The very-low-fluence and high-irradiance responses of the phytochromes have antagonistic effects on germination, mannan-degrading activities, and \textit{DfGA3ox} transcript levels in \textit{Datura ferox} seeds

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Abstract

Seed germination can be promoted by the modes of action of two of the phytochromes: the low-fluence response (LFR), which is the classical red (R)–far-red (FR) reversible response and the very-low-fluence response (VLFR) that can be saturated by extremely low levels of Pfr, which can be elicited by a saturating FR pulse. The \textit{Datura ferox} seed population used in this work had acquired the capacity to germinate through a VLFR after pretreatment in a water-saturated atmosphere (WSA) at constant 25 °C. After 12 d in WSA germination after a FR pulse was 82%, while it was less than 10% in darkness. It was found that the VLFR of germination is associated with increments in the embryo growth potential (EGP) and in the activity of two enzymes related to the weakening of the micropylar region of the endosperm (ME); endo-\(\beta\)-mannanase and \(\beta\)-mannosidase. The FR pulse also significantly stimulated the expression of \textit{DfGA3ox}, a \(\beta\)-hydroxylase, suggesting that the promotion of germination by the VLFR is associated with an increase in the synthesis of active gibberellins. The promotive action of the VLFR on germination is reduced when the FR pulse is immediately followed by a continuous FR treatment for 24 h (FRc). The effect of FRc cannot be reproduced by hourly FR pulses during the same period, showing that the antagonistic effect of FRc is a high-irradiance response (HIR). The action of the HIR in germination is associated with a decrease of both the mannan-degrading enzyme activity and the expression of \textit{DfMan} in the ME, whereas no changes in the EGP were observed. The HIR also inhibits the accumulation of \textit{DfGA3ox} in embryos, indicating that its action on germination is mediated, at least in part, through the modulation of active GA contents in seeds. This is the first report of a gene that participates in the VLFR-HIR antagonism in seeds.

Key words: Coat-imposed dormancy, embryo growth potential, endosperm softening, germination, phytochromes.

Introduction

Light is an important ecological factor that modulates germination in a large number of species. Depending on spectral composition and photon fluence, temperature, water availability, and the physiological condition of the seeds, it can promote or inhibit germination (Bewley and Black, 1994; Shinomura et al., 1994; Botto et al., 1995, 1998; de Miguel et al., 2000; Shichijo et al., 2001). In many cases, the stimulus of germination shows a biphasic response to the fluence of R light (R) (Cone et al., 1985; Botto et al., 1995). The first phase, called the very-low-fluence response (VLFR), is present in seeds with extreme sensitivity to light (Casal and Sánchez, 1998). VLFR is
mediated by phytochrome A (Botto et al., 1996; Shinomura et al., 1996) and is usually saturated by Pfr/Ptot ratios lower than 0.1%, which can also be established by a far-red light (FR) pulse (Botto et al., 1995, 1996). Thus, they do not show the classic R–FR reversibility. In natural conditions, establishment of a VLFR may occur when the seeds remain for a period of time in the soil (Scopel et al., 1991; Derkx and Karssen, 1993; Botto et al., 1998). VLFRs can also be achieved in dry stored seeds by laboratory treatments like chilling (VanDerWoude and Toole, 1980; VanDerWoude, 1985), heating (Taylorson and Dinola, 1989), temperature shifts (Cone et al., 1985) or incubation in the presence of GA (Rethy et al., 1987). By contrast with VLFR, the second-phase or low-fluence response (LFR) is saturated by moderate to high Pfr/Ptot ratios, such as those established by R, and are reversed when R is immediately followed by a FR treatment (Casal and Sánchez, 1998). The main photoreceptor involved in LFR responses is phytochrome B (Shinomura et al., 1994; Botto et al., 1995).

