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REVIEWS

DESICCATION TOLERANCE IN DEVELOPING SEEDS

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Summary. This review summarizes recent studies on the mechanisms and compounds taking part in acquisition and maintenance of desiccation tolerance of seeds. The role of ABA in regulating gene expression in either developmental or stress situation is discussed. Special attention is given to LEA proteins as protectors helping seeds to tolerate desiccation at maturity as well as following imbibition and germination. The physiological significance of desiccation in redirection of primary metabolism of seeds from development to germination is emphasized. Studies on the determination of the functions of ABA-regulated gene products and on the identification of genes that contain information of desiccation (dehydration) tolerance are also reviewed. This kind of information is essential for crop breeding purposes in order to improve stress tolerance or the productivity of crop plants under field conditions.

Key words: maturation of seeds, seed desiccation, desiccation tolerance, premature drying, germination of seeds, dehydration stress

Abbreviations: ABA – abscisic acid, DAP – days after pollination, GA – gibberellic acid, LEA-proteins – late-embryogenesis-abundant proteins

Introduction

Dehydration of plant cells can be imposed environmentally (drought, cold, heat shocks) or developmentally (seed maturation). Dehydration tolerance has been investigated using three main approaches: 1) examining tolerant systems, such as seeds and resurrection plants, 2) analyzing mutants from genetic model species, 3) analyzing the effects of different kinds of stress on agriculturally relevant plants (Ingram and Bartels, 1996; Bartels et al., 1996).

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The orthodox seeds undergo maturation drying (desiccation) on the parent plants as a terminal event in their development (Black, 1991; McCarty, 1995; Kermode, 1995). During seed maturation the embryo reaches its lowest water content (as much as 90% of the original water is removed), prepares for desiccation, becomes desiccated, and enters a period of developmental and metabolic arrest (Leprince et al., 1993; Bartels et al., 1996). Recalcitrant seeds cannot sustain desiccation even when they reach maturity, and they do not survive drying which normally occurs with seed development and maturation (Chandler and Robertson, 1994). Cereal seeds are an interesting model system for investigating the mechanisms of desiccation tolerance as their embryos can sustain reductions in water content of about 80% whereas such severe desiccation kills cells from vegetative parts of the plant (Bartels et al., 1996; Jensen et al., 1996).

Seed mutants with defects in embryo maturation are of a particular interest concerning desiccation tolerance acquisition (Koornneef et al., 1989; Meinke, 1995). These seeds are desiccation intolerant at maturity because they fail to activate embryo-specific programmes later in development. The immature embryos of these seeds enter prematurely in a germination pathway (Meinke, 1992).

Acquisition of desiccation tolerance

The desiccation period is most likely initiated by the loss of vascular supply of water and assimilates from mother plant to seed as a result of funiculus detachment between 40 and 50 days after pollination (DAP) and senescence of the covering tissues (Greenwood and Bewley, 1982). The embryo of dry seeds is dormant and highly tolerant to desiccation but it cannot withstand desiccation at all developmental stages. Tolerance is usually acquired well before the maturation drying stage (the absolute time varying among species) but is lost as germination progresses (Ingram and Bartels, 1996; Blum, 1996). Seeds are desiccation-intolerant (incapable of surviving the imposed stress of premature drying) at early stages of development and a dehydration treatment (drying) at this stage does not lead to germination upon rehydration. However within a few days (4–5) the seeds acquire both a tolerance to slow desiccation and an ability to germinate upon subsequent rehydration. Probably at early stages of development seeds are not capable of recovering their full cellular and metabolic integrity following premature desiccation (Dasgupta and Yeung, 1982). In the vast majority of seeds the transition to a desiccation-tolerant state occurs approximately midway (30–40 DAP) trough their development (Kermode and Bewley, 1985 a, b; Black, 1991; Kermode, 1995).

Desiccation damage

When water leaves a cell that does not tolerate desiccation many events occur: solutes (sugars, salts, amino acids) become more concentrated, possibly increasing the

rate of destructive chemical reactions. Some solutes may crystallize, changing the ionic strength and pH of the intracellular solution, proteins become denatured irreversibly, membranes become disrupted leading to the loss of compartmentation (Koster, 1991). Dehydration stress causes also alterations in the chemical composition and physical properties of the cell wall, cell elongation stops and lignification processes begin (Peleman et al., 1989; Nonami and Boyer, 1990; Ingram and Bartels, 1996).

