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The reduction of seed-specific dehydrins reduces seed longevity in *Arabidopsis thaliana*

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Abstract

Dehydrins are late embryogenesis abundant (LEA) proteins that accumulate during seed maturation and in response to abiotic stresses in vegetative tissues. They are thought to protect cellular components from dehydration stress. However, whether they play a role in survival in the dry state is not clear. In this study, an RNA interference (RNAi)-construct against the seed-expressed dehydrin of *Arabidopsis thaliana*, *LEA14* (At2g21490), was introduced to wild-type plants, which led to a strong reduction in transcript abundance of the target gene as well as that of two other seed-expressed dehydrin homologues, *XERO1* (At3g50980) and *RAB18 (responsive to abscisic acid 18*, At5g66400) in the transformants. Mature, dry seeds from the RNAi plants germinated to at least 95% after rehydration, indicating that seed desiccation tolerance was not affected, while they exhibited a twofold reduction in longevity. When stored at 75% relative humidity and 35°C, the seeds of two independent RNAi lines lost 50% of their viability in 10 d and 5 d, respectively, while it took 17 d for wild-type seeds to lose 50% viability. In addition, when seeds were imbibed in the presence of 100 mM NaCl, the seeds of RNAi plants exhibited reduced germination compared to wild-type seeds, suggesting that at least one of the three seed-specific dehydrins plays a role both against deterioration during storage at low moisture content and when imbibed tissues are submitted to salt stress at high moisture.

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Keywords: *Arabidopsis thaliana*, dehydrin, desiccation tolerance, late embryogenesis abundant (LEA) protein, RNAi, seed storage

Introduction

Orthodox seeds are capable of surviving the removal of most of their cellular water and can be stored in the dry state for long periods, ranging from decades to centuries, depending on the species (Priestley *et al.*, 1985; Walters *et al.*, 2005) and storage conditions (Roberts, 1960; Walters, 1998). Remarkable survival for several hundreds of years has been observed for seeds of *Canna compacta* (Lerman and Cigliano, 1971), sacred lotus *Nelumbo nucifera* (Shen-Miller, 2002) and the date palm *Phoenix dactylifera* L. (Sallon *et al.*, 2008). Seed longevity is strongly influenced by environmental factors such as temperature and seed moisture content (Boswell *et al.*, 1940; El-Shishiny, 1953), which has been attributed to the thermodynamic properties of the residual water present in seeds (reviewed in Walters *et al.*, 1997) and the intracellular glass properties under the same storage conditions (reviewed in Buitink and Leprince, 2004). Typically, seed deterioration is severely slowed down when the tissues are in the glassy state (Buitink and Leprince, 2004). Conditions such as high temperature and high relative humidity, which bring seeds out of their glassy state, result in a dramatic increase in the ageing rate. This is often referred to as accelerated ageing. Moreover, comparison of the ageing rates with the molecular mobility in intracellular glasses reveals a linear relationship between these two parameters over a wide range of temperatures and water contents (Buitink *et al.*, 2000), which indicates that deteriorative chemical reactions leading to loss of seed viability are controlled by the kinetics of molecular mobility.

Seed ageing is accompanied by the deterioration of cellular components, such as membranes (Perez and Argüello, 1995; Roqueiro *et al.*, 2010), proteins (Oge *et al.*, 2008; Rajjou *et al.*, 2008) and DNA (Rao *et al.*, 1987). It has been proposed that this deterioration originates from oxidative reactions during storage (Hendry, 1993; Roqueiro *et al.*, 2010) and that the oxidative stress defence plays an important role in seed longevity. The seeds of the *Arabidopsis vte (vitamin E)* mutants, which are deficient in several forms of tocopherols, lipophilic antioxidants, exhibit decreased germination compared to wild-type seeds after accelerated ageing for 3 d at 40°C and 100% relative humidity (RH) (Sattler *et al.*, 2004). In addition, the loss and/or oxidation of gluthathione (GSH) during storage is correlated with a decrease in the viability of seeds of different species (Kranner *et al.*, 2006).

In contrast to the existing knowledge about the oxidative stress defence, it is unclear how the abundance of stress proteins which is correlated with desiccation tolerance influences seed longevity. Heat shock proteins (HSPs) are chaperones that protect proteins from unfolding-induced aggregation and may assist refolding into the native conformation. Their abundance is strongly reduced in *abi3 (abscisic acid sensitive 3)*, *fus3 (fusca 3)* or *lec1 (leafy cotyledon 1) Arabidopsis* mutants which are desiccation sensitive (Wehmeyer and Vierling, 2000). Overexpression of the sunflower *HaHSFA9* heat stress transcription factor in tobacco seeds led to the accumulation of HSPs and conferred an increased resistance against accelerated ageing at 50°C with 28% seed moisture content on a fresh weight basis (Prieto-Dapena *et al.*, 2006).

