in desiccated or desiccating 'orthodox seeds'. However, those authors maintain that the presence of LEA proteins in 'recalcitrant seeds' of particular species could facilitate more effective survival to lowered water contents following extremely rapid dehydration by flash drying necessary to enable the axes to be cryopreserved (Berjak *et al.*, 1990).

Collada *et al.* (1997) showed the abundant presence of small HSPs (sHSPs) in cotyledons of 'recalcitrant' *Castenea sativa*. Those authors thus concluded that the occurrence of sHSPs

could not be linked to desiccation-tolerance. However, a variety of unidentified sHSPs are expressed in 'recalcitrant' amaryllid embryos, most of which are amenable to cryostorage (Berjak and Pammenter, 2008).

Leprince and co-workers were the first to implicate oleosins in desiccation-tolerance (Leprince *et al.*, 1998). Oleosins in hydrated cells are held to maintain oil bodiers as discrete entities. They were suggested to be lacking in or inadequate in lipid-rich 'recalcitrant seeds'. In this regard, no oleosins were detected in the 'highly recalcitrant seeds' of the tropical species, *Theobroma cacao*. However, later work involving cloning and characterization of cDNA and peptide sequencing has shown that two oleosins are present in mature *T. cacao* seeds (Guilloteau *et al.*, 2003).

Major intrinsic proteins (MIPs) are a family of channel proteins that are mainly represented by aquaporins (APs) in plants. They are generally divided into tonoplast intrinsic proteins (TIPs) and plasmalemma intrinsic proteins (PIPs) according to their subcellular localisation (reviewed by Maurel *et al.*, 1997).

For instance, the vacuolar membrane protein, α-TIP, a water channel protein accumulates during seed maturation in parenchyma cells of seed storage organs. Synthesis of this integral membrane protein does not appear to be related, in a quantitative manner, to storage protein deposition. A role in seed desiccation, cytoplasmic osmoregulation and/or seed rehydration has been suggested (Johnson *et al.*, 1989).

The water-channel activity can be regulated by phosphorylation. The protein assembly as a 60 Å X 60 Å square in which each subunit is formed by a heart-shaped ring comprised of a-helices (Daniels *et al.*, 1999). Homologues to PIPs and TIPs are controlled by dehydration and ABA in desiccation-tolerant resurrection plant *Craterostigma plantagineum* (Mariaux *et al.*, 1998). Members of a subset of PIPs (PIPa) are regulated by ABA-dependent and ABA-independent pathways.

### 3.2.5 Intracellular 'glass(y [/vitrified] state)'

There is considerable evidence for the existence of the intracellular milieu in the 'glassy or vitrified state' in 'orthodox seeds' once sufficient water has been lost (Berjak and Pammenter, 2008). Leopold and co-workers pioneered the involvement of (the) 'glass(y state), as a consequence of a supersaturated sugar solution, in desiccation-tolerance (Koster and Leopold, 1988; Williams and Leopold, 1989).

Later, there was a realization that there are many other intracellular molecules that must contribute to 'glass' (e. g. Walters, 1998). Koster (1991) was the first to show that certain properties of model systems, constituted to simulate intracellular sugar mixtures, differed from the situation in seeds.

In addition, Oliver *et al.* (2001) suggested that LEA proteins might underlie the stability of intracellular 'glasses' in the dry state. Existing as unordered random coils in solution, LEA proteins assume a far more ordered conformation upon dehydration as demonstrated, for example, by Wolkers *et al.* (2001) for a Group 3 LEA protein from desiccation-tolerant *Typha* pollen and Boudet *et al.* (2006) for both Group 1 and Group 5 LEA proteins from *M. truncutula* seeds.

Berjak (2006) proposed that intracellular 'glasses' in dry seeds may be based on coiled LEA proteins in interaction with sucrose and the residual water. However, a sugar-based phase might occur in narrow intermembrane spaces, with LEA proteins excluded on the basis of size as suggested by Koster and Bryant (2005).

While the relative stability of intracellular 'glassy state' is held to maintain viability albeit not indefinitely in the desiccated state of 'orthodox seeds', intracellular 'glasses' just would not normally form in 'recalcitrant seeds' as they require water contents of less than 0.3 g g$^{-1}$ (Berjak and Pammenter, 2008). Water concentrations of approximately 0.3 g g$^{-1}$ coincide with a marked increase in cytomatrical or cytoplamic viscosity, indicative of 'glass formation' (Buitink and Leprince, 2004).

Under slow drying conditions which would prevail in the natural environment, 'recalcitrant seeds' die at far higher water contents (reviewed by Pammenter and Berjak, 1999; Walters *et al.*, 2002)! However, it may be possible that transient intracellular 'glasses' can be formed as a consequence of flash drying of excised embryonic axes, which is a procedure intrinsic to the cryopreservation protocol for germplasm conservation of 'recalcitrant-seeded' species (Berjak and Pammenter, 2008).

### 3.2.6 (Re)active oxygen species and free radical-processing antioxidants

The free-radical theory of ageing originated in the medical sciences more than half-a-century ago (Harman, 1956). It was later introduced into seed science when Kaloyereas (1958) suggested that lipid oxidation might underlie loss of viability in seeds.

There has been a particular focus on free radicals, (re)active oxygen species (R/AOS) and antioxidant systems implicated in the acquisition and maintenance of desiccation-tolerance in both 'orthodox seeds' and vegetative tissues of 'resurrection plants' of late (Berjak and Pammenter, 2008). One of the most intriguing aspects of ROS to have emerged recently is their dual role in intracellular signaling as well as intracellular destruction (reviewed by Laloi *et al.*, 2004; Foyer and Noctor, 2005; Suzuki and Mittler, 2006).

Free radicals and AOS are held to result from metabolic imbalance in cellular respiration and photosynthesis. For example, phosphofructokinase (PFK), a rate-limiting enzyme in glycolysis, malate dehydrogenase (MDH), a key enzyme in the tricarboxylic acid (TCA) cycle and dehydrogenases of complexes I and IV of the electron transport chain all of oxidative phosphorylation are slightly, midly and highly adversely affected by desiccation (reviewed by Côme and Corbineaeu, 1996).

AOS are formed when high-energy electrons are transferred to molecular oxygen ($O_2$). They include singlet oxygen ($^1O_2$), hydrogen peroxide ($H_2O_2$) and the superoxide ($\cdot O_2^-$) and the hydroxyl (OH·) radicals. They have long been considered toxic species that can cause damage to lipids, protein and nucleic acids (e. g. Halliwell, 1987; Fridovich, 1998; Hendry, 1993; Suzuki and Mittler, 2006). Not surprisingly, the activity of a spectrum of enzymatic and non-enzymatic antioxidants is considered to be of prime importance in quenching ROS activity.

However, ROS are now considered as secondary messengers in a diversity of signal transduction cascades in metabolically active hydrated plant tissues (Foyer and Noctor

2005). Hydrogen peroxide and the superoxide radical are singled out because of their implication in many plant developmental and growth processes. Nevertheless this role does not gainsay the vital necessity of their control by a spectrum of antioxidants (Berjak and Pammenter, 2008).

While strict control of AOS is taken for granted in hydrated cells, possession and effective operation of a suite of both enzymatic and non-enzymatic antioxidants is of prime importance during dehydration of 'orthodox seeds' and desiccation-tolerant vegetative tissues (e. g. Kranner *et al.*, 2002; reviewed by Pammenter and Berjak, 1999; Bailly, 2004; Kranner and Birtić, 2005; Berjak, 2006; Berjak *et al.*, 2007; Berjak and Pammenter, 2008). This scenario prevails in the dry state and as soon as water uptake by desiccated cells commences.

Antioxidants are either enzymic or non-enzymic. Enzymic oxidants include ascorbate free radical reductase (AFRR), ascorbate and guaicol peroxidase (A/GPO[D]), catalse (CAT), dehydroascorbate reductase (DHAR), glutathione reductase (GR) and superoxide dismutase (SOD). Non-enzmic oxidants consists of ascorbate/ic acid (AsA) (vitamin C), reduced and di-/oxidized glutathione (GSH and GSSG), retinol (vitamin A) and $\alpha$, $\beta$ and $\gamma$-tocopherol (vitamin E).

It is possible that certain antioxidants may be operative within localized regions of higher water activity within desiccated cells. As an example, 1-cys-peroxiredoxin (CPR) has been localized to nuclei in imbibed dormant barley embryos (Stacey *et al.*, 1999). It has been suggested to provide antioxidant protection to DNA.

However, it has been suggested that there are localized regions with water activity adequate to facilitate molecular mobility in the desiccated state – 'localised water pools' (Rinne *et al.*, 1999; Leubner-Metzger, 2005). Berjak (2006) argues that it is possible that CPR can function to protect the genome against ROS in desiccated seeds if some such regions occur in the mileu of the chromatin. In this respect, it should be remembered that the cysteinyl residue of CPR can be regenerated ultimately by electron donors such as thioredoxins and glutaredoxin (Dietz, 2003).

In this regard, Rinne *et al*. (1999) conjectured that enzyme activity continues to occur in dehydrin-associated areas of greater water activity in the otherwise dehydrated cells of buds. Leubner-Metzger (2005) showed localized $\beta$-1,3-glucanase activity in the inner testa to be instrumental in after-ripening in air-dry tobacco seeds. A similar argument may be advanced for the activity of other enzymic, as well as non-enzymic, antioxidants (Bailly, 2004) in localized regions of greater water activity within intracellular 'glasses' in dehydrated seeds (see above).

Unlike the situition in 'orthodox seeds' during the latter stages stages of development, metabolism is sustained at measurable levels in 'recalcitrant seeds' (Farrant et al., 1993a, b). When water is lost, and especially when dehydration proceeds slowly, metabolism is considered unbalanced. This situation can result in considerable intracellular metabolic damage and death of seed/embryos at surprisingly high WCs (Pammenter and Berjak, 1999 and Walters *et al.*, 2002; Ntuli, 2011a) (see below). Metabolic damage in 'recalcitrant seeds' is thought to be intimately associated with the generation of AOS where the intracellular antioxidant defences are inadequate to quench them.

Recent data for 'recalcitrant' *Araucaria bidwilli*, *Quecus robur*, *Trichilia dregeana* and germinating *Pisum sativum* embryos show that there is a transient increase in antioxidant activity upon initial dehydration. However, with further water loss, activity declines, accompanied by an increase in free radicals and thio-barbituric acid-reactive substances (TBARS) (Francini *et al*. 2006; Ntuli *et al*., 2011; Song et al., 2004; Ntuli, 2011b, respectively).

### 3.2.7 Osmolytes/protectants

It is now established that compatible organic solutes play a central role in plant drought tolerance (Ashraf and Foolad, 2007). Overproduction of compatible organic osmotic is one of the responses of plants exposed to osmotic stress (Serraj and Sinclair, 2002; Ashraf *et al*., 2008).