Mechanisms of desiccation tolerance

The ability to survive the desiccated state is a result of adaptations which prevent cellular destruction during the withdrawal of water. Well before the onset of seed desiccation a number of physiological and biochemical changes occur, that prevents the damage of cellular components by water loss (Koster, 1991). It is not yet known what critical cellular changes occur during the brief period (4–5 days) when a seed changes from being intolerant of desiccation to being tolerant. Probably during this time the initial induction of protective components occurs (McKersie and Leshem, 1994; Kermode, 1995). It has been suggested that some compounds (hormones, proteins, enzymes, sugars) or cell structures (membranes) play a key role in this transition (Williams and Leopold, 1989; Crowe et al., 1992; Leprince et al., 1993).

Protection against lethal damage in seeds is correlated with the accumulation of sugars (Amuti and Pollard, 1977; Blackman et al., 1992) and proteins, i.e. dehydrins (Bradford and Chandler, 1992; Close, 1996). Sugars may protect the cell during severe desiccation by glass formation (Williams and Leopold, 1989; Koster, 1991), by alterations in properties of cell membranes or by replacing water molecules at their binding site on the membranes (Crowe et al., 1992). The presence of disaccharides (trehalose or sucrose) have been found to help stabilization of cell membranes during drying. Sugars may contribute to the stabilization of protein structure by hydrogen bonds formed between carbohydrate hydroxyl groups and polar residues in proteins (Crowe et al., 1992). High concentrations of sugars inhibit molecular mobility and therefore restrict biochemical reactions (Williams and Leopold, 1989).

Sugar accumulation is not the only way in which plants cope with desiccation. Enzymes of sugar metabolism, e. g. sucrose-phosphate syntase, sucrose synthase are of great significance for desiccation tolerance (Ingram and Bartels, 1996).

Proteolytic enzymes with functions in degradation of abnormal proteins and in protein repair could be particularly important during desiccation, when protein turnover rates are low (Mudgett and Clarke, 1994; Ingram and Bartels, 1996).

Enzymes concerned with removal of toxic intermediates produced during oxygenic metabolism and lipide peroxydation of cell membranes (e.g. glutathione reductase, superoxide dismutase) are probably very important in dehydration tolerance. The positive regulation (up-regulation) of genes, encoding enzymes that detoxify active

oxygen species has been established under stress conditions (Ingram and Bartels, 1996).

The cell membrane is considered as a primary site of dehydration damage (Senaratna and McKersie, 1983). The changes in the structure and/or composition of cell membrane are related to the acquisition of desiccation tolerance. These changes may provide a resistance against disruption of the membrane bilayer organization (Senaratna and McKersie, 1983). High degree of unsaturation of membrane fatty acids facilitates their oxidation and leads to membrane disorder. On the other hand an increased amount of lipid soluble antioxidant may protect membrane lipides during desiccation (Senaratna et al., 1987).

Obviously the cells of orthodox seeds and desiccation tolerant plants are developed mechanisms which make them able to limit damages during drying, to maintain their physiological integrity in the dry state, and then to mobilize repair mechanisms upon rehydration (Bewley, 1979).

ABA and desiccation

In seeds, ABA influences and perhaps regulates several processes including reserve material synthesis (Quatrano, 1986), dormancy (Karssen, 1983), water loss (Koornneef et al., 1989) and desiccation tolerance acquisition (Black, 1991; Chandler and Robertson, 1994). It may modulate gene expression allowing maturation to proceed, while precocious germination is inhibited (King, 1976; Quatrano, 1986). The major action of ABA in seeds and other plant parts is the regulation of gene expression. ABA induces the synthesis of several different kinds of proteins that may play important physiological role during seed development (Skriver and Mundy, 1990; Hetherington and Quatrano, 1991; Chandler and Robertson, 1994). The most typical example for ABA-modulated gene expression are LEA genes, expressed in late maturation phase of embryos and also in plants in response to various environmental stresses (Skriver and Mundy, 1990; Close, 1996). The mRNAs encoding late-embryogenesis abundant (LEA) proteins first appear at the onset of seed desiccation in middevelopment of seeds. These LEA transcripts are abundant in dehydrated tissues and gradually fall several hours after embryos begin to imbibe water (Baker et al., 1988; Bray, 1993; Close, 1996; Ingram and Bartels, 1996). It was shown that LEA-proteins confer resistance to desiccation due to their unique chemical properties – sequestration of ions, protection of other proteins or membranes, and renaturation of unfolded proteins (Dure et al., 1989; Dure, 1993). Exogenous ABA promotes the synthesis of these proteins in early stage of immature barley embryos and at the same time also confers desiccation tolerance on them (Skriver and Mundy, 1990).

