



## Research article

Weisiensin B inhibits primary and lateral root development by interfering with polar auxin transport in *Arabidopsis thaliana*

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## ABSTRACT

Weisiensin B, a new *ent*-kaurene diterpenoid isolated from *Isodon weisiensis* (C. Y. Wu) H. Hara, exhibited phytotoxic effects on root growth and lateral root development in *Arabidopsis thaliana* seedlings. Primary root growth and lateral root formation in *A. thaliana* seedlings were significantly inhibited by 10–20  $\mu$ M weisiensin B. Additionally, the role of weisiensin B in response to polar auxin transport in *A. thaliana* roots was investigated using a *PIN* promoter (*PIN::GUS*), a green fluorescent protein (GFP) fusion protein reporter (*PIN::PIN::GFP*), and *DR5::GUS* and *DR5::GFP* reporter genes. The results indicated that weisiensin B reduced the expression of *PIN2*, *PIN3*, *PIN4*, *PIN7*, and *AUX1* genes and significantly decreased the abundance of PIN2-GFP, PIN3-GFP, PIN4-GFP, PIN7-GFP, and AUX1-GFP fusion proteins at their respective cellular locations, simultaneously causing auxin accumulation in the root apex. These results suggest that weisiensin B interferes with polar auxin transport in *A. thaliana* roots, resulting in auxin accumulation in the root meristematic cells and the inhibition of root growth and lateral root development.

## 1. Introduction

Plant secondary metabolites can affect ecosystem processes and structures, and their allelopathy is regarded as a natural strategy for protecting against environmental antagonists and competing plants (Prince and Pohnert, 2010). Allelopathy may play an important role in natural ecosystems by altering the equilibrium between competition and facilitation (Callaway and Walker, 1997). Secondary metabolites also are considered as a promising source of natural herbicides due to their different modes of action, structural diversity, and new target sites in comparison to the synthetic herbicides currently used in agricultural practices (Dayan and Duke, 2014).

To date, there are approximately 200,000 known secondary metabolites in plants (Templeton et al., 2005), in which terpenes are the largest group. A large number of terpenoids act as important allelochemicals in pollination, defense, and antifeedancy and also affect the growth of other plants in ecosystems (Bleeker et al., 2011; Himanen et al., 2010). Certain terpenoids have been identified as phytohormones that regulate plant growth and development, such as gibberellins (diterpene) (Urbanova et al., 2011), abscisic acid (sesquiterpene) (Kernode, 2005), and strigolactones (sesquiterpene) (Yoneyama et al., 2007). Numerous mono- and sesquiterpenes are proved to be highly active allelochemicals, and their modes of action have been

investigated (Araniti et al., 2017; Cantrell et al., 2007; Nishida et al., 2005). The monoterpenes camphor, thymol, geraniol, menthol, and 1,8-cineole were found to induce oxidative stress, thereby inhibiting root growth in *Zea mays* L. seedlings (Zunino and Zygadlo, 2004). Camphor,  $\alpha$ -pinene, and limonene influence the respiratory activity of the mitochondria and oxidative metabolism (Abraham et al., 2003, 2000). Citral alters auxin content, cell division, and cell ultra-structure in *Arabidopsis thaliana* seedlings (Graña et al., 2013). The sesquiterpene farnesene was found to cause significant tissue alterations, cellular damage, microtubule alterations, and hormonal imbalances in *A. thaliana* seedlings (Araniti et al., 2016). There are few studies concerning the phytotoxic mechanism of diterpenoids, although their role in allelopathy is supported (Macias et al., 2008; Moralesflores et al., 2007). A glycosylated diterpenoid helikauranoside A, isolated from *Helianthus annuus* L., is associated with allelopathic behavior (Macias et al., 2008). Xu et al. (2012) reported that rice diterpenoid momilactones directly mediate antagonistic plant–plant interactions, or allelopathy, by suppressing the growth of the widespread rice paddy weed, barnyard grass. Momilactone A and B may inhibit the germination of *A. thaliana* seeds by preventing the degradation of cruciferin and cruciferina proteins (Katonoguchi et al., 2013). *Ent*-kaurene diterpenoids (of which there are over 400) are the main secondary metabolites in the genus *Isodon* and are abundant in the leaves (Sun et al., 2006).

