


SHORT COMMUNICATION



## Glycosyltransferase UGT76F1 is involved in the temperature-mediated petiole elongation and the BR-mediated hypocotyl growth in Arabidopsis

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### ABSTRACT

The signaling network formed by external environmental signals and endogenous hormone signals is an important basis for the adaptive growth of plants. We recently identified a UDP-glucosyltransferase gene, *UGT76F1*, which controls the glucosylation of auxin precursor IPyA and mediates light-temperature signaling to regulate auxin-dependent hypocotyl elongation in Arabidopsis. However, it is unclear whether *UGT76F1* is involved in the adaptive growth of other tissues and whether it is related to the signaling of other hormones besides auxin. Here we investigated the petiole elongation of *UGT76F1* overexpression lines and knockout mutant lines, and also studied the effects of *UGT76F1* on BR signaling. Experimental results indicated that *UGT76F1* is involved in the PIF4-mediated petiole growth under high temperature and that *UGT76F1* is also related to the BR signaling in controlling hypocotyl growth. These results suggest that *UGT76F1* may have a wider significance in the plant adaptations to surrounding environments.

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Glycosyltransferase; petiole elongation; BR signaling; growth regulation

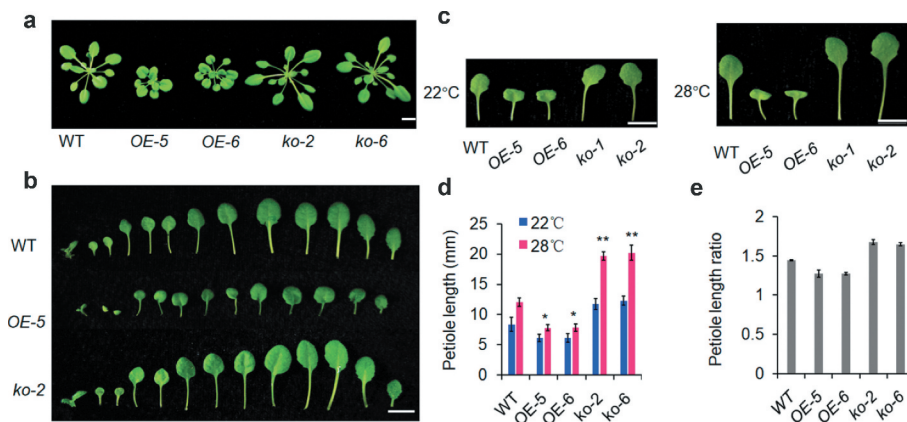
In the long evolutionary process of plants, the surrounding environmental factors provide motivation for the adaptive growth of plants under natural selection. When sensing external environments such as changing light signals and temperature signals, plants can make alterations in the growth and development in order to adapt to these changes. For example, exposure to high temperature will result in dramatic changes in Arabidopsis growths, including rapid extension of plant axes, leaf hyponasty, and early flowering.<sup>1,2</sup> For optimal growth and development, the perception and processing of surrounding environmental information are crucial to plant performance. Besides external environment, plant hormones play another key regulatory role in this process. It has been revealed that the coordinated regulation between ambient temperature and hormones, especially auxin, is important in the temperature-promoted hypocotyl elongation.<sup>1,3</sup> In addition, Phytochrome-interacting factor 4 (PIF4) is believed to be a crucial component of environmental signaling and integrate multiple hormone signals during plant development.<sup>4–8</sup> Thus, the signal network formed by the transmission of external environmental signals through endogenous hormone signals is the basis for the adaptive growth of plants.

We recently identified a UDP-glucosyltransferase gene, *UGT76F1*, which controls the glucosylation of auxin precursor IPyA and mediates light-temperature signaling to regulate auxin-dependent hypocotyl elongation in Arabidopsis.<sup>9</sup> However, it is unclear whether *UGT76F1* is involved in the adaptive growth of other tissues and whether it is related to the signalings of other hormones besides auxin. To answer these questions, we investigated the petiole elongation under 22°C and 28°C using *UGT76F1* overexpression lines and knockout

