Possible roles of LEA proteins and sHSPs in seed protection: 
a short review

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Abstract: Late embryogenesis abundant (LEA) proteins and small heat shock proteins (sHSPs) are produced in seeds during maturation and under various stress conditions. Their expression is developmentally regulated or induced by drought, salinity, cold, oxidative stress, and abscisic acid (ABA). Protective functions of sHSPs and LEA proteins have been widely analysed in many plant seeds, including seeds of the orthodox, recalcitrant and intermediate category. Moreover, both the sHSPs and LEA proteins are intensively synthesized during seed development, as a part of the embryogenesis program. Numerous investigations have proved that LEA proteins are involved in complex processes that permit seeds to survive in the dry state, such as glass formation and cryoprotection mechanisms. Considering all LEA groups, dehydrins are major proteins associated with conditions affecting the water status in cells. Either LEA proteins or sHSPs are strongly correlated with seed longevity. Due to the ability of sHSPs to interact with other chaperone proteins, they can attach to denatured proteins and therefore stabilize their conformation, but they also assist in protein folding and refolding, intracellular transportation, marking proteins for degradation, and possibly glassy state formation. Thus the presence of protective proteins and their proper action as an essential factor for seed protection is discussed here.

Keywords: seed, abiotic stress, LEA proteins, dehydrin, small heat shock proteins

INTRODUCTION

Plant species diversity is important for preserving the natural relations in ecosystems. Currently in seed genebanks various seeds can be stored for decades or even centuries, but tree propagation from seeds is not easy, especially for species that produce seeds irregularly or rarely. It is even more difficult if the seeds cannot be stored for a long time, or at least no effective storage conditions have been identified yet. There is usually a reserve of dormant seeds in the soil in habitats where the plants grow. However, seed production can be seriously limited by various environmental stresses.

Some environmental stresses, such as drought stress, cold stress and salinity stress, result in cellular dehydration. Water is one of the factors that limit seed de-
velopment, storage, and germination, so seeds must have some mechanisms that allow them to withstand the loss of water. It is more interesting if we consider that storage is not the only situation when seeds are exposed to limited water availability. The shortage of water in seeds activates specific processes and can lead to a loss of viability and decreased vigour or even death. Metabolism reduction, distribution of amphiphilic elements in the cellular membrane, and glass structure formation, enable cells to survive the time of desiccation.

Seeds are able to survive dehydration, but to a varied extent. Two types of tolerance can be distinguished on the basis of moisture content: drought tolerance and desiccation tolerance (Hoekstra et al. 2001). Drought tolerance is the tolerance to dehydration down to 23% of fresh weight, which is about 0.3 g H₂O per 1 g of dry weight. That means a lack of bulk water in the cytoplasm, so the mechanisms that stabilize intracellular structures by preferential hydration are launched. Desiccation tolerance can be defined as the ability to survive stronger dehydration and to restore normal function when rehydrated. Then mechanisms connected with water replacement by compounds able to form hydrogen bonds are activated. The organisms resistant to desiccation have structural, physiological and molecular mechanisms enabling survival at the time of water deficit. But the mechanisms are still not fully explained. Morphological changes formed as a result of the water loss, cause physiological changes and hence modifications at the molecular level: accumulation of protective compounds, changes in enzymatic activity and in gene expression (Close 1996). Considering the classification of seeds into orthodox, recalcitrant and intermediate categories, predominantly orthodox seeds are resistant to desiccation, and recalcitrant ones are only drought-tolerant (Roberts 1973, Ellis et al. 1990). The resistance to desiccation is possible in the presence of specific mono-, di- and oligosaccharides, late embryogenesis abundant (LEA) proteins, and small heat shock proteins (sHSPs) (Derocher & Vierling 1994), soluble sugars (Koster & Leopold 1998), and mechanisms related to ROS removal (Hendry 1993) and glass formation (Buitink et al. 1998).