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These diterpenoids are released from fallen leaves into the environment where they interfere with neighboring plants. Our previous research demonstrated the potential phytotoxic effects of three *ent*-kaurane diterpenoids (leukamenin E, epinodosin, and rabdosin B) on primary root growth and root hair development in lettuce seedlings, offering a preliminary mechanism for this action—that is, that they affect both the cell length in the mature region and the division of meristematic cells by cell cycle arrest (Ding et al., 2010a; 2010b, 2008; Liu et al., 2015). In addition, these *ent*-kaurane diterpenoids also significantly affect lateral root development in *A. thaliana* (Cheng et al., 2017).

Previous studies have shown that plant secondary metabolites with different structures, such as coumarins, flavonoids, alkaloids, and terpenes, may alter the distribution of auxin (IAA) in plant tissues and organs, thereby affecting plant growth and development (Araniti et al., 2017; Cheng et al., 2017; Lupini et al., 2014; Hu et al., 2012, 2015; Na et al., 2011; Faulkner and Rubery, 1992; Jacobs and Rubery, 1988), suggesting that the interfering auxin pathway may be their critical mode of action. Auxin and its polar transport play significant roles in the regulation of plant growth and development, such as in embryonic axis formation, postembryonic organ formation, and the tropistic growth response (Jürgen et al., 2010; Kim et al., 2007; Sauer et al., 2006). Auxin directly mediates the elongation and division of root cells in plants (Teale et al., 2006). Hence, it is necessary that the roles of natural compounds in the regulation of the auxin pathway are determined. Weisiensin B, an *ent*-kaurene diterpenoid, was isolated as the most abundant compound from the leaves of *Isodon weisiensis* C. Y. Wu (Ding et al., 2005). The phytotoxic effects and modes of action of this diterpenoid are unknown. In this study, we investigated the effects of weisiensin B on several auxin-mediated physiological response processes in *A. thaliana*, including primary root growth and lateral root formation. The purpose of our experiments was: (1) to determine the phytotoxic effects of weisiensin B on primary and lateral root growth in *A. thaliana* seedlings, and (2) to investigate possible modes of action of the auxin pathway in response to weisiensin B.

## 2. Materials and methods

### 2.1. Purification of weisiensin B

Weisiensin B (Fig. 1) was isolated and purified from *I. weisiensis* as previously described.

### 2.2. Plant materials and growth conditions

The *A. thaliana* ecotype Columbia (Col-0) was used as the wild-type (WT). The mutants *pin1*, *pin2*, *pin3-4*, *pin4-3*, *pin7-2*, *pin2,3,4*, *pin3,4,7*, and *aux1-7*, and the transgenic lines *DR5::GUS*, *PIN1::GUS*, *PIN2::GUS*, *PIN3::GUS*, *PIN4::GUS*, *PIN7::GUS*, *AUX1::GUS*, *DR5::GFP*, *PIN1::PIN1-GFP*, *PIN2::PIN2-GFP*, *PIN3::PIN3-GFP*, *PIN4::PIN4-GFP*, *PIN7::PIN7-GFP*, and *AUX1::AUX1-YFP* used in the study had the Col-0 background.

The *A. thaliana* seeds were surface-sterilized in 2% NaClO for 5 min and extensively rinsed with sterilized water, following which they were placed on plates of half-strength Murashige and Skoog ( $1/2$  MS) agar

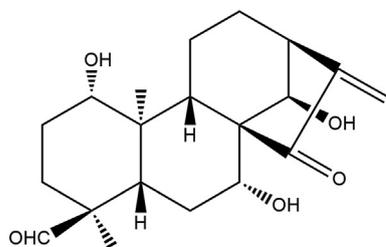


Fig. 1. Chemical structure of weisiensin B.

medium (pH 5.7) containing 1% (w/v) sucrose. The plates were kept at 4 °C for 2 days before being transferred to a growth chamber. The seedlings were maintained at 22 °C under a 16/8 h photoperiod (light intensity  $120 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for 3 days, following which they were transferred to agar medium containing weisiensin B.

