J. Plant Biol. (2011) 54:425–429
DOI 10.1007/s12374-011-9183-6

ORIGINAL RESEARCH

High Temperature Stimulates *DWARF4 (DWF4)* Expression to Increase Hypocotyl Elongation in *Arabidopsis*

Puna Maya Maharjan · Sunghwa Choe

Received: 26 July 2011 / Revised: 9 September 2011 / Accepted: 11 September 2011 / Published online: 24 September 2011

Abstract Hypocotyl growth occurs as a result of an interaction between environmental factors and endogenous phytohormones. In *Arabidopsis*, high temperature promotes auxin synthesis to increase hypocotyl growth. We previously showed that exogenously provided auxin stimulates expression of the brassinosteroid (BR) biosynthetic gene *DWARF4*. To determine whether temperature-induced hypocotyl elongation depends on BR biosynthesis, we examined the morphological responses to high temperature and the expression pattern of *DWF4pro:GUS* in different genetic backgrounds, which are as follows: Ws-2 wild-type, *iaa19/msg2, bri1-5*, and *dwf7-1*. In contrast to the wild-type, growth of the three genotypes at 29°C did not significantly increase hypocotyl length; whereas, with the exception of *iaa19/msg2*, the roots were elongated. These results confirm that BR biosynthesis and signaling pathways are required for hypocotyl growth at high temperature. Furthermore, a GUS histochemical assay revealed that a temperature of 29°C greatly increased *DWF4pro:GUS* expression in the shoot and root tips compared to a temperature of 22°C. Quantitative measurements of GUS activity in *DWF4pro:GUS* revealed that growth at 29°C is similar to the level of growth after addition of 100 nM IAA to the medium. Our results suggest that temperature-dependent synthesis of free auxin stimulates BR biosynthesis, particularly via the key biosynthetic gene *DWF4*, and that the BRs thus synthesized are involved in hypocotyl growth at high temperature.

Keywords Auxin · Brassinosteroids · Plant hormone · Temperature · *DWF4* · *Arabidopsis*

P. M. Maharjan · S. Choe (✉)
School of Biological Sciences, College of Natural Sciences,
Seoul National University,
Seoul 151-747, South Korea
e-mail: shchoe@snu.ac.kr

S. Choe
Plant Genomics and Breeding Institute, Seoul National University,
Seoul 151-921, South Korea

Introduction

Throughout their life cycles, plants receive environmental signals of various types, including temperature, light, air quality, water, and touch. Temperature influences most of the physiological processes in plants, including photosynthesis, transpiration, respiration, germination, and flowering. Depending on the species, plants respond to temperatures in essentially three different ways. Firstly, plants adjust their growth and development according to changing temperature, thus enabling them to adapt to the conditions (Clarke et al. 2004; Yamaguchi-Shinozaki and Shinozaki 2006). Secondly, they take advantage of changing temperatures to enhance their fitness in growth and development (Blazquez et al. 2003; Halliday and Whitelam 2003; Gray et al. 1998). Thirdly, plants may fail to adjust, and therefore be subjected to cold stress or heat shock. The second type of response has been of considerable interest to scientists, particularly those engaged in the field of crop production. Examples of taking advantage of changing temperatures include the winter squashes that increase their sweetness when ripened in a cooler temperature in the fall, by reducing energy use in vegetative tissues but increasing sugar storage in the roots. Furthermore, strawberries grown under high temperature increase the flavonoid and antioxidant contents in their fruits (Wang and Zheng 2001). The first type of response is possible due to the developmental plasticity of plants. As a part of adaptive adjustment, plants

respond to high temperatures by altering their morphologies: hypocotyl elongation (Gray et al. 1998), early flowering (Balasubramanian et al. 2006), petiole elongation, and leaves hyponasty (Koini et al. 2009). The hypocotyl is a good model for studying hormonal interaction in developmental plasticity via cell elongation; this is because hypocotyls can increase their length by up to 10-fold by elongation of approximately 20 cells in the absence cell division (Gendreau et al. 1997).