Investigations with various mutants of species that produce orthodox seeds (mainly Arabidopsis thaliana (L.) Heynn) have proved that modification of several genes can be sufficient to lose the resistance to desiccation (Ooms et al. 1993). However, no species with recalcitrant seeds was reported to produce orthodox seeds after any mutation. Nevertheless, the resistance to desiccation can be induced externally. Embryonic axes from the normally recalcitrant seeds of silver maple (Acer saccharinum L.) can be made more tolerant to desiccation (moisture content 10%) and low temperature (-196°C) by pretreatment with abscisic acid (ABA) and the growth-retardant tetcyclacis, which enhances endogenous ABA concentrations and induces synthesis of some LEA proteins (Beardmore & Whittle 2005). Considering that desiccation tolerance is a complex multigene process, the mechanisms by which various organisms can survive the time of desiccation must have some common, universal elements.

Both LEA proteins and sHSPs appear and accumulate during seed development, and then gradually disappear during germination, indicating that desiccation tolerance is at first achieved and then lost. Moreover, their expression can be induced or enhanced by various stress conditions. Desiccation-tolerant organisms have the
ability to form glasses. Below 0.1 g H₂O per 1 g of dry weight, the cytoplasm undergoes glass transition. The glass is a metastable and amorphous structure, which thermodynamically behaves as a liquid. In that state the diffusion of chemical compounds and reactions are slowed down (Buittink et al. 2000). Tₓ, the temperature of transition from the liquid to glassy state, depends on the moisture content, temperature and chemical composition of the cytosol. Carbohydrates (such as sucrose, raffinose and stachyose) can effectively help in osmotic pressure adaptation during water loss and participate in glass formation. They generate the highly reactive hydrogen bonds, so the mechanical properties of glass are similar to those of solid bodies. Tₓ values correlate with the molecular mass of carbohydrates: the higher the molecular mass, the higher the Tₓ value. In seeds, LEA proteins (Wolkers et al. 2001), together with oligosaccharides and possibly sHSPs (Vierling 1997), participate in glass formation and stabilization. Research on functions of LEA proteins and sHSPs in seeds is important and will be discussed here.

LATE EMBRYOGENESIS ABUNDANT (LEA) PROTEINS

Water-protein interactions are one of the main forces in protein folding and stabilizing the conformation, but also in determining the dynamics and properties of bonds in proteins and their catalytic activity. LEA proteins were first identified in seeds, when mRNA and proteins that occur in large quantities were investigated (Dure 1993). LEA proteins are located in the cytoplasm, nucleus, mitochondria, vacuoles (Houde et al. 1995, Egerton-Warburton et al. 1997), near the cellular membrane (Danyluk et al. 1998), as well as in amyloplasts (Rinne et al. 1999), but primarily they accumulate in the cytoplasm and nucleus. After synthesis they are transported to cell organelles and membranes, where they stabilize cell structures and molecules. The partition of LEA proteins into various cell compartments determines their possible function in the protection or regulation of essential biochemical processes, like replication or respiration.

Classification of LEA proteins

LEA proteins can be classified into 3 or 6 groups. The classification is based on sequence similarity and properties (Cuming 1999, Wise 2003). Proteins of group 1 contain a 20-amino-acid (aa) region, which was first identified in the wheat Em protein (osmoprotective molecule) (Cuming 1999). LEA proteins of group 2 are widespread and widely analysed in respect of water stress. Group 3 of LEA proteins is characterized by the 11-aa motive ΦΦE/QXΦKE/QKΦXE/D/Q, where Φ represents a hydrophobic aa. It has been predicted that this region forms an amphipathic α-helix, which probably participates in structural interactions (Baker et al. 1988). LEA proteins of group 3 are involved in response to cold stress. Usually they are located in the cytoplasm, nucleus and mitochondria, where they can be associated with membranes. Groups 4 and 5 have a less conserved structure and are possibly involved in the protection of membrane stability.

