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The responses of trichome mutants to enhanced ultraviolet-B radiation in *Arabidopsis thaliana*

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ABSTRACT

To gain a better understanding of the protective function of the trichome in *Arabidopsis* against UV-B radiation, we performed a study using several *Arabidopsis* trichome mutants (*gl1*, *gis*, *gis2*, *zfp8*, *try82*, and *gl3*), overexpressing trichome positive regulator lines (*35S:GIS* and *35S:GIS2*), and wild-types (WT) under simulated enhanced UV-B radiation conditions. The flowering time, height, diameter of rosette, leaf size, trichome density, and expression levels of GL3 gene were measured. Significant decreases in height, diameter of rosette, leaf size, and a notable delay in flowering time were observed in all mutants and wild-types after exposure to UV-B. Moreover, the trichome density showed a significant increase, suggesting a clear induction of trichome formation by UV-B. Comparing the mutants and WT, we found that the mutants that had more trichomes showed a lower sensitivity to UV-B than the WT, whereas the mutants that had fewer trichomes were more sensitive to UV-B. These results indicated that the trichome plays a key shielding role against UV-B radiation. qRT-PCR analysis indicated that UV-B radiation induced expression of *GL3* and an increase in *GL3* transcript level correlated with the increase in trichome density and, suggesting a possible role of *GL3* by integrating the environmental signal to control trichome initiation.

1. Introduction

Ultraviolet B (UV-B) radiation (wavelength from 280 nm to 320 nm) is a component of solar radiation. As a result of ozone layer depletion, the intensity of UV-B radiation reaching the earth's surface has increased during recent decades [1,2]. UV-B radiation has a variety of impacts on growth and development in plants. It causes damage to DNA and proteins [3], affects growth processes of sensitive plants, such as flowering time and pollen germination [4], and is a repressive factor for morphology [5].

Plants have developed effective strategies to avoid the harmful UV-B radiation. Plants growing in environments with high ambient UV-B radiation possess a notable tolerance against enhanced UV-B radiation [6–9], which is mainly attributed to tissues with high ability to absorb UV-B. The UV-B absorbing ability is assigned to UV-B absorbing compounds, such as flavonoids and other phenolics, which are mainly located in superficial tissues [10–12].

Previous studies demonstrated the existence of flavonoid compounds and other UV-screening pigments within the leaf hair [13,14]. A considerable decrease of photosystem II photochemical efficiency in dehaired olive leaves and *Quercus ilex* was reported [15,16]. Liakoura et al. [17] found that the trichome density of leaves exposed to UV-B was higher than those of shaded leaves, and leaf hairs in the exposed region showed a considerably increased UV-B absorbing capacity [17].

UV-B absorbing compounds accumulate in the trichome layer, which thus posses a UV-B absorptance profile, acting as an effective filter in screening out the penetration of UV-B to protect the underlying tissues against UV-B radiation damage [14–16,18–20].

The *Arabidopsis* trichome is a unicellular structure that develops on the surface of the plant, which is differentiated from epidermal cell. The trichomes are widely spread on the surfaces of the cauline leaves, rosette leaves, stem, petal, sepal, and root [21]. The regulation of *Arabidopsis* trichome initiation is a complex process, involving a number of genes [21–23]. Genetic analyses have revealed that GLABRA1 (GL1), TRANSPARENT TESTA GLABRA1 (TTG1), and GLABRA3 (GL3) form a trichome-promoting trimeric complex. The GL1 gene encodes a MYB transcription factor that is essential for *Arabidopsis* trichome initiation [24]. Mutations in GL1 induced an almost glabrous trichome phenotype. GL3 encodes a bHLH factor that is essential in initiation of the trichome complex; *gl3* mutants have a decreased trichome number [25]; TRIPTYCHON (TRY), another MYB transcription factor, negatively regulate trichome formation. Mutations in TRY result in increased branches of the trichomes. GLABROUS INFLORESCENCE STEMS (GIS) encodes a C2H2 transcription factor that is a positive regulator of trichome

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initiation, and which regulates the trichome initiation program during inflorescence development [26]. Loss of GIS function led to a decrease in trichome formation on inflorescence organs. Overexpression of GIS lead to an increase in trichome production on inflorescence organs. GLABROUS INFLORESCENCE STEMS 2 (GIS2) and ZINC FINGER PROTEIN8 (ZFP8) also encode C2H2 transcription factors and act by in a functionally redundant manner with GIS1 to control inflorescence trichome development through hormonal signalling [22,26].

Although some reports have demonstrated that UV-B radiation could influence trichome density [17], few studies have focused on the molecular events underlying UV-B induced trichome formation. In this study, we investigate the response of different *Arabidopsis* trichome mutants to enhanced UV-B radiation and its relation with trichome density. More importantly, we used qRT-PCR analysis as a tool to explore the molecular mechanism of how UV-B radiation influences the trichome initiation and development.