In high temperature-mediated hypocotyl elongation, the direct involvement of auxin was determined from observations of the reduced response of auxin mutants and by the direct measurement of IAA levels in seedlings grown at 29°C (Gray et al. 1998). It was found that high temperature induces auxin synthesis, and that auxin plays an important role in hypocotyl elongation. In addition to auxin, it was shown that brassinosteroids (BRs) are also involved in this temperature-mediate growth of hypocotyls. The BR biosynthetic mutant *de-etiolated2 (det2)* failed to respond to high temperature (Gray et al. 1998). Similarly, Nemhauser et al. (2004) reported that *det2* responds to high temperature only when BRs are supplied exogenously, whereas a BR signaling mutant, *brassinosteroid insensitive1-5 (bri1-5)* did not respond at all. These results suggest that functional biosynthesis and signaling of BRs are required for the temperature-mediated elongation of hypocotyls (Nemhauser et al. 2004).

In spite of genetic evidence that BRs are involved in the temperature-mediated hypocotyl elongation process, the molecular mechanism underlying this process has yet to be established. To address this lack of knowledge, we examined how the BR biosynthetic gene *DWARF4 (DWF4)* responds to high temperature. In this paper, we describe the results of a GUS histochemical analysis as well as in vivo GUS activity tests of a *DWF4pro:GUS* line, which support the idea that temperature-dependent auxin synthesis induces the expression of the BR biosynthetic gene in *Arabidopsis*, which in turn results in hypocotyl growth.

Results and Discussion

Previously, it was reported that growing *Arabidopsis* seedlings at an elevated temperature induces hypocotyl elongation due to elevated auxin biosynthesis (Gray et al. 1998; Nemhauser et al. 2004). Furthermore, we have shown that auxin stimulates BR biosynthesis through upregulation of the key BR biosynthetic gene *DWF4* (Chung et al. 2011; Maharjan et al. 2011). To investigate whether this temperature-elevated auxin increases *DWF4* expression, we examined a *DWF4pro:GUS* line in different genetic backgrounds, namely, the wild-type, a biosynthetic mutant, *dwf7-1*; and a receptor mutant, *bri1-5*.

The hypocotyl length of the control, *DWF4pro:GUS*, when grown at 29°C was increased by approximately 1.75-fold compared with that grown at 22°C (Fig. 1a). However, the BR biosynthetic mutant *dwf7-1* and the BR receptor mutant *bri1-5* did not respond to the high temperature; seedlings grown at 29°C had hypocotyls lengths similar to those grown at 22°C. In addition, a dominant mutant for the *IAA19/MSG2* gene did not elongate at 29°C. These results confirm that proper biosynthesis and signaling pathways for BRs are required for hypocotyl elongation at high temperature. Root responses to high temperature were also measured (Fig. 1b). Whereas both *dwf7-1* and *bri1-5* mutants elongated their roots when grown at high temperature, the iaa19/msg2 mutant did not. In contrast to hypocotyls, high temperature induced root elongation in the BR mutants, suggesting that the hypocotyls and roots of the BR mutants may employ different mechanisms in response to high temperature.

In order to visualize the effects of temperature on *DWF4pro:GUS* expression, we germinated and grew seedlings for 3 days at 22°C, and then incubated these seedlings at 29°C for a further 3 days before performing GUS histochemical analysis (shown in Fig. 1c). Compared with the *DWF4pro:GUS* seedlings grown continuously at 22°C (Fig. 1c, top left), the seedlings that were grown at 29°C for the final 3 days displayed increased GUS staining in the root and shoot tips as well as in marginal tissues of the emerging leaves (Fig. 1c, top right). To demonstrate this effect more clearly, we performed the same experiment with *bri1-5 DWF4pro:GUS* seedlings (Fig. 1c, bottom row). Because feedback downregulation toward *DWF4* regulation is disrupted in the weak BR receptor mutant bri1-5, the GUS staining pattern is stronger and easier to detect (Kim et al. 2006). Relative to the seedlings grown continually at 22°C (Fig. 1c, bottom left), the seedlings transferred from 22°C to 29°C clearly exhibited an increased GUS staining pattern in the tip and elongating zone of the root, shoot tip, leaf blade, and margins of the cotyledons (Fig. 1c, bottom right). These stronger GUS staining patterns at high temperature suggest that *DWF4* expression is increased in response to high temperature. Given that the auxin level increases at high temperature (Gray et al. 1998), it is likely that auxin is responsible for the upregulation of *DWF4* at 29°C (Chung et al. 2011).