Group 2 of LEA proteins is also called the D-11 family (Dure et al. 1989) or dehydrins (Close 1997). They are glycine- and lysine-rich proteins with molecular weight of 9–200 kDa. Glycine, histidine, lysine and threonine comprise 56% of all
aa in LTI30 (water stress-responsive protein). K, S and Y segments are characteristic of dehydrins (CLOSE 1996). The K segment sequence is conserved and consists of 14 aa: EKKGIMDKIKEELPG. Substitutes and structural modifications of aa are possible in the K segment, which can be repeated 1–12 times in a single protein and always is situated near the C end (CLOSE 1997). Many dehydrins contain a serine-rich S segment, which might be the phosphorylation site of the protein. The S segment phosphorylation might be an important posttranslational modification, which is required for proper protein location, as it is observed during Rab 17 placing in the nucleus (GODAY et al. 1994), and a signal in stress transduction pathway.

The classification of dehydrins is based on the presence of conserved segments (CLOSE 1997). Five classes of dehydrins can be distinguished: YnSK2, Kn, KnS, SKn, and YnK. The YnSK2 dehydrins consist of 1–3 Y segments, a serine-rich segment, and 2 K segments. Their expression is induced mainly by ABA (RAB type) and drought, less often by low temperature. Some YnSK dehydrins have an ability to bind lipid vesicles containing acidic phospholipids (KOBAG et al. 2003). In Kn dehydrins, the occurrence of 1–9 serine-rich segments is observed. They are acidic or neutral proteins, mainly induced by low temperature and somewhat by ABA and drought. The K segment of class KnS begins with sequence (H/Q) KEG instead of EKKG. Their expression is induced by ABA and dehydration. KnS dehydrins can bind various metal ions or scavenge the hydroxyl radicals (ASHGAR et al. 1994). YnK proteins usually consist of 2 Y and 2 K segments. They are expressed during seed germination and related with resistance to low temperature. The SKn dehydrins contain 1 S and 1–3 K segments and are preferentially induced by low temperature. Moreover, they can be induced by drought, salinity, injury and jasmonate treatment. Dehydrins lacking the Y segment were identified in many other plant tissues, such as stem tissue, vegetative and reproductive buds, needle tissue, unstressed leaf and root tissue. Its expression was induced by water, salt, cold and osmotic stress (CARUSO et al. 2002). The K segment was considered to appear in all classes of dehydrins (CLOSE 1997), but recently a protein lacking the K segment (ROD 25 from cold acclimated Cornus sericea, syn. C. stolonifera) was detected (SARNIGHAUSEN et al. 2002). The role of the conserved segments is to exert their biological function upon recognition and interaction with specific biological targets. The combination and number of repeated segments is related with the possible protein functions displayed in seeds under stress conditions.

**LEA gene expression and protein structure**

LEA genes are sensitive to ABA because of the presence of ABREs (ABA-responsive elements), i.e. regulatory elements in promoter regions, which contain the ACGT sequence called cassette G. Sensitivity to ABA also depends on the presence of MYC elements that include the sequence CACCTG, and MYB elements that include the TAACTG motive. ABA-independent expression of dehydrins is based on activation of DREs (drought-responsive elements), also known as CRT (C-repeat) elements (STOCKINGER et al. 1997). The ABREs can interact with the bZIP transcription factors. ABRE motifs are also present in the promoter regions in drought-stress-inducible regulatory genes that are involved in modulation of the expression of multiple genes related to plant response to stress.
Only one spatial structure of dehydrin is deposited in the PDB (Protein Data Bank) database. It is the 1YYC Arabidopsis thaliana protein, whose structure was solved by using the NMR method (Singh et al. 2005). Analyses of the wheat Em protein and maize G50 protein suggest that up to 75% of the native protein can be unordered (McCubbin et al. 1985). The lack of an ordered structure results in resistance to high temperature. The 3-dimensional structure can be achieved after protein-target interaction. The role of the conserved segments in the promotion of tertiary structure is not clear (Mouillon et al. 2006). Formation of the secondary structure of LEA proteins was examined during gradual water removal from solutions. Formation of α-helix structures was detected in the dehydrin-related DSP16 protein from Craterostigma plantagineum Hochst. (Lisse et al. 1996) and another dehydrin-related protein from Vigna unguiculata L. in the presence of sodium dodecyl sulfate (Ismail et al. 1999). In water solutions, LEA proteins do not display any spatial structure, but various secondary structures appear depending on the solvent type the proteins are put in. In saline solutions, α-helices are always formed. In sucrose solutions, both α-helices and β-sheets are created during slow drying, but only α-helices during rapid drying (Goyal et al. 2003). Various stress conditions seem to be a signal for protein folding and for achieving the tertiary structure.