### 2.3. Root phenotyping analysis

The 3-day-old WT seedlings and mutants grown on  $1/2$  MS agar medium were transferred to new medium containing the indicated concentration of weisiensin B or the polar auxin transport inhibitor *N*-1-naphthylphthalamic acid (NPA). After 7 days, the primary root length (PRL) was recorded using a Nikon digital camera D60 and analyzed by Image J software (<https://imagej.nih.gov/ij/>). The lateral root number (NLR) was counted under an optical microscope.

### 2.4. Fluorescence microscopy and confocal microscopy

Fluorescent sections were visualized using a Leica DM4000B fluorescent microscope and a Leica TCS SP5 laser scanning confocal microscope. Argon laser excitation at 488 nm was used for green fluorescent protein (GFP), while 514 nm was used for yellow fluorescent protein (YFP) fluorescence observation. All fluorescence images were obtained with the same parameter settings.

### 2.5. $\beta$ -Glucuronidase (GUS) staining

GUS assays were performed according to the method of Na et al. (2011) with slight modifications. The 7-day-old seedlings were immersed in GUS staining buffer containing phosphate-buffered saline, 10% Triton X-100, 100 mM  $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ , 100 mM  $\text{K}_3\text{Fe}(\text{CN})_6$ , and methanol. The GUS staining solution included 5-bromo-4-chloro-3-indoyl- $\beta$ -D-glucuronide (X-Gluc), GUS staining buffer, and dimethyl sulfoxide (DMSO). Tissues were incubated at 37 °C from 15 min to 3 h. The tissues were then fixed in 70% (v/v) ethanol for approximately 12 h. The representative seedlings were photographed with a Leica inverted microscope.

### 2.6. Statistical analysis

All data are presented as means  $\pm$  standard deviation (SD). Data were evaluated for normality using the Kolmogorov-Smirnov test, while homogeneity of variance was assessed with Levene's test. The data were then subjected to analysis of variance (ANOVA) with significant differences among means identified by least significant difference (LSD) multiple range tests using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Differences were considered significant at  $P \leq 0.05$ . All experiments were performed with at least three repetitions.

## 3. Results

### 3.1. Weisiensin B inhibits primary root growth and lateral root development

To elucidate the effects of weisiensin B on the postembryonic development of *A. thaliana*, 3-day-old WT *A. thaliana* seedlings were grown on medium containing different concentrations of weisiensin B for 7 days. The results showed that the roots of the seedlings responded to weisiensin B in an inhibitory manner. PRL and NLR were significantly decreased compared with the control after treatment with weisiensin B (Fig. 2A, B and C). When the seedlings were respectively treated with 10  $\mu\text{M}$  and 20  $\mu\text{M}$  weisiensin B, the PRL remained at about 84.5% and 80.1% of the control, whereas the NLR was approximately 80.2% and 73.0% of the control. The effects of NPA on the primary and lateral roots were similar to that of weisiensin B (Fig. 2B and C), being significantly inhibited (78.2% and 70% of the control, respectively) at 0.3  $\mu\text{M}$ . In addition, weisiensin B treatment showed a slight decrease in