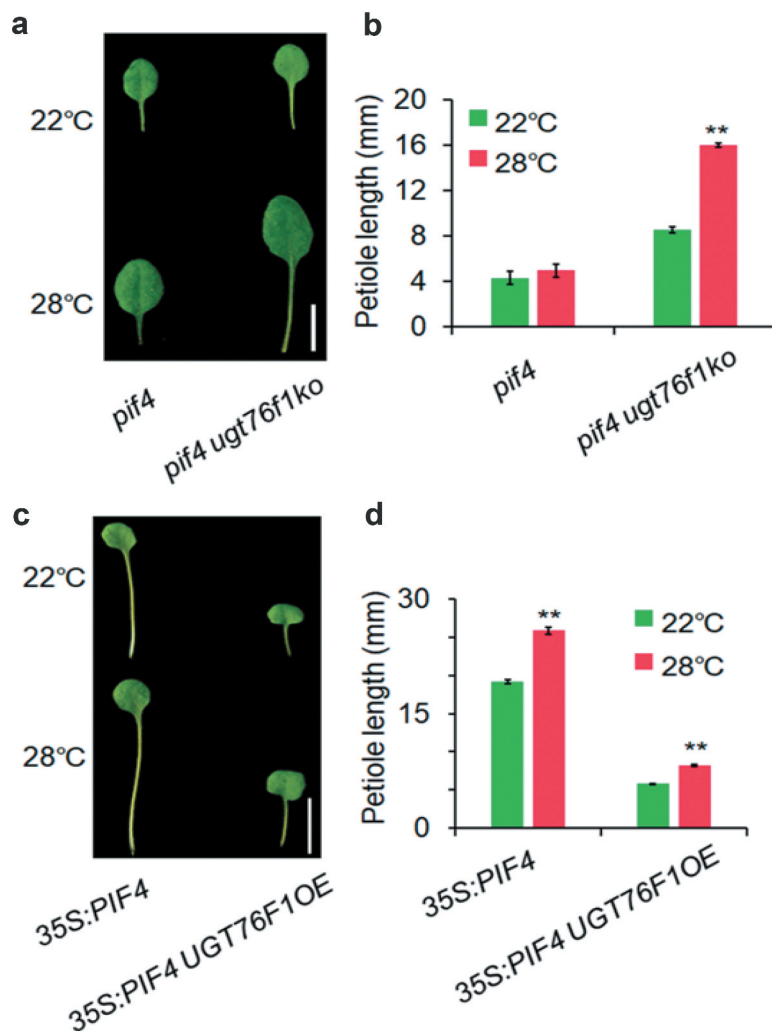
mutant lines. In addition, we also studied the effects of *UGT76F1* on BR signaling in controlling hypocotyl elongation.

After growing under 22°C for 4 weeks, *ugt76f1* knockout mutant (KO) lines displayed longer petioles whereas *UGT76F1* overexpression (OE) lines exhibited shorter petioles than WT (Figure 1a,b). Petiole extension of *UGT76F1* transgenic seedlings was further investigated at 28°C after 2 weeks grown at 22°C (Figure 1c). The petioles of *ugt76f1* mutant lines were markedly increased than that of WT at 28°C (Figure 1d,e), suggesting *ugt76f1* mutants were more sensitive to elevated temperature. However, the petiole elongation ratio of 28°C/22°C in *UGT76F1OE* lines was decreased than that of WT (Figure 1e). It is known that PIF4 acts as a negative regulator of *UGT76F1* transcription in high-temperature-induced hypocotyl elongation.<sup>9</sup> Here, we thus genetically analyzed the role of *UGT76F1* in PIF4-mediated and high-temperature-induced petiole elongation by comparing petiole length between *pif4* and *pif4ugt76f1ko* as well as between 35 S:*PIF4* and 35 S:*PIF4UGT76F1OE* lines. Intriguingly, while petiole growth response to high temperature was abolished in *pif4* mutants, the *pif4ugt76f1ko* double mutants complemented the petiole growth phenotypes of *pif4* (Figure 2a,b). Conversely, the 35 S:*PIF4UGT76F1OE* developed much shorter petioles than 35 S:*PIF4* under high temperature (Figure 2c,d). These observations suggest that *UGT76F1* genetically acts down-stream of PIF4 in high-temperature-induced petiole growth.

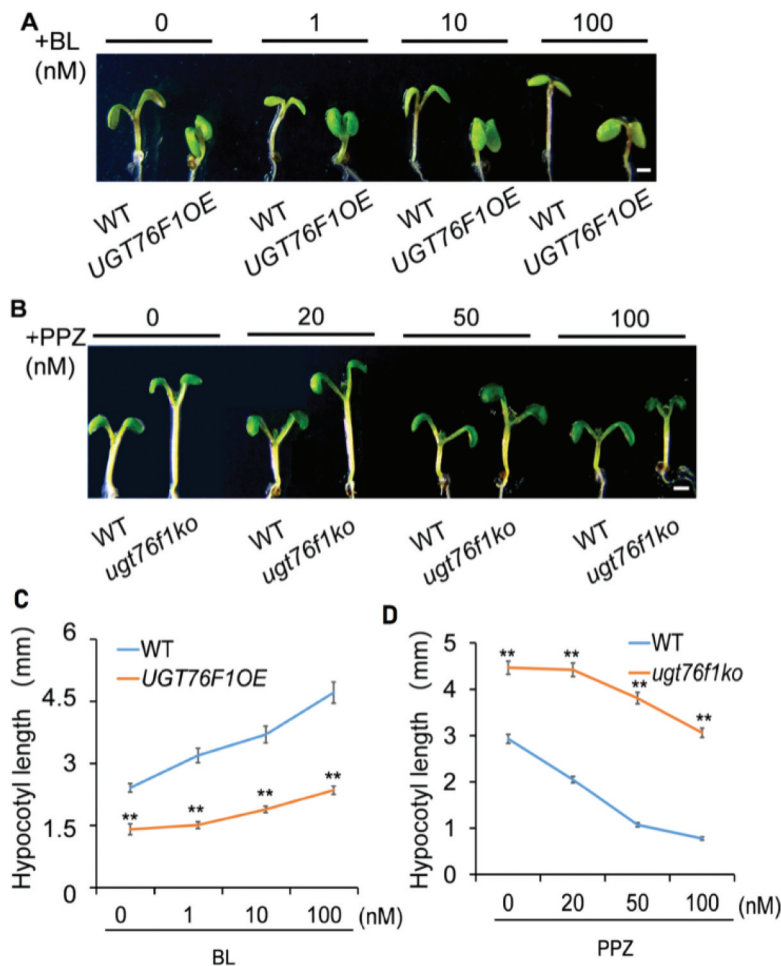
From our recent research, we know that *UGT76F1* regulates auxin-dependent hypocotyl elongation.<sup>9</sup> It has also been revealed in several studies that auxin, BR, and PIF4 interplay to control the elongation of hypocotyl cells.<sup>10–12</sup> Here, we



