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Low Temperature Induces the Accumulation of *Phenylalanine Ammonia-Lyase* and *Chalcone Synthase* mRNAs of *Arabidopsis thaliana* in a Light-Dependent Manner[1]

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Anthocyanins, which accumulate in leaves and stems in response to low temperature and changes in light intensity, are synthesized through the phenylpropanoid pathway that is controlled by key enzymes that include phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS). In this work we demonstrate that *PAL* and *CHS* mRNAs accumulate in leaves of *Arabidopsis thaliana* (L.) Heynh. upon exposure to low temperature in a light-dependent manner. The regulation of the *PAL1* gene expression by low temperature and light was examined by analyzing the expression of the β-glucuronidase (*uidA*) reporter gene in transgenic Arabidopsis plants containing the *uidA* gene of *Escherichia coli* under the control of the *PAL1* promoter. The results indicate that the accumulation of *PAL1* mRNA is transcriptionally regulated. Histochemical staining for β-glucuronidase activity showed that the *PAL1* promoter is preferentially activated in photosynthetically active cells, paralleling anthocyanin accumulation. Moreover, we show that light may also be implicated in the regulation of the *CHS* gene in response to bacterial infiltration. Finally, using two *transparent testa Arabidopsis* mutants that are unable to accumulate anthocyanins, we demonstrate that these pigments are not required for successful development of freezing tolerance in this species.

Many plant species from temperate regions are able to increase freezing tolerance when exposed to low, nonfreezing temperatures in a process known as CA (Guy, 1990). Physiological and biochemical analyses of the CA process have revealed that low temperatures induce changes in lipid, protein, and carbohydrate composition (Guy, 1990; Thomashow, 1990; Tognetti et al., 1990). In addition, changes in gene expression have been shown during CA, and many cold-inducible genes have been cloned from different species (see Jarillo et al., 1994, for refs.), although their involvement in chilling and/or freezing tolerance is still poorly understood.

One of the most important effects of low-temperature exposure in plant cells is the alteration of membrane fluidity. This alteration directly affects membrane-bound metabolic processes such as respiration and photosynthesis in chilling-sensitive plants (Levitt, 1980; Kimmerer and Kozlowski, 1982). Reduction of photosynthetic activity caused by a combination of light and low temperature (see Huner et al., 1993, for refs.) has been correlated with low-temperature sensitivity, since chilling-sensitive plants are not adversely affected by low temperature in the dark (Hodgson and Raison 1989). Anthocyanins, acting as light-screening pigments, are believed to accumulate in leaves and stems in response to low temperatures and changes in light intensity (Mancinelli, 1983). These pigments are phenylpropanoids and their synthesis is controlled in part by the key enzymes PAL and CHS, which are transcriptionally regulated (see Dangl, 1992, for refs.). PAL activity has been shown to increase in response to low temperature in different species (Rhodes and Wooltorton, 1977; Tanaka and Uritani, 1977; Graham and Patterson, 1982), and the accumulation of PAL protein during CA has been demonstrated in *Brassica napus* (Parra et al., 1990). Furthermore, a correlation between low-temperature-induced anthocyanin synthesis and the accumulation of *PAL* and *CHS* mRNA has recently been demonstrated in maize (Christie et al., 1994).

In *Arabidopsis thaliana*, a chilling-tolerant plant able to acclimate to the cold (Gilmour et al., 1988), cold stress also causes the accumulation of anthocyanins in leaves and stems. To understand how this accumulation is regulated and to further elucidate the role of anthocyanins during CA in Arabidopsis, we analyzed the expression of *PAL* and *CHS* genes during low-temperature exposure. The results indicate that low-temperature exposure increases the steady-state levels of *PAL* and *CHS* mRNAs only in the light. Furthermore, using transgenic Arabidopsis plants containing the *uidA* gene encoding the GUS of *Escherichia coli* under the control of the *PAL1* promoter, we demonstrate that the accumulation of *PAL1* mRNA is transcriptionally regulated. Histochemical staining of GUS activity in cross-sections of low-temperature-exposed stems revealed that the *PAL1* promoter is preferentially activated in cortical cells, which are photosynthetically active and accumulate anthocyanins during CA. Moreover, we show that the accumulation of *CHS* mRNA by other stimuli, such as bacterial infiltration, also requires the presence of light, suggesting that light may be indispensable for *CHS* mRNA accumulation in Arabidopsis. Finally, we demonstrate that

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Abbreviations: CA, cold acclimation; CHS, chalcone synthase; PAL, phenylalanine ammonia-lyase.