LEA genes belong to the class of genes that can express in responce to developmental and environmental stimulus (Thomas et al., 1991). It have been considered

that the signals for controlling the expression of these genes are the changes in ABA level during seed development (Hughes and Galau, 1991; Bartels et al., 1996).

The concentrations of LEA proteins in the cells are usually very high, e.g. in mature cotton embryo cells, the D7-class of LEA proteins represent about 4% of cytosolic protein content (Roberts et al., 1993). LEA proteins can help in maintaining the minimum cellular water requirement because some of them may bind water molecules; they can act as ion traps, sequestering ions that are concentrated during desiccation (Baker et al., 1988). LEA proteins may support the possible role of sugars in maintaining the structure of the cytoplasm in the absence of water. These proteins are considered as better protectants than sucrose because they cannot crystallize.

The most studied LEA proteins are dehydrins. They (a.k.a. LEA D-11 family) are heat stable, soluble, immunologically distinct proteins. They have been observed in over 100 independent studies of different kinds of stress that have a dehydrative component, embryo development and responses to exogenous ABA (Close and Chandler, 1990; Bradford and Chandler, 1992; Close, 1996). Dehydrins range in size from 9 kDa for rice (Takahashi et al., 1994) to 200 kDa for wheat (Oullet et al., 1993). Their precise role is not known, but it has been proposed that they stabilize the basic cell architecture by interaction of dehydrin α-helical domains with the surface of stabilized molecules, and interaction of hydrophilic domains with the solution (Close, 1996). A direct relationship between these proteins and desiccation tolerance has not yet been proven. Further studies have to elucidate the correlation between presence of dehydrins and ability of seeds to withstand desiccation.

Besides its function in the regulation of LEA genes expression, ABA takes part also in the inhibition of genes encoding certain reserve-mobilizing enzymes (Jacobsen and Chandler, 1987). For example, ABA participates in the suppression of α-amylase activity in developing seeds of some species, presumably by arresting enzyme synthesis (Black, 1991; Jiang et al., 1996; Chandler et al., 1997). From this point of view ABA is probably one of the main factors which is responsible for the absence of mobilizing activity in premature seed.

Understanding of the molecular mechanisms of desiccation tolerance involves the study of how ABA regulates gene expression. An important question is whether desiccation or ABA act directly on the regulatory region of genes and where the responsive sequences are located (Bartels et al., 1996).

In our opinion the following evidence from the literature can be summarized in support of the role of ABA in desiccation tolerance:

1) A good correlation between the onset of desiccation tolerance and the endogenous ABA levels has been established (King, 1982; Henson, 1984; Rock and Quatrano, 1995). During seed development ABA concentrations increase parallel to the increase in fresh weight and then decrease as the seed begins to desiccate (Zeevart and Creelman, 1988; McCarty, 1995; Quatrano et al., 1997).

2) Treatment of ABA-deficient seeds of *Arabidopsis* with ABA during their development effectivelly conferred desiccation tolerance up to 100% (Koornneef et al., 1989).

3) Excised embryos of cotton seeds incubated in ABA to prevent precocious germination, expressed LEA genes at very high levels (Ihle and Dure, 1972).

