The overexpression of specific heat shock proteins contributes to seed longevity

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Few examples of genetically modified seeds with increased longevity:

• In a model plant (*Arabidopsis thaliana*) by suppression of Phospholipase D (Devaiah *et al.* 2007)

• In tobacco by overexpression of a single transcription factor (HSFA9) from sunflower (Prieto-Dapena *et al.* 2006). This work identified a molecular pathway for seed longevity controlled by HSFA9, which involves specific small heat stress proteins (sHSPs).
Small Heat Shock Proteins (chaperones, 16-30 kD related to α-crystallin)

<table>
<thead>
<tr>
<th>Class</th>
<th>Protein IDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI</td>
<td>AtHsp17.6-CII, AtHsp17.7-CII, LpHsp17.3-CII, PsHsp17.1-CII, TaHsp17.4-CII</td>
</tr>
<tr>
<td>CII</td>
<td>AtHsp17.4-CIII, LpHsp16.1-CIII</td>
</tr>
<tr>
<td>CIII</td>
<td>AtHsp17.8-CI, AtHsp17.6A-CI, AtHsp17.6B-CI, AtHsp17.4-CI, AtHsp17.6C-CI, AtHsp18.1-CI, LpHsp17.7-CI, PsHsp18.1-CI, TaHsp16.9A-CI</td>
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<tr>
<td>ER</td>
<td>AtHsp22.0-ER, GmHsp22.0-ER, LeHsp21.5-ER, PsHsp22.7-ER</td>
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<tr>
<td>P</td>
<td>AtHsp25.3-P, LeHsp26.1-P, PsHsp26.2-P, TaHsp26.6-P</td>
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<tr>
<td>M</td>
<td>AtHsp23.6-M, AtHsp23.5-M, LeHsp23.8-M, PsHsp22.9-M, TaHsp23.5-M</td>
</tr>
</tbody>
</table>

**Seeds**
- Tobacco (Prieto-Dapena et al., 2006)
- Sunflower (Coca et al., 1994)
- Rice (mRNA data) (Guan et al., 2004)

**Seedlings after Heat Stress**
- Seeds (Scharf et al., 2001)

**Classifications**
- CI, CII, CIII: cytosolic/nuclear
- ER: endoplasmic reticulum
- P: plastid
- M: mitochondrial
Precedents tentatively linking sHSP expression and seed longevity?

- A rapid aging treatment that reduced the germination of *Brassica oleracea* seeds also reduced the amount of a CI sHSP (Betley & Finch-Savage, 1998).

- Arabidopsis mutants (i.e., *abi3-6*) that show reduced longevity produce desiccation-intolerant seeds that show low accumulation levels of seed sHSPs (Wehmeyer *et al.*, 1996; Clerkx *et al.*, 2004).

Other studies with maturing seeds indicate association between longevity and the ability of seeds to tolerate desiccation (Ellis & Hong, 1994; Hay & Probert, 1995; Bruggink *et al.*, 1999).
Our approach

Identification of crucial transcription factors that specifically regulate sHSP expression in developing seeds. (Functional analyses of CI sHSP promoters)

Gain of function in transgenic seeds using these master regulators
A Seed-specific Heat-shock Transcription Factor Involved in Developmental Regulation during Embryogenesis in Sunflower*

(Almoguera et al., 2002)

HaHSFA9

Unique functional properties

Seed-Specificity
Improved Resistance to Controlled Deterioration In Transgenic Seeds


Seed-specific overexpression of A9 using the promoter and other 3’- regulatory sequences from Hads10 (DS10, a LEA-1 gene from sunflower)
Controlled Deterioration Treatments (CDT) for Tobacco Seeds

Mature seeds (10mg, ≈150 seeds) → Germination above 98% before CDT

MCFW = 4.8% ± 0.2%

+20 µl H₂O
2h @ 25°C

MCFW = 28.1% ± 0.5%

48h @ 50°C
(Within prewarmed sealed box in a water bath)
MCFW = 27.9% ± 0.5%

Germination reduced (to ≤ 20%)
Most seeds die after CDT
Resistance to CDT of transgenic *DS10:A9* seeds (homozygous lines versus NT siblings).
Increased longevity (LD$_{50}$) in transgenic $DS10:A9$ seeds

#19-4 (T) : $LD_{50} = 2.11$ d
#22-11 (T): $LD_{50} = 2.33$ d
#19-10 (NT) : $LD_{50} = 1.78$ d
#22-7 (NT): $LD_{50} = 1.25$ d

F$= 122.3$, $P < 0.0001$

F$= 14.67$, $P = 0.0003$

#19-4 (T) : $LD_{50} = 2.11$ d
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#22-7 (NT): $LD_{50} = 1.25$ d
The seed yield, size, and seed morphology was unaltered in the *DS10:A9* lines.

10 lines each type, (F = 0.204, P = 0.66, 1 and 14 df).

#6-5 (NT)

#6-7 (T)
The seeds of *DS10:A9* lines show overaccumulation of different HSPs, in particular higher accumulation of specific CI sHSPs.
The seeds of DS10:A9 lines show overaccumulation of specific CII sHSPs.
Dehydrin protein accumulation and soluble sugar content are unaltered in seeds (*DS10:A9* homozygous lines versus NT siblings)
• Specific protein overaccumulation induced by A9:
  CI sHSPs (seed, seed-specific)
  CII sHSPs (seed)
  HSP101

• Unchanged:
  LEAs: groups 1, 2 (DHN), 3 and 4
  RFOs, Sucrose, Glucose

Seed sHSPs (and perhaps different proteins) contribute to improved seed longevity

Hypothesis: the same genetic program (induced by HSFA9) could also be also involved in seed desiccation tolerance

Hypothesis tested by ectopic overexpression of A9 in 35S:A9 lines
CONCLUSION

- *HaHSFA9* is a master gene of seed sHSP expression that could be useful for the efficient improvement of seed longevity in crop seeds

...at least in species evolutionarily close to tobacco and sunflower
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