Among the many organic osmolytes known to play a substantial role in stress tolerance, glycine betaine (GB), a quaternary ammonium compound, occurs richly in response to dehydration stress (Mansour, 2000; Mohanty *et al*., 2002; Yang *et al*., 2003; Ashraf and Foolad, 2007). Choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BDH) are two key enzymes for the biosynthesis of GB in higher plants.

Proline, like GB, is also an important compatible organic osmolyte that plays a key role in stress tolerance (reviewed by Ashraf, 2010). Pyrroline-5-carboxylate synthetase (PCS) is the key enzyme for proline biosynthesis.

Trehalose, a nonreducing sugar, is also a potential organic osmoticum which has a substantial role in the protection of plants against stresses (reviewed by Ashraf, 2010). Trehalose-6-phosphate synthase (TPS) is a key enzyme involved in trehalose biosynthesis.

Mannitol, a polyol, is one of the most important osmoprotectants that play a vital role in plant stress tolerance (reviewed by Ashraf, 2010). Mannitol-1-phosphate dehydrogenase (MPD) is involved in mannitol biosynthersis.

### 3.2.8 Lipid composition

Liu *et al*. (2006) showed that the proportion of saturated fatty acids in membrane phospholipids was significantly higher in 'recalcitrant' than in 'orthodox seeds'! In addition, Nkang *et al*. (2003) found that mature seeds of *Telfairia occidentalis*, which were characterized by predominantly saturated fatty acids, increased accumulation of both mono- and polyunsaturated fatty acids when dried at 28 °C when total lipid was evaluated. In contrast, high levels of saturated fatty acids were retained and the marked decline in viability was delayed when seeds were dried at 5 °C. Interestingly, Ajayi *et al*. (2006) reported that *T. occidentalis* seeds lost viability at 6 °C within a relatively short time, suggesting chilling sensitivity, despite the retention of saturated fatty acids during desiccation at 5 °C reported by Nkang *et al*. (2003).

Lipid peroxidation has been shown to be associated with deterioration of seeds exhibiting 'intermediate' post-harvest physiology, particularly in terms of chilling sensitivity (Berjak and Pammenter, 2008). Crane *et al*. (2006) have shown that crystallization of the predominantly saturated storage lipid occurs in *Cuphea carthagenensis* seeds at both high and very low WCs after maintenance at 5 °C. Those authors showed that rehydration without a preceding melting of crystallized triglycerides was lethal. Similarly, Neya *et al*. (2004)

showed that hydrating lipid-rich 'non-orthodox seeds' of *Azadirachta indica* in warm water alleviated the effects of imbibitional stress that occurred when cold water was used.

### 3.2.9 Repair

Osborne and co-workers were the first to advocate the role of DNA repair in desiccation-tolerance (e. g. Osborne, 1983). 'Orthodox seeds' must apparently repair any damage accumulated in the dry state soon after imbibition is initiated. Repair occurs in the lag phase of water uptake before radical protrusion.

This requirement demands unimpaired operation of repair mechanisms and restitution of normal cell structure and function. Simultaneoulsy, the presence and efficient operation of appropriate antioxidants is vital (reviewed by Pammenter and Berjak, 1999).

Newly harvested *A. marina* embryos were capable of repair when DNA fragmentation was induced by radiation but this ability was increasingly compromised if embryos had first been dehydrated (Boubriak *et al.*, 2000). In addition, Connor and Sowa (2003) showed that a reversible shift occurred between the gel and liquid crystalline phases on rehydration after initial dehydration of *Q. alba* acorns form Fourier transform infra-red analyses of membrane lipids.

### 3.2.10 Endogenous amphiphilic substances

Hoekstra and co-workers pioneered the implication of amphiphilic substances in desiccation-tolerance (Golovina *et al.*, 1998). Those authors showed that dehydration of tolerant pollen and seeds has the potential to cause certain amphiphilic molecules to migrate into membranes, with migration back into the cytomatrix upon rehydration. The amphiphiles were suggested to play a role in maintaining core fluidity of membranes in the dry state. However, subsequent investigations could not confirm this phenomenon. Although the amphiphiles may fluidise membranes, correlation with desiccation-tolerance was uncertain (Goloviva and Hoekstra, 2002).

### 3.2.11 Control of acquisition and maintenance of desiccation-tolerance

The phenomenon of the control of the acquisition and maintenance of desiccation-tolerance in 'orthodox seeds' is suggested to be pre-programmed and developmentally regulated and initiated by maternal factors rather than directly via environmental signals and later to be under the control of the embryo (reviewed by Bewley and Black, 1994; Bewley, 1979, 1997). A major point of confusion is that desiccation-tolerance overlaps with other maturation processes and the development of dormancy where it occurs.

Studies on viviparous mutants and those characterized by impairment of the maturation process in *Zea mays* (*VP* series mutants) and *Arabidopsis thaliana* (*LEC1*, *LEC2*, *FUS3* and *ABI3*) have indicated both ABA-independent and ABA-dependent pathways of gene regulation (e. g. Bray, 1993; Kermode, 1990, 1995; Kermode and Finch-Savage, 2002; Bartels, 2005; Vincente-Carbajosa and Carbonero, 2005).

Current understanding of the control of seed maturation and acquisition of desiccation-tolerance in *A. thaliana* suggests that *LEC1*, *LEC2*, *FUS3* and *ABI3* are the four 'master genes'

involved, with the latter three implicated in desiccation-tolerance (To *et al.*, 2006). Another pivotal factor is ABA which, in the context of seed development, is probably best known for its role in regulating *lea* gene transcription (e. g. Bray, 1993; Kermode, 1990, 1995; Cuming, 1999; Kermode and Finch-Savage, 2002).

Furthermore, recent evidence suggests that a delicate balance between various ROS, as secondary messengers, and antioxidants may be intimately involved with seed maturation and the acquisition of desiccation-tolerance (Berjak *et al.*, 2007). In addition, there is interaction and cross-talk in the operation of the factors involved!

## 4. Desiccation-sensitivity

Besides producing short-lived seeds, many of the 'recalcitrant-seeded species' are threatened by overexploitation, indiscriminate harvesting and habitat loss (Berjak, 2005). Hence, understanding the phenomenon of 'seed recalcitrance', and consequently developing sound conservation practices for species producing such seeds, is of major scientific and practical importance (Berjak and Pammenter, 2008). Such importance is underscored in Target VIII of the Global Strategy for Plant Conservation of the Convention on Biodiversity which calls for 60% of all threatened species to be in accessible *ex situ* collections by 2010 (Berjak and Pammenter, 2008)!

'Recalcitrant seeds' remain sensitive to drying both during development and after they are shed from the parent plant. However, the range of WCs of the embryonic axes when seeds are shed varies markedly among species – 0.4 to 4.4 g g$^{-1}$ (Chin and Roberts, 1980 ; Berjak and Pammenter, 2004). Some decline in WC prior to shedding has been recorded for several temperate species (e. g. *Acer pseudoplatanus* [Hong and Ellis, 1990], *Aesculus hippocastum* [Tompsett and Pritchard, 1993] and *Quercus robur* [Finch-savage and Blake, 1994]) and some (sub-)tropical provenance (e. g. *Machilust hunbergii* [Lin and Chen, 1995]) and species (e. g. *Ekerbergia capensis* [Berjak and Pammenter, 2008]), leading to the suggestion that a measure of desiccation-tolerance might be acquired during development (Finch-Savage and Blake, 1994), with no further importation of water (Berjak and Pammenter, 2000).

Nevertheless, even for those seeds that are shed at axis WCs towards the lower end of the range, further dehydration is deleterious, indicating that at least some of the mechanisms necessary for complete desiccation-tolerance are not entrained (Berjak and Pammenter, 2008). In contrast, WCs of axes of recalcitrant seeds of most of (sub-)tropical species which have been investigated lie at the high end of the range (> 1.5 g g-1),  and the axes are damaged after only slight drying, particularly if water loss is slow (Berjak and Pammenter, 2008).  Those authors argue that this observation indicates that few, if any, of the mechanisms putatively affording 'orthodox seeds' tolerance to desiccation are operational.

The degree of 'recalcitrance' may be difficult to quantify (Pammenter *et al.*, 2002a). Nonetheless, seeds of *Avicennia marina* (Farrant *et al.*, 1993a, b) and *Hopea* species (e. g. Chin and Roberts, 1980 ; Sunilkumar and Sudhakara, 1998) are considered highly 'recalcitrant'. In this regard, studies on *Zizania* spp. indicated that, despite differences in WCs at which desiccation damage occurred in embryos at different developmental status, all equated to a common water activity value of 0.90 (Vertucci and Farrant, 1995) ! These observations may be related to a common spectrum of metabolic events that are impaired, which has been

suggested to occur in particular water potential ranges, as 'recalcitrant seeds' are dried (Vertucci and Farrant, 1995).

Until relatively recently, seed screening for non-orthodox behaviour was based initially on water content of ostensibly mature seeds at shedding, followed by ascertaining viability retention upon sequential removal of increasing proportions of tissue water (Hong and Ellis, 1996 ; International Plant Genetic Resources Insitute [IPGRI]/DFSC, 2004. However, because frequently only small numbers of recalcitrant seeds are able to be harvested at any one time, Pritchard *et al.* (2004a) developed the 100-seed test which proved to be a reliable indicator of the desiccation responses of seeds of eight tropical palm species. Berjak and Pammenter (2008), nonetheless, recommend modofcations of the IPGRI/DFSC protocol (2004) whenever seed numbers allow. The IPGRI/DFSC protocol includes an assessment of viability retention in storage at a range of temperatues. This approach is time-consuming, but give a reliable indication of whether seeds are 'orthodox', 'recalcitrant' or fall somewhere between 'orthodoxy' and 'recalcitrance'.

Nevertheless, a variety of studies (reviewed by Daws *et al.*, 2006b) have indicated that there could be a correlation among 'recalcitrance', seed characteristics and variables characterising individual habitats. Acting on these indications, and analysing several of the parameters across 104 tropical species from 37 families deriving from Panama, Daws *et al.* (2006b) developed a relaibly predicitve model based on the just two of the traits: seed mass and seed coat/coverings ratio ([SCR] i. e. the ratio of seed coverings mass to mass of the whole seed). Desiccation-sensitivity was found to be significantly related to relatively low SRCs, typified by large seed size coupled with thin coverings. The predictive value of the model was convincingly shown when it was further applied to seeds of 28 African species and ten species from Europe, showing in all cases that the prediction was in agreement with published data on responses of the seeds to drying (Berjak and Pammenter, 2008).