To quantify the temperature-mediated increase in GUS activity, we performed an in vivo GUS assay using *DWF4pro:GUS* seedlings grown at two different temperatures for 5 days (Fig. 1d). To confirm the effect of auxin on *DWF4pro:GUS* expression, we first examined the GUS activity in response to IAA at ambient temperature. IAA at a concentration of 10^{-7} M induced an approximate 2-fold increase in *DWF4*-driven GUS activity. When *DWF4pro:*

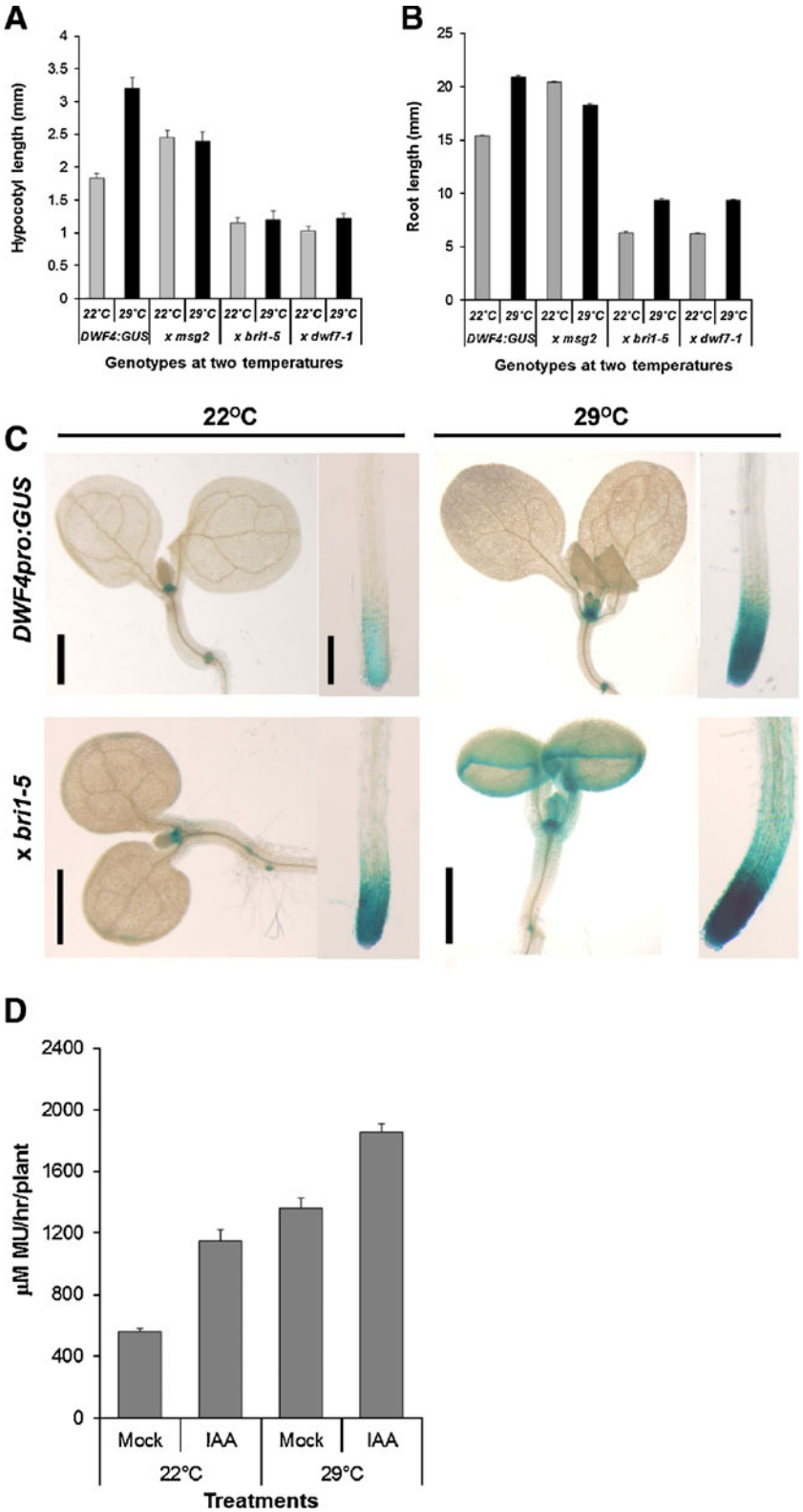

Fig. 1 Responses of *Arabidopsis* seedlings to high temperature. **a** Changes in hypocotyl length after growth for 7 days at the designated temperatures. The hypocotyl length of the *DWF4pro:GUS* control plants elongated by approximately 75% at 29°C relative to 22°C-grown seedlings. However, an auxin signaling mutant, *iaa19/msg2*; a BR biosynthetic mutant, *dwf7-1*; and a BR signaling mutant, *bri1-5*, failed to elongate at the elevated temperature. **b** Root response to high temperature. In contrast to the hypocotyl response, the *dwf7-1* and *bri1-5* genotypes elongated their roots when grown at high temperature, although the *iaa19/msg2* mutant did not show root elongation. **c** Histochemical GUS staining pattern of the 6-day-old *DWF4pro:GUS* and *bri1-5 DWF4pro:GUS* lines. The GUS staining was increased at high temperature in the shoot tip, root tip, and newly emerging leaf margins of the *DWF4pro:GUS* line. The GUS staining pattern was more obvious in the *bri1-5 DWF4pro:GUS* line; leaf blades, the upper parts of the hypocotyls, and root tips were densely stained due to lack of a feedback downregulation mechanism in this loss-of-function mutant for BR receptor. *Bar*=2 mm. **d** Quantitative analysis of the GUS response. At ambient temperature, IAA induced *DWF4*-driven GUS activity by approximately 2-fold. 29°C-grown seedlings exhibited a 2.4-fold increase in GUS activity relative to the 22°C-grown control. When seedlings were grown at 29°C in the presence of IAA, the activity was further increased to approximately 3.3-fold relative to the 22°C-grown seedlings

GUS seedlings were grown at 29°C, the GUS activity increased 2.4-fold relative to the 22°C-grown controls. This result suggests that the effects of a temperature of 29°C are almost the same as the effects of 10^{-7} M IAA. In order to investigate the effects of auxin at high temperature, seedlings were grown at 29°C in the presence of 10^{-7} M IAA. Under these conditions, the activity was further increased: approximately 3.3-fold relative to the 22°C-grown seed-

