**American Journal of Plant Sciences, 2014, 5, 3440-3455**
Published Online November 2014 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2014.522360

Scientific Research

# Insights into Late Embryogenesis Abundant (LEA) Proteins in Plants: From Structure to the Functions

**Imen Amara[1,2], Ikram Zaidi[2], Khaled Masmoudi[2,3], M. Dolors Ludevid[1], Montserrat Pagès[1], Adela Goday[1], Faiçal Brini[2*]**

[1]Department of Molecular Genetics, Center for Research in Agricultural Genomics (CSIC-IRTA-UABUB), Campus Universitat Autonoma de Barcelona, Bellatera (Cerdanyola Del Valles), Barcelona, Spain
[2]Plant Protection and Improvement Laboratory, Centre of Biotechnology of Sfax (CBS)/University of Sfax, Sfax, Tunisia
[3]International Center for Biosaline Agriculture (ICBA), Dubai, UAE
Email: *faical.brini@cbs.rnrt.tn

Received 23 September 2014; revised 22 October 2014; accepted 8 November 2014



## Abstract

**Late Embryogenesis Abundant (LEA) proteins, a group of hydrophilic proteins, have been linked to survival in plants and animals in periods of stress, putatively through safeguarding enzymatic function and prevention of aggregation in times of dehydration/heat. Yet despite decades of effort, the molecular-level mechanisms defining this protective function remain unknown. In this paper, we summarize and review research discoveries of the classification of the LEA protein groups based on their amino acid sequence similarity and on the presence of distinctive conserved motifs. Moreover, we focus on high correlation between their accumulation and water deficit, reinforcing their functional relevance under abiotic stresses. We also discuss the biochemical properties of LEA proteins arising from their hydrophilic nature and by amino acid composition. Although significant similarities have not been found between the members of the different groups, a unifying and outstanding feature of most of them is their high hydrophilicity and high content of glycine. Therefore, we have highlighted the biotechnological applications of LEA genes, and the effects of over-expressing LEA genes from all LEA groups from different species of origin into different plant hosts. Apart from agronomical purposes, LEA proteins could be useful for other biotechnological applications in relation to their capacity to prevent aggregation of proteins.**

*Corresponding author.

**How to cite this paper:** Amara, I., Zaidi, I., Masmoudi, K., Ludevid, M.D., Pagès, M., Goday, A. and Brini, F. (2014) Insights into Late Embryogenesis Abundant (LEA) Proteins in Plants: From Structure to the Functions. *American Journal of Plant Sciences*, **5**, 3440-3455. http://dx.doi.org/10.4236/ajps.2014.522360

## Keywords

**LEA Proteins, Abiotic Stress, Protein Aggregation, Intrinsically Unstructured Proteins (IUPs)**

## 1. Introduction

Plants are exposed to multiple environmental stresses along their life cycle. Abiotic stresses such as drought, high salinity and freezing temperatures affect most areas of the world and they impact in plants by directly reducing its survival in the natural environment and its productivity in agriculture. About half of the annual world crop production is lost due to abiotic stresses, especially drought [1] [2]. Most plants encounter transient decreases in relative water content at some stages of their life, and many produce a highly desiccation tolerant structure, such as seeds, spores or pollen. Although in a few vascular plants *i.e.* resurrection plants, desiccation tolerance also occurs in the vegetative tissues [3], in general plant vegetative tissues, leaves and roots, are very sensitive to water deficit and they can only withstand reduced and transient water losses [4] [5]. Drought stress induces a range of physiological, cellular and molecular responses in plants towards stress tolerance.

Cellular turgor is not the only important transducer of plant water stress. The growth regulating hormone abscisic acid (ABA) is produced in response to desiccation, causing many of the known expressions and consequences of plant water deficit such as arrested growth, stomatal closure and reproductive failure [4]. Drought and high salinity result in strong increases of plant ABA levels which induce the expression of stress-related genes and adaptive physiological responses [6] [7]. Abiotic stress produces extensive changes in the regulation of gene expression, gene activation/gene suppression, signal transduction pathways biochemical modulation and proteomic machinery which lead to the survival or death of the affected plants [8]-[10].

Upon water deficit a common mechanism is the synthesis of osmotically active compounds compatible with metabolism. These are low-molecular weight solutes that accumulate in the intracellular compartment for osmotic adjustment. Among the many existing osmoprotectants, the non-reducing disaccharide trehalose is most widespread in nature, in anhydrobiotic bacteria, fungi, invertebrates and resurrection plants. However, in most plants systems, sucrose and certain oligosaccharides such as raffinose, stachyose and cyclitols, instead of trehalose, accumulate in large quantities during seed maturation. Non-reducing disacharides function as water replacement molecules and vitrification agents and contribute to the formation of bioglasses [11]. The LEA proteins are a family of hydrophilic proteins that are presumed to play a protective role during exposure to different abiotic stresses [12]. The study of results of physiological assays under drought and salt treatments revealed that the LEA gene confers significantly enhanced tolerance to drought and salt in transgenic *Arabidopsis* plants [13].

## 2. Late Embryogenesis Abundant (LEA) Proteins

As the name suggests, Late Embryogenesis Abundant proteins were originally discovered in the late stages of embryo development in cotton seeds [14] [15]. In plants, most of LEA proteins and their mRNAs accumulate to high concentrations in embryo tissues during the last stages of seed development when desiccation occurs [3] [16]-[20]. Embryogenesis in flowering plants represents a series of stages to develop a miniature plant within the seed. In this process the formed embryo will undergo a cellular expansion stage with dry mass increase in order to provide energy for the process of germination. In most plants the final stage of seed development, maturation, is initiated by a reduction in seed water content, which will eventually drop to about 10%. During this stage and preceded by an increase in ABA content, gene expression and protein profiles change greatly and are associated with the acquisition of desiccation tolerance and development of capacity for seed germination [21] [22]. LEA proteins are accumulated in this final stage, in contrast to storage proteins which appear earlier. Moreover, their mRNAs are maintained at high levels in the dehydrated mature embryos, while transcripts of storage protein genes are completely degraded during the last embryogenesis stage [21]. Since orthodox seeds acquire the ability to withstand severe dehydration at this stage, LEA proteins have been associated with desiccation tolerance [14] [15] [23]. LEA proteins accumulate in vegetative tissues exposed to dehydration, osmotic, and/or low temperature stress [5] [17] [18] [24]. They are also found in anydrobiotic resurrection plants upon drying [3] [25]. Members of the LEA family seem to be ubiquitous in the plant kingdom. Since their first description, hundreds of LEA proteins from vascular to nonvascular plants have been isolated. Their presence has been confirmed not

only in angiosperms and gymnosperms [5] but also in seedless vascular plants (e.g. Selaginella) [26] [27], bryophytes (e.g. *Tortula*, *Physcomitrella*) [28], pteridophytes (e.g. ferns) [29] and algae [30]. Some LEA-like genes are only induced by ABA or other environmental clues [19] [20] [31]. In addition they have now also been identified in some microorganisms [32] [33], fungi [34] [35], protozoa, rotifers, nematodes [36] [37], insects and crustacean [20] [38] [39]. The correlation of LEA proteins in seed maturation stages, during water stress in vegetative plant organs, and in anydrobiotic animals suggests that LEA proteins represent a widespread adaptation to water deficit; however, their precise functions remain unclear.

## 3. Classification of LEA Proteins: Sequence Motifs

LEA proteins were initially classified in six subgroups on the basis of specific domains [24]. With increasing information available on new described members, differences on expression profiles, description in organisms other that plants and especially with the new bioinformatic tools, the classification has been subjected to different rearrangements [17]-[19] [40] [41]. Here, we will adopt a modification by Covarrubias's group [19] of the classification initially introduced by Dure *et al.* [24] in which LEA proteins from cotton were categorized by virtue of similarities in their deduced amino acid sequences. This classification is based on the presence of specific motifs conserved across species which are unique to each family. Based on these characteristics and considering all available sequence information from different plant species LEA proteins are grouped into seven distinctive groups or families; nevertheless, groups 1, 2 and 3 are considered the major LEA groups containing most members of the protein family. The first inventory of LEA proteins was performed in the *Arabidopsis thaliana* genome where fifty LEA genes have been identified [17]. In rice the lea genome comprises 33 genes, 36 in grapevine and 33 in poplar [18]. Over 100 entries for each group 1, 2 and 3 are found in public databases, Gen Bank (http://www.ncbi.nlm.nih.gov) and TIGR database (http://planta.tigr.org) [31]. LEA protein database (LEAPdb) is publicly available at http://forge.info.univ-angers.fr/~gh/Leadb/index.php, [42]. These authors resume the different classification for LEA protein (**Table 1**). Jaspard *et al.* [43] using physico-chemical properties of LEAPs and amino acid computed and statistically analyzed, led to the classification of 710 LEAPs into 12 non-overlapping classes with distinct properties.

### 3.1. LEA Group 1

This LEA group (Pfam PF00477) originally represented by the D-19 and D-132 proteins from cotton seeds contain an internal 20-mer sequence (TRKEQ [L/M] G [T/E] EGY [Q/K] EMGRKGG [L/E]). This motif may be

**Table 1.** Main classifications of LEAP with time-introduction of class nomenclature.

| Pfam | Dure *et al.* | Bray | Tunnacliffe and wise | Battaglia *et al.* | Bies-Esthève *et al.* | Hundermark and Hincha | LEAPdb |
|---|---|---|---|---|---|---|---|
| | 1989 | 1993 | 2007 | 2008 | 2008 | 2008 | 2010 |
| PF00257 | D11 | Group 2 | Group 2 | Group 2 | Group 2 | dehydrin | Classes 1 to 4 |
| PF00477 | D19 | Group 1 | Group 1 | Group 1 | Group 1 | LEA_5 | Classes 5 |
| | D132 | | | | | | |
| PF02987 | D7 | Group 3 | Group 3 | Group 3A | Group 6 | LEA_4 | Classes 6 |
| | D29 | Group 5 | | Group 3B | | | |
| PF03168 | D95 | | | Group 5C | Group 7 | LEA_2 | Classes 7 and 8 |
| PF03242 | D73 | | LEA_5 | Group 5B | Group 6 | LEA_3 | Classes 9 |
| PF03760 | | Group 4 | Group 4 | Group 4A | Group 4 | LEA_1 | Classes 10 |
| | D113 | | | Group 4B | | | |
| PF04927 | D34 | Group 6 | Group 6 | Group 5A | Group 5 | SMPO | Classes 11 |
| PF03168 | | | | Group 6 | Group 8 | PvLEA18 | Classes 12 |
| | | | | Group 5A | | | |

present in several copies arranged in tandem, from one to four in plant species, and up to eight in other organisms [18] [44]-[46]. The wheat Em proteins belong to this group. In the maize EmB564, and EMB5 proteins belong to this group [47] [48]. Group 1 LEA proteins have also been found in *Bacillus subtilis* [33], in other soil bacterial species and in the crustacean *Artemia franciscana*.

