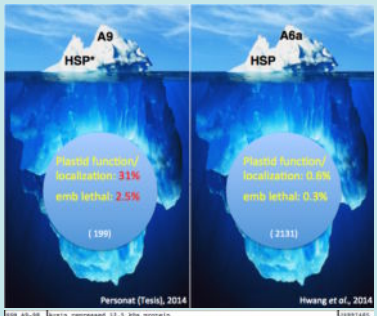
Seed-specific transcription factor HSFA9 links embryogenesis and photomorphogenesis

Pilar Prieto-Dapena[1], Concepción Almoguera[1], José-María Personat[1], Francisco Merchan[2] and Juan Jordano[1]

[1]Departamento de Biotecnología Vegetal, Instituto de Recursos Naturales y Agrobiología de Sevilla. Consejo Superior de Investigaciones Científicas (CSIC). 41012 Seville, Spain. [2]Departamento de Microbiología y Parasitología, Facultad de Farmacia, Universidad de Sevilla, 41012 Seville, Spain.

I.- Photomorphogenic effect of HSFA9?

HSFA9 (A9) is a seed-specific transcription factor involved in desiccation tolerance (1) and longevity (2). In sunflower, A9 disappears shortly after seed germination (3). Constitutive overexpression of A9 in the 35S:A9 tobacco seedlings revealed upregulation of genes related to the photosynthetic apparatus. This included components of both photosystems and genes involved in the biogenesis of chloroplasts. For example, A9 enhanced the expression of a *NADPH:protochlorophyllide oxidoreductase* (*POR*), a gene that is crucial for light-dependent biosynthesis of chlorophylls. This suggested that A9 might enhance photomorphogenesis (**Fig. 1**).



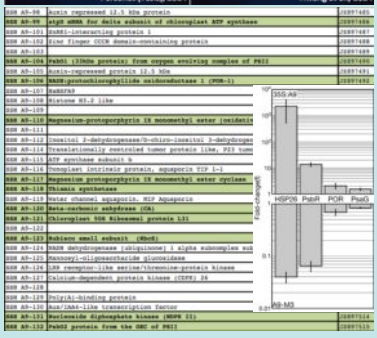


Fig. 1. Unusual target genes downstream of A9.
Potential target genes of A9 were identified by Supressive Subtractive Hybridization (SSH) cloning. **Top**. Comparison of results for A9 with that of a different HSF (A6) involved in water stress response in Arabidopsis. Note that in both cases Heat Shock Proteins (HSP) represent only a small subset of the targets. **Bottom**. Example of SSH A9 clones: marked in green those related to chloroplasts and photosynthesis (31% of total). **Inset:** RT-qPCR analyses of transcripts from selected SSH-A9 clones in gain-of-function (35S:A9) and loss-of-function (A9-M3) lines.

References

(1)! Prieto-Dapena, *et al.* (2008) Plant J. 54(6): 1004-14.
(2)! Prieto-Dapena, *et al.* (2006) Plant Phys. 142: 1102-12.
(3) Almoguera, *et al.* (2002) J. Biol. Chem. 277: 43866-72.
(4) Scharf, *et al.* (2012) Biochim. Biophys. Acta. 1819: 104-119.
(5) Carranco, *et al.* (2010) Proc. Nat. Acad. Sci. (USA). 107: 21908-21913.

Acknowledgments

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II.- Effects of A9 in seeds and seedlings before the first exposure to light.

In dark-imbibed seeds of DS10:A9 lines, where A9 was overexpressed under a seed-specific promoter, A9 augmented expression of genes that are relevant for early light signaling and for modulating photomorphogenesis. This included the phytochromes *PhyA* and *PhyB*, and activators *(HY5)* or repressors (*COP1*) of photomorphogenesis. Converse effects on gene expression were observed using A9-M3, a dominant-negative form of A9 used to achieve loss-of-function (**Fig. 2**).