Another group of stress-related proteins, the late embryogenesis abundant (LEA) proteins, is suggested to play an important role in seed desiccation tolerance (reviewed by Tunnacliffe and Wise, 2007). Some *in vitro* experiments provide evidence that at least some LEA proteins stabilize membranes and/or proteins against the deleterious effects of drying. LEAs are able to prevent protein aggregation during freezing and drying (Chakrabortee *et al.*, 2007; Boucher *et al.*, 2009), and interact with and stabilize liposomes in the dry state (Pouchkina-Stantcheva *et al.*, 2007; Tolleter *et al.*, 2007; Thalhammer *et al.*, 2010). In addition, some LEAs can stabilize sugar glasses (Wolkers *et al.*, 1999, 2001; Shimizu *et al.*, 2010), suggesting that they play a role in longevity. However, evidence for a role of LEA proteins in seed desiccation tolerance and longevity *in vivo* remains scarce. Manfre *et al.* (2006, 2009) showed that the absence of the group 1 LEA protein AtEM6 (PF00477) in *Arabidopsis* altered the timing for the establishment of desiccation tolerance. However, this protein was not essential for desiccation tolerance itself since the mutants produced viable dry seeds (Manfre *et al.*, 2009). The presence of group 2 LEA proteins, also called dehydrins (PF00257), is not sufficient to confer desiccation tolerance as these proteins were also found in desiccation-sensitive, recalcitrant seeds (Finch-Savage *et al.*, 1994; Gee *et al.*, 1994; Greggains *et al.*, 2000). In a recent study on *Medicago truncatula*, the putative link between desiccation tolerance and the heat-stable proteome, to which most LEAs belong, was analysed comprehensively (Boudet *et al.*, 2006). The survey revealed that the abundance of some, but not all, LEAs is associated with desiccation tolerance of germinating radicles. For example, some dehydrin isoforms increased in abundance upon re-induction of desiccation tolerance. However, while a function of dehydrins in low temperature and/or osmotic stress tolerance in vegetative tissues has been demonstrated experimentally (Figueras *et al.*, 2004; Brini *et al.*, 2007; Tunnacliffe and Wise, 2007 and references therein), little is known about the impact of dehydrins on seed longevity in orthodox seeds.

In this study, we address the question whether dehydrins contribute to the longevity of *Arabidopsis* seeds in the dry state using an RNAi knockdown approach. We analysed the impact of a seed-specific dehydrin gene, At2g21490, on the desiccation tolerance and longevity during storage and on salt stress tolerance upon germination. The expression of the target gene as well as two dehydrin homologues was reduced in mature seeds from RNAi plants which exhibited a reduced longevity and reduced tolerance to NaCl during germination.

Materials and methods

Cloning of LEA genes, transformation of A. thaliana *and seed production*

LEA14 (At2g21490; PF00257) was cloned from the RIKEN full-length cDNA clone RAFL09-38-N20 (Seki *et al.*, 1998, 2002). The coding region was amplified by polymerase chain reaction (PCR) and inserted into the vector pENTR.SD.D-TOPO (Invitrogen, Carlsbad, California, USA). The identity of the insert was checked by sequencing. The gene was transferred to the pJawohl8 vector obtained from Max Planck Institute für Zuechtungsforschung, Cologne, Germany, for RNAi-mediated suppression of transcript abundance *in planta*. *Arabidopsis thaliana* accession Col-0 plants were transformed with the vector via *Agrobacterium*-mediated gene transfer by floral dipping (Bechtold *et al.*, 1993; Clough and Bent, 1998). Soil-grown transgenic lines were selected based on their phosphinotricin (BASTA, Bayer, Leverkusen, Germany) resistance. Two independent single-insertion lines with a large reduction in *LEA14* expression were chosen for physiological characterization. Homozygous T3 seeds and wild-type seeds were sown in

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soil and plants were grown at 16 h day length with a light intensity of 200 $\mu E\,m^{-2}\,s^{-1}$ and a temperature regime of 20°C during the day and 18°C during the night, as described before (Rohde *et al.*, 2004). For each line, seeds of three individual plants were pooled and stored for 2 months at ambient conditions to release dormancy.

Accelerated ageing of Arabidopsis *seeds*

Approximately 200 fully afterripened seeds of each genotype were spread evenly in a 4-cm-diameter glass Petri dish that was put in an air-tight container with 75% RH adjusted by a saturated NaCl solution. One container was prepared per time point of storage. The containers were stored at 35°C in the dark for up to 4 weeks. At different intervals, seeds were removed from the containers and left to cool for 1 h at ambient conditions before germination tests. To calculate the P_{50} (the time necessary to obtain loss of 50% viability during storage), a sigmoidal equation was fitted to the data using the Sigmaplot Software (SPSS Inc., Chicago, Illinois, USA).

Germination assay

Square Petri dishes (12 cm) containing 25 ml half-concentrated modified Hoagland medium (for composition, see Hundertmark and Hincha, 2008) with 1% (w/v) agar (bacteriological agar, ultrapure, Carl Roth GmbH, Karlsruhe, Germany) were prepared. For germination under salt stress, 100 mM NaCl was added to the Hoagland solution. Approximately 150 seeds were taken up in 0.5 ml of 0.1% (w/v) agar containing 0.002% (v/v) Tween 20 and spread in three rows on the plates. Plates were placed upright in shallow translucent boxes at 23°C under continuous light (~50 $\mu E\,m^{-2}\,s^{-1}$). Seeds were considered germinated when the radicle protruded from the seed coat. Germination percentage was assessed after 3 d and 7 d of imbibition.