Seed development, desiccation and storage – apparent functions of LEA proteins

LEA proteins have been widely analysed in plants. They play an important role in seed maturation and osmotic stress. LEA proteins in seeds are related to desiccation tolerance (Xu et al. 1996). In seeds the germination rate (Koster & Leopold 1998), electrolyte leakage (Sun & Leopold 1993), presence of heat-stable proteins and LEA accumulation (Close 1997) can be measured to detect the moment of desiccation tolerance acquisition. The mRNA of LEA genes appears in maturing seeds and becomes the most abundant mRNA species in the dry seed, but disappears shortly after imbibition. LEA proteins are hydrophilic. In drought-tolerant seeds, numerous polar groups situated on the surface of LEA proteins participate in preferential hydration. During water deficit in desiccation-tolerant seeds, they can interact with other groups and replace water in the cell. LEA proteins occur mostly in orthodox but also in recalcitrant and intermediate seeds, indicating their key functions, and possible protective roles could be displayed in all categories. Constitutive expression of some LEA genes was detected in A. thaliana, e.g. dhnx (Welin et al. 1994) and ERD14 (Nylander et al. 2001), and in pea (Robertson & Chandler 1992). LEA gene expression can be induced both in desiccation-resistant and sensitive tissues after ABA treatment, except some tropical species that produce orthodox seeds (Farrant et al. 1996, Kermode 1997).

Dehydrins or dehydrin-related proteins were detected in many species, with seeds classified into various categories: recalcitrant (Quercus robur L., Castanea sativa L., Acer saccharinum L.), orthodox (Acer platanoides L., Pismum sativum L., Zea mays L.), and intermediate (Coffeea arabica L.) (Robertson & Chandler 1992, Finch-Savage et al. 1994, Campbell et al. 1998, Hinniger et al. 2006). Differences observed in dehydrin occurrence in embryonic axes and cotyledons of mature and immature seeds of diverse organisms show differential desiccation sensitivity of seed tissues and variations in desiccation tolerance between species (Finch-Savage et al. 1994, Close 1997).
The glass matrix formed in sucrose solutions containing LEA proteins is characterized by higher $T_g$ values and stronger hydrogen bonds than those observed in a sucrose solution without LEA proteins. Such properties are typical of glass structure formed in seeds during storage (OLIVER et al. 2001). The glass structure assures cell stability at the time of dehydration. Intracellular glass, due to its high viscosity, can prevent the crystallization of chemical compounds (in spite of their high concentrations), membrane fusion, and conformational changes of proteins. There is a linear dependence between the limited mobility of the cytoplasm and cell life span, therefore intracellular glass has a significant effect on the storage life of seeds (LEOPOLD et al. 1994, BUITINK et al. 2000). The existence of glassy state was detected in many orthodox seeds, e.g. in *Pisum sativum* L. (BUITINK et al. 1998), *Lactuca sativa* L. (BUITINK et al. 2000), *Zea mays* L. (WILLIAMS & LEOPOLD 1989), *Glycine max* (L.) Merr. (SUN & LEOPOLD 1993), and also in the intermediate seeds of *Fagus sylvatica* L. (PUKACKA et al. 2003). Glassy state is essential for seeds to stay alive during storage and grow when they are needed.