### 3.2. LEA Group 2

This group of LEA proteins (Pfam PF00257), also known as "dehydrins", was originally identified as the D-11 family in cotton embryos. Group 2 LEA protein is the most characterized group of LEA proteins. A distinctive feature of group 2 LEA proteins is a conserved, Lys-rich 15-residue motif, EKKGIMDKIKEKLPG, named the K-segment [49] which can be found in one to 11 copies within a single polypeptide. An additional motif also found in this group is the Y-segment, whose conserved consensus sequence is VTD [E/Q] YGNP [19], usually found in one to 35 tandem copies in the N-terminus of the protein; this motif has similar amino acid sequence to the nucleotide binding domain found in chaperones of plants and bacteria. Many proteins of this group also contain a tract of Ser residues, called the S-segment, acting as a site for protein phosphorylation [50]. Less conserved motifs, the ø-segments are usually rich in polar amino acids and lay interspersed between K-segments [24] [51]. The K-segments of the wheat dehydrin DHN-5 are essential for the protection of lactate dehydrogenase and *β*-glucosidase activities *in vitro* [52]. The presence and arrangement of these different motifs in a single polypeptide allow the classification of group 2 LEA proteins into five subgroups [53] [54]. Proteins that only contain the K-segment are in the K-subgroup, and those that include the S-segment followed by K-segment are in the SK-subgroup. In addition, there are the YSK-, YK-, and KS-subgroups [31].

### 3.3. LEA Group 3

Group 3 LEA proteins (Pfam PF02987) are characterized by a repeating motif of 11 amino acids [55]. Differences found in the molecular mass in this group of proteins are usually a consequence of the number of repetitions of this 11-mer motif. The variability in the 11-mer motif leads to a sub-classification of the group 3 LEA proteins into two subgroups: 3A, represented by the cotton D-7 LEA protein; and 3B, represented by the cotton D-29 LEA protein. The first subgroup is highly conserved; two of the motifs characteristic of these proteins correspond to almost the same 11-mer described originally for this subgroup, with some variation at positions 9 and 10 (TAQ [A/S] AK [D/E] KT[S/Q] E). The other subgroup (3B) is more heterogeneous [19]; four variations of the 11-mer were found. Interestingly, proteins similar to plant group 3 LEA proteins accumulate in several non plant organisms in response to dehydration, in fungi, microbial and animal kingdoms. They have been found in prokaryotes *Deinococcus radiodurans* [56], *Haemophilus influenza* [57] and in *Caenorhabditis elegans* (Ce-LEA-1), whose expression is correlated with the survival of this nematode under conditions of desiccation, osmotic, and heat stress [58]. Other anhydrobiotic organisms such as the nematodes *Steinernema feltiae* [36] [59] and *Aphelencus avenae* [37], as well as the bdelloid rotifer *Philodina roseola* [60], the chironomid *Polypedilum vanderplanki* [61] and the crustacean *A. franciscana* [62] [63] also accumulate group 3 LEA proteins in their desiccated states.

### 3.4. LEA Group 4

Group 4 LEA proteins (Pfam PF03760) are of widespread occurrence in the plant kingdom, including nonvascular plants and vascular plants. A motif that has characterized the proteins in this group is motif 1, located at the N-terminal region with the following consensus sequence: AQEKAEKMTA [R/H] DPXKEMAHERK [E/K] [A/E] [K/R] [19]. However, four additional motifs can be distinguished in many group 4 LEA proteins. The presence or absence of motif defines two subgroups within the family. The first subgroup (group 4A) consists of small proteins (80 - 124 residues long). The other subgroup (group 4B) has longer representatives (108 - 180 residues). D-113 protein from cotton, the first discovered of this group [64].

### 3.5. LEA Group 5

The first proteins described for this group (Pfam PF04927) were D-34, D-73, and D-95 from cotton [16] [23]. They represent an atypical LEA subgroup because they contain a higher proportion of hydrophobic residues. These proteins are not soluble after boiling, suggesting that they adopt a globular conformation [16] [23] [44].

They include maize Rab28 [65], carrot ECP31 [66] and *Medicago trunculata* MtPM25 [67] among others.

### 3.6. LEA Group 6

PvLEA18 protein from bean (*Phaseolus vulgaris*) was the first protein described from this group (Pfam PF03168) [68]. To date, 36 genes of this family have been described from vascular plants. The proteins in this group are characterized by their small size (approximately 7 - 14 kD) [19]. A distinctive characteristic of the group 6 LEA protein characterized is its accumulation in the elongation or growing regions of common bean roots and hypocotyls, which showed lower water potentials than the non-growing regions [68].

### 3.7. LEA Group 7

The ASR proteins, considered to be members of the LEA family, are small, heat-stable, and intrinsically unstructured proteins [69] [70]. The group of this gene has been identified from various species of dicotyledonous and monocotyledonous plants [70] as well as from gymnosperm species. However, no ASR-like genes are found in *Arabidopsis*. They share physiochemical properties with other LEA proteins and they accumulate in seeds during late embryogenesis and in response to water-limiting conditions [70].

## 4. Biochemical Properties and Structure of LEA Proteins

Most of the biochemical properties of LEA proteins arise from their hydrophilic nature and based amino acid composition. Although significant similarities have not been found between the members of the different groups, a unifying and outstanding feature of most of them is their high hydrophilicity and high content of Gly and a lack or low proportion of Cys and Trp residues, and a preponderance of certain amino acid residues such as Ala, Glu, Lys/Arg, and Thr [16] [32] [55] [71]. The high hydrophilicity is likely to be responsible for their lack of conventional secondary structure in the hydrated state. Most of them exist principally as randomly coiled proteins in solution. While structure modeling and structure prediction programs suggest that at least some LEA proteins from particular families contain defined conformations [51] [55] [57] all hydrophilic LEA proteins studied experimentally have revealed a high degree of unordered structure in solution. This has led them to be considered as intrinsically unstructured proteins [72]-[81]. Similarly, the ability of LEA proteins to remain soluble at elevated temperatures can be attributed to their hydrophilic, unstructured nature. Heat-induce aggregation of proteins results from partial denaturation and association through exposed hydrophobic regions, something that cannot occur in hydrophilic and natively unfolded protein [39] [82].

LEA proteins are variable in size, ranging from 5 to 77 kD among most groups. They can be acid, neutral or basic. Group 1 proteins are acidic to neutral; group 2 comprises proteins with different pIs and groups 3 are neutral to basic [31]. The aberrant mobility of this type of proteins in SDS-PAGE is rather caused by the less interaction between SDS and charged amino acid residues [83]. Since the discovery than group 1 protein Em, from wheat, was flexible in conformation with little secondary structure (*i.e.* $\alpha$-helice or $\beta$-sheet) and 70% of the protein behaving as random coil [84] most LEA proteins have been found to have an unfolded structure in the hydrated state [39]. LEA proteins from groups 1, 2 and 3 are predicted to be at least 50% unfolded. Lack of conventional secondary structure means that members of the major LEA protein groups are included in the large class of protein variously called “natively unfolded”, “intrinsically disordered” or “intrinsically unstructured” [85]-[87] and it is thus not surprising that attempts to crystallize purified LEA proteins for X-ray crystallography have reportedly been unsuccessful [72] [84]. Disorder prediction programs (http://www.pondr.com) suggest that 27% - 41% of all eukaryotic proteins contain unstructured region ≥50 residues long and that 6% - 17% of polypeptides are wholly disordered [85]; therefore, many proteins are natively unfolded or contain natively unfolded domains. The disordered proteome is likely to be a rich source of unforeseen activities, highlighting new functions of both biological and potentially pharmaceutical significance [88]. Nevertheless, some LEA proteins show some secondary structure and they have some structural elements in equilibrium with unstructured states. Thus, a polyproline-type II (PPII) extended, left-handed helices have been described in groups 1 and 2 from soybean [76], group 2 from *Arabidopsis* [89] and in group 3 from an anydrobiotic nematode [72]. Many natively unfolded proteins are known to undergo increased folding under some conditions, usually when they bind a partner molecule or cation [87]. Environmental conditions can also affect folding and several LEA proteins become more structured when dried [39].

LEA proteins exhibit the remarkable ability to become more ordered and to develop secondary structure as dehydration proceeds. For animal LEA proteins, Tunnacliffe's group first demonstrated this phenomenon by using Fourier-transform infrared (FTIR) spectroscopy [72]. FTIR spectroscopy allows for the assessment of protein secondary structure in the dry state by using the profile of the amide-I band, which provides information on the relative contributions of $\alpha$-helix, $\beta$-sheet, and turn structures. A group 3 LEA protein from *Typha latifolia* become largely $\alpha$-helical when dried rapidly; slow drying resulted in intermolecular $\beta$-sheet formation, as well as $\alpha$-helix [80]. Similarly, the group 3 LEA proteins AavLEA1 from nematode *Aphelenchus avenae* [72] and LEAM from pea mitochondria [90] also gain structure on drying. Boudet *et al*. (2006) [91] used FITR to study both group 1 (MtEm6) and group 5 (MtPm25) proteins from *M. trunculata* and found them both to have increased folding in the dried state. Koag *et al*. (2003) [92] have also reported gain of structure, $\alpha$-helical, when cowpea DHN1 is incubated with small unilamellar vesicles (SUVs).