Most light-requiring seeds have tissues (e.g. the endosperm) that offer considerable resistance to the expansion of the embryo. In these seeds, the control of germination lies in the balance between the capacity of the embryo to grow and the constraint imposed by the tissue that surrounds it (Watkins and Cantliffe, 1983; Groot and Karssen, 1987; Bewley and Black, 1994; Sánchez and Mella, 2004). In Datura ferox, the stimulus of germination by a LFR involves the weakening of the micropylar region of the endosperm (ME) (de Miguel and Sánchez, 1992). Endosperm softening mediated by a LFR is associated with an increment in mannan-degrading activities in ME of tomato and D. ferox (Nomaguchi et al., 1995; Sánchez and de Miguel, 1997). In D. ferox, R action on ME involves both a promotion of endo-β-mannanase and β-mannosidase activities and the accumulation of endo-β-mannanase and expansins transcripts (Sánchez and de Miguel, 1997; Mella et al., 2004; Sánchez and Mella, 2004). Pfr formation also promotes embryo growth potential (EGP) in lettuce and D. ferox and this is associated with germination (Carpita et al., 1979; de Miguel and Sánchez, 1992). LFR-dependent changes in embryos and ME of D. ferox require gibberellins (GAs) biosynthesis (Sánchez and de Miguel, 1997; Arana et al., 2006) and there is an increment of GA 3β-hydroxylase transcript levels in the embryo after R, indicating that the embryo is the origin of the gibberellins required for germination in this species (Sánchez and de Miguel, 1997; Sánchez and Mella, 2004). Modulation of gibberellin metabolism is a main feature in the promotion of germination by LFR in lettuce and Arabidopsis seeds (Finch-Savage and Leubner-Metzger, 2006). By contrast with LFR, physiological mechanisms involved in the promotion of germination by a VLFR have not been explored. In view of the fact that LFR and VLFR depend on different photoreceptors (Shinomura et al., 1994; Botto et al., 1995, 1996) and that the physiological status of the seeds that display them are quite different, the action of LFR and VLFR in the promotion of germination might not involve the same regulatory targets. However, no information is available on this subject.

On the other hand, prolonged periods of exposure to continuous FR (FRc) can inhibit germination in several species (Negbi and Koller, 1964; Hartmann, 1966; Hendricks et al., 1968; Hendrick and Frankland, 1969; Bartley and Frankland, 1982; de Miguel et al., 2000). This third mode of action of the phytochromes is called the high-irradiance response (HIR) and, as VLFR, is mediated by phytochrome A (Shichijo et al., 2001). The action spectrum of the inhibition of germination by a HIR has a maximum near 717 nm (Hendricks et al., 1968). HIR requires prolonged exposure to FR. In D. ferox, the HIR antagonizes the LFR-mediated promotion of germination. The HIR significantly inhibits the LFR-mediated promotion of endo-β-mannanase activity in ME, indicating that endosperm weakening is a regulatory point in the LFR–HIR antagonism in germination (de Miguel et al., 2000). Whether HIR might antagonize the promotion of germination by a VLFR has not, so far, been explored.

While a VLFR participates in the promotion of germination by brief exposures to light during soil disturbances, such as tillage operations, it has been proposed that an HIR participates in the inhibition of germination under green canopies (Casal and Sánchez, 1998), even under sparse canopies typical of the early phases of development of annual crops (Batlla et al., 2000). Moreover, the germination of the seeds of several species on the uncovered soil surface can also be inhibited by an HIR, a response that may help to prevent seedling production in the soil layer where water is lost very rapidly, placing the survival of the new plant at risk (Górski and Górska, 1979). To inhibit germination of a large proportion of the seeds in the soil under these conditions it would be expected that the HIR should override the potential stimulating effects of the other light modes of action. It is therefore of interest to examine the physiological bases of the interaction between the HIR and the VLFR. One of the most frequently used seed models, Arabidopsis thaliana, is not useful for this purpose because, so far, an HIR has not been found in these seeds. Another model system for studying seed germination is tomato, but the seeds of this species are not suitable for VLFR studies. On the other hand both HIR (de Miguel et al., 2000) and VLFR (Scopel et al., 1991) can be elicited in D. ferox seeds. The photocontrol of germination in D. ferox seeds has been extensively studied (Sánchez and Mella, 2004) and is a model species for endosperm weakening, a key process in seeds with physiological dormancy (Finch-Savage and Leubner-Metzger, 2006). In this work physiological and molecular
aspects of the promotion of germination through a VLFR were investigated by evaluating the effects of FR pulses on germination, EGP, and ME weakening of *D. ferox* seeds. It was also studied whether the induction of germination by a VLFR can be inhibited by a HIR, and some molecular aspects of this antagonism.

