Journal of Plant Physiology **164** (2007) 263—273

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JOURNAL OF PLANT PHYSIOLOGY
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Light induces expression of a dehydrin-encoding gene during seedling de-etiolation in sunflower (*Helianthus annuus* L.)

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Received 14 November 2005; accepted 24 January 2006

KEYWORDS
De-etiolation;
Dehydrin;
Light;
Photoreceptors;
Transcription;
Sunflower

Summary

The effects of light quality on the expression of a sunflower dehydrin-encoding gene, *HaDhn1*, were studied during seedling de-etiolation. Seeds were germinated in the dark and, after 5 days, seedlings were maintained well watered and de-etiolated under different lights for 3, 6, 12, and 24 h. Exposure to white light stimulated *HaDhn1* transcript accumulation in the cotyledons of these seedlings, contrary to seedlings grown in the dark. *HaDhn1* transcripts increased also treating plantlets with monochromatic lights, especially red light. The increase of *HaDhn1* transcripts is provoked by the formation of the active form of phytochrome. Further experiments, performed saturating active phytochrome by yellow light, in combination or not with blue light, showed that also cryptochrome can increase *HaDhn1* transcripts accumulation after exposure to light. In situ analysis of *HaDhn1* expression domains in cotyledons of light-treated seedlings showed a hybridisation signal spread in all mesophyll cells, especially in the basal portion and in the vascular tissue. In the distal portion of the cotyledons, less intense signal was observed. Western blot analysis indicated that *HaDhn1* transcription is not followed by dehydrin-protein accumulation.

The isolated putative promoter sequence of the *HaDhn1* gene showed that different putative *cis*-elements recognisable by transcription factors occur in the isolated sequence, including a putative light-responsive G-box. On the whole, our

Abbreviations: ABA, abscisic acid; WL, white light; R, red light; B, blue light; FR, far red light; UV, UVB light; Y, yellow light

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0176-1617/$ - see front matter
doi:10.1016/j.jplph.2006.01.015

results indicate that *HaDhn1* gene expression is induced by light during de-etiolation, in absence of water stress.

Introduction

Dehydrins are an immunologically distinct family, also known as the *Lea D11* subgroup of late-embryogenesis-abundant (*Lea*) proteins (Dure et al., 1989) and have been described and classified in many angiosperm and gymnosperm species (Close, 1997). They are characterised by several domains, including one or more putative amphipathic α-helix forming consensus regions at the C-terminus (K-domain), and, in the majority of dehydrins, a region at the N-terminus with homologies to a portion of the nucleotide binding site of chaperones of plants and bacteria (Y-domain); many dehydrins contain also a tract of serine residues, possibly phosphorilatable (S-domain; see Natali et al., 2003).

Many studies indicate that dehydrins are associated with macromolecules such as nucleoprotein complexes in the nucleus (Godoy et al., 1994) and endomembranes in the cytoplasm (Schneider et al., 1993). This suggests that these proteins are surfactants that inhibit the coagulation of a range of macromolecules and preserve their structural integrity, stabilising proteins and membranes through detergent or reverse chaperone activities (Close, 1996; Ismail et al., 1999; Hara et al., 2001). Recently, dehydrins have been demonstrated to confer freezing, drought and salt stress tolerance in *Arabidopsis* and rice (Cheng et al., 2001; Puhakainen et al., 2004) and to be implicated in metal binding (Alsheikh et al., 2005).

Dehydrins are usually produced by plants in the late stages of embryo development (Dure et al., 1989) and also following any environmental stimulus involving dehydration, such as drought or cold stress and salinity, as key components of dehydration tolerance (Close, 1996; Zhu et al., 2000). Moreover, modulation of transcripts by light has been reported for many dehydrin-encoding genes in drought- or cold-stressed plants (Chauvin et al., 1993; Crosatti et al., 1999; Panta et al., 2001; Ohno et al., 2003).

An YSK-type dehydrin cDNA, *HaDhn1*, induced by drought stress, has been isolated and sequenced in sunflower (Ouvrard et al., 1996); the accumulation of *HaDhn1* transcripts correlates with drought tolerance (Cellier et al., 1998). This gene was found to be expressed in the latest stages of *Helianthus annuus* embryogenesis, depending on abscisic acid (ABA) accumulation; moreover, *HaDhn1* transcripts accumulated after drought stress even in ABA deficient sunflower mutants (Giordani et al., 1999). The expression of this gene is modulated by dark/light cycle during drought stress (Cellier et al., 2000).

With the aim to clarify mechanisms of expression regulation of this gene by light, we have analysed *HaDhn1* expression during seedling de-etiolation, which provides an ideal experimental condition to evaluate the effects of different photoreceptors on gene activity. Recent data on expression profiling during seedling de-etiolation in *Arabidopsis*, showed that, beside many transcription factors, also different stress- or defence-protein encoding genes are expressed within 24 h of light exposure, due to phytochrome A activity (Tepperman et al., 2001) and many of these genes are regulated by more than one phytochrome (Tepperman et al., 2004). Activation of such genes should enable seedling to respond rapidly to different abiotic stresses by the accumulation of mRNA (even before stress events occur) with the subsequent production of protective molecules and/or proteins.

In this sense, we have analysed *HaDhn1* induction during de-etiolation and observed that light can induce the expression of this gene in absence of water stress and established the classes of photoreceptors involved in *HaDhn1* induction.

Materials and methods

Plant material and light treatments

Seeds of an inbred line of *Helianthus annuus*, HCM, were used in the experiments. Seeds were germinated at 25 °C in the dark in Petri dishes on filter paper moistened with distiled water.

Light treatments were performed in the same growth chamber, at 25 °C, and manipulations were made in the dark or under dim green safelight. After 5 days, when 4–5 cm in length, seedlings were maintained in the dark or transferred under different lights or a UV source. Red light (R) at 15.0 μmol m^{-2} s^{-1} was obtained from Philips TL 20 W/15 fluorescent tubes filtered with Roscolux