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Arabidopsis mutants defective in anthocyanin accumulation are able to acclimate to the cold and develop similar levels of freezing tolerance as the wild type, indicating that anthocyanin accumulation is not a requirement for this adaptive response.

MATERIALS AND METHODS

Plant Material

The *Arabidopsis thaliana* (L.) Heynh. plants used in this study were ecotype Columbia obtained from Lehle Seeds (Tucson, AZ). For the freezing-tolerance experiments we used the *tt4* and *ttg* mutants isolated in the Landsberg *erecta* ecotype (Koornneef, 1990) that were kindly provided by M. Koornneef (Wageningen, The Netherlands). Transgenic Arabidopsis plants ecotype Columbia containing the pSO-1 construction, a transcriptional fusion of the *PAL1* promoter (−1816 to +70 bp) to the *Escherichia coli uidA* coding sequence (Ohl et al., 1990), were kindly provided by C.J. Lamb (La Jolla, CA).

Growth Conditions, Freezing-Tolerance Analyses, and Bacterial Infiltrations

Plants were grown at 20°C under continuous illumination of 100 μmol m^{-2} s^{-1}, provided by cool-white fluorescent lamps, in pots containing a mixture of perlite, vermiculite, and sphagnum (1:1:1), and irrigated with mineral nutrient solution (Haughn and Somerville, 1986). CA of 3-week-old plants was performed at 4°C under the same light conditions, for different periods of time. Light treatments of 3-week-old plants were performed with the same light conditions after 4 d of dark adaptation. Freezing tolerance was analyzed by a survival assay following the method described by Jarillo et al. (1993).

Leaves of Arabidopsis, ecotype Columbia, were inoculated with *Pseudomonas syringae* pv *phaseolicola* strain race 6, with or without the avirulence gene *avrRpm1* (Debener et al., 1991), in the manner described by these authors. Plants were grown either under continuous light or dark adapted for 4 d, and leaves were infiltrated with high-density (10^8 colony-forming units/mL in 10 mM $MgCl_2$) bacterial suspensions for different periods of time. Infiltration with 10 mM $MgCl_2$ for the same periods was used as a wounding control.

RNA Extraction and Blot Hybridization Analyses

Total RNA was prepared from leaves following the protocol described by Ausubel et al. (1987). RNA was electrophoresed through formaldehyde gels, transferred by capillarity to Hybond N^+ membranes (Amersham) as recommended by the manufacturer, and hybridized with random-primed ^{32}P-labeled DNA probes (Feinberg and Vogelstein, 1983) following standard protocols (Ausubel et al., 1987). To detect *PAL* transcripts, a 1.0-kb *Pst*I/*Hin*dIII fragment containing part of the PAL1 coding region (Ohl et al., 1990) was used as a probe. *CHS* transcripts were detected using a 1.2-kb *Hin*dIII fragment containing part of the Arabidopsis *CHS* gene (Feinbaum and Ausubel, 1988) as a probe. To detect GUS (*uidA*) transcripts, a 2.2-kb *Sma*I/*Eco*RI fragment from PBI101 (Jefferson et al., 1987) was used as a probe. *ELI3* transcripts, corresponding to a family of genes whose expression is induced by bacterial infiltration in Arabidopsis, were detected by using a 1.3-kb *Eco*RI fragment containing part of the Arabidopsis *ELI3–1* gene (Kiedrowski et al., 1992) as a probe. To check for equal RNA loading, total RNA samples were electrophoresed in agarose gels and stained with ethidium bromide to compare the rRNA intensities. To quantify the intensity of hybridization bands, autoradiographs were scanned with a Howteck Scanmaster 3+ scanner and analyzed with Bioimage 3.3 software from Millipore. RNA samples from each experiment were analyzed in at least two independent blots, and each experiment was repeated at least twice.

Histochemical Staining of GUS Activity

Histochemical staining of GUS activity in transgenic Arabidopsis plants, containing the *PAL1-uidA* promoter fusion, was performed as previously described (Liang et al., 1989), except that stem sections were embedded in 4% agarose for hand sectioning. Since Arabidopsis stems show constitutive anthocyanin accumulation in the most basal internodes, the third internode was always used to prepare the sections.

