

remain largely unstructured in their hydrated state. Their proposed functions include protecting cellular membranes and proteins during dehydration and helping cells tolerate the stresses associated with water loss.

Keywords: LEA proteins; Desiccation tolerance; Seed maturation; Intrinsically disordered proteins; Orthodox seeds; Recalcitrant seeds; ABA signalling; Seed longevity; Seed priming; Abiotic stress tolerance.

Introduction

Orthodox seeds the type produced by the vast majority of crop and wild plant species are unusual among living tissues in their capacity to survive drying to moisture contents as low as 5% or lower, remaining metabolically quiescent for months, years, or even decades, and then resuming normal growth almost immediately upon rehydration. This trait, called desiccation tolerance, is acquired progressively during the later stages of seed development and is essential for long-term seed storage, germplasm conservation and the seed trade. Recalcitrant seeds, produced by many tropical and some temperate tree species, never acquire this tolerance and die if dried below a comparatively high critical moisture content, which is why they cannot be stored conventionally.

Among the molecular players implicated in desiccation tolerance including non-reducing sugars such as sucrose and raffinose-family oligosaccharides, antioxidants, and small heat-shock proteins Late Embryogenesis Abundant (LEA) proteins stand out for their striking correlation with the developmental window in which tolerance is acquired. First described in cotton seeds in the 1980s, LEA proteins are now known to be present across the plant kingdom and in a range of other desiccation-tolerant organisms, from bacteria and yeast to nematodes, rotifers, and tardigrades, pointing to a deeply conserved protective role under water-limiting conditions.

Seed Desiccation Tolerance: A Developmental Acquisition

Desiccation tolerance in orthodox seeds is not a passive consequence of drying; it is actively built during the late maturation phase, under the control of the plant hormone abscisic acid (ABA) and transcription factors such as ABSCISIC ACID INSENSITIVE 3 (ABI3). As the seed transitions from reserve accumulation to maturation drying, a coordinated programme switches on genes encoding LEA proteins, raffinose-family oligosaccharide biosynthesis enzymes, and antioxidant systems, while metabolism is progressively down-regulated. If this programme is disrupted for example in *abi3* mutants seeds fail to accumulate a normal complement of LEA proteins and become desiccation sensitive even though they otherwise appear mature, directly implicating this regulatory pathway, and the LEA proteins it controls, in the tolerance phenotype (Delahaie et al., 2013).

Classification of LEA Proteins

LEA proteins constitute a diverse group of proteins that differ considerably in sequence and structure. They are commonly classified into several groups according to characteristic sequence motifs and other structural features. Major groups include Group 1, dehydrins or Group 2, Group 3, Group 4 and other less common groups. Dehydrins, for example, are characterized by conserved lysine-rich K-segments. Genome-wide studies have identified numerous LEA genes in plants, including *Arabidopsis thaliana*, where the genes are distributed among several LEA groups (Hundertmark and Hinch, 2008).

A common feature of many LEA proteins is their high hydrophilicity and tendency to remain intrinsically disordered when hydrated. Rather than maintaining a rigid three-dimensional structure, many of these proteins can change their conformation in response to dehydration. This flexibility is considered important for their interaction with membranes, proteins and other cellular components during water loss.

Molecular Mechanisms of LEA Protein Action

Despite three decades of research, no single mechanism fully explains how LEA proteins protect cells during drying, and current evidence points instead to several complementary modes of action operating together.

Disorder-to-Order Transition and the Molecular Shield Hypothesis

Most hydrophilic LEA proteins exist as intrinsically disordered proteins in solution, adopting no fixed three-dimensional structure. As water is progressively removed, many of them undergo a striking conformational change, folding into amphipathic α -helices. This was elegantly demonstrated for LEAM, a mitochondrial LEA protein from pea seeds, which is natively unfolded when hydrated but reversibly folds into a class-A amphipathic helix structurally analogous to apolipoproteins upon drying, allowing it to associate with and stabilise membrane surfaces specifically under water-limited conditions (Tolter et al., 2007). This disorder-to-order transition is thought to underlie a broader 'molecular shield' function, in which the unfolded protein sterically hinders the aggregation of other proteins during drying without needing to bind them in a specific, folded manner.

Water Replacement and Preferential Hydration

Because of their high content of charged and polar residues, LEA proteins can form an extensive hydrogen-bonded shell around macromolecules and membranes, in effect substituting for the structural water lost during drying. This water-replacement activity helps to preserve the spacing and conformation of membrane phospholipids and protein surfaces that would otherwise collapse and aggregate as bulk water is removed.

Vitrification and Glass-State Stabilisation

Dry orthodox seed cytoplasm enters a glassy, highly viscous state that dramatically slows diffusion-dependent deteriorative reactions. LEA proteins, together with soluble sugars, are believed to contribute to the formation and mechanical stability of this intracellular glass, increasing its glass-transition temperature and reducing molecular mobility within it, thereby extending the period over which the dry seed can remain viable in storage.

Ion Sequestration and Direct Chaperone-like Protection

Some LEA proteins bind free ions that would otherwise reach damaging concentrations as cellular water is lost, while in vitro assays show that several hydrophilins can directly protect the activity of dehydration-sensitive enzymes, such as lactate dehydrogenase, when co-dried with them sometimes even at near-equimolar ratios, suggesting a more specific interaction than simple bulk hydration effects alone.