lings. This result implies that growth at 29°C was not saturated by auxin and seedlings have the capacity to respond to exogenously applied auxin.

In conclusion, it is likely that hypocotyl elongation in *Arabidopsis* at high temperature depends on BR biosynthesis, particularly through the expression of the key biosynthetic enzyme *DWF4*. Previously, it has been reported that a high temperature induces auxin biosynthesis and that hypocotyl elongation at high temperature is an auxin-specific response in *Arabidopsis* (Gray et al. 1998). Furthermore, we have shown that exogenous treatment with auxin induces *DWF4* expression and elevates the flux of BR biosynthetic pathways (Chung et al. 2011). Taken together with the findings of previous studies, our results strongly suggest that the temperature-dependent elongation of the hypocotyl requires functional BR biosynthesis and signal transduction pathways. We accordingly propose that the temperature-dependent increase in free auxin may have stimulated BR biosynthesis, and that the BRs thus synthesized are indeed responsible for the observed hypocotyl growth.

Materials and Methods

Plant Growth

The effects of elevated temperature on hypocotyl elongation and *DWF4* expression were determined. The lengths of the hypocotyls and roots were measured at 7 days after germination (Fig. 1a, b). In order to demonstrate the GUS histochemical patterns after treatment with high temperature, two sets of seedlings were prepared. Both sets of seedlings were initially grown at 22°C on 1×MS agar-solidified media. After 3 days, one set was transferred to 29°C and incubated at this temperature for a further 3 days before taking the photographs.

Histochemical GUS Assay

GUS staining was performed according to a standard method (Jefferson et al. 1987), with minor modifications. Briefly, seedlings were incubated at 37°C in GUS staining buffer [1 mM 5-bromo-4-chloro-3-indoyl-β-D-GlcUA, 100 mM sodium phosphate (pH 7), 0.5 mM potassium ferrocyanide, 0.5 mM potassium ferricyanide, 10 mM EDTA, and 0.1% (*v*/*v*) Triton X-100]. The chlorophyll in the tissue was removed by serial treatments with 50%, 70%, and 100% ethanol.