**LEA proteins in relation to environmental stresses**

Various LEA proteins have been found to accumulate in plants during cold acclimation. Therefore, LEA proteins might participate in cryoprotection mechanisms, by accumulation in the tissues where primary ice nucleation occurs. Enzyme protection is important during long-term seed storage in seedbanks (usually low temperature) and in the soil during winter. In various plant tissues LEA proteins are able to prevent the inactivation of cyclic frozen and unfrozen lactate dehydrogenase (HOUDE et al. 1995), so they probably display the same protective role in seeds. Moreover, during germination, LEA proteins may regulate $\alpha$-amylase activity and starch grain degradation throughout the time of water deficit (RINNE et al. 1999). The regulatory role in relation to enzymatic activity also can be detected at the reaction level, because in dry seeds and whole plants LEA proteins can store the water molecules needed to catalyse biochemical reactions, especially in wintertime. A high salt concentration affects many important processes, such as seed development and germination. Due to saline stress, diverse compatible solutes (osmotically active compounds) and polyamines are produced, and the antioxidant defence mechanism, ion transport and compartmentalization of toxic ions are initiated. The expression of LEA proteins of all groups is responsive to salinity, as it was shown many times in salt-stressed seedlings and mature plants (NAOT et al. 1995). During osmotic stress, dehydrins can act as antioxidants scavenging the toxic quantities of metal ions in whole plants (HARA et al. 2005).

Using the POPP program, additional possible functions of LEA proteins have been proposed. LEA proteins may have enzymatic or chaperone activity and act as nucleus proteins that unwind or repair DNA, regulate transcription, and might be associated with chromatin or cytoskeleton (WISE & TUNNACLIFE 2004).

**SMALL HEAT SHOCK PROTEINS (sHSPs)**

Eukaryotic organisms have 5 classes of heat shock proteins: Hsp90, Hsp70 (DnaK), GroEL and Hsp60, Hsp100 (Clp), and sHSPs (WANG et al. 2004). Various
sHSPs are widespread in all kingdoms, but are absent in several pathogenic organisms. Bacteria and unicellular eukaryotes contain 1 or 2 sHSP genes, while 4 genes are present in Drosophila melanogaster, 16 in the nematode C. elegans, and 19 in A. thaliana (HASLBECK et al. 2005). In plants, sHSPs are a numerous and diverse protein group. Classification into 6 groups is based on the similarity of gene sequence, immunological reactivity, and intracellular location (WATERS et al. 1996). These proteins usually are undetectable in vegetative tissues, but in seeds their expression is induced by stress conditions and development stage (WEHMEYER & VIERLING 2000). Classes CI, CII and CIII are represented by cytosolic and nuclear proteins, CIV are found in plastids (VIERLING 1997), CV are associated with endoplasmic reticulum (HELM et al. 1993), and CVI are mitochondrial (LENE et al. 1995). Their broad distribution implicates that these proteins can protect practically all cellular compartments (KOTAK et al. 2007). Specific organelle localization of sHSPs is characteristic for plants, except for HSP22, which occurs also in other eukaryotic mitochondria. Proteins that belong to the same class show a high sequence similarity, even if they come from different plant species. Conserved elements of protein sequences render almost identical core structures and therefore the same functions. The structural organization of sHSPs is evolutionarily conserved. They contain an α-crystalline domain, which consists of 90 aa and can undergo dimerization, and conserved N and C ends. The α-crystalline domain functions like a molecular chaperone, in preventing the aggregation of various proteins under a wide range of stress conditions by selective interactions of its hydrophobic surface with non-native proteins (REDDY et al. 2006). The spatial structure of sHSPs is very dynamic and plastic. The crystal structure of several sHSPs is known, mainly bacterial and fungal. They all can associate in oligomeric structures with various numbers of subunits: 24 in yeasts (Hsp26), and 12 in wheat (Hsp16.9) (VAN MONTFORT et al. 2001).

**Induction of sHSP gene expression and protein synthesis in seeds**

Expression of some sHSPs can be induced by osmotic stress. In sunflower (Helianthus annuus L.) seeds, the HaHSP17.6 (CI) and HaHSp17.9 (CII) gene expression is induced by dehydration, and the mRNA level correlates with the rate of dehydration. Also mannitol and ABA can induce HaHsp17.9 expression (ALMOGUERA et al. 1993). HaHsp17.6 and HaHsp17.9 are similar to cytosolic sHSPs of C. plan-tagineum and accumulate after dehydration (ALAMILLO et al. 1995).