The original categorisation of seeds according to their post-harvest storage responses, which embodies the idea of two distinctive groupings – 'orthodox' and 'recalcitrant', was pioneered by Roberts (1973) in a landmark paper! A further category – intermediate – was later introduced (Ellis *et al.*, 1990a). Intermediate storage behaviour implies that the seeds are shed at relatively high WCs, but will withstand considerable dehydration, although not to the extent tolerated by 'orthodox seeds', especially at low temperatures.

Although categorising seeds into the three distinct categories is useful, it is noteworthy that desiccation-sensitivity can be further subcategorised into three subcategories – highly, moderately and minimally – 'recalictrant' (Farrant *et al.*,1988). Berjak and co-workers and many now favour an open-ended continuum of seed behaviour, subtended by extreme 'orthodoxy' at the one end and the highest degree of 'recalcitrance' at the other (Berjak and Pammenter, 1994, 1997a, b, 2000, 2001, 2004, 2008; Pammenter and Berjak, 1999 ; Sun, 1999 ; Kermode and Finch-Savage, 2002, Berjak, 2005, 2006).

The continuum concept accomodates the marked variability occuring both between and within species and is supported by evidence indicating that provenance has a significant effect on seed development and the degree of drying tolerated for individual species (Daws *et al.*, 2004a,b 2006a). In terms of the continuum concept, it is considered pertinent that even 'orthodox seeds' are not equally desiccation-tolerant!

## 4.1 Occurrence

Chin and Roberts (1980) published the first list of species recorded as producing 'recalcitrant seeds', and collated what was then known about their post-harvest behaviour. The species those authors listed produce seeds important in agroforestry (e. g. species of *Quercus* and *Shorea*), as crops for seed or fruit consumption (e. g. *Castanea* species and *Artocarpus heterophyllus*), or commodity production (e. g. *Elais guineensis*, *Havea brasiliensis* and *Theobroma cacao*).

Since then, the seed biology of a range of lesser known and generally (sub-)tropical tree species has been studied, revealing many more produce 'recalcitrant or otherwise non-orthodox seeds' (reviewed by Berjak and Pammenter, 2004; Sacandé *et al.*, 2004; Flynn *et al.*, 2006). In a single year, a screening programme focusing on southern African species revealed that seeds of at least 17 herbaceous geophytic amaryllids are 'recalcitrant' – which is unusual in indicating a familial trait – while seeds of a further 13 unrelated trees species have also proved 'recalcitrant' (Erdey *et al.*, 2007)!

While the majority of the species producing 'recalcitrant seeds' are endemic to the humid (sub-)tropics, such seeds are also produced by a small spectrum of mainly trees of temperate provenance, while certain dryland species (Danthu *et al.*, 2000 ; Gaméné *et al.*, 2004 ; Pritchard *et al.*, 2004b) have also been identified as being 'recalcitrant seeded'. Studies have also identified seeds of some cycads  (e. g. *Encephalartos* spp.) to be 'recalcitrant' (Woodenberg *et al.*, 2007), while Daws *et al.* (2007) reported a considerable incidence of desiccation-sensitive seeds among palms.

## 4.2 Variability

There are marked differences in the degree of drying that 'recalcitrant seeds' will tolerate, although the lowest WC survived depends on other parameteres, especially the rate at which water is lost (see below) (reviewed by Berjak and Pammenter, 2008). Comparisons of published data on individual species are often not helpful, because of the differing conditions under which dehydration was carried out.

However, a similar pattern of ultrastructural events terminating in cell lysis was recorded as occuring at markedly different WCs when 'recalcitrant seeds' of three unrelated species – a gymnosperm: *Araucaria angustifolia*, a dicotyledonous vine: *Landolphia kirkii* and a herbaceous monocot: *Scadoxus membranaceous* - were dried under identical conditions (Farrant *et al.*, 1989). Significantly, the rate at which the seeds of the three species lost water was inversely related to the WC at which viability was lost!

### 4.2.1 Genera

Differences in the crtical/lethal/lowest 'safe' WC which 'recalcitrant seeds' will withstand are not confined to disparate genera (reviewed by Berjak and Pammenter, 2008). They have also been noted for different species of individual genera.

A thought-provoking finding is that seeds of different species of a single genus may be differently categorised (reviewed by Berjak and Pammenter, 2008). For example, species of *Acer* and *Coffea* are variously categorised (Hong and Ellis, 1990 ; Eira *et al.*, 1999).

### 4.2.2 Provenance

Interestingly, recent data indicate that seeds of *A. hippocastum* and *A. pseudoplatanus* from different provenances differ in their response to dehydration (Daws *et al.*, 2004b ; 2006a, respectively).

### 4.2.3 Developmental status

While developing seeds of *Machilus thunbergii* lost viability within thirty days when dried at 73% RH and 25 °C, those that were mature were able to tolerate a 19% loss of water before germinability declined (Li and Chen, 1995). Differing degrees of desiccation sensitivity have been similarly correlated with developmental status for *Landolphia kirkii* and *Camellia sinensis* (Pammenter *et al.*, 1991; Berjak *et al.*, 1992; 1993).

It appears that the least desiccation-sensitive stage generally occurs when the metabolic rate is the lowest which usually but not invariably coincides with shedding (reviewed by Berjak and Pammenter, 2008). However, desiccation sensitivity increaeses markedly as germinative metabolism progresses (Farrant *et al.*, 1986 ; Berjak *et al.*, 1989).

### 4.2.4 Tissues

Zygotic embryonic axes and storage tissues seldom have if ever have the same WC (e. g. *Acer hippocstum* [Tompsett and Pritchard, 1993]). There is also an uneven water distribution between component tissues in *Araucaria hunsteinii* (Pritchard *et al.*, 1995).

Frequently, axes are at higher WCs, and more desiccation-sensitive than cotyledons (reviewed by Berjak and Pammenter, 2008). However, cotyledons have been reported to be more sensitive to dehydration than axes in *Castanea sativa* (Leprince *et al.*, 1999).

### 4.2.5 Season

A further contribution to the variability among seeds of individual species is that their characterisitcs differ both intra- and interseasonally (reviewed by Berjak and Pammenter, 2008). Intraseasonal variation includes differing WCs of the component tissues of ostensibly mature seeds depending on the time of harvest (reviewed by Berjak and Pammenter, 1997a,b).

An additional feature that has been consistently been observed is the poor quality of seeds produced late in the season, which are often severely fungally infected. In this regards, an enhanced rate of deterioration upon dehydration has been reported for late-harvested seeds of *Machilus kanoi* (Chien and Lin, 1997). It has also been observed that late-season fruits of *Avicennia marina* and *Syzygium cordatum* have a tendency either to abort or not abscise.

It is probable that the poor quality of late-season seeds may be explained in terms of the cumulative heat sum during development (Daws *et al.*, 2004b). Those authors monitored *Acer hippocastum* seed development along a latitudinal gradient and reported that the greater the cumulative heat sum, the more robust, further developed and less desiccation sensitive were the seeds.

Berjak and Pammenter (2008) argue that a similar interpretation for poor seed quality can be applied to fruits and seeds produced in the latter part of the season in non-equitorial zones.

Temperatures decline as the summer wanes and, accompanied by by shortening day-lengths, results in a sob-optimal heat sum to late-developing fruits. This phenomenon is proposed to influence fruit and seed development negatively, resulting in their poor quality, which includes lowered resistance to fungal establishment.

Interseasonal varation among seeds of the same spcies may be similary rationalised (Berjak and Pammenter, 2008). For example, *Camellia sinensis* seeds harvested showed axial WCs as disparate as 2.0 ± 0.3 to 4.4 ± 2.4 g g$^{-1}$ (Berjak *et al.*, 1996).

### 4.2.6 Individual seeds

There are usually marked differences in axial WCs among individual seeds (reviewed by Berjak and Pammenter, 1997a,b)! These differences persist even when seeds are harvested simultaneously.

### 4.3 Experimental conditions

A number of experimental parameters determine survival during drying, cooling, warming or thawing and imbibition or rehydration of seed tissues.They include drying rate and temperature, cooling rate and imbibition or rehydration rate and temperature.

Disparate opinions have been expressed about the effect of drying rate on the critical/lethal/lowest WC tolerated by 'recelacitrant seeds without compromising viability (reviewed by Berjak and Pammenter, 2008). However, it is apparent that the actual rates described as raspid or slow can pertain to very different time scales.

For example, rapid desiccation can be achieved in a matter of as little as fifteen minutes for some species and as much as twent-four hours for others when dehydrating excised axes by 'flash drying' (e. g. Ntuli and Pammenter, 2009). Both these examples are rapid relative to axes within whole seeds which require a matter of days to attain similarly low WCs (e. g. Pammenter *et al.*, 1998).

Zygotic embryonic axes generally but not invariably consitute a very small proportion of the total mass or volume of a 'recalcitrant seed'. When excised, they can be dried very rapidly in a laminar air-flow or using the technique of 'flash-drying' (reviewed by Pammenter et al, 2002b; Ntuli, 2011c).

Rapidly-dried axes and, occassionally, seeds will survive to far lower WCs than can be attained on slow dehydration (reviewed by Berjak and Pammenter, 2008). This phenomenon has been shown for a variey of species. Fast desiccation facilitates axis viability retention well into hydration level III and, occassionally, just into level II (Vertucci and Farrant, 1995), at which extreme, generally lethal damage is associated with slow water loss.

It is not that 'flash-drying' renders 'recalcitrant seeds' desiccation-tolerant (reviewed by Berjak and Pammenter, 2008). On the contrary, they will rapidly lose viability at ambient or refridgerator temperatures if allowed to remain at the low WCs attained. 'Flash-drying' achieves the rapid passage through the intermediate WC ranges at which aqueous-based metabolism-linked - metabolic damage - occurs.

'Recalcitrant seeds' are metabollically active and initiate germination around shedding (reviewed by Berjak and Pammenter, 2008). As a result, their developmental status is

becoming more advanced and their desiccation sensitivity is increasing. Thus, desiccation-sensivity could be increasing as drying is proceeding if germination is occurring at the same time frame as drying so reducing the water loss tolerated (Berjak *et al.*, 1984, 1989; Farrant *et al.*, 1986).

The drying rate is markedly affected by the nature of the coverings, size and developmental status (reviewed by Berjak and Pammenter, 2008). There is a lower WC limit below which 'recalcitrant seeds' will not survive. This WC is generally at or near the level at which all the remaining water is structure associated. Desiccation damage *sensu stricto* ensues when structure-associated or non-freezable water. A major differnce between orhtodx and recalcitrant seeds' is that the former can lose a considerable proportion of this water.

## 4.4 Storage

Seed storage is imperative, not only to provide good-quality planting material and feedstock from season to season in agriculture, as well as interseasonal food reserves, for food security, but also as base and active collections in the long term conserving of genetic resourses for biodiversity conservation (reviewed by Berjak and Pammenter, 2008). As long as 'orthodox seeds' are of high quality after harvest, the period for which they can be stored without deterioration is predictable under defined conditions of low temperature and RH that will maintain low WC.