Expression analysis by quantitative RT-PCR

RNA was isolated from T4 seeds and quantitative two-step real-time (RT)-PCR was performed as described previously (Hundertmark and Hincha, 2008). The *UBC9* gene (ubiquitin-conjugating enzyme 9, At4g27960) was used as a reference. Primers for the reference and *LEA* genes are listed in Hundertmark and Hincha (2008). Data were expressed as the cycle number necessary to reach a threshold fluorescence value (Ct). ΔCt values ($Ct_{reference} - Ct_{gene}$) were calculated. The fold change in gene expression was calculated using the following equation:

$$\text{fold change} = 1/[2(\Delta Ct_{RNAi} - \Delta Ct_{WT})]$$

where WT = wild type.

Results

Seed-expressed and stress-expressed dehydrins in Arabidopsis

Ten different genes encoding dehydrins were identified in the *Arabidopsis* genome (Hundertmark and Hincha, 2008). Five distinct groups of dehydrins can be distinguished based on the presence and number (*n*) of the signature amino acid motifs: YnSKn, SKn, Kn, YnKn and KnS (Rorat, 2006). They differ in the number

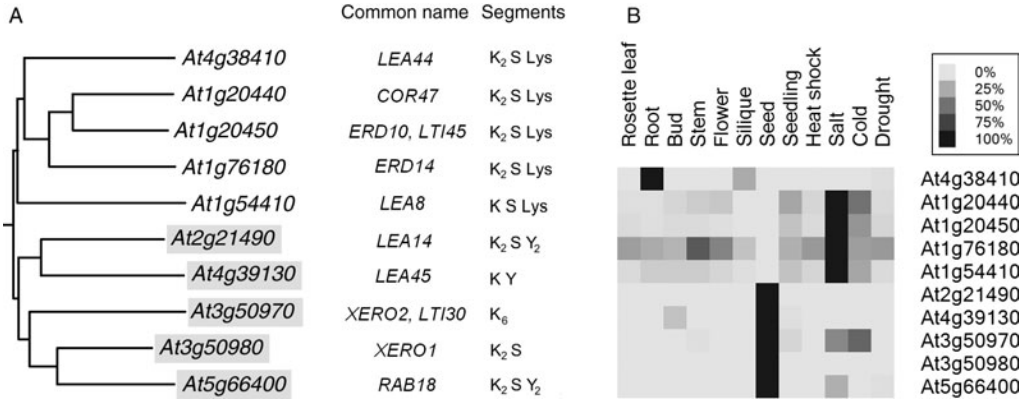

Figure 1. Phylogenetic tree of *Arabidopsis* dehydrins and their expression profiles. (A) The ten dehydrin genes of *Arabidopsis thaliana* were aligned with clustalW and a dendrogram was drawn. Seed-expressed genes are highlighted in grey. The content of K, S, Y and Lys-rich segments, which are typical for dehydrins, in the gene products is given, as well as the attributed gene names. (B) Expression of the *Arabidopsis* dehydrin genes in various organs without stress and in leaves in response to abiotic stresses. Data are shown as a heatmap that is normalized per gene to indicate where maximal expression occurs. The heatmap was calculated using our quantitative RT-PCR data from Hundertmark and Hincha (2008). COR, cold-regulated; ERD, early response to dehydration; LTI, low-temperature induced; RAB, responsive to abscisic acid.

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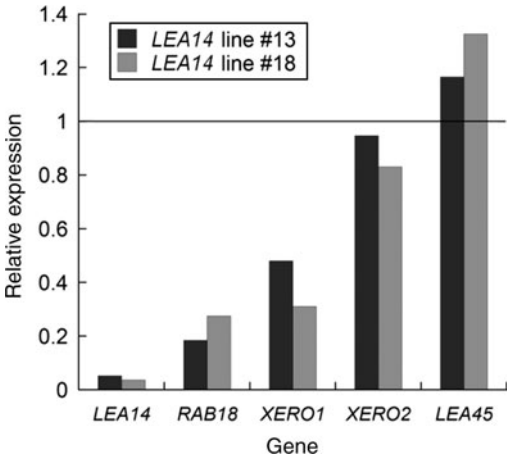

Figure 2. Expression of seed-specific *LEA*s in the two *LEA14* RNAi lines. Expression of dehydrin genes in seeds was monitored by quantitative RT-PCR. Data are expressed as relative expression levels in relation to those in wild-type seeds (horizontal bar at 1). RAB, responsive to abscisic acid.