**Materials and methods**

**Source of seeds and general incubation conditions**

*D. ferox* seeds were collected from plants invading soybean fields in Junín, Province of Buenos Aires, Argentina. After harvest, the seeds were stored in dark glass jars at room temperature. In order to obtain seeds with extreme sensitivity to light, dormancy was reduced by the storage of the seeds in a water-saturated atmosphere (WSA) in complete darkness at 25 °C for 10–12 d (de Miguel and Soriano, 1974). The seeds were then sown on cotton wool saturated with distilled water (5 ml) in clear plastic boxes and exposed to the different light treatments. After the exposure to light, the plastic boxes were wrapped in black plastic sheets and incubated in darkness at alternating temperatures [15 h at 20 °C and 9 h at 30 °C (20/30 °C)] until sampling. Control seeds were sown and kept in complete darkness under alternating temperatures (20/30 °C) until sampling.

**Light sources and treatments**

Red light was provided by Philips 40/15 40 W fluorescent lamps (Philips, Eindhoven, The Netherlands). Far-red light treatments were provided by light from 125 W incandescent internal reflector lamps filtered through an RG9 Schott glass filter (Mainz, Germany) and a 10 cm water filter. Prolonged FR treatments (FRc, FRp) were provided by light from 125 W incandescent internal reflector lamps filtered through a combination of two red acetate filters, six 2 mm thick blue acrylic filters (La Casa del Acetato, Buenos Aires, Argentina), and a 10 cm water filter. The calculated Pfr/Ptot proportions were 0.87 and 0.03 for the R and FR sources, respectively. Light treatments were: a single saturating FR pulse (FR: 30 min, 15 μmol m⁻² s⁻¹); hourly FR pulses (FRp: 3 min, 300 μmol m⁻² s⁻¹); continuous FR for 24 h (FRc: 15 μmol m⁻² s⁻¹). Far-red continuous and FRp treatments were performed under the alternating temperature regime (20/30 °C). Controls were kept in absolute darkness until germination counting or sampling for enzyme activities, embryo growth potential measurements, or the RNA assays.

**Germination tests**

After the end of the pretreatment in WSA, seeds were sown on cotton wool saturated with water and exposed to the different light treatments, or maintained in darkness (dark controls). After light treatments, seeds were incubated in darkness at alternating temperatures (20/30 °C).

Germination was measured 72 h after the beginning of the incubation on water. A total of 30 seeds per sample were used in four independent experiments. One-way analysis of variance (ANOVA) of the angular transformation of the germination counts (%) was used to analyse the data.

**Embryo growth potential measurements**

Twenty-four hours after the beginning of incubation on water, seeds were decoated and detipped by excising the conical 0.5 mm micropyral end with a surgical blade. The detipped seeds were treated with a FR pulse in order to eliminates the Pfr formed during the dissection and then incubated in darkness under alternating temperatures until the embryo length measurements were performed. The embryo growth potential was measured as described in de Miguel and Sánchez (1992). A total of 25 embryos for each sample were measured in three independent experiments. All the incubations and the FRc treatments were performed under alternating temperatures (20/30 °C).

**Enzyme activity measurements and RT-PCR reactions**

Forty-five hours after the beginning of the incubation on water, seeds were decoated under white light. The degree of softening of the ME of those seeds that received the FRc treatment was evaluated under a dissecting microscope by applying gentle force on the centre of the micropyral endosperm with a rounded graphite bar 0.6 mm in diameter. This procedure allowed us to distinguish clearly two groups of seeds; one group consisting of c. 50% of the whole population, that showed softened micropyral endosperms, termed soft seeds, and another pool of seeds with hard micropyral endosperms termed hard seeds. The proportion of hard seeds (c. 50% of the population) agreed with the proportion of the seeds that did not germinate under FRc. Both mannan-degrading activities and RNA analysis were performed separately for each group. Endo-β-mannanase and β-mannosidase activities were measured as described in Sánchez and de Miguel (1997). One-way analysis of variance (ANOVA) was used to test for treatments effects on enzyme activities. RT-PCR reactions were performed as described in Arana et al. (2006). PCR calibration curves with different cycle numbers were performed for each gene, in order to determine the number of amplification events that yield RT-PCR reactions in the linear phase. The optimal cycle number ranges used for the RT-PCR reactions were: *Actin* 23–25 cycles (embryos), 30–32 cycles (micropyral endosperms); *DfExpa1* and *DfExpa2*: 29–32 cycles (embryos); *DfExp1*: 32–35 cycles (micropyral endosperms); *DfGA3ox* and *DfGA20ox*: 25–27 cycles; *DfMan*: 23–27 cycles. The results were expressed as the ratio of *DfMan*, *DfExpa1*, *DfExpa2*, *DfGA3ox*, or *DfGA20ox* to actin, and normalized using expression in FR as 1. Paired *t* tests; using angular transformations of the normalized values were used to examine treatment effects. Differences between treatments were considered significant with *P* values lower than 0.05.