Storage longevity of 'orthodox seeds' increases logarithmically with decreasing WC (Ellis and Roberts, 1980) although there appear to be limits of drying beyond which no further advantage is gained (Ellis *et al.*, 1990b) and, in fact, if exceeded, may be damaging (e.g. Walters, 1998 ; Walters and Engels, 1998 ; reviewed by Berjak, 2006). However, Berjak and Pammenter (2008) noted that there is no unanimity about this issue (Hong *et al.*, 2005). Nonetheless, 'orthodox seeds' have finite lifespans – years, decades or centuries – even under ideal conditions (Walters *et al.*, 2005b).

In contrast, 'recalcitrant seeds' are characterised by post-harvest lifespans of the order of days to months, or for temperate species, a year or two, as long as such seeds will tolerate low but not sub-zero temperatures (e. g. Chin and Roberts, 1980). The inexoreable progress of germinative metabolism – which occurs with no requirement for additional water – is one of the major factors hampering short- to medium-term storage of 'recalcitrant seeds'. The developmental status of 'recalcitrant seeds' changes rapidly after they are shed because they not only hydrated but metabolically active.

Enzymic antioxidants have been found to be inadequate in counteracting oxidative stress during storage (Tommasi *et al.*, 2006) in 'recalcitrant seeds' of *Ginkgo biloba* (Liang and Sun, 2002). The viability of the seeds at 25 °C declined from 80 to 46% between three and six months, accompanied by a decline in WC from *c.* 2.0 to 1.0 g g$^{-1}$. The seeds stored at 4 °C lost viability precipitously between six and nine months in storage during which the reduction in WC was insignificant.

It is suggested that the viability at 25°C was the direct result of metabolic damage (Pammenter and Berjak, 1999; Walters *et al.*, 2001, 2002) (see above). Generation of free radicals/ROS and accumulation of thiobarbituric acid-reactive substances (TBARS), along with the decreasing ability of antioxidants to modulate the situation, is consistent with the

water stress-induced damage in both cases. It is proposed that death occurred at 4 °C because metabolism progressed albeit slowly to the stage at which exogenous water supply was needed (e. g. Berjak *et al*., 1989; Pammenter *et al*. 1994; Ntuli, 2011d). Working with 'recalcitrant' *Acer saccharinum* seeds, Ratajczak and Pukacka (2006) concluded from changes in enzymes of the ascorbate-glutathione cycle and levels of ascorbate and glutathione that viability of stored hydrated seeds could be maintained only when a vigorous antioxidant system was operational.

### 4.4.1 Wet storage

The only way in which vigour and viability of 'recalcitrant seeds' can be maintained is to keep them under conditions not permitting water loss at the lowest temperature they will withstand and to eliminate or, at least, minimise seed-associated mycoflora (reviewed by Berjak and Pammenter, 2008). Nevertherless, storage of whole seeds is strictly short- to medium-term. This limitation is due to the fact that 'recalcitrant seeds' are metabolically active, and will progress from development into germination at shedding.

'Recalcitrant seeds' of some species are shed consireably before development is complete (reviewed by Berjak and Pammenter, 2008). For example, *Trichilia dregeana* seeds can be stored for several months at 16 °C (Goveia *et al*., 2004).

Storage longevity may be further optimised in the case of 'recalcitrant seeds' that are not chilling sensitive which would be expected for temperate species (reviewed by Berjak and Pammenter, 2008). Chilling sensitivity may be provenance related, and there appears to bedistinct genetic differences among plants from different provenances (Bharuth *et al*., 2007).

It has been sporadically suggested that lowering 'recalcitrant seed' WC to levels permitting basal metabolism but precluding germination in storage might extend their longevity (reviewed by Berjak and Pammenter, 2008). This means has proved to be deleterious to both life span and quality of seeds for a range of species (Corbineau and Côme, 1986a, b, 1988; Drew *et al*., 2000 ; reviewed by Eggers *et al*., 2007).

Not only did storage life span decline in the 'sub-imbibed condition' relative to that of seeds stored at the shedding WCs, but fungalproliferation was exacerbated. In this respect, it is noteworthy that mild desiccation stress paradoxically stimulates germination of recalcitrant seeds before the damaging effects set in (reviewed by Eggers *et al*., 2007).

Seeds will have been stimulated to entrain germinative metabolism sooner, when they are placed into storage after loss of a small proportion of the water originally present (reviewed by Eggers *et al*., 2007). Hence, they become increasingly desiccation sensitive more rapidly than if not dried. This situation results in a greater water stress and thus seed debilitastion (Pammenter *et al*., 1994), and favours more rapid fungal proliferation from seed-associated inocuulum (Calistru *et al*., 2000; Anguelova-Merhar *et al*., 2003; Dos Santos *et al*., 2006).

In this regard, 'recalcitrant seeds' appear able to elaborate antifungal enzymes and other compounds (Calistru *et al*., 2000; Anguelova-Merhar *et al*., 2003; Dos Santos *et al*., 2006). These defences become decreasingly effective during storage.

Fungicide treatment has been shown to be highly effective in extending storage life span of 'recalcitrant seeds' in hydrated storage (e. g. Sunilkumar and Sudharata, 1998 ; Calistru *et al*.,

2000). However, application of non-penetrative fungicides will be effective only in situations where the inoculum is primarily located on the seed surfaces !

### 4.4.2 Cryostorage

However effectively the storage life span of 'recalcitrant seeds' can be extended, it remains a short- to medium-term option (reviewed by Berjak and Pammenter, 2008). This situation is because of the fact that germination at the shedding WC will virtually inevitably occur!

Seedling slow growth offers an alternative to wet storage of seeds (Chin, 1996). This means of long-term conservation is less than ideal. Hence cryostorage – generally in liquid nitrogen at 196 °C or, less ideally, at below -80 ° - presently appears to offer the only option for long-term storage (reviewed by Berjak and Pammenter, 2008)!

It would be ideal if whole seeds could be cryopreserved. This event is generally not possible because 'recalcitrant seeds' of most species are large, and at high WCs when shed. As discussed above, large seeds cannot be dried rapidly, and slow dehydration to WCs commensurate with efficient cooling or freezing is lethal.

WC must be reduced to a level obviating lethal ice crystallisation during cooling for survival at ctyogenic temperatures. Successful cryopreservation of small 'non-orthodox seeds' has been achieved in caes where desiccation could be achieved rapidly (e. g. *Azadiachta indica* [Berjak and Dumet, 1996], *Warburgia salutaris* [Kioko *et al.*, 1999, 2003] and *Wasubia japonica* [Potts and Lumpkin, 2000]).

If whole seeds are optimally warmed or thawed and imbibed or rehydrated after retrieval from cryostorage, seedlings should, theoretically, be able to be generated in a greenhouse without an intervening *in vitro* stage. However, 'recalcitrant seeds' are far too large in the great majority of cases necessitating the use of the excised embryonic axes as explants for cryopresearvation.

## 5. Concluding remarks, future perspectives and prospects

Although a number of phenomena and mechanisms, as discussed above, have been implicated in the acquisition and maintenance of desiccation-tolerance, it seems likely that the picture is not yet complete according to Berjak and Pammenter (2008). This situation makes unequivocal identification of the differences underlying 'recalcitrant seed' behavior presently unattainable.

Similarly, it is not yet possible to present a coherent view integrating the control of the acquisition of desiccation-tolerance. In view of its complexity and our presently fragmentary understanding of the events at the control level, and also of the expression of the many phenomena characterising the acquisition and retention of desiccation-tolerance, it is perhaps not surprising that we have a long way to go before comprehending the basis of the 'recalcitrant condition' (Berjak and Pammenter, 2008).

The use of axes complicated the cryopreservation procedure. They may be injured on excission (e. g. Goveia *et al.*, 2004). Potentially injurious treatments are also required to eliminate seed–associated microorganism inoculum (Berjak *et al.*, 1999). In addition, the extent of 'flash-drying' and cooling rates must be determined (e. g. Wesley-Smith *et al.*,

2001a, b, 2004a, b). Furthermore, the desirability of using cryoprotectants needs to ascertained. Moreover, the *in vitro* technologyensuring excised axes will establish vigorous seedlings must be developed. Additionally, thawing and especially rehydration must be optimised (e. g. Berjak *et al.*, 1999; Berjak and Mycock, 2004). Further, the means for efficient dissemination of explants retrieved from cryostorage must be established (e. g. Peran *et al.*, 2006). A further aspect that could be profitably pursued is to induce a measure of axis desiccation and chilling tolerance prior to cryopreservation (e. g. Beardmore and Whittle, 2005).

## 6. Acknowledgements

## 7. References

Ajayi, S. A., Berjak, P., Kioko, J., Dulloo, M. E. and Vodouhe, R. S. (2006). Responses of fluted pumpkin (*Telfairia occidentalis* Hook.f.) seeds to desiccation, chilling and hydrated storage. *South African Journal of Botany* 72: 544-550.

Alpert, P. (2005). The limits and frontiers of desiccation-tolerant life. *Integrative and Comparative Biology* 45: 685-695.

Anguelova-Merhar, V. S., Calistru, C. and Berjak, P. (2003). A study of some biochemical and histopathological responses of wet-stored recalcitrant seeds of *Avicennia marina* infeceted with *Fusarium moniliforme*. *Annals of Botany* 92: 1-8.

Ashraf, M. (2010). Inducing drought tolerance in plants: recent advances. *Biotechnology Advances* 28: 169-183.

Ashraf, M. and Foolad, M. R. (2008). Role4s of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* 59: 206-216.

Ashraf, M., Atlar, H. R., Harris, P. J. C. and Kwon, T. R. (2007). Some perpective strategies for improving salt tolerance. *Advances in Agronomy* 97: 45-110.

Bailly, C. (2004). Active oxygen species and antioxidants in seed biology. *Seed Science Research* 4: 93-107.

Bartels, D. (2005). Desiccation tolerance studied in the resurrection plant *Craterostigma plantagineum*. *Integrative and Comparative Biology* 45: 696-701.

Beardmore, T. and Whittle, C. A. (2005). Induction of tolerance to desiccation and crypopreservation in silver maple (*Acer saccharinum*) embryonic axes. *Tree Physiology* 25: 965-972.

Berjak, P. (2005). Protector of the seeds: seminal reflections from southern Africa. *Science* 307: 47-49.

Berjak, P. (2006). Unifying perspectives of some mechanisms basic to desiccation tolerance across life forms. *Seed Science Research* 16: 1-15.

Berjak, P. And Dumet, D. (1996). Cryopreservation of seeds and isolated embryonic axers of neem (*Azidirachta indica*). *CryoLetters* 17: 99-104.

Berjak, P and Mycock, D. J. (2004). Calcium, with magnesium, is essential for normal seedling development from partially-dehydrated recalcitrant axes: a study on *trichilia dregeana* Sond. *Seed Science Research* 14: 217-231.