**Figure 1.** Temperature-mediated petiole growth in *UGT76F1* overexpression lines (OE) and mutant lines (KO) under 22°C and 28°C. (a-b) Petiole lengths of WT, *UGT76F1OE* lines, and *ugt76f1* mutants grown at 22°C. Four-week plants were photographed (Scale bar = 1 cm). (c) Petiole lengths of WT, *UGT76F1OE* lines and *ugt76f1* mutants grown at 22°C for 4 weeks or at 22°C for 2 weeks and then transferred to 28°C for another 2 weeks (Scale bar = 1 cm). (d) Petiole lengths of seedlings shown in (c). Data are means  $\pm$  SD,  $n = 30$ . Significant difference was compared to respective value at 22°C. Student's *t*-test was performed (\* $P < 0.05$ , \*\* $P < 0.01$ ). (e) Petiole length ratios (28°C/22°C) of the quantified petiole length in (d). Experiments were conducted for three biological replicates, yielding similar results.



**Figure 2.** *UGT76F1* is involved in *PIF4*-mediated and temperature-promoted petiole elongation. (a) Phenotypes of *pif4* and *pif4ugt76f1 ko* seedlings grown at 22°C for 4 weeks or at 22°C for 2 weeks and then transferred to 28°C for another 2 weeks (Scale bar = 1 cm). (b) Petiole lengths of seedlings shown in (a). Data are means  $\pm$  SD,  $n = 30$ . Significant difference was compared to respective value at 22°C. Student's *t*-test was performed (\*\* $P < 0.01$ ). (c) Phenotypes of 35S:*PIF4* and 35S:*PIF4UGT76F1OE* seedlings grown at 22°C for 4 weeks or at 22°C for 2 weeks and then transferred to 28°C for another 2 weeks (Scale bar = 1 cm). (d) Petiole lengths of seedlings shown in (c). Data are means  $\pm$  SD,  $n = 30$ . Significant difference was compared to respective value at 22°C. Student's *t*-test was performed (\*\* $P < 0.01$ ).



**Figure 3.** UGT76F1 affects BR-mediated hypocotyl elongation. (a and c) The *UGT76F1* overexpression lines reduced the sensitivity to BL. Seedlings were grown on media containing various concentrations of BL at 28°C for 4 d. Representative seedlings grown on either mock (0 nM BL) or BL (1–100 nM BL) media are shown in (a). (b and d) The *ugt76f1* mutant lines reduced the sensitivity to PPZ. Seedlings were grown either on mock (0 nM PPZ) or PPZ (20–100 nM PPZ) at 28°C for 4 d. Data are means  $\pm$  SD,  $n = 30$ . Significant difference was compared to respective WT. Student's *t*-test was performed (\*\* $P < 0.01$ ).

determined whether UGT76F1 is involved in BR signaling in hypocotyl elongation. *UGT76F1OE* lines and *ugt76f1* mutants were exposed to various concentrations of exogenous brassinolide (BL, the most active form of BR) or BR biosynthesis inhibitor propiconazole (PPZ) at 28°C for 4 d. We found that overexpression of *UGT76F1* reduced the sensitivity of seedlings to BR treatment in hypocotyl elongation compared to WT (Figure 3a,c). Likewise, the knockout of UGT76F1 reduced the sensitivity of seedlings to BR inhibitor in hypocotyl elongation (Figure 3b,d). These results suggest that UGT76F1 is also related to the BR signaling in controlling plant growth.

Previously, it has been shown that BR-regulated BZR1, auxin-regulated ARF6, and light/temperature-regulated PIF4, interact with each other and cooperatively regulate hypocotyl cell elongation.<sup>11</sup> Based on the observation in this study and our recent research,<sup>9</sup> we propose that UGT76F1 functions in the adaptive growth of plants to external environments likely downstream of the network regulation including auxin, BR, and PIF4. Since UGT76F1 is a glycosyltransferase toward auxin precursor IPyA, the glycosylation toward the metabolites of other plant hormones may also play important roles in the plant adaptations to surrounding environments.

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