The primers used for *DfExpa1*, *DfExpa2*, and *actin* amplification were described in Mella et al. (2004) whereas the primers used for *DfMan* amplification were described in Arana et al. (2006). Primers for *DfGA3ox* and *DfGA20ox* were: lower primer: 5'-CTTTTTTCGATAAAGCTATGTTGG-3', upper primer: 5'-CTTTAGTTCCCCAAATACCTACCTCC-3', and lower primer: 5'-GGTTTTTCATCTTAAGTGGCTTTG-3', upper primer: 5'-GTATACCAGCTGCTAGTGCAA-3', respectively.

**Accession numbers**

The GenBank accession numbers for the *DfExpa1*, *DfExpa2*, *DfMan*, *DfGA3ox*, and *DfGA20ox* are AF442773, AF442772, AF32949, AF32950, and AF321085, respectively.

**Results and discussion**

*Datura ferox* seeds are deeply dormant at maturity. After a period in the soil or dry afterripening, primary dormancy is reduced and seed germination can be promoted by moderate-to high Pfr/Ptot ratios, through an LFR of the phytochromes (Soriano et al., 1964; Botto et al., 1998). However, at this stage of dormancy, seed sensitivity to light is relatively low, and a VLFR is not detected.
Promotion of germination by a VLFR requires a stronger reduction in the level of dormancy. Such a decrease in dormancy is observed under natural conditions when the seeds remain buried in the soil for long periods (months) (Scopel et al., 1991; Botto et al., 1998). In this work, a similar decrease in dormancy has been induced under controlled conditions by exposing the seeds to a WSA pretreatment.

After this pretreatment, exposing the seeds to 30 min of FR dramatically promoted germination. Values of germination in response to FR were similar to those for R: 81.9±4.1% and 84.9±4.6%, respectively. By contrast, the seeds incubated in darkness germinated less than 10% (Fig. 1). Therefore, the FR pulse promoted germination through a phytochrome VLFR. If the seeds were continuously exposed to FR for 24 h (FRc), instead of single 30 min FR pulse, germination was only c. 40%. This shows that the FRc decreased the promotion by the VLFR. Since this effect of FRc could not be reproduced by hourly FR pulses over the same period (FRp) (Fig. 1), these results show that after a dormancy reduction pretreatment in WSA germination of D. ferox seeds can be promoted by a VLFR and a subsequent HIR significantly antagonizes the VLFR.

Given that the operation of VLFR requires a change in dormancy and involves a different photoreceptor than LFR, some functional differences between these two modes of action of the phytochromes are to be expected. It is known that the LFR promotes EGP and softening of the micropylar endosperm through processes involving the action of gibberellins (Sánchez and de Miguel, 1997; Arana et al., 2006). The reduction in dormancy required to sensitize the seeds to VLFR could eliminate the light requirements for some of these processes. Consequently, the effect of a FR pulse on processes related to the embryo growth potential and to the weakening of the micropylar endosperm was explored.

A VLFR induced by a FR pulse significantly promoted the EGP, an effect which was not altered by a subsequent treatment with 24 h of FRc (Fig. 2B), although the FRc treatment sharply reduced the germination response (Fig. 1). Expansins are thought to be involved in the embryo axis expansion both in the LFR-promoted D. ferox seeds (Mella et al., 2004) and in GA-treated tomato seeds (Chen et al., 2001). By contrast, none of the expansin genes found in the embryo of D. ferox were promoted by a FR pulse (Fig. 2C). At the end of the WSA pretreatment, DfExpa2 already showed a high level of transcripts, while the transcripts of DfExpa1 increased significantly (P <0.001) in the first 45 h of dark incubation following the WSA pretreatment. Therefore, the same conditions that allowed the seeds to display a VLFR eliminated the light requirement for the induction of the expansin gene expression in the embryo. This requirement was present in more dormant seeds that require a LFR for germination.