In Arabidopsis leaves, sHSPs of class I do not appear after dehydration, but in seeds the expression of AtHsp17.7 (CII) and AtHsp17.6 (CII) is induced by osmotic stress (WEHMEYER et al. 1996). Some cytosolic CI and CII sHSPs (ALAMILLO et al. 1995), mitochondrial sHSPs (BANZET et al. 1998) and chloroplast sHSPs (LEE & VIERLING 2000) can be induced by oxidative stress. Besides, sHSPs can be induced by cold stress (SABEHAT et al. 1998), heavy metals (GYÖRGYEVY et al. 1991), ozone (ECKEY-KAL TENBACH et al. 1997), UV and γ radiation (BANZET et al. 1998). This suggests that sHSPs can be associated with the general mechanism of cell response to abiotic stress. Changes in sHSPs expression are similar to those in LEA proteins at the time of dehydration and crucial developmental stages. sHSPs are synthesized during embryogenesis, germination, pollen production, and fruit maturation (WATERS et al. 1996, SUN et al. 2002). In A. thaliana embryos, the cytosolic AtHsp17.4 (CI),
AtHsp17.6 (CI) and AtHsp17.7 (CII) accumulate during the middle phase of seed maturation. Their concentration remains high during the late phase and in dry mature seeds (Wehmeier et al. 1996). Similarly, the accumulation of class I and class II sHSPs is observed in pea and sunflower seeds (Almoguera et al. 1993, DeRocher & Vierling 1994). Moreover, OsHsp16.9A abundantly accumulates in mature dry rice seeds (Guan et al. 2004). The synthesis of sHSPs during seed maturation indicates their probable role in cell component protection mechanisms. Mutants sensitive to desiccation contain smaller amounts of sHSPs during maturation (Wehmeier et al. 1996, Wehmeier & Vierling 2000). In seeds, the appearance of sHSPs is species-specific, and an increase in their content is always observed before the acquisition of desiccation tolerance (Zur Nieden et al. 1995). During germination, sHSPs are present in large quantities for several days, and next disappear rapidly (DeRocher & Vierling 1994, Wehmeier et al. 1996). Additionally, during germination, the sHSPs disappearance is parallel to storage protein degradation (Lubaretz & Zur Nieden 2002). Their activity is induced and does not depend on the presence of ATP. After activation by stress conditions or phosphorylation, sHSPs cooperate with other chaperone proteins that have ATPase activity (Haslbeck et al. 2005, van Montfort et al. 2001).

Role of sHSPs

These proteins may act as protective proteins in vitro (Lee et al. 1997) and in vivo (Löw et al. 2000). Their universal protective role, low tissue specificity, and participation in glass structure formation (HSP 17.4 in Arabidopsis) have been proposed (Wehmeier & Vierling 2000). They can attach to denatured proteins and thus stabilize their conformation, also can help in protein folding, oligomer formation, intracellular transportation, and marking for degradation (Hendrick & Hartl 1995). Conformation changes, such as dimerization, oligomer formation (Haslbeck et al. 1999), and alternations in hydrophobic interactions on the surface of proteins (Lee & Vierling 2000), are essential for binding the substrate.