Berjak, P. and Pammenter, N. W. (1994). Recalcitrance is not an all-or-nothing situation. *Seed Science Research* 4: 263-264.

Berjak, P. and Pammenter, N. W. (1997a). Progress in the understanding and manipulation of desiccation-sensitive (recalcitrant) seeds. In: Ellis, R. M., Black, M., Murdoch, A. J. and Hong, T. D. (eds) *Basic and applied aspects of seed biology*. Kluwer Academic Press, Dordrecht. pp. 689-703.

Berjak, P. and Pammenter, N. W. (1997b). Important considerations pertaining to desiccation sensitivity of seeds. In: Edwards, D. G. W. and Naithani, S. C. (eds) *Seed and nursery technology of forest trees*. New Age International (P) Limited Publishers, New Dehli. pp. 43-69.

Berjak, P. and Pammenter, N. W. (2000). What ultrastructure has told us about recalcitrant seeds.*Revista Brasieleira de Fisiogia Vegetal* 12: 22-55.

Berjak, P. and Pammenter, N. W. (2001). Seed recalcitrance – current perspectives. *South African Journal of Botany* 67: 79-89.

Berjak, P. and Pammenter, N. W. (2004). Recalcitrant seeds. In: Benech-Arnold, R. L. and Sánchez, R. A. (Editors). *Handbook of seed physiology*: *application to agriculture*. Harworth Press, New York. pp. 305-345.

Berjak, P. and Pammenter, N. W. (2008). From *Avicennia* to *Zizania*: seed recalcitrance in perspective. *Annals of Botany* 101: 213-218.

Berjak, P., Dini, M. and Pammenter, N. W. (1984). Possible mechanisms underlying the differing dehydration responses in recalcitrant and orthodox seeds. Desiccation-associated subcellular changes in propagules of *Avicennia marina*. *Seed Science and Technology* 12: 365-384.

Berjak, P., Farrant, J. M., Mycock, D. J. and Pammenter, N. W. (1990). Recalcitrant (homoiohydrous) seeds: The enigma of their desiccation sensitivity. *Seed Science and Technology* 18: 297-310.

Berjak, P., Farrant, J. M. and Pammenter, N. W. (1989). The basis of recalcitrant seed behavior. In: Taylorson, R. B. (Editor). *Recent advances in the development of and germination of seeds*. Plenum Press, New York. pp. 89-108.

Berjak, P., Farrant, J. M. and Pammenter, N. W. (2007). Seed desiccation tolerance mechanisms. In: Jenks, M. A. and Wood, A. J. (Editors). *Plant desiccation tolerance*. Blackwell Publishing, Ames, Iowa. pp. 51-90 .

Berjak, P., Mycock, D. J., Wesley-Smith, J., Dumet, D. and Watt, M. P. (1996). Strategies for *in vitro* conservation of hydrated germplasm. In: Normah, N. M., Narimah, M. K. and Clyde, M. M. (Editors). *In vitro conservation of plant genetic resources*. Percetakan Watah Sdn. Bhd, Kuala Lumpur, Malaysia. pp. 19-52.

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

Berjak, P., Vertucci, C. W. and Pammenter, N. W. (1993). Effects of developmental status and dehydration rate on characteristics of water and desiccation sensitivity in recalcitrant seeds of *Camellia sinensis*. *Seed Scoience Research* 3: 155-166.

Berjak, P., Walker, M., Watt, M. P. and Mycock, D. J. (1999). Experimental parameters underlying failure or success in plant germplasm conservation: a case study on zygotic axes of *Quercus robur* L. *CryoLetters* 20: 251-262.

Bewley, J. D. (1979). Physiological aspects of desiccation tolerance. *Annual Review of Plant Physiology* 30: 195-238.

Bewley, J. D. (1997). Seed germination and dormancy. *Plant Cell* 30: 1055-1066.

Bewley, J. D. and Black, M. (1994). *Seed physiology of development and germination, Second Edition*. Plenum Press, New York.

Bharuth, V, Berjak, P., Pammenter, N. W. and Naidoo, T. (2007). Responses to chilling of recalcitrant seeds of *Eklebergia capensis* from different provenances. Abstracts from the Fifth International Workshop on Desiccation Tolerance and Sensitivity of Seeds and Vegetative Plant Tissues. *South African Journal of Botany* 73: 498.

Billi, D. and Potts, M. (2002). Life and death of dried prokaryotes. *Research in Microbiology* 153, 7-12.

Black, M., Corbineau, F., Gee, H. and Cóme, D. (1999). Water content, raffinose and dehydrins in the induction of desiccation tolerance in immature wheat embryos. *Plant Physiology* 120: 463-471.

Blackman, S. A., Obendorf, R. L. and Lepold, A. C. (1995). Desiccation tolerance in developing soybean seeds: the role of stress proteins. *Physiologia Plantarum* 93: 630-638.

Boubriak, I., Dini, M. Berjak, P and Osborne, D. J. (2000). Desiccation and survival in the recalcitrant seeds of *Avicennia marina*: DNA replication, DNA repair and protein synthesis. *Seed Science Research* 10: 307-315.

Boudet, J., Buitink, J., Hoekstra, F. A., Rogniaux, Larré, C., Satour, P. and Leprince, O. (2006). Comparative analysis of the heat stable proteome of radicles of *Medicago truncatula* seeds during germination identifies late embryogenesis abundant proteins associated with desiccation tolerance. *Plant Physiology* 140: 1418-1436.

Bray, E. A. (1993). Molecular responses to water deficit. *Plant Physiology* 103: 1035-1040.

Browne, J., Turnacliffe, A. and Burnell, A. (2002). Anhydrobiosis: plant desiccation gene found in a nematode. *Nature* 416: 38.

Brunori, A. (1967). A relationship between DNA synthesis and water content during ripening *Vicia faba* seeds. *Caryologia* 20: 333-338.

Bryant, G., Koster, K. L. and Wolfe, J. (2001). Membrane behavior in seeds and other systems at low water content: the various effects of solutes. *Seeds Science Research* 11: 17-25.

Buitink, J. and Leprince, O. (2004). Glass formation in plant anhydrobiotes: survival in the dry state. *Cryobiology* 48: 215-218.

Buitink, J., Hoekstra, F. A. and Leprince, O. (2002). Biochemistry and biophysics of tolerance systems. In: Black, M. and Pritchard, H. W. (Editors). *Desiccation and survival in plants: drying without dying*. CABI Publishing, Wallingford, Oxford. pp. 293-318.

Biutink, J., Leger, J. J., Guisle, I., Vu, B. L., Wuillemse, S., Lamirault, G., Le Bars, A., Le Meur, N., Becker, A., Küster, H. and Leprince, O. (2006). Transcriptome profiling uncovers metabolic and regulatory processes occurring during the transition from desiccation-sensitive to desiccation-tolerant stages of *Medicago truncatula* seed. *Plant Journal* 47: 735-750.

Calistru, C., McLean, M., Pammenter, N. W. and Berjak, P. (2000). The effects of mycofloral infection on the viability and ultrastructure of wet-stored recalcitrant seeds of *Avicennia marina* (Forssk.) Vierh. *Seed Science Research* 10: 341-353.

Caprioli, M., Katholm, A. K., Melone, G., Ramlov, H., Ricci, C. and Santo, N. (2004). Trehalose in desiccated rotifers: a comparison between a bdelloid and a monogonont species. *Comparative Bichemistry and Physiology A – Molecular and Integrative Physiology* 139: 527-532.

Chien, C. T and Lin, P. (1997). Effects of harvest date on the storability of desiccation-sensitive seeds of *Machilus kusanoi* Hay. *Seed Science and Technology* 25: 361-371.

Chin, H. F. (1996). Strategies for conservation of recalcitrant species. In: Normah, M. N., Marimah, M. K. and Clyde, M. M. (Editors). *In vitro conservation of plant genetic resources*. Percetakan watan Sdn. Bdh, Kuala Lumpur, Malaysia. pp. 203-215.

Chin, H. F. and Roberts, E. H. (1980). *Recalcitrant crop seeds*. Tropical Press Sdn Bhd, Kaula Lumpur, Malaysia.

Clegg, J. S. (1986). The physical properties and metabolic status of *Artemia* cysts at low water contents: the 'water replacement hypothesis'. In: Leopold, A. C. (Editor). *Membranes, metabolism and dry organisms*. Comstock Publishing Associates, Ithaca, New York. pp. 167-187.

Clegg, J. S. (2005). Desiccation tolerance in encysted embryos of the animal extremophile, *Artemia*. *Integrative and Comparative Biology* 45: 715-724.

Close, T. J. (1996). Dehydrins: emergence of a biochemical role for a familyof plant dehydration proteins. *Physiologia Plantarum* 97: 795-803.

Close, T. J. (1997). Dehydrins: a commonality in the response plants to dehydration and low temperature. *Physiologia Plantarum* 100: 291-296.

Collada, C., Gomez, L., Casado, R. and Aragoncillo, C. (1997). Purification and *in vitro* chaperone activity of a class I small heat shock protein abundant in recalcitrant chestnut seeds. *Plant physiology* 115: 71-77.

Côme, D and Cornineau, F. (1996). Metabolic damage related to desiccation sensitivity. In: Ouédraogo, A. S., Poulsen, K. and Stibsgaard, F. (Editors). *Intermediate/recalcitrant tropical forest tree seeds*. International for Genetic Plant Resources Insitute, Rome and DANIDA, Humlebaek. pp. 107-120.

Connor, K. F. and Sowa, S. (2003). Effects of desiccation on the physiology and biochemistry of *Quercus alba* acorns. *Tree Physiology* 23: 1147-1152.

Corbineau, F. and Côme (1986a). Experiments of germination and storage of seeds of two dipterocarps: *Shorea roxburghii* and *Hopea odorata*. *Seed Science and Technology* 14: 585-591.

Corbineau, F. and Côme (1986b). Experiments on the storage of seeds and seedlings of *Symphonia lgobulifera* L. f. (Guttiferrae). *The Malaysian Forester* 49: 371-381.

Corbineau, F. and Côme (1988). Storage of recalcitrant seeds of four tropical species. *Seed Science and Technology* 16: 97-103.

Crane, J., Kovach, D., Gardner, C. and Walters, C. (2006). Triacylglycerol phase and 'intermediate' seed storage physiology: a study of *Cuphea carthagenensis*. *Planta* 223: 1081-1089.

Cuming, A. C. (1999). LEA proteins. In: Shewry and P. R., Casey, R. (Editors). *Seed proteins*. Kluwer Academic Publishers, Dordrecht, The Netherlands. Pp. 753-779.