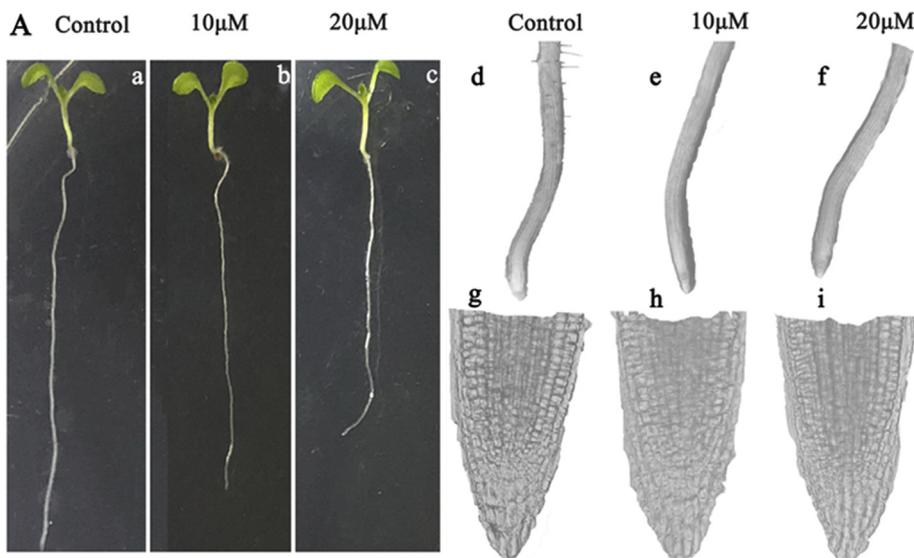
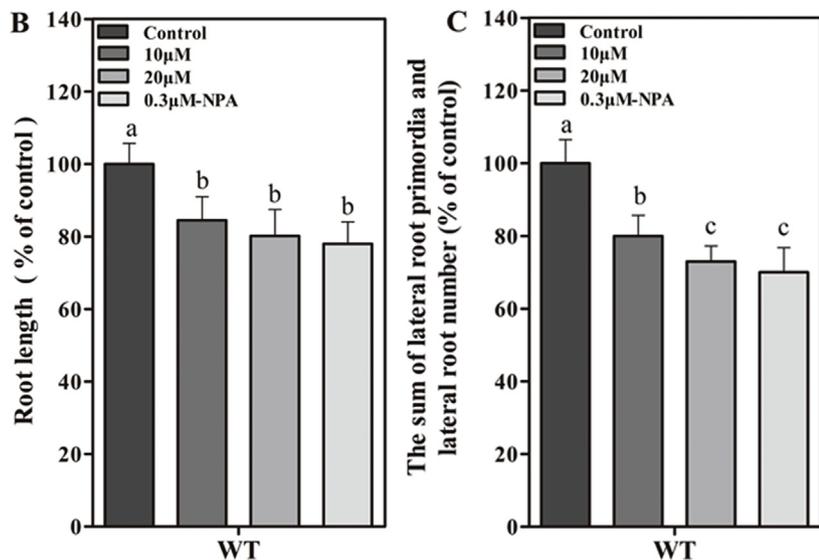


Fig. 2. Effect of weisiensin B or NPA on root growth in *A. thaliana* seedlings. (A) *Arabidopsis* seedlings grown on agar medium untreated (a) and treated with 10  $\mu$ M (b) or 20  $\mu$ M (c) weisiensin B. Morphology of *Arabidopsis* root tips untreated (d, g) and treated with 10  $\mu$ M (e, h) or 20  $\mu$ M (f, i) weisiensin B. (B) Inhibition of weisiensin B or NPA on the length of the primary roots in *A. thaliana* seedlings 7 days after treatment. (C) Inhibition of weisiensin B or NPA on the sum of the lateral root primordia and lateral root number in *A. thaliana* seedlings 7 days after treatment. Different letters indicate significant values ( $P \leq 0.05$ ). WT: wild-type *A. thaliana*.



the number of root hair (Fig. 2A d–f). However, 10  $\mu$ M and 20  $\mu$ M weisiensin B had no significant impact on the morphological characteristics of the root apex (Fig. 2A a–i), as well as on hypocotyl and cotyledon growth in the seedlings (Fig. 2A a–c).