In addition, these results show that for the increase in EGP associated with germination; changes other than those involved in expansins expression are required.

As is the case when germination is induced by a LFR (de Miguel and Sánchez, 1992), the VLFR provokes the softening of the micropylar endosperm and large changes in the activity of mannan-degrading enzymes. In WSA pretreated seeds, softening was clearly detected 45 h after the FR pulse treatment (data not shown), and significant increments in endo-β-mannanase and β-mannosidase activities were observed compared with controls kept in darkness (Fig. 3A). These results show that a VLFR promotes endosperm weakening and mannan-degrading activities in the micropylar endosperms, in agreement with its action on germination (Fig. 1). It was shown in previous work that the promotion of germination by a LFR is associated with a matching promotion of the expression of two genes of cell-wall-related proteins DfMan and DfExpa1 (Sánchez and Mella, 2004). When dormancy was decreased by the WSA pretreatment, the
accumulation of transcripts of these two genes in the ME was no longer stimulated by light and was high after 45 h of dark incubation (Fig. 3B). Interestingly, endo-β-mannanase activity was significantly enhanced by the FR pulse (Fig. 3A). This suggests some phytochrome A-dependent post-transcriptional control of endo-β-mannanase. Therefore, although both phytochrome modes of action increase endo-β-mannanase activity in the ME, there is an important difference that depends upon dormancy status and the photoreceptor involved. In more deeply dormant seeds, dependent on the stable phytochromes, light is required to enhance the amount of DfMan transcripts (Sánchez and Mella, 2004) whereas in seeds with shallower dormancy that can be induced to germinate by a VLFR, the transcript level of DfMan is not enhanced by light (Fig 3B). In the latter, post-transcriptional processes triggered by phyA are apparently required for increasing endo-β-mannanase activity.

The stimulus of germination by a VLFR was significantly inhibited by a HIR (Fig. 1). This inhibition was observed only in half of the seeds, indicating that the response to the FRc treatment was not homogeneous within the population; c. 50% of the seeds were susceptible to the inhibition of germination by a HIR. In the other fraction, the stimulus of germination by the VLFR could not be antagonized by the HIR. Because only a part of the population responded to the HIR it was considered more informative to analyse mannan-degrading enzyme activities and transcript levels of cell wall proteins separately in the responsive and non-responsive seeds. Twenty-one hours after the end of the FRc treatment, long before the radicle protruded, two groups of seeds were distinguished on the basis of their micropylar endosperm hardness: hard seeds and soft seeds. Softening of the ME was easily seen after gently pressing in the centre of the endosperm with a rounded graphite bar 0.6 mm in diameter; pressure markedly deformed the softened ME whereas no change in shape was noticed in the hard seeds. The percentage of hard seeds in the population 21 h after beginning the treatment with FRc was around 50%, coinciding with the proportion of seeds in which the HIR inhibited germination (Fig. 1), while the remainder were soft seeds that showed clearly weakened micropylar endosperms. Endo-β-mannanase and β-mannosidase activities were significantly smaller in the hard seeds than in the soft seeds (Fig. 3A). The activities of both enzymes in the hard seeds were similar to the controls which were kept in darkness. Therefore the HIR antagonized the promotive effect of the VLFR on endo-β-mannanase and β-mannosidase activities and there

![Fig. 2. Embryo growth potential is promoted by a VLFR and unaffected by a HIR.](https://academic.oup.com/jxb/article-abstract/58/14/3997/463452)
was a clear association between HIR inhibition of mannan-degrading activities and its effect on germination. The same relationship was found between the HIR action on the transcript levels of DfMan, inhibition of endosperm softening and germination (Fig. 3B). By contrast, no difference was found in the amounts of DfExp1 transcripts between hard and soft seeds (Fig. 3B).