Li and He (2009) [93] utilized a 66-amino-acid fragment of AavLEA1 and documented, through molecular dynamics simulation, many of these properties. Water was removed from 83.5 wt% to 2.4 wt%. As water is removed, the protein assumes progressively a more folded conformation. At 83.5 wt% LEA protein is completely solvated. At 50% water between 83.5% and 50.4%, the protein is unstructured. In this range and below this point, water molecules no longer are sufficient to fully solvate the protein. At less than 20% water the protein becomes more dehydrated and begins to adopt a significant amount of secondary structure. $\alpha$-helical structure is apparent, and hairpin-like structures are formed. At 2.4% water the structure is very similar to that in the complete absence of water. The propensity of some LEA proteins to gain structure under some conditions, may be a general property of these proteins, and may have important functional implications in their physiological roles [94].

## 5. Sub Cellular Localization and Expression Profiles

In plants, LEA proteins have been found localized in cytoplasm, nucleus, mitochondrion, chloroplast, endoplasmic reticulum, vacuole, peroxisome and plasma membrane [39]. The different LEA groups show no preference for a specific subcellular localization. Most LEA proteins from the different groups are accumulated during embryo development in the dry seed.

In plants, group 1 LEA proteins are found mostly in seeds and they are not induced by stress conditions in vegetative tissues. They accumulate during seed development and they are considered as embryo-specific LEA proteins [95] [96].

Group 2 LEA proteins accumulate during seed desiccation and in response to water deficit induced by drought, low temperature or salinity [73] [97] [98]. Most of them accumulate in all tissues upon water deficit although there are those that preferentially respond to particular stress conditions. Some dehydrins are strongly accumulated in response to low-temperature treatments but not to drought or salinity [99]; other group 2 members are not induced in response to low temperatures, while a small number of dehydrins show an unusual constitutive expression [100]-[102]. However, it is not possible to assign a specific accumulation pattern to particular group or subgroup. In addition, not all are ABA-induced and in some cases their response to stress is mediated by more than one pathway, one of which may be ABA dependent [97] [103] [104]. Many group 2 LEA proteins accumulate in the cytoplasm, and some of them are also localized to the nucleus. For nucleus directed SK2 proteins, the phosphorylated S-segment and the RRKK sequence are relevant for their nuclear localization [105] [106]. Some dehydrins are also found in other cell compartments, including the vicinity of the plasma membrane, mitochondria, vacuole, and endoplasmic reticulum [35] [100] [101] [107] [108].

Expression and transcriptomic analysis of plant group 3 LEA proteins show their accumulation in mature seeds and in response to dehydration, salinity, or low temperatures [109]-[112]. Some members also respond to hypoxia [113] or to high-excitation pressure imposed by high light [112].

As for LEA proteins from other groups, the expression of group 3 LEA proteins appears to be regulated by ABA during specific developmental stages and/or upon stress conditions [114] [115]. Group 3 D-7 LEA protein from cotton accumulates to a concentration of about 200 mM in mature cotton embryos [116]. Studies of seeds have localized group 3 LEA proteins to the cytoplasm and protein storage vacuoles, as is the case for HVA1 from barley *Hordeum vulgare* [117] whereas PsLEAm is distributed within the mitochondrial matrix of pea seeds [118]. Group 3 proteins are also detected in vegetative tissues; WAP27A and WAP27B are abundantly accumulated in endoplasmic reticulum of cortical parenchyma cells of the mulberry tree (*Morus bombycis*) during winter [119]; and WCS19 accumulates specifically in wheat leaves and rye (*Secale cereale*) during cold ac-

climation, where it was localized within the chloroplast stroma [112].

LEA proteins from group 4 in some classifications are partially included in group 3. Cotton D-113 was found homogeneously distributed in all embryo tissues at a concentration of nearly 300 mM [116]. Later, similar proteins were found to accumulate in vegetative tissues in response to water deficit. In tomato (*Solanum lycopersicum*) plants, group 4 LEA transcripts (LE25) accumulated in leaves in response to water deficit and ABA [120]. In *Arabidopsis* vegetative tissues, the transcripts of the group 4 LEA proteins also accumulated in response to water-deficit treatments [64] [121].

Transcripts from group 5 LEA proteins accumulate during the late stage of seed development and in response to stress conditions, such as drought, UV light, salinity, cold, and wounding [33] [122]-[124]. In maize, Rab28 has been found accumulated in the nucleolus of scutellar cells of mature dry embryos [125]. In *Medicago truncatula*, MtPM25 is highly induced by drought [91].

## 6. LEA Protein Functions

There is an extensive bibliography showing the correlation between the expression of LEA proteins or their genes with stress resistance [20] [31] [60]. Many studies show the protection conferred by LEA proteins during salt and osmotic stress. Introduction of heterologous LEA proteins into plants and microorganisms results in an enhanced stress tolerance. Transgenic approaches have shown that over-expression of LEA proteins from different species in *Arabidopsis*, tobacco, rice, wheat, maize, lettuce or cabbage produces improve abiotic stress resistant phenotypes [126]-[131]. However, the precise molecular function of LEA proteins is still unclear and so far LEA proteins have been suggested to act as stabilizers, hydration buffers, membrane protectants, antioxidants, organic glass formers and/or ion chelators [39].

### 6.1. Protein Protection

LEA proteins have the capacity to protect target proteins from inactivation and aggregation during water stress. A role in protein stabilization is supported by the fact that some LEA proteins preserve enzyme activity *in vitro* after partial dehydration, desiccation or freezing [72] [132] [133]. One mechanism of protection is the prevention of water stress induced aggregation of proteins [74] [134]-[137].

Many proteins, including the enzymes citrate synthase and lactate dehydrogenase, form insoluble aggregates when dried or frozen, but aggregation is reduced in the presence of LEA proteins from groups 1, 2 and 3. Group 2 proteins also prevent protein aggregation from heat stress [74]. This protein anti-aggregation activity extends to the protection of complex mixtures of proteins, such as the water-soluble proteomes of human and nematode cells [134]. Due to their hydrophilic, unstructured nature, LEA proteins themselves are not susceptible to aggregation on desiccation, freezing or boiling [60]. The antiaggregation properties of group 3 LEA proteins have been demonstrated also in living cells; mammalian cells overexpressing aggregation-prone proteins reduce the formation of aggregates in the presence of nematode *A. avena* LEA protein [134]. The group of Tunnacliffe has proposed that LEA proteins may exert a “molecular shield” activity [41] [135]. In the increasing crowded environment of the dehydrating cytoplasm LEA proteins could decrease the interaction between partially denatured polypeptides and avoid their aggregation. The shield proteins might also have a space-filling role and help to prevent collapse of the cell as its water is lost. Another functional hypothesis is the chaperone activity [74]. The anti-aggregation activity of LEA proteins resembles a “holding” molecular chaperone, which function in the cell would be to stabilize passively protein species in a partially unfolded state, preventing aggregation while the stress lasts. They resemble holding chaperones, in their functioning without ATP, in contrast to classical folding chaperones which require ATP. But they are distinct in that they lack structure and do not form transient complexes with their client proteins through hydrophobic surfaces, since they are hydrophilic [64] [133]. The two functional mechanisms may not be so distinct and in the context of desiccation tolerance both activities may contribute to damage avoidance [60].

### 6.2. Membrane Protection

During desiccation membrane protection is essential to preserve the cellular and organellar integrity. Some LEA proteins could contribute with sugars to H-bonding networking and protect membranes in the dry state [38]. LEA proteins being highly hydrophilic are not expected to interact with cellular membranes in hydrated condi-

tions, but interaction cannot be excluded through $\alpha$-helices in a dehydrating cell. Among group 2 LEA proteins, wheat WCOR414 and *Arabidopsis* LTi29 acidic dehydrins were immunodetected in the vicinity of the plasma membrane during cold acclimation [100] [138] and maize dehydrins were observed in association to membranes of protein and lipid bodies [139]. Maize native and recombinant dehydrin DHN (Rab17) binds *in vitro* to anionic lipid vesicles and binding produces an increase in amphipathic $\alpha$-helix (a structural element that interacts to membranes and proteins); both binding and gain in conformation were attributed to the K-segment [140] [141]. Other dehydrins, ERD10 and ERD14, bind *in vitro* to acidic phospholipid vesicles [74]. Recently, the contribution of dehydrin K-segments from group 2 Lti30 LEA protein has been analyzed *in vitro* in the binding to membranes and the relevance of the flanking His side chains as regulators of the interaction between the K-segments and membranes in a pH-dependent manner has been shown [142].

Group 3 LEA protein, LEAM, is located in pea mitochondria and is able to interact with a membrane and afford protection in the dry state [90]. When drying, this unstructured polypeptide is able to fold into amphipathic $\alpha$-helices. The interaction between LEAM and phospholipids and the protective effect of LEAM was demonstrated by differential scanning colorimetry using a liposome desiccation assay [90]. To summarize, although their hydrophylicity and lack of structure suggest that LEA proteins should act in soluble compartments of cells, specific LEA proteins upon folding during desiccation or freezing may contribute to membrane protection [60].

### 6.3. Ion Binding and Antioxidant Function

One consequence of dehydration is the increase in concentration of intracellular components, including ions. Increased ionic concentration can affect macromolecular structure and function. It has been proposed that LEA proteins, because of their many charged amino acid residues might act to sequester ions [55] [100]. A dehydrin-like protein from celery is located in the vacuole and binds $Ca^{++}$ when phosphorylated [108]. Acidic group 2 proteins, ERD10, COR47 and ERD14 also exhibit phosphorylation-dependent $Ca^{++}$ binding. Phosphorylation sites are located in the serine motif [143]. Group 2 LEA proteins also bind a number of other metal ions; the interaction was proposed to occur through His residues which are over-represented in most group 2 LEA proteins [144]. Binding of metals ions by group 2 LEA proteins may be linked to the antioxidant properties reported for citrus CuCOR19 protein and *in vitro* scavenging activity for hydroxyl radicals [145]. LEA proteins might reduce oxidative stress in dehydrating cells by scavenging ROS and/or by sequestering metal ions that generate ROS [39].