of K-segments (KIKEKLPG), the signature motif of dehydrins (Fig. 1A). The other conserved motifs, Y-segment, the S-stretch and the Lys-rich segment, which differs from the K-segment and mainly consists of a stretch of lysine residues, are not systematically present in all *Arabidopsis* dehydrin sequences. Expression profiles of the ten genes show that their expression depends on the plant developmental stage or abiotic stress factors (Fig. 1B). For four dehydrin genes, no transcript was detected in seeds. Instead the genes were highly up-regulated in leaves under salt and cold stress conditions, suggesting an important role of these dehydrins in abiotic stress responses. One dehydrin gene (At4g38410) was expressed in roots and developing siliques at very low levels (Hundertmark and Hincha, 2008), and was not stress-responsive. The other five genes showed the highest transcript abundance in dry mature seeds. Interestingly, the dendrogram shows that the seed-expressed dehydrins form a separate cluster from those expressed in vegetative tissues (Fig. 1A). The Y-segment was found in three out of the five seed-expressed dehydrins whereas the Lys-segment is absent in the seed-expressed dehydrins. Among the seed-expressed dehydrins, At3g50970 (*XERO2*) and At5g66400 (*RAB18*) also respond to cold and salt stress in leaves. These expression data were consistent with the previous *in silico* analysis of available microarray data on seeds, resurrection plants and vegetative tissues subject to abiotic stress (Illing *et al.*, 2005). Those authors suggested that only a specific group of *LEA* genes was activated as part of the desiccation tolerance programme. To elucidate the possible role of seed-expressed dehydrins in desiccation tolerance and seed longevity, we focused on *LEA14* (At2g21490), which is a so far uncharacterized, seed-specific gene that was not induced in vegetative tissue by stress, and performed experiments using a reverse genetics approach.

Expression analysis using quantitative RT-PCR

We verified the reduction of *LEA14* transcripts in seeds of the two RNAi lines by quantitative RT-PCR. The transcript level of *LEA14* was reduced to 5% and 3% in seeds of the independent RNAi-lines #13 and #18, respectively, compared to that in wild-type seeds (Fig. 2), indicating that the introduction of the RNAi construct was efficient. We monitored the transcript levels of the four other seed-expressed dehydrins in the RNAi lines (Fig. 2). While *XERO2* and *LEA45* showed hardly any change in expression, the transcript levels of *XERO1* and *RAB18* were reduced to approximately 40% and 20%, respectively. This likely reflects the high homology of the nucleotide sequences of *LEA14*, *XERO1* and *RAB18*, which share regions that are highly similar (data not shown). Thus, the expression of three seed-specific dehydrin genes was affected by our RNAi approach.

Desiccation tolerance of LEA14 RNAi lines

Dry afterripened seeds of both RNAi lines exhibited high germination percentages under optimal conditions

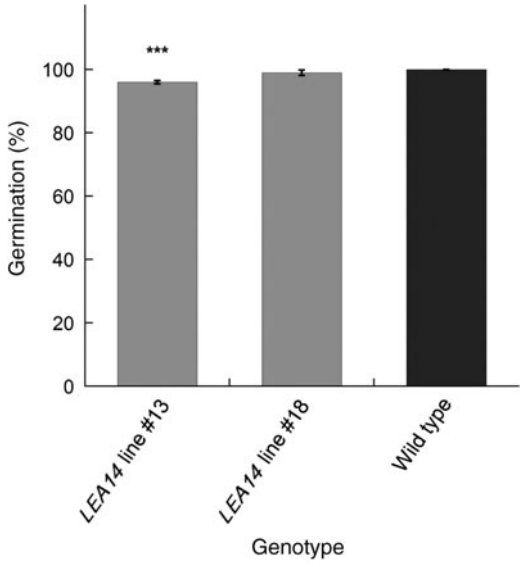

Figure 3. Germination of mature dry seeds of the two *LEA14* RNAi lines and wild type. Approximately 150 seeds per line were imbibed and the germination percentages were determined after 7 d at 23°C. Bars indicate standard errors ($n = 5$). Asterisks indicate the significant difference between the line *LEA14* #13 and wild type according to an ANOVA test ($P \leq 0.0001$).

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(Fig. 3), indicating that the reduction in dehydrin transcript abundance had no major influence on the desiccation tolerance of *Arabidopsis* seeds. While the slight reduction of germination of seeds from the RNAi line #13 compared to wild-type seed germination was statistically significant (96.0% versus 99.8%, respectively, $P \leq 0.0001$ in an ANOVA test), the germination percentage of seeds from the RNAi line #18 (98.9%) was not significantly different from that of the wild type. The reason for the slight reduction in line #13 is not clear.

Seed germination after ageing

To assess whether the partial silencing of the three dehydrin genes had an influence on seed longevity, accelerated ageing tests were performed at 35°C and 75% RH over 4 weeks. Samples were taken at weekly intervals and imbibed to assess germination, which provided the ageing curves shown in Fig. 4A. The wild-type seeds showed 76% and 51% germination after 14 d and 21 d of ageing, respectively. In contrast, seeds of both RNAi lines showed much lower germination percentages at the same time points (<30% and <3% for 14 d and 21 d, respectively). The enhanced susceptibility to ageing conditions was reflected in the P_{50}, the time of ageing necessary to obtain loss of 50% viability (Fig. 4B). While it took 17 d of ageing to diminish the germination percentage of wild-type seeds to 50%, it took only 5 d and 10 d for seeds of line #13 and #18, respectively.