2. Materials and methods

2.1. Plant Material and growth conditions

Arabidopsis thaliana ecotype *Columbia* (*Col-0*) and *Landsberg erecta* (*Ler*) were used as the wild type controls in this study. The *gl1*, *gis*, *gis2*, *and zfp8* mutants, and overexpressed lines 35S:GIS and 35S:GIS2 used in this study are in the *Col-0* background. The *try82* and *gl3* mutants are in the *Ler* background. These mutants and overexpressing lines are classified into two groups in different wild-type background. One is the trichome-increased phenotype mutants and overexpressed lines, including overexpressing lines 35S:GIS, 35S:GIS2 (Col-0 background) and the try82 mutant (Ler background), the other is the trichome-decreased phenotype mutants, including gis, gis2, zfp8, gl1 mutants (Col-0 background) and gl3 (Ler background). Before sowing, all seeds were imbibed with water and placed at 4 °C for 3 days to eliminate dormancy. Plants were grown under 16-h-light (95 μmol m^{-2} s^{-1}, 23 °C) and 8-h-dark (19 °C) cycles at 65% humidity. The experiments were performed in a growth chamber and repeated at least once with similar results.

2.2. UV-B radiation treatments

All plants were exposed to ambient background intensities of photosynthetically active radiation (220 μmol m^{-2} s^{-1}). Enhanced UV-B radiation was provided when the fourth rosette leaf had expanded fully by filtered Qin brand 30 W lamps (290–320 nm) (Qin brand, Baoji Lamp Factory, China) suspended above the plants, the lamps were filtered with 0.13 mm thick cellulose deacetate (transmission down to 290 nm) for UV-B irradiance and 0.13 mm polyester plastic films (absorbs all radiation below 320 nm) as a control. The cellulose deacetate filters were presolarized. The desired irradiation was obtained by altering the distance between the lamps and the plants. The spectral irradiance from the lamps was measured with an Optronics Model 742 (Optronics Labs, Orlando, FLUSA) spectroradiometer. The spectral irradiance was weighted with a generalized plant response action spectrum [27] and normalized at 300 nm to obtain the level of biologically effective UV-B radiation (UV-B_{BE}). Plants received a daily exposure of 3.24 kJ m^{-2} UV-B irradiation for 20 days.

2.3. Morphologic parameters and trichome enumeration

The flowering time, height, and diameter of vegetative rosettes of plants were recorded at the plant flowering stage. During the flowering period, the 8th rosette leaf and the first cauline leaf trichomes were counted under a dissecting microscope when the leaves had fully expanded; the leaf sizes were measured by image analysis software (Image-Pro Plus 5.0.2) after the leaves were scanned into images through a scanner. The number of trichomes of the first internode was recorded when the main stem reached about 15 cm in height, and the length of the internode was measured, as described before [26].

2.4. RNA extraction and real-time RT-PCR

Plant RNA was extracted from 50 mg leaf samples using TRIzol reagent, according to the manufacturer's protocol. About 5 μg of total RNA were reverse-transcribed using the Script RT Kit (TIANGEN, China), after first strand cDNA synthesis using random hexamer primers, according to the manufacturer's instructions, and the samples were diluted to 50 μl. For Quantitative real-time RT-PCR analysis, 1.6 μl cDNA was added to 9 μl SYBR-Green mix (TIANGEN, China) and 0.2 μl of each primer (200 nM final concentration) in triplicate 20 μl reactions. Gene specific primer sequences used for the analysis of the trichome initiation related gene *GL3* were as follows: GL3: GL3F5, 5′-GGTACCACAGAACATATTACGGAAGA-3′ and GL3R3, 5′- CAAGAACGTTGTCGAT-GTGATAATC-3′; UBQ10: UBQ10F5, 5′-GGTTCGTACCTTTGTCCAAGCA-3′ and UBQR3, 5′-CCTTCGTTAAACCAAGCTCAGTATC-3′. Real-Time PCR was performed using an Bio-rad CFX 96 thermal cycler, using the following cycling conditions: 95 °C for 10 min; followed by 40cycles of 95 °C for 15 s and 60 °C for 1 min. Melting curve analysis was performed following every run to ensure a single amplified product for every reaction. UBQ10 was used as an endogenous control gene to normalize transcriptional level of the other genes. UBQ10 was chosen as the housekeeping gene for its consistent expression pattern between different tissues and treatments [28]. Relative quantification of the RNA expression levels were calculated by subtracting the threshold cycle (*Ct*) value for UBQ10 from those of the target gene (to obtain ΔCt) and then calculating $2^{-\Delta\Delta Ct}$ [26]. The real time PCR analyses were repeated at least once with the similar results.

2.5. Data analysis and statistics

Data were tested using SPSS software (version 15.0; SPSS Inc., Chicago, IL USA). A one-way analysis of variance (ANOVA) was used to determine significant differences between the UV-B radiation treatments and controls.

3. Results

3.1. Effects of UV-B radiation on growth and development

To investigate the effects of UV-B radiation on growth and development in plant, we observed the changes in flowering time and several morphologic parameters in *Arabidopsis* after UV-B radiation. Compared to the control plants, a significant delay ($P < 0.01$) in flowering time in plants exposed to UV-B radiation was observed in all the experiments (Fig. 1A). The flowering time of mutant *gl1* was the most delayed (by almost 62.5%), from an average value of 41 days to 66.7 days in comparison to its wild-type control. The relative percentage changes between UV-B radiation treated plants and the controls are summarized in Table 1. Comparing the relative percentage changes of the mutants with wild-types for flowering time, we found that different responses to UV-B radiation occurred. The trichome-decreased phenotype mutants, *gl1, gis, gis2*, and *zfp8*, showed a significant delay ($P < 0.01$) in flowering time in comparison to the wild type control *Col-0*; *gl3* in *Ler* background showed a similar pattern (Table 1). By contrast, the trichome-increased phenotype mutant *try82* dis-