### 6.4. Other Functions

LEA proteins might act as hydration buffers, slowing down the rate of water loss during dehydration; during partial drought, osmotic or freezing stress, hydration buffers allow sufficient water activity for proteins to retain function [23] [32]. Using a knockout mutant of *Arabidopsis*, whose seed exhibited premature dehydration, a role for group1 LEA protein Atm6 as hydration buffer was proposed [95]. In a desiccating cell, when the water content falls below 10% on a dry weight basis, the cytoplasm vitrifies and enters in the “glassy state” [146]. In plants the formation of intracellular glasses is indispensable for survival in the dry state (seeds and pollens). LEA proteins accumulate to high levels in seeds (2% - 4% of the water soluble proteome) [116] and they increase the density of the sugar glasses by strengthening the hydrogen-bonding of the sucrose/LEA mixture [146]. Thus a potential role of LEA proteins is their contribution to the formation of biological glasses.

While among LEA proteins, some proteins which display similar sequences show different structure and *in vitro* properties, a prevalent issue is that a single LEA protein might have more than one function. The chloroplast LEA-like protein COR15am protects both membranes and proteins [137] [147]; the mitochondrial group 3 protein LEAM, also exhibits both functions [90]; group 2 from *Citrus* shows ionic binding, antioxidant and nucleic acid binding properties [145] [148]. This versatility may be a general feature of LEA proteins. Performance of more than one function, “moonlightening”, is not uncommon among proteins and it is more likely to evolve in unfolded, rather than in folded proteins [78].

## 7. Biotechnological Applications of LEA Genes

Numerous transgenic studies revealed a positive effect of LEA gene expression on plant stress tolerance. In general, the phenotypes of the transgenic plants show enhanced stress tolerance, often related to drought or salt

stress. Most studies report enhanced growth rates and reduced wilting of the aerial parts under stress under laboratory conditions and in some field trials, demonstrating a real potential of LEA proteins in engineering crops more tolerant to stress [126]. Apart from agronomical purposes, LEA proteins could be useful for other biotechnological applications in relation to their capacity to prevent aggregation of proteins. The use of a group 3 LEA protein as a fusion partner facilitates recombinant expression of recalcitrant proteins in *E. coli* in a soluble form [149]. Moreover, the anti-aggregation properties of another group 3 LEA protein have been applied to reduce the formation of *in vivo* aggregates; thus, coexpression of aggregation-prone proteins containing long polyglutamine (polyQ) or polyalamine (polyA) sequences, with group 3 AavLEA1 LEA protein in mammalian cells reduces substantially the expansion of protein aggregates associated to neurodegenerative diseases [150].

## 8. Conclusion and Future Prospects

With increasing data from diverse research fields, LEA appears to be an amazingly versatile family of proteins presumably due to their intrinsically unstructured character. They exhibit myriads of functions (e.g., chaperone, cryoprotective, antifreeze, radical-scavenging, ion-binding functions) when exposed to various stress factors, including drought, high-salinity stress, low-temperature stress, heavy-metal stress, and perhaps also to biotic stresses. Despite the relevant progress made toward understanding the role of LEA, the molecular mechanisms through which they can enhance stress tolerance remain unknown. Future work should broadly examine other LEA to learn whether LEA-dependent regulatory mechanisms modulate stress responses.

## Acknowledgements

This work was supported jointly by grants from the Ministry of Higher Education and Scientific Research, Tunisia and the Agence Espagnole de cooperation Internationale (AECI) Officina Técnica de Cooperacion, Spain.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

[1] Boyer, J.S. (1982) Plant Productivity and Environment. *Science*, **218**, 443-448. http://dx.doi.org/10.1126/science.218.4571.443

[2] Vinocur, B. and Altman, A. (2005) Recent Advances in Engineering Plant Tolerance to Abiotic Stress: Achievements and Limitations. *Current Opinion in Biotechnology*, **16**, 123-132. http://dx.doi.org/10.1016/j.copbio.2005.02.001

[3] Ingram, J. and Bartels, D. (1996) The Molecular Basis of Dehydratation Tolerance in Plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **47**, 377-403. http://dx.doi.org/10.1146/annurev.arplant.47.1.377

[4] Blum, A. (2011) The Interdrought Conference in Perspective. *Journal of Experimental Botany*.

[5] Bray, E. (1993) Molecular Responses to Water Deficit. *Plant Physiology*, **103**, 1035-1040.

[6] Cramer, G.R., Urano, K., Delrot, S., Pezzotti, M. and Shinozaki, K. (2011) Effects of Abiotic Stress on Plants: A Systems Biology Perspective. *BMC Plant Biology*, **11**, 163. http://dx.doi.org/10.1186/1471-2229-11-163

[7] Raghavendra, A.S., Gonugunta, V.K., Christmann, A. and Grill, E. (2010) ABA Perception and Signalling. *Trends in Plant Science*, **15**, 395-401. http://dx.doi.org/10.1016/j.tplants.2010.04.006

[8] Ahuja, I., de Vos, R.C., Bones, A.M. and Hall, R.D. (2010) Plant Molecular Stress Responses Face Climate Change. *Trends in Plant Sciences*, **15**, 664-674. http://dx.doi.org/10.1016/j.tplants.2010.08.002

[9] Xiong, L., Schumaker, K.S. and Zhu, J.K. (2002) Cell Signaling during Cold, Drought, and Salt Stress. *The Plant Cell Online*, **14**, S165-S183.

[10] Yamaguchi-Shinozaki, K. and Shinozaki, K. (2006) Transcriptional Regulatory Networks in Cellular Responses and Tolerance to Dehydration and Cold Stresses. *Annual Review of Plant Biology*, **57**, 781-803. http://dx.doi.org/10.1146/annurev.arplant.57.032905.105444

[11] Cacela, C. and Hincha, D.K. (2006) Monosaccharide Composition, Chain Length and Linkage Type Influence the Interactions of Oligosaccharides with Dry Phosphatidylcholine Membranes. *Biochimica et Biophysica Acta* (*BBA*)—*Biomembranes*, **1758**, 680-691. http://dx.doi.org/10.1016/j.bbamem.2006.04.005

[12] Amara, I., Capellades, M., Ludevid, M.D., Pagès, M. and Goday, A. (2013) Enhanced Water Stress Tolerance of Transgenic Maize Plants Over-Expressing LEA Rab28 Gene. *Journal of Plant Physiology*, **170**, 864-873.

http://dx.doi.org/10.1016/j.jplph.2013.01.004

[13] Liang, J., Zhou, M., Zhou, X., Jin, Y., Xu, M. and Lin, J. (2013) JcLEA, a Novel LEA-Like Protein from *Jatropha curcas*, Confers a High Level of Tolerance to Dehydration and Salinity in *Arabidopsis thaliana*. *PLoS ONE*, **8**, e83056. http://dx.doi.org/10.1371/journal.pone.0083056

[14] Dure, L., Greenway, S. and Galau, G. (1981) Developmental Biochemistry of Cotton Seed Embryogenesis and Germination: Changing Messenger Ribonucleic Acid Populations as Shown by *in Vitro* and *in Vivo* Protein Synthesis. *Biochemistry*, **20**, 4162-4168. http://dx.doi.org/10.1021/bi00517a033

[15] Galau, G.A., Hughes, D.W. and Dure, L. (1986) Abscisic Acid Induction of Cloned Cotton Late Embryogenesis-Abundant (Lea) mRNAs. *Plant Molecular Biology*, **7**, 155-170. http://dx.doi.org/10.1007/BF00021327

[16] Baker, J., Steele, C. and Dure, L. (1988) Sequence and Characterization of 6 Lea Proteins and Their Genes from Cotton. *Plant Molecular Biology*, **11**, 277-291. http://dx.doi.org/10.1007/BF00027385

[17] Bies-Etheve, N., Gaubier-Comella, P., Debures, A., Lasserre, E., Jobet, E., Raynal, M., Cooke, R. and Delseny, M. (2008) Inventory, Evolution and Expression Profiling Diversity of the LEA (Late Embryogenesis Abundant) Protein Gene Family in *Arabidopsis thaliana*. *Plant Molecular Biology*, **67**, 107-124. http://dx.doi.org/10.1007/s11103-008-9304-x

[18] Hundertmark, M. and Hincha, D.K. (2008) LEA (Late Embryogenesis Abundant) Proteins and Their Encoding Genes in *Arabidopsis thaliana*. *BMC Genomics*, **9**, 118. http://dx.doi.org/10.1186/1471-2164-9-118

[19] Battaglia, M., Olvera-Carrillo, Y., Garciarrubio, A., Campos, F. and Covarrubias, A.A. (2008) The Enigmatic LEA Proteins and Other Hydrophilins. *Plant Physiology*, **148**, 6-24. http://dx.doi.org/10.1104/pp.108.120725

[20] Hand, S.C., Menze, M.A., Toner, M., Boswell, L. and Moore, D. (2011) LEA Proteins during Water Stress: Not Just for Plants Anymore. *Annual Review of Physiology*, **73**, 115-134. http://dx.doi.org/10.1146/annurev-physiol-012110-142203

[21] Goldberg, R.B., Barker, S.J. and Perez-Grau, L. (1989) Regulation of Gene Expression during Plant Embryogenesis. *Cell*, **56**, 149-160. http://dx.doi.org/10.1016/0092-8674(89)90888-X

[22] Skriver, K. and Mundy, J. (1990) Gene Expression in Response to Abscisic Acid and Osmotic Stress. *Plant Cell*, **2**, 503-512. http://dx.doi.org/10.1105/tpc.2.6.503

[23] Cuming, A. (1999) LEA Proteins. In: Casey, R. and Shewry, P.R., Eds., *Seed Proteins*, Kluwer Academic Publishers, Dordrecht, 753-780. http://dx.doi.org/10.1007/978-94-011-4431-5_32

[24] Dure, L., Crouch, M., Harada, J., Ho, T.H., Mundy, J., Quatrano, R., Thomas, T. and Sung, Z. (1989) Common Amino Acid Sequence Domains among the LEA Proteins of Higher Plants. *Plant Molecular Biology*, **12**, 475-486. http://dx.doi.org/10.1007/BF00036962

[25] Bartels, D. and Sunkar, R. (2005) Drought and Salt Tolerance in Plants. *Critical Reviews in Plant Sciences*, **24**, 23-58. http://dx.doi.org/10.1080/07352680590910410

[26] Alpert, P. (2005) The Limits and Frontiers of Desiccation-Tolerant Life. *Integrative and Comparative Biology*, **45**, 685-695. http://dx.doi.org/10.1093/icb/45.5.685

[27] Oliver, M.J., Tuba, Z. and Mishler, B.D. (2000) The Evolution of Vegetative Desiccation Tolerance in Land Plants. *Plant Ecology*, **151**, 85-100. http://dx.doi.org/10.1023/A:1026550808557

[28] Oliver, R.P. and Solomon, P.S. (2004) Does the Oxidative Stress Used by Plants for Defence Provide a Source of Nutrients for Pathogenic Fungi? *Trends in Plant Science*, **9**, 472-473. http://dx.doi.org/10.1016/j.tplants.2004.08.006

[29] Reynolds, T.L.B. and Derek, J. (1993) Abscisic Acid Enhances the Ability of the Desiccation-Tolerant Fern *Polypodium virginianum* to Withstand Drying. *Journal of Experimental Botany*, **44**, 1771-1779. http://dx.doi.org/10.1093/jxb/44.12.1771

[30] Honjoh, K.I., Yoshimoto, M., Joh, T., Kajiwara, T., Miyamoto, T. and Hatano, S. (1995) Isolation and Characterization of Hardening-Induced Proteins in *Chlorella vulgaris* C-27: Identification of Late Embryogenesis Abundant Proteins. *Plant and Cell Physiology*, **36**, 1421-1430.