Progress in understanding the role of ABA in desiccation tolerance has been achieved by characterizing ABA-deficient or ABA-insensitive mutants (Black, 1991; Leon-Kloosterziel et al., 1996). It was shown that a strong positive correlation exists between endogenous ABA levels and desiccation tolerance in the aba/abi3 recombinants of *Arabidopsis* seeds. These seeds retain a high water content throughout development. They are desiccation intolerant and fail to produce the LEA proteins found in the wild type. These seeds can overcome desiccation intolerance by simple treatments with ABA that also restore production of LEA proteins which are present in normal seeds (Koornneef et al., 1989). Viviparous maize ABA-deficient or ABA-insensitive mutants do not dehydrate, nor do they contain LEA gene products (Kriz et al., 1990). Recalcitrant seeds which are desiccation-intolerant cannot produce LEA proteins. One of the explanations for this failure is connected with ABA deficiency, insensitivity to this hormone or absence of LEA genes.

The source of the seed's ABA is not yet clear, but it has been proposed that the embryo and endosperm can synthesize ABA (Milborrow et al., 1973; Black, 1991).

ABA is undetectable in developing seeds until about 16 days after pollination, thereafter the level steeply rises, reaches a peak around the onset of maturation (King, 1982), then disappears by full maturity (Black, 1991; Thomas, 1993; Chandler and Robertson, 1994). For example in wheat grains ABA levels increase before any appreciable water loss occurs, i.e. before seed desiccation (Milborrow, 1974; Radley, 1976). During the later stages of growth of wheat grains there is a dramatic increase (up to 40 fold) in the content of abscisic acid. This level remains high from 25 to 40 DAP. Then in association with natural or artificial drying of the grains, there is a rapid drop (5–10 fold) in the ABA content and a brief increase in the content of bound ABA (King, 1976). These high ABA levels are thought to prevent immature embryo from germination and to promote embryo tolerance to desiccation (McCarty et al., 1991; Thomas, 1993).

Data in the literature show that two mechanisms change consecutively in order to prevent premature germination of developing seeds. In immature seeds the high ABA levels prevent precocious germination. In dry seeds the desiccation plays this function. ABA and low water potentials seem to be interchangeable in their effects on seed germination control (Black, 1991).

Physiological significance of seed desiccation

The biological significance of seed desiccation at a whole plant level is to ensure species survival. The desiccated seed can successfully survive extreme environmental conditions and to achieve wide dispersal (Leprince et al., 1993; McCarty, 1995; Bartels et al., 1996).

Desiccation ensures seed dormancy even in the absence of ABA. Seed dormancy is associated with or induced by the phenomenon of drying during seed maturation (Trewavas and Jones, 1991).

Desiccation serves as the boundary between seed maturation and germination. It prepares the embryo for germination trough redirection the metabolism from a developmental to a germinative mode. Data in the literature show that this redirection of programme could also be elicited at earlier stages of development, before the seed reaches full maturity (Kermode and Bewley, 1985a, b; Kermode, 1990; Kermode, 1995). When desiccation is imposed to seeds before maturation a switch of metabolic events is elicited: synthesis unique to development is terminated, while this associated with germination and growth is initiated (Evans et al., 1975; Oishi and Bewley, 1990). This is demonstrated by a change in the pattern of protein synthesis within many seeds upon subsequent rehydration (Kermode and Bewley, 1985a, b; Kermode and Bewley, 1986; Kermode and Bewley, 1988). Besides desiccation/rehydration treatment developing seeds can be induced to germinate precociously by isolation of immature embryos from seed tissues.

Desiccation, premature or natural, is also necessary for the induction of synthesis of enzymes involved in protein breakdown and lipid utilization during the postgerminative stage of seedling development (Chandler et al., 1997). Such is the case in castor been endosperm in which premature drying induces isocitrate lyase and L-leicyll-B-naphthylamidase to levels comparable to those produced following germination of mature seeds (Kermode, 1995; Jiang et al., 1996). Adams et al. (1983) demonstrated that immature soybean seeds can produce malate synthase and isocitrate lyase activities only after they are first subjected to a slow drying regime.

Desiccation may alter the responsiveness of aleurone layer to hormones. Chandler et al. (1997) speculate that the sensitivity of the aleurone layer to GA had been changed as a result of premature drying. These authors explain the reduced capacity of aleurone layer to bind this hormone with changes in the levels, availability or conformation of hormone receptors. In many cereal seeds the sensitivity to this hormone can be induced prematurely following imposed drying (Armstrong et al., 1982; Oishi and Bewley, 1990). Similar results are reported by Adams et al. (1983) for tenfold reduction of castor been seeds responsiveness to ABA following drying.