RESULTS

PAL and *CHS* mRNAs Accumulate during Low-Temperature Exposure in a Light-Dependent Manner

PAL and *CHS* mRNA levels were analyzed in leaves of 3-week-old Arabidopsis plants exposed to 4°C for different times. Figure 1 shows that the steady-state levels of *PAL* and *CHS* mRNAs started to increase after 10 h of low-temperature exposure, reaching maximum levels (30-fold) after 2 d and declining by the 7th d of exposure. These results indicate that both *PAL* and *CHS* mRNAs accumulate coordinately in response to low temperatures.

Since low-temperature induction of anthocyanin synthesis is modulated by light (Mancinelli, 1983; Rabino and Mancinelli, 1986), we analyzed whether the accumulation shown in Figure 1 was also light dependent. For this purpose, 3-week-old Arabidopsis plants were dark adapted for 4 d and then transferred to 4°C in the dark. Leaves were harvested after 48 h of cold treatment and used to prepare total RNA. RNA-blot hybridization analyses using *PAL1* and *CHS* probes showed no accumulation of the corresponding transcripts in the dark (Fig. 2). These transcripts were not detected in a 7-d time-course experiment (data not shown). In contrast, a strong hybridization signal was observed when the same experiment was performed in the light (Fig. 2), demonstrating that the cold-induced accumulation of *PAL* and *CHS* mRNAs is light dependent. Similar results were obtained when using transgenic Arabidopsis plants containing the *uidA* reporter gene fused to the full-length *PAL1* promoter and the *uidA* gene as a probe (Fig. 2). These results suggest that the accumulation of *PAL1* transcripts by low temperature in the presence of light may be regulated at the transcriptional level.

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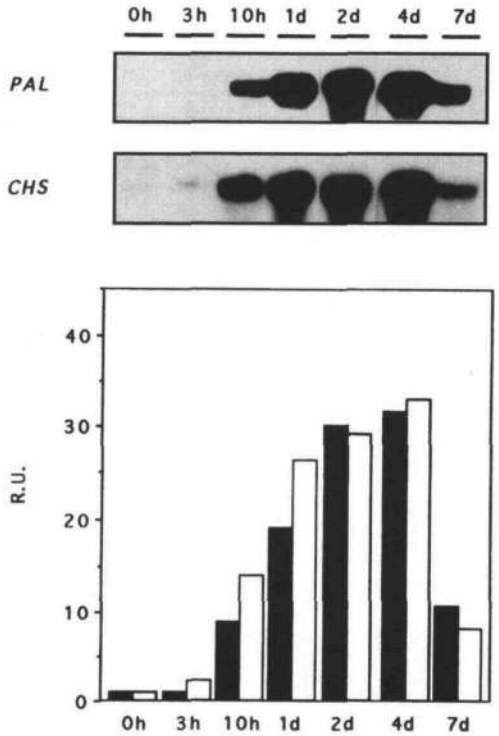

Figure 1. Time-course accumulation of *PAL* and *CHS* mRNAs during low-temperature exposure. Ten micrograms of total RNA from leaves of Arabidopsis were fractionated by gel electrophoresis, blotted, and hybridized with *PAL1* and *CHS* probes as described in "Materials and Methods." Plants were grown at 20°C for 3 weeks (0 h) and shifted to 4°C for 3 h, 10 h, and 1, 2, 4, and 7 d. Histograms represent the relative quantification of the hybridization signals as obtained by densitometric analysis. Black and white bars represent the results obtained for the PAL and CHS probes, respectively. R.U., Relative units.

Because it is well known that *PAL* and *CHS* genes are light inducible (Feinbaum and Ausubel, 1988; Ohl et al., 1990), we studied the effect of low temperature in this induction. Three-week-old plants were dark adapted for 4 d at 20°C and then transferred to continuous illumination at 20 or 4°C. Total RNA was isolated from leaves harvested at different times after these treatments and subjected to RNA-blot hybridizations using *PAL1* and *CHS* probes. We found that, in response to light, both *PAL* and *CHS* mRNAs accumulate more at 4°C than at 20°C (Fig. 3, B and A, respectively), indicating that low temperature has an additive effect on the light-induced accumulation. In addition, we compared the low-temperature accumulation of *PAL* and *CHS* mRNAs in dark-adapted plants to that taking place in continuous-light-grown plants. The results showed that the level of accumulation of these two transcripts during low-temperature exposure was less in dark-adapted than in light-grown plants (Fig. 3, B and C, respectively).