[31] Shih, M.D., Hoekstra, F.A. and Hsing, Y.I.E. (2008) Late Embryogenesis Abundant Proteins. *Advances in Botanical Research*, **48**, 212-240. http://dx.doi.org/10.1016/S0065-2296(08)00404-7

[32] Garay-Arroyo, A. Colmenero-Flores, J., Garciarrubio, A. and Covarrubias, A.A. (2000) Highly Hydrophilic Proteins in Prokaryotes and Eukaryotes Are Common during Conditions of Water Deficit. *Journal of Biological Chemistry*, **275**, 5668-5674. http://dx.doi.org/10.1074/jbc.275.8.5668

[33] Stacy, R.A.P., Nordeng, T.W., Francisco, A. and Aalen, R.B. (1999) The Dormancy Related Peroxiredoxin Anti-Oxidant, PER1, Is Localized to the Nucleus of Barley Embryo and Aleurone Cells. *The Plant Journal*, **19**, 1-8. http://dx.doi.org/10.1046/j.1365-313X.1999.00488.x

[34] Abba, S., Ghignon, S. and Bonfante, P. (2006) A Dehydration-Inducible Gene in the Truffle *Tuber borchii* Identifies a

Novel Group of Dehydrins. *BMC Genomics*, **7**, 39. http://dx.doi.org/10.1186/1471-2164-7-39

[35] Borovskii, G.B., Stupnikova, I.V., Antipina, A.I., Downs, C.A. and Voinikov, V.K. (2000) Accumulation of Dehydrin-Like-Proteins in the Mitochondria of Cold-Treated Plants. *Journal of Plant Physiology*, **156**, 797-800. http://dx.doi.org/10.1016/S0176-1617(00)80250-3

[36] Solomon, A., Salomon, 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. http://dx.doi.org/10.1017/S0031182099006563

[37] Browne, J.A., Dolan, K.M., Tyson, T., Goyal, K., Tunnacliffe, A. and Burnell, A.M. (2004) Dehydration-Specific Induction of Hydrophilic Protein Genes in the Anhydrobiotic Nematode *Aphelenchus avenae*. *Eukaryotic Cell*, **3**, 966-975. http://dx.doi.org/10.1128/EC.3.4.966-975.2004

[38] Hoekstra, F.A., Golovina, E.A. and Buitink, J. (2001) Mechanisms of Plant Desiccation Tolerance. *Trends in Plant Science*, **6**, 431-438. http://dx.doi.org/10.1016/S1360-1385(01)02052-0

[39] Tunnacliffe, A. and Wise, M. (2007) The Continuing Conundrum of the LEA Proteins. *Naturwissenschaften*, **94**, 791-812. http://dx.doi.org/10.1007/s00114-007-0254-y

[40] Battaglia, M. and Covarrubias, A.A. (2013) Late Embryogenesis Abundant (LEA) Proteins in Legumes. *Front Plant Science*, **25**, 190.

[41] Tunnacliffe, A., Lapinski, J. and McGee, B. (2005) A Putative LEA Protein, but No Trehalose, Is Present in Anhydrobiotic *Bdelloid rotifers*. *Hydrobiologia*, **546**, 315-321. http://dx.doi.org/10.1007/s10750-005-4239-6

[42] Hunault, G. and Jaspard, E. (2010) LEAPdb: A Database for the Late Embryogenesis Abundant Proteins. *BMC Genomics*, **11**, 221. http://dx.doi.org/10.1186/1471-2164-11-221

[43] Jaspard, E., Macherel, D. and Hunault, G. (2012) Computational and Statistical Analyses of Amino Acid Usage and Physico-Chemical Properties of the Twelve Late Embryogenesis Abundant Protein Classes. *PLoS ONE*, **7**, 1-5. http://dx.doi.org/10.1371/journal.pone.0036968

[44] Galau, G.A., Hughes, D.W. and Dure, L. (1986) Abscisic Acid Induction of Cloned Cotton Late Embryogenesis-Abundant (Lea) mRNAs. *Plant Molecular Biology*, **7**, 155-170. http://dx.doi.org/10.1007/BF00021327

[45] Goday, A., Sánchez-Martínez, D., Gómez, J., Domènech, P.P. and Pagès, M. (1988) Gene Expression in Developing *Zea mays* Embryos: Regulation by Abscisic Acid of a Highly Phosphorylated 23- to 25-kD Group of Proteins. *Plant Physiology*, **88**, 564-569. http://dx.doi.org/10.1104/pp.88.3.564

[46] Campos, F., Cuevas-Velazquez, C., Fares, M., Reyes, J.L. and Covarrubias, A.A. (2013) Group 1 LEA Proteins, an Ancestral Plant Protein Group, Are Also Present in Other Eukaryotes, and in the Archeae and Bacteria Domains. *Molecular Genetics & Genomics*, **288**, 503-517. http://dx.doi.org/10.1007/s00438-013-0768-2

[47] Amara, I., Odena, A., Oliveira, E., Moreno, A., Masmoudi, K., Pagès, M. and Goday, A. (2012) Insights into Maize LEA Proteins: From Proteomics to Functional Approaches. *Plant Cell Physiology*, **53**, 312-329. http://dx.doi.org/10.1093/pcp/pcr183

[48] Wu, X., Gong, F. and Wang, W. (2013) Functional Assignment to Maize Group 1 LEA Protein EMB564 within the Cell Nucleus Using Computational Analysis. *Bioinformation*, **9**, 276-280. http://dx.doi.org/10.6026/97320630009276

[49] Campbell, S.A. and Close, T.J. (1997) Dehydrins: Genes, Proteins, and Associations with Phenotypic Traits. *New Phytologist*, **137**, 61-74. http://dx.doi.org/10.1046/j.1469-8137.1997.00831.x

[50] Campbell, S.A., Crone, D.E., Ceccardi, T.L. and Close, T.J. (1998) A ca. 40 kDa Maize (*Zea mays* L.) Embryo Dehydrin Is Encoded by the Dhn2 Locus on Chromosome 9. *Plant Molecular Biology*, **38**, 417-423. http://dx.doi.org/10.1023/A:1006037308167

[51] Close, T. (1996) Dehydrins: Emergence of a Biochemical Role of a Family of Plant Dehydration Proteins. *Physiologia Plantarum*, **97**, 795-803. http://dx.doi.org/10.1111/j.1399-3054.1996.tb00546.x

[52] Drira, M., Saibi, W., Brini, F., Gargouri, A., Masmoudi, K. and Hanin, M. (2013) The K-Segments of the Wheat Dehydrin DHN-5 Are Essential for the Protection of Lactate Dehydrogenase and *β*-Glucosidase Activities *in Vitro*. *Molecular Biotechnology*, **2**, 643-650. http://dx.doi.org/10.1007/s12033-012-9606-8

[53] Scott, A.C. and Close, T.J. (1997) Dehydrins: Genes, Proteins, and Associations with Phenotypic Traits. *New Phytologist*, **137**, 61-74. http://dx.doi.org/10.1046/j.1469-8137.1997.00831.x

[54] Vaseva, I.I., Anders, I. and Feller, U. (2013) Identification and Expression of Different Dehydrin Subclasses Involved in the Drought Response of *Trifolium repens*. *Journal of Plant Physiology*, **171**, 213-224.

[55] Dure, L. (1993) A Repeating 11-Mer Amino Acid Motif and Plant Desiccation. *The Plant Journal*, **3**, 363-369. http://dx.doi.org/10.1046/j.1365-313X.1993.t01-19-00999.x

[56] Battista, J., Park, M.J. and McLemore, A. (2001) Inactivation of Two Homologues of Proteins Presumed to Be Involved in the Desiccation Tolerance of Plants Sensitizes *Deinococcus radiodurans* R1 to Desiccation. *Cryobiology*, **43**,

133-139. http://dx.doi.org/10.1006/cryo.2001.2357

[57] Dure, L. (2001) Occurrence of a Repeating 11-Mer Amino Acid Sequence Motif in Diverse Organisms. *Protein and Peptide Letters*, **8**, 115-122. http://dx.doi.org/10.2174/0929866013409643

[58] Gal, T., Glazer, I. and Koltai, H. (2004) An LEA Group 3 Family Member Is Involved in Survival of *C. elegans* during Exposure to Stress. *FEBS Letters*, **577**, 21-26. http://dx.doi.org/10.1016/j.febslet.2004.09.049

[59] Boswell, L.C., Moore, D.S. and Hand, S.C. (2013) Quantification of Cellular Protein Expression and Molecular Features of Group 3 LEA Proteins from Embryos of *Artemia franciscana*. *Cell Stress Chaperones*, **19**, 329-341. http://dx.doi.org/10.1007/s12192-013-0458-3

[60] Tunnacliffe, A., Hincha, D., Leprince, O. and Macherel, D. (2010) LEA Proteins: Versatility of Form and Function. In: Lubzens, E., Cerda, J. and Clark, M., Eds., *Dormancy and Resistance in Harsh Environments*, Springer, Heidelberg, 91-108. http://dx.doi.org/10.1007/978-3-642-12422-8_6