Desiccation eliminates the influence of factors which prevent germination of seeds in two ways – through influence upon ABA level itself or decreasing the sensitivity of the embryo to ABA (Kermode and Bewley, 1986). In the first case drying may affect the hormonal balance of the seed, ABA breakdown may be promoted either by depletion of endogenous ABA pool, or by conversion of ABA to conjugated forms (Ackerson, 1984). In the second case the content or conformation of ABA receptors is probably changed, which leads to a decrease in embryo competence to hormone (Finkelstein et al., 1985).

Desiccation appears to act primarily at the transcriptional and posttranscriptional levels (mRNA processing, transcript stability, translation efficiency, and protein modification or turnover). As it regards transcription, desiccation affects an off- or negative (down-) regulation of the synthesis of developmental mRNAs and an positive (on-) regulation of mRNA synthesis associated with germination and growth (Kermode, 1990; Jiang and Kermode, 1994; Kermode, 1995). At gene level it is proposed that cis-acting promoter elements probably serve as binding sites for transcription factors and are therefore involved in stress (desiccation) specific gene activation (Bartels et al., 1996). For example, it has been found that the promoter regions of storage protein genes contain the information for their down regulation during seed desiccation (Jiang et al., 1995).

Comparisons between mechanisms underlying drought and desiccation tolerance

A relationship between mechanisms of drought tolerance of plants and desiccation tolerance of seeds has been considered. It has been proposed that plants may possess a general mechanism of resistance to any stress that results in desiccation of the protoplasm (Levitt, 1958). This hypothesis could be supported by the following evidence:

1) A close similarity exists between the effects of desiccation and dehydration (drought) on cell metabolism.
2) Several genes which are expressed during drought in vegetative tissues are also expressed during desiccation of developing seeds (Close, 1996).
3) Many genes which respond to ABA and water deficit, and are considered to function in the protection of cells from dehydration, are also expressed at the late stages of seed embryogenesis (Skriver and Mundy, 1990).
4) ABA may play similar roles during periods of environmentally and developmentally imposed water deficit.
5) Genes originally identified in drought-stressed vegetative tissue are related to the LEA genes that accumulate during the maturation stage of seeds (Ihle and Dure, 1972).

In our opinion however there are some significant differences between drought and desiccation tolerance. They can be summarize as follow:

1) Leaves, stems and roots of plants are less susceptible to large reductions in water content compared to seeds (Mullet and Whitsitt, 1996).
2) At a whole plant level, dehydration is environmentally imposed. Several mechanisms exist to avoid cell desiccation under adverse conditions which all lead to

reduced water flux through the plant or increased water uptake – reduced leaf growth, stomatal closure, increased root growth (Tardieu, 1996).

3) Tolerance to drought is rarely observed in vegetative parts of plants, while numerous other organisms such as yeast cells, bacterial and fungal spores, angiospermous seeds and pollen are able to survive extreme dehydration (Leopold et al., 1992; Bartels et al., 1996).
4) In orthodox seeds embryo desiccation is a normal part of the seed maturation process (McKersie and Leshem, 1994; Kermode, 1995; Close, 1996; Mullet and Whitsitt, 1996).
5) Several genes which show ABA-regulated expression following dehydration stress in vegetative tissues, do not seem to require ABA for expression in seeds. Probably in seeds they are under a different developmental programme independant of ABA (Chandler and Robertson, 1994). These genes induce tissue protection against severe dehydration, and are frequently expressed in the dehydrating embryos of agronomic crops (Jensen et al., 1996).
6) Processes in developing seeds are very sensitive to ABA in comparison with those in drought stressed plants (Bray, 1991).
7) Desiccation tolerance in embryos occurs in a metabolically quiescent tissue, whereas water stress in plant occurs during active metabolism and growth (Bartels et al., 1996).

Knowledge about the desiccation tolerance mechanism in seeds can help to elucidate the factors and components required for dehydration tolerance in plants. The information provided by such studies is essential for crop breeding purposes in order to improve stress tolerance or the productivity of crop plants under field conditions.

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