Germination under salt stress conditions

Overexpressing dehydrin genes may lead to increased tolerance to abiotic stress during seedling establishment (Figueras *et al.*, 2004). We tested whether the tolerance to salt stress was affected by the suppression of the three dehydrin genes. We chose 100 mM NaCl, a concentration where the wild-type seeds showed a germination percentage (90.4%) comparable to that observed in the absence of salt (99.8%). Germination of seeds from the RNAi-lines was affected by the salt treatment: seeds of RNAi-lines #13 and #18 germinated only 61% and 65%, respectively, after incubation on agar containing 100 mM NaCl for 7 d (Fig. 5).

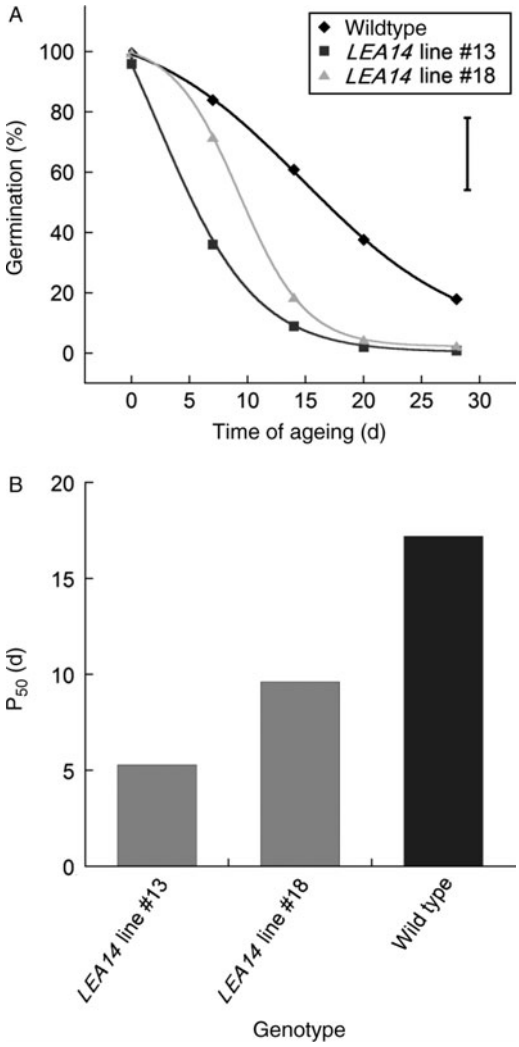

Figure 4. Loss of viability in wild-type seeds and seeds from *LEA14* RNAi lines during storage at 35°C and 75% RH. (A) Germination percentages during ageing. Germination tests (7 d, 23°C) on approximately 150 seeds per line were carried out after indicated time points during storage. Data were fitted with sigmoidal curves. The bar indicates the least significant difference ($P < 0.05$) calculated from the Poisson distribution ($n = 150$). (B) Time necessary to obtain 50% viability during storage (P_{50}). Data were calculated from the curves shown in (A) and are expressed in days.

Discussion

This study addresses the question whether dehydrins are involved in the desiccation tolerance and storability of orthodox seeds. Because of the small sizes of *LEA* genes, not many suitable T-DNA insertion lines exist in the *Arabidopsis* mutant collections. Therefore, we used an RNAi approach which led to a strong reduction in the expression of *LEA14* and other homologous dehydrins, *LEA34* (*XERO1*) and *LEA51* (*RAB18*). The seeds of RNAi plants exhibited reduced longevity while their tolerance to desiccation was not affected. There are several reports showing that enhanced dehydrin expression leads to improved germination or seedling growth under stress (Ismail *et al.*, 1999; Figueras *et al.*, 2004; Brini *et al.*, 2007). Our results indicate that the reduction in dehydrins affects the storability of *Arabidopsis* seeds. Wechsberg *et al.* (1994) did not find a correlation between the abundance of dehydrin-like proteins and

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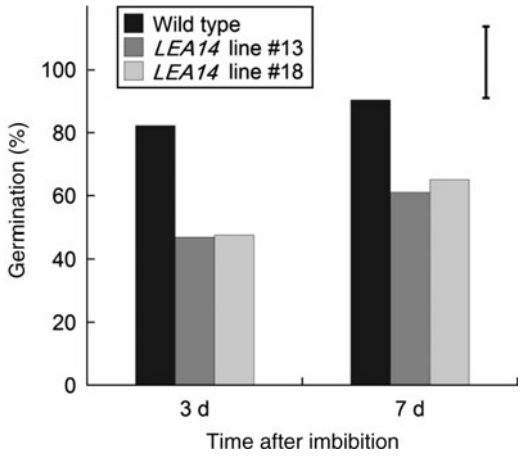

Figure 5. Germination of seeds from the *LEA14* RNAi lines and wild-type plants under salt stress conditions. Approximately 150 seeds per line were imbibed in the presence of 100 mM NaCl and germination percentages were determined after 3 d and 7 d at 23°C. The error bar indicates the least significant difference ($P < 0.05$) calculated from the Poisson distribution ($n = 150$).

storability of developing *Ranunculus sceleratus* seeds. The discrepancy between our data and the previous finding is probably due to the technical differences between the two studies: the previous study used polyclonal antibodies raised against a consensus sequence of the K-segment which is present in all dehydrins (Fig. 1), resulting in the recognition of all dehydrins, which may have masked possible correlations between certain key dehydrins and seed longevity. We have demonstrated here that in *Arabidopsis* the down-regulation of three out of five seed-expressed dehydrins was sufficient to affect seed longevity and performance under salt stress. A decreased ability to tolerate accelerated ageing and abiotic stress conditions during germination is a symptom of low seed vigour (McDonald, 1999). Therefore, our data indicate that seed-specific dehydrins play a role in seed performance as previously suggested by Ismail *et al.* (1999).