[61] Wise, M.J. and Tunnacliffe, A. (2004) POPP the Question: What Do LEA Proteins Do? *Trends in Plant Science*, **9**, 13-17. http://dx.doi.org/10.1016/j.tplants.2003.10.012

[62] Hand, S., Jones, D., Menze, M. and Witt, T. (2007) Life without Water: Expression of Plant LEA Genes by an Anhydrobiotic Arthropod. *Journal of Experimental Zoology*, **307A**, 62-66. http://dx.doi.org/10.1002/jez.a.343

[63] Wang, X.S., Zhu, H.B., Jin, G.L., Liu, H.L., Wu, W.R. and Zhu, J. (2007) Genome-Scale Identification and Analysis of LEA Genes in Rice (*Oryza sativa* L.). *Plant Sciences*, **172**, 414-420. http://dx.doi.org/10.1016/j.plantsci.2006.10.004

[64] Olvera-Carrillo, Y., Campos, F., Reyes, J.L., Garciarrubio, A. and 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**, 373-390. http://dx.doi.org/10.1104/pp.110.158964

[65] Pla, M., Gómez, J., Goday, A. and Pagès, M. (1991) Regulation of the Abscisic Acid-Responsive Gene rab28 in Maize Viviparous Mutants. *Molecular and General Genetics*, **230**, 394-400. http://dx.doi.org/10.1007/BF00280296

[66] Kiyosue, T., Yamaguchi-Shinozaki, K., Shinozaki, K., Higashi, K., Satoh, S., Kamada, H. and Harada, H. (1992) Isolation and Characterization of a cDNA That Encodes ECP31, an Embryogenic-Cell Protein from Carrot. *Plant Molecular Biology*, **19**, 239-249. http://dx.doi.org/10.1007/BF00027345

[67] Boucher, V., Buitink, J., Lin, X., Boudet, J., Hoekstra, F.A., Hundertmark, M., Renard, D. and Leprince, O. (2010) MtPM25 Is an Atypical Hydrophobic Late Embryogenesis-Abundant Protein That Dissociates Cold and Desiccation-Aggregated Proteins. *Plant, Cell & Environment*, **33**, 418-430. http://dx.doi.org/10.1111/j.1365-3040.2009.02093.x

[68] Colmenero-Flores, J.M.C., Garciarrubio, F. and Covarrubias, A.A. (1997) Characterization of *Phaseolus vulgaris* cDNA Clones Responsive to Water Deficit: Identification of a Novel Late Embryogenesis Abundant-Like Protein. *Plant Molecular Biology*, **35**, 393-405. http://dx.doi.org/10.1023/A:1005802505731

[69] Maskin, L., Frankel, N., Gudesblat, G., Demergasso, M.J., Pietrasanta, L.I. and Iusem, N.D. (2007) Dimerization and DNA-Binding of ASR1, a Small Hydrophilic Protein Abundant in Plant Tissues Suffering from Water Loss. *Biochemical and Biophysical Research Communications*, **352**, 831-835. http://dx.doi.org/10.1016/j.bbrc.2006.11.115

[70] Silhavy, D., Hutvágner, G., Barta, E. and Bánfalvi, Z. (1995) Isolation and Characterization of a Water-Stress-Inducible cDNA Clone from *Solanum chacoense*. *Plant Molecular Biology*, **27**, 587-595. http://dx.doi.org/10.1007/BF00019324

[71] Oliveira, E., Amara, I., Bellido, D., Odena, M.A., Dominguez, E., Pages, M. and Goday, A. (2007) LCMSMS Identification of *Arabidopsis thaliana* Heat-Stable Seed Proteins: Enriching for LEA-Type Proteins by Acid Treatment. *Journal of Mass Spectrometry*, **42**, 1485-1495. http://dx.doi.org/10.1002/jms.1292

[72] Goyal, K., Tisi, L., Basran, A., Browne, J., Burnell, A., Zurdo, J. and Tunnacliffe, A. (2003) Transition from Natively Unfolded to Folded State Induced by Desiccation in an Anhydrobiotic Nematode Protein. *The Journal of Biological Chemistry*, **278**, 12977-12984. http://dx.doi.org/10.1074/jbc.M212007200

[73] Ismail, A., Hall, A. and Close, T. (1999) Purification and Partial Characterization of a Dehydrin Involved in Chilling Tolerance during Seedling Emergence of Cowpea. *Plant Physiology*, **120**, 237-244. http://dx.doi.org/10.1104/pp.120.1.237

[74] Kovacs, D., Agoston, B. and Tompa, P. (2008) Disordered Plant LEA Proteins as Molecular Chaperones. *Plant Signaling & Behavior*, **3**, 710-713. http://dx.doi.org/10.4161/psb.3.9.6434

[75] Shih, M.D., Lin, S.C., Hsieh, J.S., Tsou, C.H., Chow, T.Y., Lin, T.P. and Hsing, Y.I. (2004) Gene Cloning and Characterization of a Soybean (*Glycine max* L.) LEA Protein, GmPM16. *Plant Molecular Biology*, **56**, 689-703. http://dx.doi.org/10.1007/s11103-004-4680-3

[76] Soulages, J., Kim, K., Arrese, E., Walters, C. and Cushman, J. (2003) Conformation of a Group 2 Late Embryogenesis Abundant Protein from Soybean. Evidence of Poly (L-Proline)-Type II Structure. *Plant Physiology*, **131**, 963-975.

http://dx.doi.org/10.1104/pp.015891

[77] Soulages, J., Kim, K., Walters, C. and Cushman, J. (2002) Temperature-Induced Extended Helix/Random Coil Transitions in a Group 1 Late Embryogenesis-Abundant Protein from Soybean. *Plant Physiology*, **128**, 822-832. http://dx.doi.org/10.1104/pp.010521

[78] Tompa, P. (2005) The Interplay between Structure and Function in Intrinsically Unstructured Proteins. *FEBS Letters*, **579**, 3346-3354. http://dx.doi.org/10.1016/j.febslet.2005.03.072

[79] Tompa, P. and Kovacs, D. (2010) Intrinsically Disordered Chaperones in Plants and Animals. *Biochemistry and Cell Biology*, **88**, 167-174. http://dx.doi.org/10.1139/O09-163

[80] Wolkers, W., McCready, S., Brandt, W., Lindsey, G. and Hoekstra, F. (2001) Isolation and Characterization of a D-7 LEA Protein from Pollen That Stabilizes Glasses *in Vitro*. *Biochimica et Biophysica Acta*, **1544**, 196-206. http://dx.doi.org/10.1016/S0167-4838(00)00220-X

[81] Kushwaha, R., Downie, A.B. and Payne, C.M. (2013) Uses of Phage Display in Agriculture: Sequence Analysis and Comparative Modeling of Late Embryogenesis Abundant Client Proteins Suggest Protein-Nucleic Acid Binding Functionality. *Computational and Mathematical Methods in Medicine*, **2013**, 1-11. http://dx.doi.org/10.1155/2013/470390

[82] Wise, M. (2003) LEAping to Conclusions: A Computational Reanalysis of Late Embryogenesis Abundant Proteins and Their Possible Roles. *BMC Bioinformatics*, **4**, 52. http://dx.doi.org/10.1186/1471-2105-4-52

[83] Gentile, F., Amodeo, P., Febbraio, F., Picaro, F., Motta, A., Formisano, S. and Nucci, R. (2002) SDS-Resistant Active and Thermostable Dimers Are Obtained from the Dissociation of Homotetrameric Glycosidase from Hyperthermophilic *Sulfolobus solfataricus* in SDS: Stabilizinz Role of the A-C Intermonomeric Interface. *The Journal of Biological Chemistry*, **277**, 44050-44060. http://dx.doi.org/10.1074/jbc.M206761200

[84] McCubbin, W., Kay, C. and Lane, B. (1985) Hydrodynamic and Optical Properties of the Wheat Germ $E_m$ Protein. *Biochemistry and Cell Biology*, **63**, 803-811. http://dx.doi.org/10.1139/o85-102

[85] Dunker, A.K., Lawson, J.D., Brown, C.J., Williams, R.M. and Romero, P. (2001) Intrinsically Disordered Protein. *Journal of Molecular Graphics and Modelling*, **19**, 26-59. http://dx.doi.org/10.1016/S1093-3263(00)00138-8

[86] Tompa, P. (2002) Intrinsically Unstructured Proteins. *Trends in Biochemical Sciences*, **27**, 527-533. http://dx.doi.org/10.1016/S0968-0004(02)02169-2

[87] Uversky, V., Gillespie, J. and Fink, A. (2000) Why Are "Natively Unfolded" Proteins Unstructured under Physiologic Conditions? *Proteins*, **41**, 415-427. http://dx.doi.org/10.1002/1097-0134(20001115)41:3<415::AID-PROT130>3.0.CO;2-7

[88] Chakraborte, S., Meersman, F., Schierle, G.S., Bertoncini, C.W., McGee, B., Kaminski, C.F. and Tunnacliffe, A. (2010) Catalytic and Chaperone-Like Functions in an Intrinsically Disordered Protein Associated with Desiccation Tolerance. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 16084-16089. http://dx.doi.org/10.1073/pnas.1006276107

[89] Mouillon, J.M., Gustafsson, P. and Harryson, P. (2006) Structural Investigation of Disordered Stress Proteins. Comparison of Full-Length Dehydrins with Isolated Peptides of Their Conserved Segments. *Plant Physiology*, **141**, 638-650. http://dx.doi.org/10.1104/pp.106.079848

[90] Tolleter, D., Jaquinod, M., Mangavel, C., Passirani, C., Saulnier, P., Manon, S., Teyssier, E., Payet, N., Avelange-Macherel, M.H. and Macherel, D. (2007) Structure and Function of a Mitochondrial Late Embryogenesis Abundant Protein Are Revealed by Desiccation. *The Plant Cell Online*, **19**, 1580-1589. http://dx.doi.org/10.1105/tpc.107.050104

[91] Boudet, J., Buitink, J., Hoekstra, F., Rogniaux, H., Larre, 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. http://dx.doi.org/10.1104/pp.105.074039

[92] Koag, M.-C., Fenton, R.D., Wilkens, S. and Close, T.J. (2003) The Binding of Maize DHN1 to Lipid Vesicles. Gain of Structure and Lipid Specificity. *Plant Physiology*, **131**, 309-316. http://dx.doi.org/10.1104/pp.011171