In Vivo GUS Assay

A quantitative in vivo GUS assay was performed according to a previously published method (Blazquez et al. 1998; Chung et al. 2011). A seedling was transferred to each well of a 96-well plate pre-filled with 100 μL of a substrate solution [50 mM sodium phosphate (pH 7), 10 mM β-mercaptoethanol, 10 mM EDTA, 0.1% (*w*/*v*) SDS, 0.1% (*w*/*v*) triton X-100, 2% isopropanol, and 440 mg/L 4-methylumbelliferyl β-D-glucuronide], and the plate was incubated at 37°C for 12 h. The reaction was terminated by adding 100 μL ice-cold stop buffer (0.2 M Na_2CO_3). Fluorescent products were quantified using a fluorescence spectrophotometer (Varian, USA) with the excitation and emission wavelengths set at 360 and 465 nm, respectively. A standard curve was obtained by using 4-methylumbelliferol solutions of known concentration.

Acknowledgments This research was supported in part by grants from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center No. PJ008051) and the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ906910), Rural Development Administration, Republic of Korea; the Technology Development Program (110033-5) for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea, and the Basic Science Research Program through the National Research Foundation of Korea (NRF) as funded by the Ministry of Education, Science and Technology (2010-0012736) (to SC); and the BK21 Research Fellowships funded by the Ministry of Education, Science, and Technology of the Korean Government (to PM) S.D.G.

References

Balasubramanian S, Sureshkumar S, Lempe J, Weigel D (2006) Potent induction of Arabidopsis thaliana flowering by elevated growth temperature. PLoS Genet 2:980–989

Blazquez MA, Ahn JH, Weigel D (2003) A thermosensory pathway controlling flowering time in Arabidopsis thaliana. Nat Genet 33:168–171

Blazquez MA, Green R, Nilsson O, Sussman MR, Weigel D (1998) Gibberellins promote flowering of Arabidopsis by activating the LEAFY promoter. Plant Cell 10:791–800

Chung Y, Maharjan PM, Lee O, Fujioka S, Jang S, Kim B, Takatsuto S, Tsujimoto M, Kim H, Cho S, Park T, Cho H, Hwang I, Choe S (2011) Auxin stimulates DWARF4 expression and brassinosteroid biosynthesis in Arabidopsis. Plant J 66:564–578

Clarke SM, Mur LA, Wood JE, Scott IM (2004) Salicylic acid dependent signaling promotes basal thermotolerance but is not essential for acquired thermotolerance in Arabidopsis thaliana. Plant J 38:432–447

Gendreau E, Traas J, Desnos T, Grandjean O, Caboche M, Hofte H (1997) Cellular basis of hypocotyl growth in Arabidopsis thaliana. Plant Physiol 114:295–305

Gray WM, Ostin A, Sandberg G, Romano CP, Estelle M (1998) High temperature promotes auxin-mediated hypocotyl elongation in Arabidopsis. Proc Natl Acad Sci USA 95:7197–7202

Halliday KJ, Whitelam GC (2003) Changes in photoperiod or temperature alter the functional relationships between phytochromes and reveal roles for phyD and phyE. Plant Physiol 131:1913–1920

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6:3901–3907

Kim HB, Kwon M, Ryu H, Fujioka S, Takatsuto S, Yoshida S, An CS, Lee I, Hwang I, Choe S (2006) The regulation of DWARF4

expression is likely a critical mechanism in maintaining the homeostasis of bioactive brassinosteroids in Arabidopsis. Plant Physiol 140:548–557

Koini MA, Alvey L, Allen T, Tilley CA, Harberd NP, Whitelam GC, Franklin KA (2009) High temperature-mediated adaptations in plant architecture require the bHLH transcription factor PIF4. Curr Biol 19:408–413

Maharjan PM, Schulz B, Choe S (2011) BIN2/DWF12 antagonistically transduces brassinosteroid and auxin signals in the roots of Arabidopsis. J Plant Biol 54:126–134

Nemhauser JL, Mockler TC, Chory J (2004) Interdependency of brassinosteroid and auxin signaling in Arabidopsis. PLoS Biol 2:1460–1471

Wang SY, Zheng W (2001) Effect of plant growth temperature on antioxidant capacity in strawberry. J Agr Food Chem 49:4977–4982

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu Rev Plant Biol 57:781–803