Danthu, P., Gueye, A., Boye, A., Bauwens, D. and Sarr, A. (2000). Seed storage behavior of four Sahelian and Sudanian tree species (*Boscia senegalensis*, *Butyrospermum parkii*, *Cordyla pinnata* and *SABA senegalensis*). *Seed Science Research* 10: 183-187.

Daniels, M. J., Chrispeels, M. J. and Yeager, M. (1999). Projection structure of a plant vacuole membrame aquaporin by electron crystallography. *Journal of Molecular Biology* 294: 1337-1349.

Daws, M. I., Gaméné, C. S., Glidewell, S. M. and Pritchard, H. W. (2004a). Seed mass varioation masks a single critical water content in recealcitrant seeds. *Seed Science Research* 14: 185-195.

Daws, M. I., Lydall, E., Chmielarz, P., Leprince, O., Matthews, S., Thamos, C. A. and Pritchard, H. W. (2004b). Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastum* across Europe. *New Phytologist* 162: 157-166.

Daws, M. I., Cleland, H., Chmielarz, P., Gorian, F., Leprince, O., Mullins, C. E., Thanos, C. A., Vandvik, V. and Pritchard, H. W. (2006a). Variable desiccation tolerance in *Acer pseudolatanus* seeds in relation to developmental conditions: a case of phenotypic recalcitrance? *Functional Plant Biology* 33: 59-66.

Daws, M. I., Garwood, N. C. and Pritchard, H. W. (2006b). Predicition of desiccation sensitivity in seeds of woody species: a probabilistic model based on two seed traits in 104 species. *Annals of Botany* 97: 667-674.

Daws, M. I., Wood, C. B., Marks, T. and Pritchard, H. W. (2007). Desiccation sensitivity in the Arecaceae: correlated and frequency. Abstracts from the Fifth International Workshop on Desiccation Tolerance and Sensitivity of Seeds and Vegetative Plant Tissues. *South African Journal of Botany* 73: 483.

Dietz, K-J. (2003). Plant peroxiredoxins. *Annual review of Plant Biology* 54: 93-107.

Dos Santos, A. L. W., Wiethölter, N. El Gueddari, N. E. and Moerschbacher, B. M. (2006). Protein expression during seed development of *Araucaria angustifolia*: transient accumulation of class IV chitinases and arabinogalactan proteins. *Physiologia Plantarum* 127: 138-148.

Drew, P. J., Pammenter, N. W. and Berjak, P. (2000). 'Sub-imbibed' storage is not an option for extending longevity of recalcitrant seeds of the tropical species, *Trichilia dregeana*. *Seed Science Research* 10: 355-363.

Eggers, S., Erdey, D., Pammenter, N. W. and Berjak, P. (2007). Storage and germination responses of recalcitrant seeds subjected to mild dehydration. In: Adkins, S. (Editor). *Seed science research: advances and applications*. CABI Publishing, Wallingford, Oxford. pp. 85-92.

Eira, M. T. S., Walters, C., Caldas, L. S., Fazuoli, L. C., Sampaio, J. B. and Dias, M. C. L. L. (1999). Tolerasnce of *Coffea* spp. Seeds to desiccation and low temperature. *Revista Brasileira de Fisiologia* 11: 97-105.

Ellis, R. H. and Roberts, E. H. (1980). Improved equations for the prediction of seed longevity. *Annals of Botany* 45: 13-30.

Ellis, R. H., Hong, T. D. and Roberts, E. H. (1990a). An intermediate category of seed storage behavior? 1. Coffee. *Journal of Experimental Botany* 41: 1167-1174.

Ellis, R. H., Hong, T. D., Roberts, E. H. and Tao, K. L. (1990b). Low-moisture-content limits to relations between seed longevity and moisture. *Annals of Botany* 65: 493-504.

Erdey, D., Sershen, Pammenter, N. W. and Berjak, P. (2007). Drying out in Africa: physical and physiological seed characteristics of of selected indigenous plant species. Abstracts

from the Fifth International Workshop on Desiccation Tolerance and Sensitivity of Seeds and Vegetative Plant Tissues. *South African Journal of Botany* 73: .

Faria, J. M. R., Van Lamme, A. A. M. and Hilhorst, H. M. W. (2004). Desiccation sensitivity and cell cycle aspects in seeds of *Inga vera* subspp. *Affinis. Seed Science Research* 14: 165-178.

Faria, J. M. R., Buitink, J., Van Lamme, A. A. M. and Hilhorst, H. M. W. (2005). Changes in DNA and microtubules during loss and re-establishment of desiccation tolerance in geminating *Medicago truncutula* seeds. *Journal of Experimental Botany* 56: 2119-2130.

Farrant, J. M., Berjak, P. and Pammenter, N. W. (1993a). Studies on the development of the desiccation-sensitive (recalcitrant) seeds of *Avicennia marina* (Forsk.) Vierh.: the acquisition of germinability and response to storage and dehydration. *Annals of Botany* 71: 405-410.

Farrant, J. M., Pammenter, N. W. and Berjak, P. (1986). The increasing desiccation sensitivity of recalcitrant *Avicennia marina* seeds with storage time. *Physiologia Plantarum* 67: 291-298.

Farrant, J. M., Pammenter, N. W. and Berjak, P. (1988). Recalcitrance - a current assessment. *Seed Science and Technology* 16: 155-166.

Farrant, J. M., Pammenter, N. W. and Berjak, P. (1989). Germination-associated events and the desiccation sensitivity of recalcitrant seeds – a study of three unrelated species. *Planta* 178: 189-198.

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

Farrant, J. M., Pammenter, N. W., Berjak, P. and Walters, C. (1996). Presence of dehydrin-like proteins and levels of abscisic acid in recalcitrant (desiccation-sensitive) seeds may be related to habitat. *Seed Science Research* 6: 175-182.

Farrant, J. M., Pammenter, N. W., Berjak, P. and Walters, C. (1997). Subcellular organization and metabolic activity during the development of seeds that attain different levels of desiccation tolerance. *Seed Science Research* 7: 135-144.

Finch-Savage, W. E. and Blake, P. S. (1994). Indeterminate development in desiccation-sensitive seeds of *Quercus robur* L. *Seed Science Research* 4: 127-133.

Finch-Savage, W. E., Pramanik, S.. and Bewley, D. (1994). The expression of dehydrin proteins in desiccation-sensitive (recalcitrant) seeds of temperate trees. *Planta* 193: 478-485.

Flynn, S., Turner, R. M. and Stuppy, H. W. (2006). Seed Information DatABAse (SID) release7-0, http://www.kew.org/data/sidOctober.

Foyer,C. H. and Noctor, G. (2005). Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell and Environment* 28: 1056-1071.

Francini, A., Galleschi, L., Saviozzi, F., Pinzino, C., Izzo, R., Sgherri, C. and Navari-Izzo, F. (2006). Enzymatic and non-enzymatic protective mechanisms in recalcitrant seeds of *Araucaria bidwilli* subjected to desiccation. *Plant Physiology and Biochemistry* 44: 556-563.

Fridovich, I. (1998). Oxygen toxicity: a radical explanation. *Journal of Experimental Biology* 201: 1203-1209.

Galau, G. A. and Hughes, D. W. (1987). Coordinate accumulation of homologous transcripts of seven cotton *lea* gene families during embryogenesis and germination. *Developmental Biology* 123: 213-231.

Galau, G. A., Bijaisorodat, N. and Hughes, D. W. (1987). Accumulation kinetics of cotton late embryogenesis-abundant mRNAs: coordinate regulation during embryogenesis and the role of abscisic acid. *Developmental Biology* 123: 198-212.

Galau, G. A., Hughes, D. W. and Dure, L. S. III (1986). Abscisic acid induction of cloned cotton late embryogenesis-abundant (*lea*) mRNAs. *Plant Molecular Biology* 123: 198-212.

Gaméné, C. S., Pritchard, H. W. and Daws, M. I. (2004). Effects of desiccation and storage on *Vitellaria paradoxa* seed viability. In: Sacandé, M., Joker, D., Dolloo, M. E. and Thomsen, K. A. (Editors). *Comparative storage of biology of tropical tree seeds*. International Plant Genetic Resources Insitute, Rome. pp. 40-56.

Gee, O. H., Probert, R. J. and Coomber, S. A. (1994). 'Dehydrin-like' proteins and desiccation tolerance in seeds. *Seed Science Research* 4: 135-141.

Golovina, E. A. and Hoekstra, F. A. (2002). Membrane behavior as influenced by parttioning of amphiphiles during drying: a compararative study on anhydrobiotic plant systems. *Comparative Biochemistry and Physiology Part A* 131: 545-558.

Golovina, E. A., Hoekstra, F. A. and Hemminga, M. A. (1998). Drying increases intracellular partitioning of amphiphilic substances into the lipid phase: impact on membrane permeability and significance for desiccation tolerance. *Plant Physiology* 118: 975-986.

Goveia, M., Kioko, J. I. and Berjak, P. (2004). Developmental staus is a critical factor in the selection of excised recalcitrant axes as explants for cryopreservation. *Seed Science Research* 14: 241-248.

Guilloteau, M., Laloi, M., Blais, D., Crouzillat, D. and McCarthy, J. (2003). Oil bodies in *Theobroma cacao* seeds: cloning and characterization of cDNA encoding the 15.8 and 16.9 kDa oleosins. *Plant Science* 164: 597-606.

Gumede, Z., Merhar, V. and Berjak, P. (2003). Effect of desiccation on the microfilament component of the cytoskeleton in zygotic embryonic axes of *Trichilia dregeana*. In: *Proceedings of the Fourth International Workshop on Desiccation Tolerance and Sensitivity of Seeds and vegetative Plant Tissues*. Blouwwaterbaai, South Africa. p. 22.

Halliwell, B. (1987). Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts. *Chemistry and Physics of Lipids* 44: 327-340).

Halperin, S. J. and Koster, K. L. (2006). Sugar effects on membrane damage during desiccation of pea embryo protoplast. *Journal of Experimental Botany* 57: 2303-2311.

Harman, D. (1956). Ageing: a theory based on free radical and radiation chemistry. *Journal of Gerontology* 11: 298-300.

Hendry, G. A. F. (1993). Oxygen and free radical processes in seed longevity. *Seed Science Research* 3: 141-153.

Hong, T. D. and Ellis, R. H. (1990). A comparison of maturation drying, germination and desiccation tolerance between developing seeds of *Acer pseudoplatanus* L. and *Acer platanoides* L. *New Phytologist* 116: 589-596.

Hong, T. D. and Ellis, R. H. (1996). *A protocol to determine seed storage behavior*. In: Engels, J. M. N. and Toll, J. (Editors). International Plant Genetic Resources Institute, Rome.

Hong, T. D., Ellis, R. H., Astley, D., Pinnegar, A. E., Groot, S. P. C. and Kraak, H. L. (2005). Survival and vigour of ultra-dry seeds after ten years of hermetic storage. *Seed Science and Technology* 33: 449-460.