In Arabidopsis, *PAL* and *CHS* mRNAs are known to accumulate in response to an array of environmental cues, with the hypersensitive response to bacterial infiltration being one of the best characterized (Dangl, 1993). Because low temperature induced *PAL* and *CHS* mRNA accumulation in a light-dependent manner (see above), we decided to analyze the light involvement in the accumulation of *PAL* and *CHS* mRNAs by bacterial infiltration. With this purpose infiltration experiments were performed, both in the light and in the dark, in leaves from transgenic Arabidopsis plants containing the *PAL1* promoter-*uidA* gene fusion. In this experiment we used a *P. syringae* pv *phaseolicola* strain carrying the avirulence gene *avrRpm1* (Debener et al., 1991). This strain produces a hypersensitive response on the Columbia ecotype (C. Ritter and J.L. Dangl, personal communication). A control experiment was performed using the *ELI3* gene as a probe. *ELI3* mRNA accumulates in Columbia leaves after being challenged with the *P. syringae*-incompatible strain carrying the *avrRpm1* gene (C. Ritter and J.L. Dangl, personal communication). The results indicated that this mRNA accumulated after 3 h of infiltration either in light-grown or 4-d dark-adapted plants (Fig. 4). Using the *uidA* gene as a probe, we found that the leaves of both light-grown and dark-adapted plants infiltrated with the incompatible *Pseudomonas* strain transiently accumulated the *uidA* mRNA, also after 3 h of inoculation (Fig. 4). Therefore, light seems not to be essential for the inducibility of *PAL1* promoter in response to bacterial infiltration.

However, when the *CHS* probe was used in the same hybridization experiments, we found that *CHS* mRNA accumulated to high levels 24 h after inoculation in light-grown plants, but not in dark-adapted plants infiltrated and maintained in the dark (Fig. 4). Similar results were obtained when using the virulent *P. syringae* strain lacking the *avrRpm1* gene (data not shown). Moreover, the same

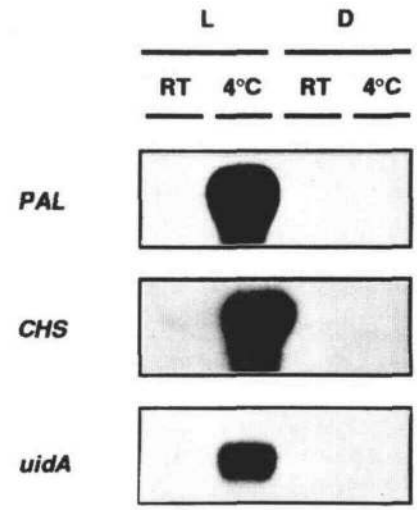

Figure 2. Light-dependent accumulation of *PAL* and *CHS* mRNAs during low-temperature exposure. Ten micrograms of total RNA from leaves of Arabidopsis Columbia ecotype and transgenic plants containing the *PAL1* promoter-*uidA* gene fusion were fractionated by gel electrophoresis, blotted, and hybridized with *PAL1*, *CHS*, and *uidA*. L, Plants grown at 20°C for 3 weeks (RT) and shifted to 4°C for 48 h (4°C). D, The same as in L but plants were dark adapted for 4 d before low-temperature treatment in the dark.

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patterns of PAL and CHS transcript accumulation were obtained when infiltration was carried out in wild-type Arabidopsis plants (data not shown). These data indicate that the accumulation of *CHS* mRNA either by bacterial infiltration or low temperature is strictly light dependent.

The Expression Pattern of *PAL1* Promoter in Response to Low Temperature Is Restricted to Photosynthetic Tissue

We have shown above that *PAL* and *CHS* mRNAs accumulate in response to low temperature in a light-dependent manner. Moreover, low temperature has been shown to induce the accumulation of anthocyanins in leaves and stems (Mancinelli, 1983), suggesting a putative role for these compounds as photoprotective pigments. To determine if the accumulation of the *PAL1* mRNA by low temperature was linked to photosynthetic tissue, we analyzed the tissue specificity of GUS expression and its relationship with anthocyanin accumulation in three independent, transgenic Arabidopsis lines containing the *PAL1* promoter-*uidA* gene fusion. Since in Arabidopsis cortical cells have photosynthetic activity, we used stem cross-sections in all the microscopic analyses. As shown in Figure 5A, in plants grown at room temperature anthocyanins are generally found in a few cortical cells, preferentially in the outer cell layers. However, after 48 h of low-temperature exposure, a drastic increase in the number of cortical cells accumulating anthocyanins could be observed (Fig. 5B). A similar pattern of purple anthocyanin accumulation was also observed in response to high light intensity (data not shown). These results indicate that anthocyanin accumulation in response to low temperature is restricted to photosynthetic cells.