The storage conditions chosen to assess longevity (~20°C and ~65% RH) were an intermediate between ambient conditions under which seed deterioration becomes measurable only after several years of storage and extremely deleterious conditions that lead to loss of viability within a few days (Walters, 1998; McDonald, 1999; Hay *et al.*, 2003). There is increasing evidence from different plant species, including *Arabidopsis*, that the accelerated ageing by high temperatures and/or high RH cannot be used to extrapolate the kinetics of deterioration during natural ageing (Clerkx *et al.*, 2004; Niedzielski *et al.*, 2009). In our study, seeds were stored at 35°C and 75% RH. The water content under these conditions is likely to be around 8.5% (Hay *et al.*, 2003). Thus, the decreased storability of the seeds of RNAi plants suggests a protective role of at least one of the three dehydrins in seeds with low moisture content. Under these conditions, the water potential of the seed matrix is likely very low and the seed matrix is close to the point of glass transition, when it is no longer in the glassy state (Buitink and Leprince, 2004). We conclude that dehydrins play a protective role in seeds when the seed matrix is out of its glassy state. Whether dehydrins are also able to play a protective role against natural ageing, similar to the group 3 LEA proteins that stabilize sugar glasses *in vitro* (Wolkers *et al.*, 2001; Shimizu *et al.*, 2010), remains to be investigated.

For the decreased tolerance of imbibed seeds of RNAi plants to salt stress (Fig. 5), the applied stress corresponds to an osmotic stress of about −0.2 MPa. This suggests that the dehydrins LEA14, XERO1 and RAB18 play a role also at high moisture contents. LEA proteins have a broad range of stabilizing functions (Tunnacliffe and Wise, 2007). It remains to be investigated whether these functions vary depending on seed moisture contents. The reduced germination of the seeds of RNAi plants under salt stress conditions observed in this study is in agreement with the finding that the overexpression of dehydrins, including *RAB18*, resulted in an increased germination rate under salt stress (Brini *et al.*, 2007).

The precise mechanisms about how the three dehydrins function and whether they act synergistically in the seed matrix remain to be elucidated. The structure of the recombinant RAB18 protein, which has been investigated by circular dichroism spectroscopy (Mouillon *et al.*, 2006), indicates that it is largely devoid of stable secondary structures such as α-helix or β-sheet, but contains a significant content of highly flexible poly-Pro II (PII) helices. Based on the current understanding of the function of the characteristic conformation of PII, Mouillon *et al.* (2006) speculated that PII helices could alter the hydration shell of macromolecules and act as a water reservoir. This idea is also in accordance with our current understanding about how the physical environment of the seed matrix responds to water content and temperature changes during storage and influences the kinetics of ageing (Walters, 1998; Buitink and Leprince, 2004). Other possible functions of dehydrins, such as the stabilization of enzymes or membranes in the dry state or binding to Fe ions (for a review see Tunnacliffe and Wise, 2007), could also contribute to seed longevity.

The RNAi-induced reduction in the transcript levels of *LEA14*, *XERO1* and *RAB18* had little influence on the desiccation tolerance (Fig. 2). Likewise, Manfre *et al.* (2009) showed that the onset of desiccation tolerance in an *Arabidopsis* mutant with a T-DNA insertion in *AtEM6*, a *LEA* gene, was altered during

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seed development, but the desiccation tolerance itself remained unaffected. In the *atem6* mutant, the homologous protein, AtEM1, was found to be more abundant than in wild-type seeds. The authors hypothesized that the accumulation of the EM1 protein probably compensated for the loss of EM6. Here we showed that none of the other seed-expressed dehydrins were significantly up-regulated. On the contrary, the down-regulation of two other dehydrins was observed due to the RNAi construct used to target *LEA14*. The reduction in dehydrin transcripts did not lead to an impaired desiccation tolerance of *Arabidopsis* seeds. These results could either indicate that these dehydrins play no important role in desiccation tolerance, or that their suppression was compensated by other mechanisms that were not investigated in this study, as the acquisition of desiccation tolerance seems to be a multigenic trait.

In conclusion, our findings show that *Arabidopsis* seeds contain an array of dehydrins, some of which have adaptive roles in both seed longevity in the dry state and the tolerance to salt stress during germination. This study also shows that an RNAi strategy is a valuable approach to dissect the roles of LEA proteins *in vivo* in relation to seed performance.

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References

Bechtold, N., Ellis, J. and Pelletier, G. (1993) In-planta agrobacterium-mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *Comptes Rendus de l'Academie des Sciences. Series III, Sciences de la Vie/Life Sciences* **316**, 1194–1199.