Relations of sHSPs with oxidative stress

Chloroplasts and mitochondria are the main organelles where ROS production is observed both in normal and stress conditions, so sHSPs localized in those organelles might be associated with their response to oxidative stress. The unique structure of chloroplast sHSPs, which have the methionine-rich amphipathic α-helix, and their ability to change the conformation, might be crucial in preventing oxidative effects (Lee & Vierling 2000, Harndahl et al. 1999). The expression of cytosolic and mitochondrial sHSPs can also be induced by oxidative stress (Banzet et al. 1998). A 22-kDa sHSP, called HSP22, is located in mitochondria and can prevent damages that might be caused by oxidative stress and ageing. HSP22 accumulation appears also after H₂O₂ and γ-radiation treatment (Lee & Vierling 2000). Moreover, for Qshsp10.4-CI, the CI class sHSP that was obtained from Quercus suber L. (cork oak), a possible protective role was proposed due to its accumulation in cork and other oxidatively stressed tissues (Jofré et al. 2003). Reactive oxygen species (ROS) attack DNA, proteins and membranes, cause lipid peroxidation and deesterification, as well as accumulation of carbonyl derivatives (Potts 1994). Therefore, increased
ROS scavenging is required during desiccation. The metabolism shut down is also necessary to avoid oxidative stress. In seeds, during drying and storage, ROS content increases and is related to ageing. Seeds of individual species have characteristic potential life spans. The variability of seed longevity during storage is correlated with the mechanisms of damage and protection during oxidative and ageing stress. Beside results of HSP gene expression, analyses in transgenic seeds indicate that sHSP are positively correlated with seed longevity (PRIETO-DAPENA et al. 2006). A detoxifying system of enzymes (superoxide dismutase, ascorbate peroxidase, dehydroascorbate reductase, glutathione reductase, and catalase) might be insufficient during water deficit (ELSTNER & OSSWALD 1994). In that case, ROS are removed by glutathione, ascorbic acid, polyols, peroxiredoxins, tocopherol, β-carotene, proline, polyamines and flavonoids (SMIRNOFF 1993, HOEKSTRA et al. 2001). Oxidative stress is the main force that causes a decrease in vitality and loss of seed vigour during storage, hence ROS removal is needed to avoid oxidative damage of proteins – so that the native structure of stress proteins could be sustained to play their protective role. In addition, oligomeric sHSPs can exchange the thiol and disulfide groups with glutathione and synergistically act as protective elements regulating redox homeostasis (ZA VYALOV et al. 1998).

Interactions of sHSPs with proteins and other compounds

In the cell, proteins are continuously degraded to aa and replaced by newly synthesized proteins. Eukaryotic cells have a mechanism of protein degradation, in which ubiquitin and HSPs are involved. Denatured proteins aggregate because of the tendency of joining damaged hydrophobic domains to other such domains. Similarly, if badly folded proteins are dominant in a cell and the mechanisms of degradation do not keep up with their removal, then damaged polypeptides form aggregates before they are removed (GOLDBERG 2003). Large amounts of damaged proteins are noticed under stress conditions. Various sHSPs have the ability to bind the non-native proteins and therefore prevent their aggregation (WATERS et al. 1996, HASLBECK et al. 1999, LEE & VIERLING 2000). Probably the hydrophobic interactions are responsible for binding denatured proteins and then protective proteins, like DnaK or ClpB/DnaK complex (LEE et al. 1997, LEE & VIERLING 2000, REDDY et al. 2006). Recently a mechanism of native protein structure restoration, involving cooperation of sHSPs and other HSPs, has been proposed (HASLBECK et al. 2005).

In plants, sHSPs can be associated into granules, and in a fully hydrated state are connected with membranes, where they can stabilize lipids and membrane fluidity (TSVETKOVA et al. 2002). This could have significant implications in seeds, as sHSPs might participate in preserving membrane integrity during thermal fluctuations in winter, and also might act as a factor that lowers electrolyte leakage during seed desiccation.

CONCLUSIONS

In this short review we summarized the possible roles of LEA proteins and sHSPs in seed protection. Both groups of stress proteins are essential for proper seed development, maturation, storage, and germination. They also function during vari-
ous environmental stresses, helping in stabilization of cell structures and compounds. The proteins are associated with the response to drought and desiccation and strongly affect seed viability, mostly by preferential hydration of diverse molecules and water replacement in dehydrated cells. Similarly, membrane integrity and protein conformation are assured by the action of sHSPs, which assist in protein folding and molecular interactions. Despite all experimentally investigated, theoretically predicted or suggested functions of LEA proteins and sHSPs, further analyses are needed to explain fully their exact roles and possible interactions with other protective compounds.

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