We next examined GUS activity in transgenic plants grown at room temperature. The results revealed a close association of *PAL1-uidA* expression with the stem vascular system, with GUS activity being confined to protoxylem cells (Fig. 5C). Some GUS activity was also detected in a few cortical cells (Fig. 5C). When plants were exposed to low temperature for 48 h, a dramatic increase in GUS activity could be detected in the cortical cells (Fig. 5D). These results show that low temperature induces a change in the expression pattern of *PAL1* promoter that is closely related to the pattern of anthocyanin accumulation.

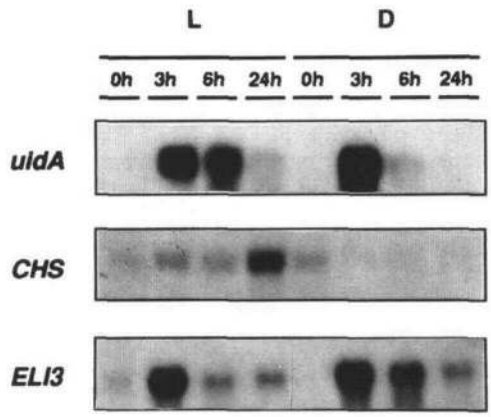

Figure 4. Effect of bacterial infiltration on the induction of *PAL1* promoter and *CHS* gene expression. Leaves of transgenic Arabidopsis plants containing the *PAL1* promoter-*uidA* gene fusion, grown in continuous light, were infiltrated with a *P. syringae*-incompatible strain and harvested at different times after inoculation. Ten micrograms of total RNA from the harvested leaves were fractionated by gel electrophoresis, blotted, and hybridized with *uidA* and *CHS* probes. L, Plants infiltrated and harvested in the light; D, plants dark-adapted for 4 d, infiltrated, and harvested in the dark. The same filter was hybridized with an *ELI3* probe as a control.

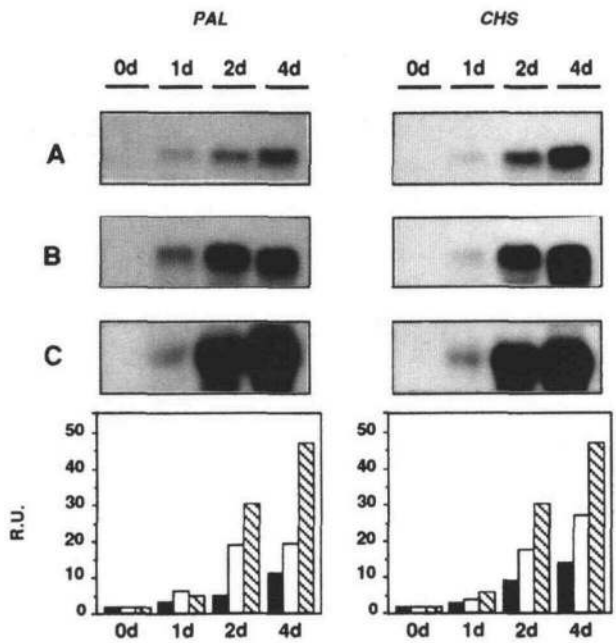

Figure 3. Effect of low temperature on the light inducibility of *PAL* and *CHS* genes. Ten micrograms of total RNA from leaves of continuous-light-grown Arabidopsis plants harvested immediately before (0 d) and 1 to 4 d after treatments were fractionated by gel electrophoresis, blotted, and hybridized with a *PAL1* and *CHS* probe. Treatments were: A, plants transferred to continuous light at 20°C after 4 d of dark adaptation at the same temperature; B, plants transferred to continuous light at 4°C after 4 d of dark adaptation at 20°C; C, continuous-light-grown plants shifted from 20 to 4°C. Histograms represent the relative quantification of the hybridization signals as obtained by densitometric analysis. Black, white, and hatched bars correspond to the results obtained in treatments A, B, and C, respectively. R.U., Relative units.