Boswell, V.R., Toole, E.H., Toole, V.K. and Fisher, D.F. (1940) A study of rapid deterioration of vegetable seeds and methods for its prevention. *US Department of Agriculture Technical Bulletin* **708**, 1–48.

Boucher, V., Buitink, J., Lin, X., Boudet, J., Hoekstra, F.A., Hundertmark, M., Renard, D. and Leprince, O. (2009) MtPM25 is an atypical hydrophobic late embryogenesis-abundant protein that dissociates cold and desiccation-aggregated proteins. *Plant, Cell and Environment* **33**, 418–430.

Boudet, J., Buitink, J., Hoekstra, F.A., 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.

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* **26**, 2017–2026.

Buitink, J. and Leprince, O. (2004) Glass formation in plant anhydrobiotes: survival in the dry state. *Cryobiology* **48**, 215–228.

Buitink, J., Leprince, O., Hemminga, M.A. and Hoekstra, F.A. (2000) Molecular mobility in the cytoplasm: An approach to describe and predict lifespan of dry germplasm. *Proceedings of the National Academy of Sciences USA* **97**, 2385–2390.

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 USA* **104**, 18073–18078.

Clerkx, E.J.M., El-Lithy, M.E., Vierling, E., Ruys, G.J., Blankestijn-De Vries, H., Groot, S.P.C., Vreugdenhil, D. and Koornneef, M. (2004) Analysis of natural allelic variation of *Arabidopsis* seed germination and seed longevity traits between the accessions Landsberg *erecta* and Shakdara, using a new recombinant inbred line population. *Plant Physiology* **135**, 432–443.

Clough, S.J. and Bent, A.F. (1998) Floral dip: A simplified method for *agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *The Plant Journal* **16**, 735–743.

El-Shishiny, E.D.H. (1953) Effect of temperature and desiccation during storage on germination and keeping quality of *Kochia indica* seeds. *Journal of Experimental Botany* **4**, 403–406.

Figueras, M., Pujal, J., Saleh, A., Save, R., Pages, M. and Goday, A. (2004) Maize *rab17* overexpression in *Arabidopsis* plants promotes osmotic stress tolerance. *Annals of Applied Biology* **144**, 251–257.

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

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

Greggains, V., Finch-Savage, W.E., Quick, W.P. and Atherton, N.M. (2000) Putative desiccation tolerance mechanisms in orthodox and recalcitrant seeds of the genus *Acer*. *Seed Science Research* **10**, 317–327.

Hay, F.R., Mead, A., Manger, K. and Wilson, F.J. (2003) One-step analysis of seed storage data and the longevity of *Arabidopsis thaliana* seeds. *Journal of Experimental Botany* **54**, 993–1011.

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

Hundertmark, M. and Hincha, D.K. (2008) LEA (late embryogenesis abundant) proteins and their encoding genes in *Arabidopsis thaliana*. *BMC Genomics* **9**, 118.

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

Ismail, A.M., Hall, A.E. and Close, T.J. (1999) Allelic variation of a dehydrin gene cosegregates with chilling

tolerance during seedling emergence. *Proceedings of the National Academy of Sciences USA* **96**, 13566–13570.

Kranner, I., Birtic, S., Anderson, K.M. and Pritchard, H.W. (2006) Glutathione half-cell reduction potential: a universal stress marker and modulator of programmed cell death? *Free Radical Biology and Medicine* **40**, 2155–2165.

Lerman, J.C. and Cigliano, E.M. (1971) New carbon-14 evidence for six hundred years old *Canna compacta* seed. *Nature* **232**, 568–570.

Manfre, A.J., Lanni, L.M. and Marcotte, W.R. Jr (2006) The *Arabidopsis* group 1 late embryogenesis abundant protein ATEM6 is required for normal seed development. *Plant Physiology* **140**, 140–149.

Manfre, A.J., LaHatte, G.A., Climer, C.R. and Marcotte, W.R. Jr (2009) Seed dehydration and the establishment of desiccation tolerance during seed maturation is altered in the *Arabidopsis thaliana* mutant *atem6*-1. *Plant and Cell Physiology* **50**, 243–253.

McDonald, M.B. (1999) Seed deterioration: physiology, repair and assessment. *Seed Science and Technology* **27**, 177–237.

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.

Niedzielski, M., Walters, C., Luczak, W., Hill, L.M., Wheeler, L.J. and Puchalski, J. (2009) Assessment of variation in seed longevity within rye, wheat and the intergeneric hybrid triticale. *Seed Science Research* **19**, 213–224.

Oge, L., Bourdais, G., Bove, J., Collet, B., Godin, B., Granier, F., Boutin, J.-P., Job, D., Jullien, M. and Grappin, P. (2008) Protein repair l-isoaspartyl methyltransferase1 is involved in both seed longevity and germination vigor in *Arabidopsis*. *The Plant Cell* **20**, 3022–3037.

Perez, M.A. and Argüello, J.A. (1995) Deterioration in peanut (*Arachis hypogaea* l. Cv. Florman) seeds under natural and accelerated ageing. *Seed Science and Technology* **23**, 439–445.