[93] Li, D. and He, X. (2009) Desiccation Induced Structural Alterations in a 66-Amino Acid Fragment of an Anhydrobiotic Nematode Late Embryogenesis Abundant (LEA) Protein. *Biomacromolecules*, **10**, 1469-1477. http://dx.doi.org/10.1021/bm9002688

[94] Olvera-Carrillo, Y., Reyes, J.L. and Covarrubias, A.A. (2011) Late Embryogenesis Abundant Proteins: Versatile Players in the Plant Adaptation to Water Limiting Environments. *Plant Signaling & Behavior*, **6**, 586-589. http://dx.doi.org/10.4161/psb.6.4.15042

[95] Manfre, A., Lanni, L. and Marcotte, W. (2006) The Arabidopsis Group 1 Late-Embryogenesis Abundant Protein ATEM6 Is Required for Normal Seed Development. *Plant Physiology*, **140**, 140-149. http://dx.doi.org/10.1104/pp.105.072967

[96] Manfre, A.J., LaHatte, G.A., Climer, C.R. and Marcotte, W.R. (2009) Seed Dehydration and the Establishment of De-

siccation Tolerance during Seed Maturation Is Altered in the *Arabidopsis thaliana* Mutant Atem6-1. *Plant and Cell Physiology*, **50**, 243-253. http://dx.doi.org/10.1093/pcp/pcn185

[97] Nylander, M., Svensson, J., Palva, E.T. and Welin, B.V. (2001) Stress-Induced Accumulation and Tissue-Specific Localization of Dehydrins in *Arabidopsis thaliana*. *Plant Molecular Biology*, **45**, 263-279. http://dx.doi.org/10.1023/A:1006469128280

[98] Brini, F., Hanin, M., Lumbreras, V., Amara, I., Khoudi, H., Hassairi, A., Pagès, M. and Masmoudi, K. (2007) Overexpression of Wheat Dehydrin DHN-5 Enhances Tolerance to Salt and Osmotic Stress in *Arabidopsis thaliana*. *Plant Cell Reports*, **11**, 2017-2026. http://dx.doi.org/10.1007/s00299-007-0412-x

[99] Rorat, T., Szabala, B., Grygorowicz, W., Wojtowicz, B., Yin, Z. and Rey, P. (2006) Expression of SK3-Type Dehydrin in Transporting Organs Is Associated with Cold Acclimation in Solanum Species. *Planta*, **224**, 205-221. http://dx.doi.org/10.1007/s00425-005-0200-1

[100] Danyluk, J., Perron, A., Houde, M., Limin, A., Fowler, B., Benhamou, N. and Sarhan, F. (1998) Accumulation of an Acidic Dehydrin in the Vicinity of the Plasma Membrane during Cold Acclimation of Wheat. *The Plant Cell Online*, **10**, 623-638. http://dx.doi.org/10.1105/tpc.10.4.623

[101] Houde, M., Dhindsa, R.S. and Sarhan, F. (1992) A Molecular Marker to Select for Freezing Tolerance in Gramineae. *Molecular and General Genetics*, **234**, 43-48.

[102] Rorat, T., Grygorowicz, W.J., Irzykowski, W. and Rey, P. (2004) Expression of KS-Type Dehydrins Is Primarily Regulated by Factors Related to Organ Type and Leaf Developmental Stage during Vegetative Growth. *Planta*, **218**, 878-885. http://dx.doi.org/10.1007/s00425-003-1171-8

[103] Giordani, T., Natali, L., D'Ercole, A., Pugliesi, C., Fambrini, M., Vernieri, P., Vitagliano, C. and Cavallini, A. (1999) Expression of a Dehydrin Gene during Embryo Development and Drought Stress in ABA-Deficient Mutants of Sunflower (*Helianthus annuus* L). *Plant Molecular Biology*, **39**, 739-748. http://dx.doi.org/10.1023/A:1006194720022

[104] Welling, A., Rinne, P., Vihera-Aarnio, A., Kontunen-Soppela, S., Heino, P. and Palva, E.T. (2004) Photoperiod and Temperature Differentially Regulate the Expression of Two Dehydrin Genes during Overwintering of Birch (*Betula pubescens* Ehrh.). *Journal of Experimental Botany*, **55**, 507-516. http://dx.doi.org/10.1093/jxb/erh045

[105] Plana, M., Itarte, E., Eritja, R., Goday, A., Pagès, M. and Martínez, M.C. (1991) Phosphorylation of Maize RAB-17 Protein by Casein Kinase 2. *The Journal of Biological Chemistry*, **266**, 22510-22514.

[106] Riera, M., Figueras, M., Lopez, C., Goday, A. and Pages, M. (2004) Protein Kinase CK2 Modulates Developmental Functions of the Abscisic Acid Responsive Protein Rab17 from Maize. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 9879-9884. http://dx.doi.org/10.1073/pnas.0306154101

[107] Borovskii, G., Stupnikova, I., Antipina, A., Vladimirova, S. and Voinikov, V. (2002) Accumulation of Dehydrin-Like Proteins in the Mitochondria of Cereals in Response to Cold, Freezing, Drought and ABA Treatment. *BMC Plant Biology*, **2**, 5. http://dx.doi.org/10.1186/1471-2229-2-5

[108] Heyen, B.J., Alsheikh, M.K., Smith, E.A., Torvik, C.F., Seals, D.F. and Randall, S.K. (2002) The Calcium-Binding Activity of a Vacuole-Associated, Dehydrin-Like Protein Is Regulated by Phosphorylation. *Plant Physiology*, **130**, 675-687. http://dx.doi.org/10.1104/pp.002550

[109] Cattivelli, L. and Bartels, D. (1990) Molecular Cloning and Characterization of Cold-Regulated Genes in Barley. *Plant Physiology*, **93**, 1504-1510. http://dx.doi.org/10.1104/pp.93.4.1504

[110] Hsing, Y., Chen, Z., Shih, M., Hsieh, J. and Chow, T. (1995) Unusual Sequences of Group 3 LEA mRNA Inducible by Maturation or Drying in Soybean Seeds. *Plant Molecular Biology*, **29**, 863-868. http://dx.doi.org/10.1007/BF00041175

[111] Romo, S., Labrador, E. and Dopico, B. (2001) Water Stress-Regulated Gene Expression in *Cicer arietinum* Seedlings and Plants. *Plant Physiology and Biochemistry*, **39**, 1017-1026. http://dx.doi.org/10.1016/S0981-9428(01)01318-3

[112] NDong, C., Danyluk, J., Wilson, K.E., Pocock, T., Huner, N.P.A. and Sarhan, F. (2002) Cold-Regulated Cereal Chloroplast Late Embryogenesis Abundant-Like Proteins. Molecular Characterization and Functional Analyses. *Plant Physiology*, **129**, 1368-1381. http://dx.doi.org/10.1104/pp.001925

[113] Siddiqui, N.U., Chung, H.J., Thomas, T.L. and Drew, M.C. (1998) Abscisic Acid-Dependent and -Independent Expression of the Carrot Late-Embryogenesis-Abundant-Class Gene Dc3 in Transgenic Tobacco Seedlings. *Plant Physiology*, **118**, 1181-1190. http://dx.doi.org/10.1104/pp.118.4.1181

[114] Curry, J., Morris, C.F. and Walker-Simmons, M.K. (1991) Sequence Analysis of a cDNA Encoding a Group 3 LEA mRNA Inducible by ABA or Dehydration Stress in Wheat. *Plant Molecular Biology*, **16**, 1073-1076. http://dx.doi.org/10.1007/BF00016078

[115] Ried, J.L. and Walker-Simmons, M.K. (1993) Group 3 Late-Embryogenesis-Abundant Protein in Desiccation-Tolerant Seedlings of Wheat (*Triticum aestivum* L.). *Plant Physiology*, **102**, 125-131. http://dx.doi.org/10.1104/pp.102.1.125

[116] Roberts, J.K., DeSimone, N.A., Lingle, W.L. and Dure III, L. (1993) Cellular Concentrations and Uniformity of

Cell-Type Accumulation of Two Lea Proteins in Cotton Embryos. *The Plant Cell Online*, **5**, 769-780. http://dx.doi.org/10.1105/tpc.5.7.769

[117] Marttila, S., Tenhola, T. and Mikkonen, A. (1996) A Barley (Hordeum *vulgare* L.) LEA3 Protein, HVA1, Is Abundant in Protein Storage Vacuoles. *Planta*, **199**, 602-611. http://dx.doi.org/10.1007/BF00195193

[118] Grelet, J., Benamar, A., Teyssier, E., Avelange-Macherel, M.H., Grunwald, D. and Macherel, D. (2005) Identification in Pea Seed Mitochondria of a Late-Embryogenesis Abundant Protein Able to Protect Enzymes from Drying. *Plant Physiology*, **137**, 157-167. http://dx.doi.org/10.1104/pp.104.052480

[119] Ukaji, N., Kuwabara, C., Takezawa, D., Arakawa, K. and Fujikawa, S. (2001) Cold Acclimation-Induced WAP27 Localized in Endoplasmic Reticulum in Cortical Parenchyma Cells of Mulberry Tree Was Homologous to Group 3 Late-Embryogenesis Abundant Proteins. *Plant Physiology*, **126**, 1588-1597. http://dx.doi.org/10.1104/pp.126.4.1588

[120] Cohen, A., Plant, Á.L., Moses, M.S. and Bray, E.A. (1991) Organ-Specific and Environmentally Regulated Expression of Two Abscisic Acid-Induced Genes of Tomato. *Plant Physiology*, **97**, 1367-1374. http://dx.doi.org/10.1104/pp.97.4.1367

[121] Delseny, M., Bies-Etheve, N., Carles, C., Hull, G., Vicient, C., Raynal, M., Grellet, F. and Aspart, L. (2001) Late Embryogenesis Abundant (LEA) Protein Gene Regulation during Arabidopsis Seed Maturation. *Journal of Plant Physiology*, **158**, 419-427. http://dx.doi.org/10.1078/0176-1617-00353

[122] Kiyosue, T., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1994) Characterization of Two cDNAs (ERD10 and ERD14) Corresponding to Genes That Respond Rapidly to Dehydration Stress in *Arabidopsis thaliana*. *Plant and Cell Physiology*, **35**, 225-231.