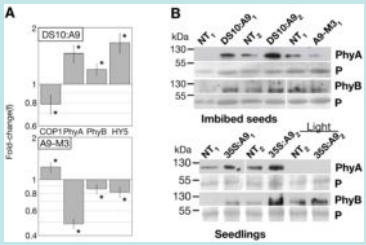


Fig. 2. (A) Changes in transcript levels for *COP1*, *PhyA*, *PhyB* and *HY5* in seeds imbibed under darkness for 24h. (B) Enhanced accumulation of phyA and phyB proteins under darkness in imbibed seeds and seedlings. The-PhyA band disappears (as expected) after illumination for 6 h with white light (Light).

III.- A9 activates the *NtphyA1* promoter.

A9 would impact -in part- upstream of phytochrome genes, perhaps directly activating *a PhyA* promoter, as suggested by transient expression assays. Effects of A9 on *PhyB* would be indirect (**Fig. 3).**

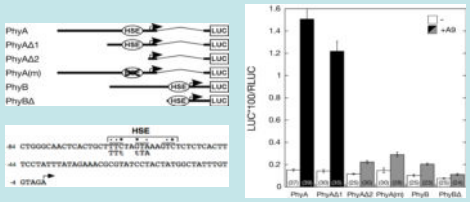


Fig. 3. Left, the different reporter genes used in the transient-expression assays. HSE denotes HSF-binding elements, including the one used by A9 in *NtphyA1*; this HSE was mutated in PhyA(m). The below-indicated nucleotide substitutions would impair DNA-binding of only A9 or similar HSF. Right, the assays in bombarded sunflower leaves.

IV.- A9 enhances phytochrome-dependent light responses.

A9 improved *PhyA*- and *PhyB*-dependent light signaling, as shown by intensified hypocotyl length reduction of the 35S:A9 seedlings under continuous far-red and red light, respectively (**Fig. 4**).

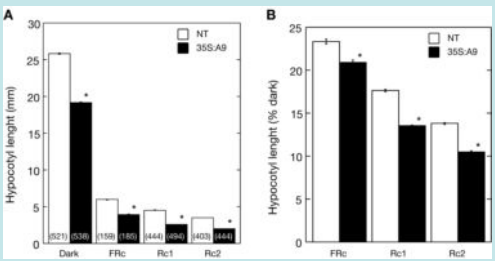


Fig. 4. Growth inhibition of 35S:A9 seedlings under monochromatic light.
(A) Hypocotyl length in seedlings grown under dark, or under continuous light: 5 µmol m^{-2} s^{-1} far-red light (FRc), 25 µmol m^{-2} s^{-1} red light (Rc1), or 35 µmol m^{-2} s^{-1}red light (Rc2). (B) Lengths from (A) are represented as the percentage of length under FRc (or Rc) in comparison to that in the dark.

Fig. 6. A9 enhances cotyledon unfolding. Top: unfolding was scored after the etiolated seedlings (in conditions as in Fig. 5) were exposed to continuous white light for 16 h. Bottom: pictures illustrate representative results of gain-of-function and loss-of-funcion line pairs. Scale bars, 2 mm.

The A9-link:

We uncover a transcriptional link, through A9, between seed maturation and early photomorphogenesis. A9 enhances gene expression relevant for photomorphogenesis in seeds in the dark. A9 also augments the initial light responses occurring after seed germination and seedling emergence from the soil.

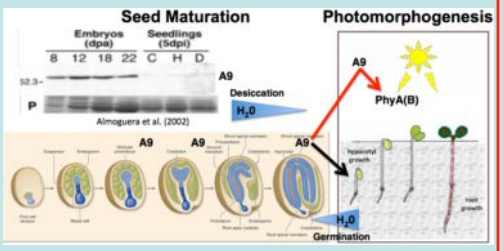


Fig. 7. A9 would operate immediately upstream of *PhyA*, and have indirect effects on *PhyB*. The gain- and loss-of-function effects of A9 reported here fit the timing of A9 expression in sunflower (3). The A9-link would be conserved at least in dicot plants similar to sunflower and tobacco (see **BOX-I**, below)

V.- A9 accelerates seedling greening

Following seedling exposure to white light, A9 accelerated the initial photosynthetic development. A9 augmented the accumulation of carotenoids, chlorophyllide and chlorophyll (**Fig. 5**), leading to earlier unfolding of the cotyledons (**Fig. 6**). Converse effects on greening, and cotyledon unfolding were observed using A9-M3. This confirmed by loss-of-function the photomorphogenic effect of A9 in the time-window where A9 is present and active in sunflower (3).