Anthocyanin Accumulation Is Not Required for the Development of Freezing Tolerance

Now that it has been demonstrated that *PAL* and *CHS* mRNAs accumulate in Arabidopsis as a result of low-temperature exposure in the presence of light, and that this accumulation is correlated to that of anthocyanins in stem cortical cells, the question remains whether anthocyanin accumulation is required for freezing tolerance in Arabidopsis. With this question in mind we characterized the freezing tolerance of two recessive *transparent testa Arabidopsis* mutants, *ttg* and *tt4*, blocked in anthocyanin accu-

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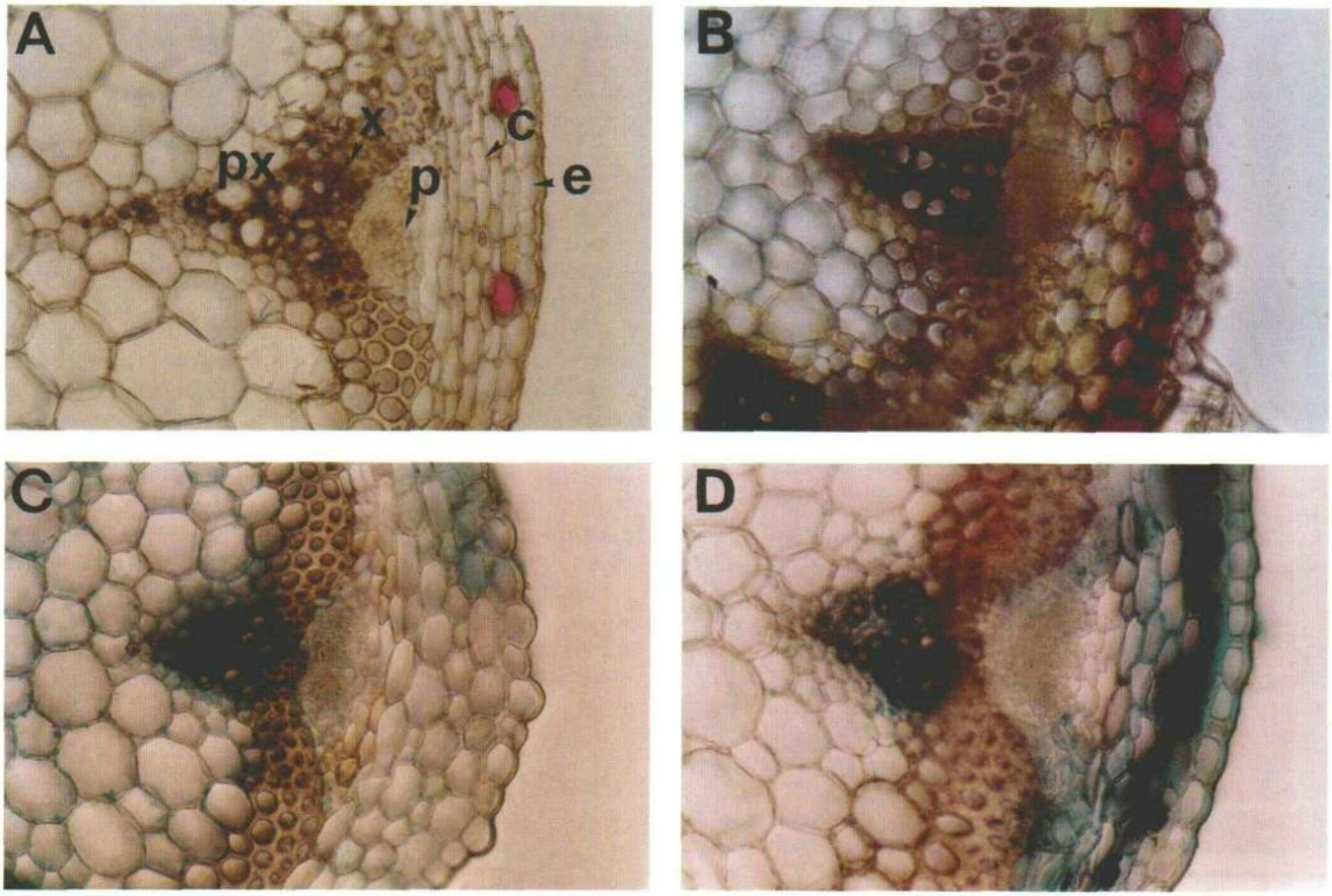