[123] Maitra, N. and Cushman, J. (1994) Isolation and Characterization of a Drought-Induced Soybean cDNA Encoding a D95 Family Late-Embryogenesis-Abundant Protein. *Plant Physiology*, **106**, 805-806. http://dx.doi.org/10.1104/pp.106.2.805

[124] Zegzouti, H., Jones, B., Frasse, P., Marty, C., Maitre, B., Latché, A., Pech, J.C. and Bouzayen, M. (1999) Ethylene-Regulated Gene Expression in Tomato Fruit: Characterization of Novel Ethylene Responsive and Ripening-Related Genes Isolated by Differential Display. *The Plant Journal*, **18**, 589-600. http://dx.doi.org/10.1046/j.1365-313x.1999.00483.x

[125] Niogret, M.F., Culiáñez-Macià, F.A., Goday, A., Albà, M.M. and Pagès, M. (1996) Expression and Cellular Localization of Rab28 mRNA and Rab28 Protein during Maize Embryogenesis. *The Plant Journal*, **9**, 549-557. http://dx.doi.org/10.1046/j.1365-313X.1996.09040549.x

[126] Leprince, O. and Buitink, J. (2010) Desiccation Tolerance: From Genomics to the Field. *Plant Science*, **179**, 554-564. http://dx.doi.org/10.1016/j.plantsci.2010.02.011

[127] Goday, A., Jensen, A.B., Culianez-Macia, F.A., Alba, M., Figueras, M., Serratosa, M., Torrent, J. and Pagès, M. (1994) The Maize Abscisic Acid-Responsive Protein Rab17 Is Located in the Nucleus and Interacts with Nuclear Localization Signals. *Plant Cell*, **6**, 351-336. http://dx.doi.org/10.1105/tpc.6.3.351

[128] Hanin, M., Brini, F., Ebel, C., Toda, Y., Takeda, S. and Masmoudi, K. (2011) Plant Dehydrins and Stress Tolerance: Versatile Proteins for Complex Mechanisms. *Plant Signaling & Behavior*, **6**, 1503-1509. http://dx.doi.org/10.4161/psb.6.10.17088

[129] Wasilewska, A., Vlad, F., Sirichandra, C., Redko, Y., Jammes, F., Valon, C., Frey, N.F.D. and Leung, J. (2008) An Update on Abscisic Acid Signaling in Plants and More. *Molecular Plant*, **1**, 198-217. http://dx.doi.org/10.1093/mp/ssm022

[130] Delahaie, J., Hundertmark, M., Bove, J., Leprince, O., Rogniaux, H. and 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*, **14**, 4559-4573. http://dx.doi.org/10.1093/jxb/ert274

[131] Guo, F., Liu, C., Xia, H., Bi, Y., Zhao, C., Zhao, S., Hou, L., Li, F. and Wang, X. (2013) Induced Expression of AtLEC1 and AtLEC2 Differentially Promotes Somatic Embryogenesis in Transgenic Tobacco Plants. *PLoS ONE*, **8**, e71714. http://dx.doi.org/10.1371/journal.pone.0071714

[132] Grelet, J., Benamar, A., Teyssier, E., Avelange-Macherel, M.-H., Grunwald, D. and Macherel, D. (2005) Identification in Pea Seed Mitochondria of a Late-Embryogenesis Abundant Protein Able to Protect Enzymes from Drying. *Plant Physiology*, **137**, 157-167. http://dx.doi.org/10.1104/pp.104.052480

[133] Reyes, J., Rodrigo, M.J., Colmenero-Flores, J., Gil, J.V., Garay-Arroyo, A., Campos, F., Salamini, F., Bartels, D. and Covarrubias, A. (2005) Hydrophilins from Distant Organisms Can Protect Enzymatic Activities from Water Limitation Effects *in Vitro*. *Plant, Cell & Environment*, **28**, 709-718. http://dx.doi.org/10.1111/j.1365-3040.2005.01317.x

[134] Chakrabortee, S., Boschetti, C., Walton, L.J., Sarkar, S., Rubinsztein, D.C. and Tunnacliffe, A. (2007) Hydrophilic Protein Associated with Desiccation Tolerance Exhibits Broad Protein Stabilization Function. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 18073-18078.

http://dx.doi.org/10.1073/pnas.0706964104

[135] Goyal, K., Pinelli, C., Maslen, S.L., Rastogi, R.K., Stephens, E. and Tunnacliffe, A. (2005) Dehydration-Regulated Processing of Late Embryogenesis Abundant Protein in a Desiccation Tolerant Nematode. *FEBS Letters*, **579**, 4093-4098. http://dx.doi.org/10.1016/j.febslet.2005.06.036

[136] Hatanaka, R., Hagiwara-Komoda, Y., Furuki, T., Kanamori, Y., Fujita, M., Cornette, R., Sakurai, M., Okuda, T. and Kikawada, T. (2013) An Abundant LEA Protein in the Anhydrobiotic Midge, PvLEA4, Acts as a Molecular Shield by Limiting Growth of Aggregating Protein Particles. *Insect Biochemistry and Molecular Biology*, **11**, 1055-1067. http://dx.doi.org/10.1016/j.ibmb.2013.08.004

[137] Nakayama, K., Okawa, K., Kakizaki, T., Honma, T., Itoh, H. and Inaba, T. (2007) Arabidopsis Cor15am Is a Chloroplast Stromal Protein That Has Cryoprotective Activity and Forms Oligomers. *Plant Physiology*, **144**, 513-523. http://dx.doi.org/10.1104/pp.106.094581

[138] Puhakainen, T., Hess, M., Makela, P., Svensson, J., Heino, P. and Palva, E. (2004) Overexpression of Multiple Dehydrin Genes Enhances Tolerance to Freezing Stress in Arabidopsis. *Plant Molecular Biology*, **54**, 743-753. http://dx.doi.org/10.1023/B:PLAN.0000040903.66496.a4

[139] Egerton-Warburton, L.M., Balsamo, R.A. and Close, T.J. (1997) Temporal Accumulation and Ultrastructural Localization of Dehydrins in *Zea mays*. *Physiologia Plantarum*, **101**, 545-555. http://dx.doi.org/10.1111/j.1399-3054.1997.tb01036.x

[140] Koag, M.C., Fenton, R.D., Wilkens, S. and Close, T.J. (2003) The Binding of Maize DHN1 to Lipid Vesicles. Gain of Structure and Lipid Specificity. *Plant Physiology*, **131**, 309-316. http://dx.doi.org/10.1104/pp.011171

[141] Koag, M.C., Wilkens, S., Fenton, R.D., Resnik, J., Vo, E. and Close, T.J. (2009) The K-Segment of Maize DHN1 Mediates Binding to Anionic Phospholipid Vesicles and Concomitant Structural Changes. *Plant Physiology*, **150**, 1503-1514. http://dx.doi.org/10.1104/pp.109.136697

[142] Eriksson, S.K., Kutzer, M., Procek, J., Grobner, G. and Harryson, P. (2011) Tunable Membrane Binding of the Intrinsically Disordered Dehydrin Lti30, a Cold-Induced Plant Stress Protein. *The Plant Cell*, **6**, 2391-404l. http://dx.doi.org/10.1105/tpc.111.085183

[143] Alsheikh, M., Svensson, J. and Randall, S. (2005) Phosphorylation Regulated Ion-Binding Is a Property Shared by the Acidic Subclass Dehydrins. *Plant, Cell & Environment*, **28**, 1114-1122. http://dx.doi.org/10.1111/j.1365-3040.2005.01348.x

[144] Svensson, J., Palva, E.T. and Welin, B. (2000) Purification of Recombinant *Arabidopsis thaliana* Dehydrins by Metal Ion Affinity Chromatography. *Protein Expression and Purification*, **20**, 169-178. http://dx.doi.org/10.1006/prep.2000.1297

[145] Hara, M., Fujinaga, M. and Kuboi, T. (2005) Metal Binding by Citrus Dehydrin with Histidine-Rich Domains. *Journal of Experimental Botany*, **56**, 2695-2703. http://dx.doi.org/10.1093/jxb/eri262

[146] Buitink, J. and Leprince, O. (2004) Glass Formation in Plant Anhydrobiotes: Survival in the Dry State. *Cryobiology*, **48**, 215-228. http://dx.doi.org/10.1016/j.cryobiol.2004.02.011

[147] Lin, C. and Thomashow, M. (1992) DNA Sequence Analysis of a Complementary DNA for Cold Regulated *Arabidopsis* Gene *cor*15 and Characterization of the COR15 Polypeptide. *Plant Physiology*, **99**, 519-525. http://dx.doi.org/10.1104/pp.99.2.519

[148] Hara, M., Shinoda, Y., Tanaka, Y. and Kuboi, T. (2009) DNA Binding of Citrus Dehydrin Promoted by Zinc Ion. *Plant, Cell & Environment*, **32**, 532-541. http://dx.doi.org/10.1111/j.1365-3040.2009.01947.x

[149] Singh, J., Whitwill, S., Lacroix, G., Douglas, J., Dubuc, E., Allard, G., Keller, W. and Schernthaner, J.P. (2009) The Use of Group 3 LEA Proteins as Fusion Partners in Facilitating Recombinant Expression of Recalcitrant Proteins in *E. coli*. *Protein Expression and Purification*, **67**, 15-22. http://dx.doi.org/10.1016/j.pep.2009.04.003

[150] Chakrabortee, S., Meersmanb, F., Schierlec, G.S.K., Bertoncinid, C.W., McGeea, B., Kaminskic, C.F. and Tunnacliffe, A. (2010) Catalytic and Chaperone-Like Functions in an Intrinsically Disordered Protein Associated with Desiccation Tolerance. *Proceedings of the National Academy of Sciences*, **107**, 16084-16089. http://dx.doi.org/10.1073/pnas.1006276107

Scientific Research Publishing (SCIRP) is one of the largest Open Access journal publishers. It is currently publishing more than 200 open access, online, peer-reviewed journals covering a wide range of academic disciplines. SCIRP serves the worldwide academic communities and contributes to the progress and application of science with its publication.

Other selected journals from SCIRP are listed as below. Submit your manuscript to us via either submit@scirp.org or Online Submission Portal.