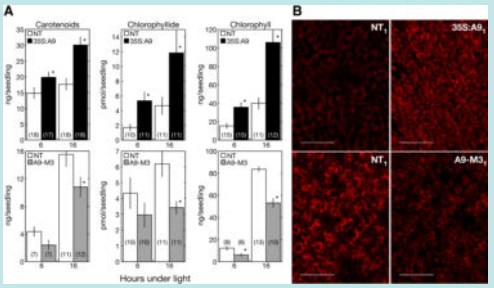
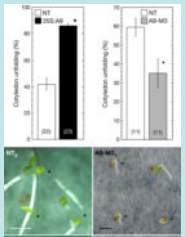


Fig. 5 (A) Top: 35S:A9 and NT seedlings were germinated and kept in darkness for 10 days. Bottom: A9-M3, and NT seeds were germinated and kept in darkness for 4 days. The samples were next transferred to continuous white light conditions for 6 h or 16 h. Photopigment formation was then quantified. (B) Confocal microscopy stacked images of cotyledon chloroplasts after illumination for 16h (for similar samples as in panel A). Scale bars, 50 µm.



BOX-I: Is the A9-link conserved in plants?

Monocot plants lack A9, and some dicot plants, for example *Eucalyptos grandis*, have at least 17 closely related A9 (4). Sunflower and tobacco (both belonging to the dicot Asterid clade) sport a single A9 with conserved regulation and functions (see 5, and references therein). In Rosid plants, as Arabidopsis, functional studies of A9 are hindered by the lack of loss-of-function lines (A9 mutants without effect), and by deleterious consequences of A9 overexpression. There are hints of functional differences in A9 between Asterid and Rosid dicot plants (please ask me for details!).

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HSFA9 is a seed-specific transcription factor involved in desiccation tolerance and seed longevity (Prieto-Dapena *et al.*, 2006; 2008). In sunflower plants, HSFA9 disappears a few days after seed germination (Almoguera *et al.*, 2002). Here we uncover a connection between HSFA9 and the initial acquisition of photosynthetic competence, which occurs following seed germination and seedling emergence. The constitutive overexpression of HSFA9 in the 35S:A9 tobacco seedlings enhanced expression of genes that are relevant for early light signaling (as the phytochromes *PhyA* and *PhyB*), and for light-dependent synthesis of chlorophylls [for example, a *NADPH:protochlorophyllide oxidoreductase* (*POR*, EC 1.3.1.33)]. The 35S:A9 seedlings also showed reduced expression of a crucial photomorphogenesis repressor (*CONSTITUTIVE PHOTOMORPHOGENIC 1, COP1*). Similar effects were observed in dark-imbibed seeds of DS10:A9 lines, where the overexpression of HSFA9 occurs in a time window similar to that for the expression of HSFA9 in sunflower. In the 35S:A9 seedlings, HSFA9 enhanced *PhyA*- and *PhyB*-dependent light signaling, as shown by intensified hypocotyl length reduction under continuous far-red and red light, respectively. Following exposure to white light, HSFA9 accelerated the initial photosynthetic development of the 35S:A9: seedlings. This occurred by augmenting the accumulation of chlorophyllide and chlorophyll, leading to earlier unfolding of the cotyledons. Converse effects on gene expression, greening, and cotyledon unfolding were observed using a dominant-negative form of HSFA9 expressed within the same time window as for DS10:A9. Our results demonstrate a transcriptional link between late embryogenesis and early photomorphogenesis that involves HSFA9, a transcription factor acting below the top hierarchical regulators of embryogenesis. We conclude that, in developing seeds and before the first exposure to light, HSFA9 enhances gene expression relevant for photomorphogenesis. HSFA9 subsequently boosts light-responses that promote early greening. HSFA9 would thus help facilitating quick seedling establishment after seedlings emerge from the soil and are exposed to light.

References:

Almoguera, et al. (2002) J. Biol. Chem. 277(46): 43866-43872.
Prieto-Dapena, et al. (2006). Plant Physiol. 142(3): 1102-1112.
Prieto-Dapena, et al. (2008). Plant J. 54(6): 1004-1014.