Biosynthesis of active gibberellins is a central point in the promotion of germination by light in several species (Casal and Sánchez, 1998; Kamiya and García-Martínez, 1999; Finch-Savage et al., 2007) and is certainly required for the stimulus by R of D. ferox seed germination (Arana et al., 2006). Exposure to R promotes the increase of DfGA3ox transcripts in embryos in a FR reversible manner (Sánchez and Mella, 2004), suggesting that gibberellins are synthesized only in the embryo where they stimulate EGP (Sánchez and Mella, 2004; Arana et al., 2006) and also move to micropylar endosperm, promoting softening (Sánchez and de Miguel, 1997). Increased expression of genes coding for GA 3β-hydroxylase has been also shown to be associated with LFR promotion of germination in Arabidopsis and lettuce seeds (Toyomasu et al., 1998; Yamaguchi et al., 1998; Finch-Savage et al., 2007). However, there has been no previous information concerning the relationship between active GA synthesis and the VLFR. These results show that a VLFR stimulates the accumulation of DfGA3ox transcripts in the embryos (Fig. 4). The level of DfGA3ox transcripts was significantly higher in embryos from seeds stimulated to germinate by a FR pulse treatment compared with the dark controls, suggesting that the VLFR-mediated promotion of germination is associated with an increase of bioactive gibberellins. While a brief exposure to FR stimulated the expression of DfGA3ox, prolonged exposure to FR inhibited it. Therefore, a HIR antagonized the VLFR-mediated effect on DfGA3ox transcript levels in embryos (Fig. 4), suggesting that the contents of bioactive gibberellins in seeds is a key regulatory point in the VLFR–HIR antagonism in germination. This effect was consistent with the VLFR–HIR interaction on mannan-degrading enzyme activities, endosperm softening, and germination. By contrast to DfGA3ox, DfGA20ox transcripts were clearly detected after the pretreatment in the WSA, and were unaffected by FR (Fig. 4). Nevertheless, transcript levels of DfGA20ox were somewhat larger in embryos from FRc-treated seeds, compared with those from FR-treated seeds (Fig. 4). This effect was observed in both of hard and soft seeds, indicating that it is

Fig. 3. Mannan degrading activity, DfMan and DfExp1 transcript levels in micropylar endosperm affected by a VLFR or a HIR of the phytochromes. (A) Endo-β-mannanase and β-mannosidase activities in micropylar endosperm. Significant (P < 0.05) differences between treatments are indicated with different letters above the bars. (B) DfMan and DfExp1 RT-PCR band densitometry normalized using FR values as 1. The experimental protocol was the same as in the Fig. 1. Samples were taken 45 h after the beginning of the incubation on water, except for the Dark 0 h treatment which was taken after the end of the incubation in WSA. Error bars show SEM of at least three independent experiments. Asterisks in (B) show significant (P <0.05) differences with respect to FR.
FRcS

SEM of three independent experiments. Asterisks show significant immediately after the end of the incubation in WSA. Error bars show incubation on water, except for the Dark 0 h treatment which was taken treatment. Samples were taken 45 h after the beginning of the treatment. FRc H: embryos from hard seeds after the FRc treatment, FRc S: embryos from soft seeds after the FRc treatment. Band densitometry normalized using FR values as 1. The experimental protocol was the same as in Fig. 1. FRc H: embryos from hard seeds independent of the susceptibility of the seeds to the inhibition of germination by a HIR.

The VLFR–HIR interaction involved in the expression of DfGA3ox described above is the first report of an antagonism between two modes of actions of the phytochromes in the control of a particular gene in which the same photoreceptor (phytochrome A) is involved. In this scenario, the interplay between transient (FR) and sustained (FRc) light signals results either in the promotion or inhibition of germination of D. ferox seeds. DfGA3ox expression is a point of convergence for the VLFR and HIR in the modulation of germination. DfGA3ox transcript levels are also under phytochrome control in LFR responses (Sánchez and Mella, 2004), indicating that the contents of active gibberellins in seeds is a common regulatory point in the modulation of germination by the three modes of actions of the phytochromes. These findings are consistent with the observations in Arabidopsis showing that GA3ox1 can integrate multiple signals like cold, nitrate, and after-ripening (Finch-Savage et al., 2007). In this case, perception of the FRc signal sustained for several hours switches the seeds from a state of extreme light sensitivity that might allow germination under a closed canopy to another state that requires high levels of Frf and would only lead to germination after the opening of a gap in the canopy.

The HIR–VLFR antagonism in the control of seed germination contrasts with the influence of these modes of action in other responses such as hypocotyl elongation or Lhcb1*2 expression where both have the same effect (Casal et al., 2003). Further analysis of the molecular bases of this unusual interaction between VLFR and HIR will most likely reveal aspects of phytochrome signalling not equally present in other processes.

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References