Figure 5. Histochemical localization of GUS activity during low-temperature exposure in stem cross-sections of transgenic Arabidopsis plants containing the *PAL1* promoter-*uidA* gene fusion. A and C, Stem cross-section from plants grown at 20°C under continuous light, unstained and stained for GUS activity, respectively. B and D, The same as in A and C but 48 h after exposure to 4°C, unstained and stained for GUS activity, respectively. e, Epidermis; c, cortical cells; p, phloem; px, protoxylem; x, mature xylem.

mulation (Koornneef, 1981, 1990). The *TTG* locus of Arabidopsis is functionally homologous to the *R* locus of maize (Lloyd et al., 1992), whereas *tt4* is a mutant at the *CHS* structural gene (Li et al., 1993). The survival of acclimated and nonacclimated *ttg* and *tt4* plants at freezing temperatures was similar to the survival of the corresponding wild-type plants (Fig. 6), indicating that anthocyanin accumulation is not required for freezing tolerance in Arabidopsis under our experimental conditions.

DISCUSSION

Anthocyanins have been proposed to function as photoprotective pigments (Li et al., 1993) and they accumulate in plants in response to low temperatures (Mancinelli, 1983). To understand how this accumulation is regulated and to elucidate the role of these compounds in the CA process, we analyzed the cold-induced accumulation of *PAL* and *CHS* mRNAs, which encode key regulatory enzymes of the anthocyanin biosynthetic pathway. Our results showed that low-temperature exposure coordinately induces the accumulation of *PAL* and *CHS* mRNAs only in the light. The accumulation of *PAL* and *CHS* mRNAs also has been described in response to other environmental stimuli (Davis and Ausubel, 1989; Ohl et al., 1990; Davis et al., 1991; Feinbaum et al., 1991; Kubasek et al., 1992). The coordinated accumulation of these two mRNAs suggests that their response to such a diverse stimuli may be regulated through a common controlling mechanism (Loake et al., 1991). In addition, the fact that low-temperature accumulation of *uidA* transcripts in transgenic Arabidopsis plants containing the *PAL1* promoter-*uidA* gene fusion is identical to the accumulation of the endogenous *PAL* mRNAs suggests that the light-dependent accumulation of *PAL1* mRNA by low temperature is regulated at the transcriptional level. Tissue-specific accumulation of *PAL1* mRNA in response to low temperature was analyzed using the same transgenic plants. The 1.8-kb *PAL1* promoter confers on the *uidA* reporter gene a specific expression pattern directly associated with the accumulation of anthocyanins. In nonstressed stems, *PAL1* promoter activity is restricted to the protoxylem cells, likely related to lignin deposition (Bevan et al., 1989; Liang et al., 1989). However, in response to low temperature, the promoter is additionally active in cortical cells. These cells are photosyntheti-

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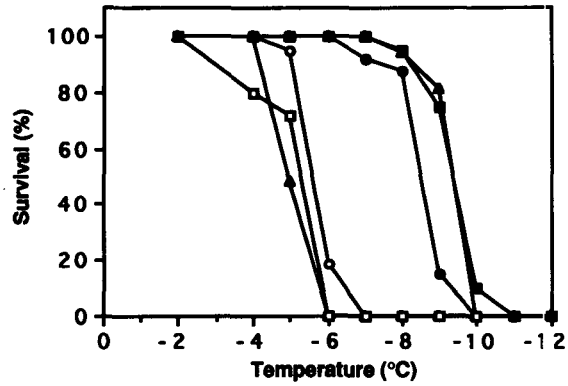

Figure 6. Effect of CA on the development of freezing tolerance in *tt4* and *ttg Arabidopsis* mutants. Plants were acclimated and frozen as described in "Materials and Methods." Freezing tolerance was estimated as the percentage of plants surviving a specific freezing temperature. Control nonacclimated plants are represented by open symbols: ○ for wild type, △ for the *tt4* mutant, and □ for the *ttg* mutant. Acclimated plants are represented by closed symbols: ● for wild type, ▲ for the *tt4* mutant, and ■ for the *ttg* mutant.

cally active in Arabidopsis and show anthocyanin accumulation during low-temperature exposure. Similarly, specific accumulation in photosythetically active tissue has been shown for a wheat cold-inducible gene (*WES19*) (Chauvin et al., 1993). Interestingly, the low-temperature inducibility of this gene in wheat is also light dependent.