Horowicz, M. and Obendorf, R. L. (1994). Seed desiccation tolerance and storability: dependence on flatulence-producing oligosaccharides and cyclitols – review and survey. *Seed Science Research* 4: 385-405.

Illing, N., Denby, K. J., Collett, H., Shen, A. and Farrant, J. M. (2005). The signature of seeds in resurrection plants: a molecular and physiological comparison of desiccation tolerance in seeds and vegetative tissues. *Integrative and Comparative Biology* 45: 771-787.

International Plant Genetic Resources Insitute/DFSC. (2004). The desiccation and storage protocol. In: Secandé, M, Jøker, D., Dulloo, M. E. and Thomsen, K. A. (Editors). *Comparative storage biology of tropical tree species*. International Plant Genetic Resources Insitute, Rome. pp. 345-351.

Janská, A., Maršík, P., Zelenková, S. and Ovesná, J. (2010). Cold stress and acclimation – what is important for metabolic adjustment? *Plant Biology* 12: 395-405.

Johnson, K. D., Herman, E M and Chrispeels, M. J. (1989). An abundant, highly conserved tonoplast protein in seeds. *Plant Physiology* 91: 1006-1013.

Kaloyereas, S. A. (1958). Rancidity as a factor in loss of viability in pine and other seeds. *Journal of Oil Chemists' Society* 35: 176-179.

Kermode, A. R. (1990). Regulatory mechanismsm involved in the transition from seed development to germination. *Critical reviews in Plant Sciences* 9: 155-195.

Kermode, A. R. (1995). Regulatory mechanismsm involved in the transition from seed development to germination: interactions between the embryo and the seed environment. In: Kigel, J. and Galili, G. (Eidtors). *Seed development and germination*. Marcel Dekker, New York. pp. 273-332.

Kermode, A. R. and Finch-Savage, W. E. (2002). Desiccation sensitivity in orthodox and recalcitrant seeds in relation to development. *Desiccation and survival in plants*: *drying without dying*. CABI Publishing, Wallingford, Oxford. pp. 149-184.

Kioko, J. I., Berjak, P., Pritchard, H. and Daws, M. (1999). Studies on the post-shedding behavior and cryopreservation of seeds of *Warburgia salutaris*, a highly endangered medicinal plant indigenous to tropical Africa. In: Marzalina, M., Khoo, K. C., Jayanthi, N., Tsan, F. Y. and Krishanpillay, B. (Editors). *Recalcitrant seeds*. CABI Publishing, Wallingford, Oxford. pp. 365-371.

Kioko, J. I., Berjak, P., and Pammenter, N. W. (2003). Responses to dehydration and cryopreservation of seeds of *warburgia salutaris*. *South African Journal of Botany*. 69: 532-539.

Koster, K. L. (1991). Glass formation and desiccation tolerance in seeds. *Plant Physiology* 96: 302-304.

Koster, K. L. and Bryant, G. (2005). Dehydration in model membranes and protoplasts: contrasting effects at low, intermediate and high hydrations. In: Chen, T. H. H., Uemura, M. and Fujikawa, S. (Editors). *Cold hardiness in plants*: *molecular genetics*, *cell biology and physiology*. CAB International, Wallingford, Oxford. pp. 219-234.

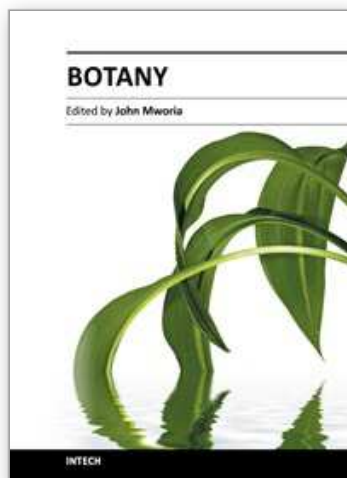Koster, K. L. and Leopold, A. C. (1988). Sugars and desiccation tolerance in seeds. *Plant Physiology* 88: 829-832.

Kranner, I. and Birtić, S. (2005). A modulating role for antioxidants in desiccation tolerance. *Integrative and Comparative Biology* 45: 734-740.

Kranner, I., Beckett, R. P., Wornik, S., Zorn, M., and Pfeifhofer, H. W. (2002). Revival of a resurrection plant correlates with its antioxidant status. *Plant Journal* 31: 13-24.

Kranner, I., Cram, W. J., Zorn, M., Wornik, S., Yoshimura, I., Stabenheimer, E. and Pfeifhofer, H. W. (2005). Antioxidant and photoprotection in a lichen as compared with its isolated symbiotic partners. *Proceedings of the National Academy of Sciences, USA* 102: 3141-3146.

Laloi, C., Apel, K. and Damon, A. (2004). Reactive oxygen signaling: the latest news. *Current Opinion in Plant Biology* 7: 323-328.

Leprince, O., Buitink, J. and Hoekstra, F. A. (1999). Axes and cotyledons of recalcitrant seeds of *Castanea sativa* Mill. exhibit contrasting responses of respiration to drying in relation to dersiccation sensitivity. *Journal of Experimental Botany* 50: 1515-1524.

Leprince, O., Hendry, G. A. F. and McKersie, B. D. (1993). The mechanisms of desiccation tolerance in developing seeds. *Seed Science Research* 3: 231-246.

Leprince, O., Van Aelst, A., Pritchard, H. W. and Murphy, D. J. (1998). Oleoosins prevent oil-body coalescence during seed imbibitions as suggested by alow-temperature scanning electron microscope styudy of desiccation-tolerant and sensitive seeds. *Planta* 204: 109-119.

Lapinski, J. and Turnacliffe, A. (2003). Anhydrobiosis without trehalose in bdelloid rotifers. *FEBS Letters* 533: 387-380.

Leubner-Mtzger, G. (2005). B-1,3-glucanase gene expression in low-hydrated seeds as a mechanism for dormancy release during tobacco after-ripening. *Plant Journal* 41: 133-145

Liang, Y. H. and Sun, W. (2002). Rate of dehydratyion and cumulative desiccation stress integrated to modulate desiccation tolerance of cocoa and ginkgo embryonic tissues. *Plant Physiology* 128: 1323-1331.

Lin, T.-P. and Chen, M-H. (1995). Biochemical characteristics associated with the development of the desiccation-sensitive seeds of *Machilus thunbergii* Sieb & Zucc. *Annals of Botany* 76: 381-387.

Liu, M.-S. Chang, C.-Y. and Lin, T.-P. (2006). Comparison of phospholipids and their fatty acids in recalcitrant and orthodox seeds. *Seed Science and Technology* 34: 443-452.

Mansour, M. M. F. (2000). Nitrogen containing compounds and adaptation of plants to salinity stress. *Biology of Plants* 43: 491-500.

Mariaux, J. B., Bockel, C., Salamini, F. and Bartels, D. (1998). Desiccation- and ABA – responsive genes encoding major intrinsic proteins (MIPs) from the resurrection plant *Craterostigma plantagineum*. *Plant Molecular Biology* 38: 1089-1099.

Maurel, C., Chrispeels, M., Lurin, C., tacnet, F., Geelen, D., Ripoche, P. and Guern, J. (1997). Function and regulation of seed aquaporins. *Journal of Experimental Botany* 48: 421-430.

Mohanty, A., Kathuria, H., Ferjani, A., Sakamoto, A., Mohanty P., Murata, N. and Tyagi, A. (2002). Trasnsgenics of an elite indica rice variety Pusa Bumati 1 harbouring the *coda* gene are highly tolerant to salt stress. *Theoretical and Applied Genetics* 106: 51-57.

Mycock, D. J., Berjak, P. and Finch-Savage, W. E. (2000). Effects of desiccation on subcellular matrix of the embryonic axes of *Quercus robur*. In: Black, M., Bradford, K. J. and

Vázquez-Ramos, J. (Editors). *Seed biology: advances and applications*. CABI Publishing, Wallingford, Oxford. pp. 197-183.

Neya, O., Golovina. E. A., Nijsse, J. and Hoekstra, F. A. (2004). Ageing increases the sensitivity of neem (*Azadirachta indica*) seeds to imbibitional stress. *Seed Science Research* 14: 205-217.

Nkang, A., Omokaro, D., Egbe, A. and Amanke, G. (2003). Variations in fatty acid proportions during desiccation of *Telfairia occidentalis* seeds harvested at physiological and agronomic maturity. *African Journal of Biotechnology* 2: 33-39.

Ntuli, T. M. (2011a). Some biochemical studies on respiratory metabolism. In: Ntuli T. M. *Aspects of the influence of drying rate and wet storage on the physiology and biochemistry of embryonic axes from desiccation-sensitive seeds*. VBM Verlag Publishing, Beau-Bassin, Mauritius. pp. 95-114.

Ntuli, T. M. (2011b). The role of free radical processes in seed deterioration. In: Ntuli T. M. *Aspects of the influence of drying rate and wet storage on the physiology and biochemistry of embryonic axes from desiccation-sensitive seeds*. VBM Verlag Publishing, Beau-Bassin, Mauritius. pp. 115-145.

Ntuli, T. M. (2011c). Aspects of water relations during desiccation and wet storage. In: Ntuli T. M. *Aspects of the influence of drying rate and wet storage on the physiology and biochemistry of embryonic axes from desiccation-sensitive seeds*. VBM Verlag Publishing, Beau-Bassin, Mauritius. pp. 56-73.

Ntuli, T. M. (2011d). Biochemical, biophysical and physiological assessment of seed quality. In: Ntuli T. M. *Aspects of the influence of drying rate and wet storage on the physiology and biochemistry of embryonic axes from desiccation-sensitive seeds*. VBM Verlag Publishing, Beau-Bassin, Mauritius. pp. 74-94.

Ntuli, T. M., and Pammenter, N. W. (2009). Dehydration kinetics of embryonic axes from desiccation-sensitive seeds: an assessment of descriptive models *Journal of Integrative Plant Biology* 51: 1002-1007.

Ntuli, T. M., Berjak, P., Pammenter, N. W. and Smith, M. T. (1997). Effects of temperature on the desiccation responses of seeds of *Zizania palustris*. *Seed Science Research* 7: 145-160.

Ntuli, T. M., Finch-Savage, W. E., Berjak, P. and Pammenter, N. W. (2011). Increased drying rate lowers the critical water content for survival in embryonic axes of English oak (*Quercus robur* L) seeds. *Journal of Integrative Plant Biology* 53: 270-280.

Obendorf, R. L. (1997). Oligosaccharides and galactosyl cyclitols in seed desiccation tolerance. *Seed Science Research* 7: 61-74.

Oliver, A. E., Leprince, O., Wolkers, W. F., Hinchta, D. K., Heyer, A/. G. and Crowe, J. H. (2001). Non-disaccharide-based mechanisms of protection during drying. *Cryobiology* 43: 151-167.