Genetic and Proteomic Evidence Linking LEA Proteins to Seed Survival

Comparative Studies of Orthodox and Recalcitrant Seeds

One of the most direct lines of evidence comes from comparing closely related species that differ in their storage behaviour. In a comparison of the heat-stable seed proteome of the desiccation-sensitive legume *Castanospermum australe* with the desiccation-tolerant model legume *Medicago truncatula*, a large majority of the LEA proteins normally abundant in *M. truncatula* seeds were absent or strongly reduced in the recalcitrant species, and this deficit tracked with disrupted ABI3-dependent regulation rather than with the loss of the LEA genes themselves (Delahaie et al., 2013). Complementary proteomic work in *M. truncatula* radicles showed that a defined subset of LEA polypeptides including members of Groups 1, 3 and 5 and a dehydrin appear and disappear in step with the acquisition and loss of desiccation tolerance during germination, and can be re-induced by an osmotic treatment that re-establishes tolerance, tightly linking their abundance to the tolerant phenotype rather than to developmental stage alone (Boudet et al., 2006).

Functional (Loss- and Gain-of-Function) Evidence in Arabidopsis

Correlative evidence has been reinforced by direct genetic tests. In *Arabidopsis*, silencing or knocking out members of the Group 4 LEA family reduced seed germination efficiency and seedling survival under osmotic and salt stress, lowered biomass and bud recovery after severe drought, and even reduced total seed yield under otherwise optimal irrigation, while overexpressing one family member (AtLEA4-5) improved recovery of adult plants from severe dehydration and increased their relative water content under stress (Olvera-Carrillo et al., 2010). Because these Group 4 proteins accumulate to their highest levels in dry seeds, this work provided some of the first direct genetic proof that a specific LEA protein family contributes causally not just correlatively to desiccation and water-deficit tolerance.

Applications for Seed Technology and Crop Improvement

Understanding LEA proteins has potential applications in seed science because desiccation tolerance is closely related to the ability of orthodox seeds to survive drying and remain viable during storage. Seed maturation, drying, storage and germination are all influenced by physiological processes that occur during the acquisition and maintenance of desiccation tolerance. Consequently, LEA proteins are of interest in studies of seed longevity, stress tolerance and seed-quality maintenance.

LEA proteins have also attracted attention beyond seed biology. Their ability to protect biological molecules during dehydration has encouraged research into their possible use for stabilizing biological materials during drying and storage. In crop improvement, LEA genes may also provide potential targets for developing plants with improved tolerance to drought and other water-related stresses. However, the practical use of individual LEA genes for improving seed storability will require further validation across crop species and under different storage environments.

Conclusion

LEA proteins are important components of the protective system that enables orthodox seeds to withstand severe water loss. Their abundance during seed maturation, hydrophilic nature, structural flexibility and ability to protect membranes and other cellular components support their role in desiccation tolerance. Evidence from comparative proteomic studies, protein-structure experiments and genetic analyses indicates that LEA proteins contribute to dehydration tolerance, although their functions differ among protein groups and are not yet completely understood. Continued investigation of LEA proteins and their interaction with sugars, membranes and other protective systems may improve our understanding of seed longevity and provide new opportunities for improving seed storability and stress tolerance in crops.

References

1. Boudet, J., Buitink, J., Hoekstra, F. A., Rogniaux, H., Larré, C., Satour, P., & 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(4), 1418–1436.
2. Delahaie, J., Hundertmark, M., Bove, J., Leprince, O., Rogniaux, H., & Buitink, J. (2013). LEA polypeptide profiling of recalcitrant and orthodox legume seeds reveals ABI3-regulated LEA protein abundance linked to desiccation tolerance. *Journal of Experimental Botany*, 64(14), 4559–4573.
3. Hundertmark, M., & Hinch, D. K. (2008). LEA (late embryogenesis abundant) proteins and their encoding genes in *Arabidopsis thaliana*. *BMC Genomics*, 9, 118.
4. Olvera-Carrillo, Y., Campos, F., Reyes, J. L., Garciarrubio, A., & Covarrubias, A. A. (2010). Functional analysis of the Group 4 Late Embryogenesis Abundant proteins reveals their relevance in the adaptive response during water deficit in *Arabidopsis*. *Plant Physiology*, 154(1), 373–390.
5. Tolleter, D., Jaquinod, M., Mangavel, C., Passirani, C., Saulnier, P., Manon, S., Teyssier, E., Payet, N., Avelange-Macherel, M. H., & Macherel, D. (2007). Structure and function of a mitochondrial late embryogenesis abundant protein are revealed by desiccation. *The Plant Cell*, 19(5), 1580–1589.
6. Tunnacliffe, A., & Wise, M. J. (2007). The continuing conundrum of the LEA proteins. *Naturwissenschaften*, 94(10), 791–812.
7. Battaglia, M., Olvera-Carrillo, Y., Garciarrubio, A., Campos, F., & Covarrubias, A. A. (2008). The enigmatic LEA proteins and other hydrophilins. *Plant Physiology*, 148(1), 6–24.
8. Olvera-Carrillo, Y., Reyes, J. L., & Covarrubias, A. A. (2011). Late embryogenesis abundant proteins: Versatile players in the plant adaptation to water limiting environments. *Plant Signaling & Behavior*, 6(4), 586–589.