Comparison of the accumulation of *PAL* and *CHS* transcripts in response to light at room temperature and at 4°C showed that cold has an additive effect on the accumulation of these mRNAs by light exposure. Low temperature reduces the photosynthetic capacity of the cells, leading to an excess of photon flux (Somersalo and Krause, 1989). This excess could produce an additional light stress, resulting in a further induction of the phenylpropanoid pathway. In this way a light-inducible factor controlling anthocyanin accumulation has recently been characterized in maize (Cone et al., 1993). Similar regulatory proteins could also be required for the accumulation of *CHS* mRNA by bacterial infiltration, which we have also shown to be light dependent. In agreement with this observation, the soybean *CHS1* promoter has *cis*-acting elements that confer both UV light and elicitor inducibility (Wingender et al., 1990). In Arabidopsis, the induction of genes controlling the phenylpropanoid pathway by bacterial infiltration has frequently been confounded by differences in the bacterial strains used (Dangl, 1993). This fact could explain why Dong et al. (1991) did not observe any induction of the *CHS* gene after bacterial infiltration, whereas we found that both the incompatible and the compatible *P. syringae* strains activate the *CHS* gene in a light-dependent manner.

Therefore, the induction of the *CHS* gene that we observed here is probably independent of the *avrRpm1* gene product. Although it has been reported that the basal levels of other defense-related genes are modulated by light (Reimann-Philipp et al., 1989), it is unexpected that light appears to be a requirement for the induction of a defense gene by bacterial infiltration. Similarly, other genes involved in plant adaptive responses, like a desiccation-inducible gene of *Craterostigma plantagineum*, have been shown to be regulated by light (Bartels et al., 1992). If this regulatory restriction of the *CHS* gene is unique for Arabidopsis, which has a single *CHS* gene, or if it is also found in other plant species, remains to be shown.

Because CA requires the plant to adjust to a combination of light and low temperature (see Huner et al., 1993, for refs.) and because photosynthesis is one of the first processes adversely affected by low-temperature exposure (Öquist et al., 1987), we speculated that anthocyanins, acting as photoprotectants, could play an important role in the acquisition of freezing tolerance. The availability of recessive *transparent testa* Arabidopsis mutants, blocked in anthocyanin accumulation, allowed a direct evaluation of the contribution of these phenolic compounds to the acquisition of freezing tolerance. Our results showed that the lack of anthocyanin accumulation does not affect the development of freezing tolerance. This indicates that under our experimental conditions Arabidopsis can stand the light stress promoted by low temperatures, even without anthocyanin synthesis. Alternatively, it has been suggested that although flavonoids may be involved in plant UV-B photoprotection, they could not be directly linked to the photoprotection of photosynthetic machinery (Middleton and Teramura, 1993).

Recently, the accumulation of *PAL* and *CHS* mRNAs by low temperature has been demonstrated in maize (Christie et al., 1994). These authors analyzed *PAL* and *CHS* mRNA accumulation only in the presence of light and suggested that the molecular mechanisms controlling CA in plants and the low-temperature induction of the phenylpropanoid pathway could have common steps. However, our results indicate that, at least in Arabidopsis, this may not be the case since, *PAL* and *CHS* mRNAs do not accumulate by low-temperature treatment in the dark but only in the presence of light. Similarly, the wheat cold-inducible gene *WES19*, mentioned above, is induced only by low temperature in a light-dependent manner (Chauvin et al., 1993). Since most studies dealing with the CA process do not consider the involvement of light in gene expression, we cannot rule out the possibility of light dependency being a more general feature of cold-induced gene expression. This light-dependent network may also be implicated in regulating the *CHS* gene expression in response to bacterial infiltration. Plant development is constantly modulated by light (Chory, 1993) and it is tempting to speculate that some adaptive mechanisms, controlled by the phenylpropanoid pathway, have conserved their light dependence during plant evolution. Further experiments are required to determine the molecular mechanisms responsible for the light-dependent transcriptional control observed here, and to elucidate if this is a more general phenomenon during the integration of plant adaptive responses to developmental programs.

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P. syringae strains distributed by the EC BRIDGE Arabidopsis T-Project DNA Stock Center in Köln; F.M. Ausubel for providing the *CHS* probe; and M. Koorneef for providing the *tt* mutant seeds. We also acknowledge the expert technical assistance of Juan Capel, Francisco Sánchez, and María José Jorquera.

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