Pouchkina-Stantcheva, N.N., McGee, B.M., Boschetti, C., Tolleter, D., Chakrabortee, S., Popova, A.V., Meersman, F., Macherel, D., Hincha, D.K. and Tunnacliffe, A. (2007) Functional divergence of former alleles in an ancient asexual invertebrate. *Science* **318**, 268–271.

Priestley, D.A., Cullinan, V.I. and Wolfe, J. (1985) Differences in seed longevity at the species level. *Plant, Cell & Environment* **8**, 557–562.

Prieto-Dapena, P., Castano, R., Almoguera, C. and Jordano, J. (2006) Improved resistance to controlled deterioration in transgenic seeds. *Plant Physiology* **142**, 1102–1112.

Rajjou, L., Lovigny, Y., Groot, S.P.C., Belghazi, M., Job, C. and Job, D. (2008) Proteome-wide characterization of seed ageing in *Arabidopsis*: A comparison between artificial and natural ageing protocols. *Plant Physiology* **148**, 620–641.

Rao, N.K., Roberts, E.H. and Ellis, R.H. (1987) Loss of viability in lettuce seeds and the accumulation of chromosome damage under different storage conditions. *Annals of Botany* **60**, 85–96.

Roberts, E.H. (1960) The viability of cereal seed in relation to temperature and moisture. *Annals of Botany* **24**, 12–31.

Rohde, P., Hincha, D.K. and Heyer, A.G. (2004) Heterosis in the freezing tolerance of crosses between two *Arabidopsis thaliana* accessions (columbia-0 and c24) that show differences in non-acclimated and acclimated freezing tolerance. *The Plant Journal* **38**, 790–799.

Roqueiro, G., Facorro, G.B., Huarte, M.G., Rubin de Celis, E., Garcia, F., Maldonado, S. and Maroder, H. (2010) Effects of photooxidation on membrane integrity in *Salix nigra* seeds. *Annals of Botany* **105**, 1027–1034.

Rorat, T. (2006) Plant dehydrins – tissue location, structure and function. *Cellular & Molecular Biology Letters* **11**, 536–556.

Sallon, S., Solowey, E., Cohen, Y., Korchinsky, R., Egli, M., Woodhatch, I., Simchoni, O. and Kislev, M. (2008) Germination, genetics, and growth of an ancient date seed. *Science* **320**, 1464.

Sattler, S.E., Gilliland, L.U., Magallanes-Lundback, M., Pollard, M. and DellaPenna, D. (2004) Vitamin E is essential for seed longevity and for preventing lipid peroxidation during germination. *The Plant Cell* **16**, 1419–1432.

Seki, M., Carninci, P., Nishiyama, Y., Hayashizaki, Y. and Shinozaki, K. (1998) High-efficiency cloning of *Arabidopsis* full-length cDNA by biotinylated cap trapper. *The Plant Journal* **15**, 707–720.

Seki, M., Narusaka, M., Kamiya, A., Ishida, J., Satou, M., Sakurai, T., Nakajima, M., Enju, A., Akiyama, K., Oono, Y., Muramatsu, M., Hayashizaki, Y., Kawai, J., Carninci, P., Itoh, M., Ishii, Y., Arakawa, T., Shibata, K., Shinagawa, A. and Shinozaki, K. (2002) Functional annotation of a full-length *Arabidopsis* cDNA collection. *Science* **296**, 141–145.

Shen-Miller, J. (2002) Sacred lotus, the long-living fruits of *China Antique*. *Seed Science Research* **12**, 131–143.

Shimizu, T., Kanamori, Y., Furuki, T., Kikawada, T., Okuda, T., Takahashi, T., Mihara, H. and Sakurai, M. (2010) Desiccation-induced structuralization and glass formation of group 3 late embryogenesis abundant protein model peptides. *Biochemistry* **49**, 1093–1104.

Thalhammer, A., Hundertmark, M., Popova, A.V., Seckler, R. and Hincha, D.K. (2010) Interaction of two intrinsically disordered plant stress proteins (COR15a and COR15b) with lipid membranes in the dry state. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1798**, 1812–1820.

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* **19**, 1580–1589.

Tunnacliffe, A. and Wise, M. (2007) The continuing conundrum of the LEA proteins. *Naturwissenschaften* **94**, 791–812.

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

Walters, C., Ried, J.L. and Walker-Simmons, M.K. (1997) Heat-soluble proteins extracted from wheat embryos have tightly bound sugars and unusual hydration properties. *Seed Science Research* **7**, 125–134.

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

Wechsberg, G.E., Probert, R.J. and Bray, C.M. (1994) The relationship between 'dehydrin-like' proteins and seed longevity in *Ranunculus sceleratus* L. *Journal of Experimental Botany* **45**, 1027–1030.

Wehmeyer, N. and Vierling, E. (2000) The expression of small heat shock proteins in seeds responds to discrete developmental signals and suggests a general protective role in desiccation tolerance. *Plant Physiology* **122**, 1099–1108.

Wolkers, W.F., Tetteroo, F.A.A., Alberda, M. and Hoekstra, F.A. (1999) Changed properties of the cytoplasmic matrix associated with desiccation tolerance of dried carrot somatic embryos. An *in situ* Fourier transform infrared spectroscopic study. *Plant Physiology* **120**, 153–163.

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

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