Osborne, D. J. (1983). Biochemical control of systems operating in the early hours of germination. *Canadian Journal of Botany* 61: 3568-3577.

Owttrim, C. W. (2006). RNA helicases and abiotic stress. *Nucleic Acid Research* 34: 3220-3330.

Pammenter, N. W. and Berjak, P. (1999). A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Science Research* 9: 13-37.

Pammenter, N. W., Berjak, P., Farrant, J. M., Smith, M. T. and Ross, G. (1994). Why do stored hydrated recalcitrant seeds die? *Seed Science Research* 4: 187-191.

Pammenter, N. W., Greggains, V., Kioko, J. I., Wesley-Smith, J., Berjak, P. and Finch-Savage, W. E. (1998). Effects of differential drying rates on viability retention of recalcitrant seeds of *Ekebergia capansis*. *Seed Science Research* 8: 463-471.

Pammenter, N. W., Naidoo, S. and Berjak, P. (2002a). Desiccation rate, desiccation response and damage accumulation: can desiccation sensitivity be quantified? In: Nicolás, N. Bradford, K. J., Cóme, D. and Pritchard, H. W. (Editors). *The biology of seeds – recent advances*. CABI Publishing, Wallingford, Oxford. pp. 319-325.

Pammenter, N. W., Berjak, P., Wesley-Smith, J. and Vander Willigen, C. (2002b). Experimental aspects of drying and recovery. In: Black, M. and Pritchard, H. W. (Editors). *Desiccation and survival in plants*: *drying without dying*. CABI Publishing, Wallingford, Oxford. pp. 93-110.

Parsegian, V. A. (2002). Protein-water interactions. *International Review of Cytology* 215: 1-31.

Perán, R., Berjak, P., Pammenter, N. W. and Kioko, J. I. (2006). Cryopreservation, encapsulation and promotion of shoot production of embnryonic axes of a recalcitrant species, *Ekebergia capansis* Sparrm. *CryoLetters* 27:1-12.

Potts, S. E. and Lumpkin, T. A. S. (2000). Cryopreservation of *Wasubia* spp. seeds. *CryoLetters* 18: 1-12.

Pritchard, H. W., Tompsett, P. B., Manger, K. and Smidt, W. J. (1995). The effect of moisture content on the low temperature responses of *Araucaria hunsteneii* seed and embryos. *Annals of Botany* 76: 79-88.

Pritchard, H. W., Wood, C. B., Hodges, S. and Vautier, H. J. (2004a). 100-seed test for desiccation tolerance and germination: a case study on 8 tropical palm species. *Seed Science and Technology* 32: 393-403.

Pritchard, H. W., Daws, M. I., Fletcher, B. J., Gaméné, Msanga, H. P. and Omondi, W. (2004b). Ecological correlates of seed desiccation tolerance in tropical African dryland trees. *American Journal of Botany* 91: 393-403.

Proctor, M. C. F. and Pence, V. C. (2002). Vegetative tissues: bryophytes, vascular resurrection plants and vegetative propagules. In: Black, M. and Pritchard, H. W. (Editors). *Desiccation and survival in plants*: *drying without dying*. Wallingford, CABI Publishing. pp. 207-237.

Ratajczak, E. and Pukacka, S. (2006). Changes in ascorbate-glutathione system during storage of recalcitrant seeds of *Acer saccharium* L. *Acta Societatis Botanicorum Poloniae* 75: 23-27.

Rinne, P. L H., Kaikuranta, P. L. M., Van Der Plas, L. H. W. and Van Der Schoot, C. (1999). Dehydrins in cold-acclimated apices of binch (*Betula pubescens* Ehrh.): production, localization and potential role in rescuing enzyme function during dehydration. *Planta* 209: 377-388.

Roberts, E. H. (1973). Predicting the storage life of seeds. *Seed Science and Technology* 1: 499-514.

Rogerson, N. E. and Matthews, S. (1977). Respiratory and carbohydrate changes in developing pea (*Pisum sativum*) seeds in relation to their ability to withstand desiccation. *Journal of Experimental Botany* 28: 304-313.

Sacandé, M., Jøker, D., Dulloo. M. E., Thomsen, K. A. (Editors) (2004). *Comparative storage biology of tropical tree seeds*. International Plant Genetic Resources Insitute, Rome.

Sen, S. and Osborne, D. J. (1974). Germination of rye embryos following hydrationdehydration treatments: enhancement of protein and RNA synthesis and earlier induction of DNA replication. *Journal of Experimental Botany* 25: 1010-1019.

Serraj, R. and Sinclair, T. R. (2002). Osmolyte accumulation:can it really increase crop yield under drought conditions. *Plant, Cerll and Environment* 25: 333-341.

Solomon, A., Solomn, R., Paperna, I. and Glazer, I. (2000). Desiccation stress of entomopathogenic nematodes induces the accumulation of a novel heat-stable protein. *Parasitology* 121: 409-416.

Song, S.-Q., Berjak, P. and Pammenter, N. (2004). Desiccation sensivity of *Trichilia dregeana* axes and antioxidant role of ascorbic acid. *Acta Botanica Sinica* 46: 803-810.

Stacey, R. A. P., Nordeng, T. W., Culiñáez-Maciá, F. A. and Aalen, R. B. (1999). The dormancy-related peroxiredoxins antioxidant PER1 is located to the nucleus in barley embyos and aleurone. *Plant Journal* 19: 1-8.

Sun, W. Q. (1999). Desiccation sensitivity of recalcitrant seeds and germinated orthodox seeds: can germinated orthodox seeds serve as model systems for studies on recalcitrance? In: Marzalina, M. Khoo, K. C., Jayanthi, N., Tsan, F. Y and Krishnapillay, B. (Editors). *Recalcitrant seeds*. Forest Research Insitute, Kuala Lumpur, Malaysia. pp 29-42.

Sunilkumar, K. K. and Sudhakara, K. (1998). Effects of temperature, media and fungicides on the storage behavior of *Hopea parviflora* seeds. *Seed Science and Technology* 26: 781-797.

Suzuki, N. and Mittler, R. (2006). Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. *Physiologia Plantarum* 126: 45-51.

Timasheff, S. N. (1982). Preferential interactions in protein-water co-solvent systems. In: Franks, F. and Mathias, S. F. (Editors). *Biophysics of water*. Marcel Dekker, New York. pp. 70-72.

To, A., Valon, C., Savino, G., Jocylyn, G., Devic, M. and Parcy, F. (2006). A network of local and redundant gene regulation governs *Arabidopsis* seed maturation. *Plant Cell* 18: 1642-1651.

Tommasi, F., Paciollla, C., De Pihno, M. C. and De Gara, L. (2006). Effects of storage temperature on viability, germination and antioxidant metabolism in *Ginkgo biloba* L. seeds. *Plant Physiology and Biochemistry* 44: 359-368.

Tompsett, P. B. and Pritchard, H. W. (1993). Water status changes during development in relation to the germination and desiccation tolerance of *Aesculus hippocastum* L. seeds. *Annals of Botany* 71: 107-116.

Vashisht, A. A. and Tuteja, N. (2006). Stree response to DEAD-box helicases: a new pathway to engineer plant stress tolerance.*Journal of Photochemistry and Photobiology B Biology* 84: 150-160.

Vertucci, C. W. and Farrant, J. M. (1995). Acquisition and loss of desiccation-tolerance. In: Kigel, J. and Galili, G. (Editors). *Seed development and germination*. Marcel Dekker Inc., New York. pp. 237-271.

Vincente-Carbajosa, J. and Carbanero, P. (2005). Seed maturation: developning an intrusive phase to accomplish a quiescent state. *International Journal of Devlopment* 49: 645-651.

Walters, C. (1998). Understanding the mechanisms and kinetics of seed aging. *Seed Science Research* 8: 223-244.

Walters, C. and Engels, J. (1998). The effects of storing seeds under extremely dry conditions. *Seed Science Research* 8 (Supplement Number 1): 3-8.

Walters, C., Farrant, J. M., Pammenter, N. W. and Berjak, P. (2002). Desiccation stress and damage. In: Black, M. and Pritchard, H. (eds) *Desiccation and survival in plants*: *drying without dying*. CAB International, Wallingford. pp. 263-291.

Walters, C., Hill, L. M. and Wheeler, L. M. (2005a). Drying while dry: kinetics and mechanisms of deterioration in desiccated organisms. *Integrative and Comparative Biology* 45: 751-758.

Walters, C., Pammenter, N. W., Berjak, P. and Crane, J. (2001). Desiccation damage, accelerated ageing and respiration in desiccation-tolerant and sensitive seeds. *Seed Science Research* 11: 135-148.

Walters, C., Wheeler, L. and Grotenhuis, J. M. (2005b). Longevity of seeds stored in a genebank: species characteristics. *Seed Science Research* 15: 1-20.

Wesley-Smith, J., Pammenter, N. W., Berjak, P. and Walters, C. (2001a). The effects of two drying rates on the desiccation tolerance of embryonic axes of recalcitrant jackfruit (*Artocarpus heterophyllus* Lumk.) seeds. *Annals of Botany* 88: 653-664.

Wesley-Smith, J., Walters, C., Pammenter, N. W. and Berjak, P. (2001b). Interactions of water content, rapid (non-equilibrium) cooling to -196 °C and survival of embryonic axesof *Aesculus hippocastum* L. seeds. *Cryobiology* 42: 196-206.

Wesley-Smith, J., Walters, C., Pammenter, N. W. and Berjak, P. (2004a). Non-equilibrium cooling of *Poncirus triofoliata* L. embryonic axes at various water contents. *CyroLetters* 25: 121-128.

Wesley-Smith, J., Walters, C., Pammenter, N. W. and Berjak, P. (2004b). The influence of water content, cooling and warming rates upon survival of embryonic axes of *Poncirus triofoliata*. *CyroLetters* 25: 129-138.

Williams, R. J. and Leopold, A. C. (1989). The glassy state in corn embryos. *Plant Physiology* 89: 977-981.

Wolkers, W. F., McCready, S., Brandt, W. F., Lindsey, G. G. and Hoekstra, F. A. (2001). Isolation and characterization of a D-7 LEA protein in pollen that stabilizes glasses *in vitro*. *Biochimica et Biophysica* 1544: 196-206.

Woodenberg W., Erdey, D., Pammenter, N. W. and Berjak, P. (2007). Post-shedding seed behaviour of selected *Encephalartos* species. Abstracts from the Fifth International Workshop on Desiccation Tolerance and Sensitivity of Seeds and Vegetative Plant Tissues. *South African Journal of Botany* 73: 496.

Yang, W. J., Rich, P. J., Axell, J. D., Wood, K. V., Bonham, C. C., Ejeta, G., Mickelbart, M. V., and Rhodes, D. (2003). Genotypic variation for glycine betainein sorghum. *Crop Science* 43: 162-169.

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