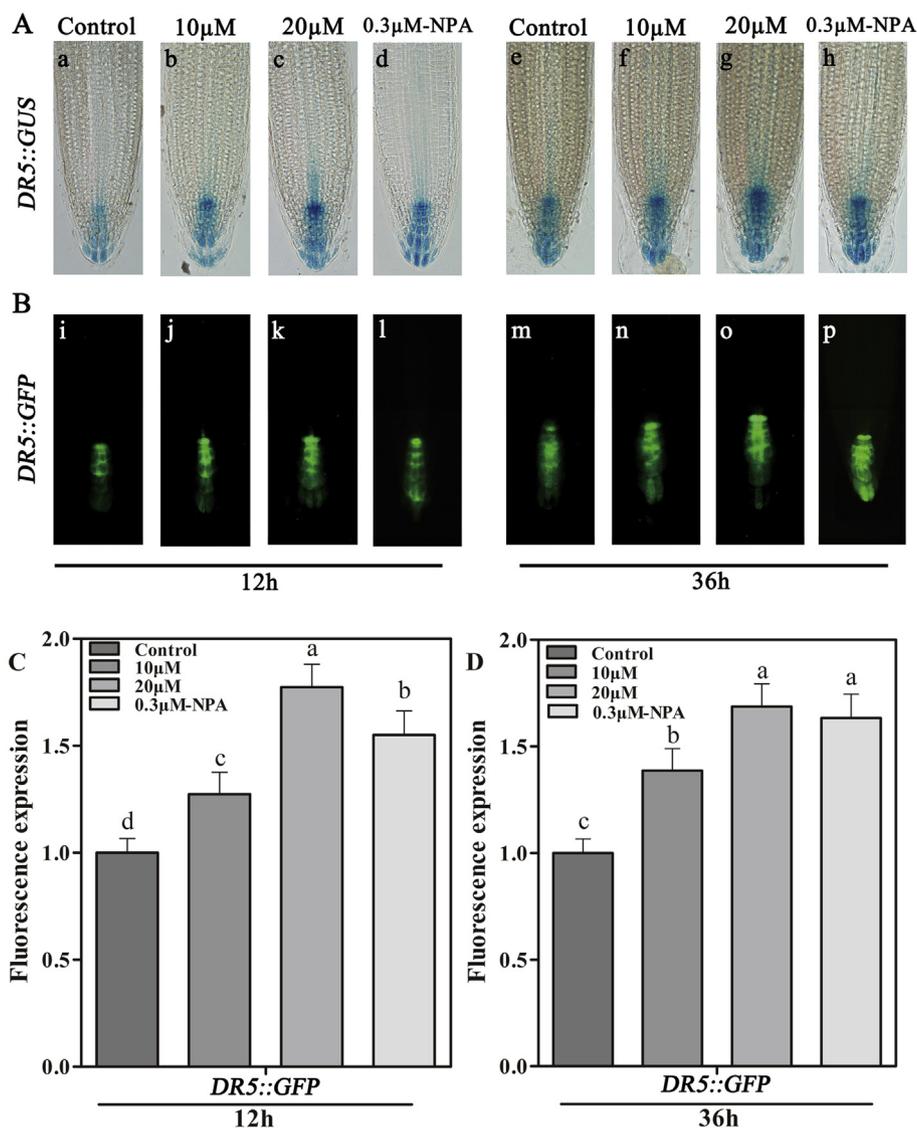
### 3.2. Weisiensin B affects the distribution of auxin in *A. thaliana* root tips

Auxin transport within the root apical tissues is reportedly involved in the regulation of root meristem activity and the development of the lateral roots (Marchant et al., 2002; Sabatini et al., 1999). The inhibition of primary root and lateral root growth by weisiensin B indicated that weisiensin B might influence the auxin distribution in the roots of the *A. thaliana* seedlings. Three-day-old *DR5::GUS* seedlings and *DR5::GFP* seedlings were transferred to medium with or without weisiensin B and NPA for 12 and 36 h. DR5 promoter activity was detected in the roots of the seedlings. Fig. 3 shows a dose-dependent increase in DR5 activity in the columella cells, quiescent center, and its surrounding cells, indicating that the content and distribution of auxin in the root tips were altered by weisiensin B. Weisiensin B treatment increased DR5:GFP fluorescence expression by 1.3–1.8 times in the root tips (Fig. 3B, i–l and C). In addition, the DR5 activity following NPA treatment showed a similar alteration with weisiensin B treatment in the root tips of the *A. thaliana* seedlings (Fig. 3A B and C).

### 3.3. Weisiensin B inhibits *A. thaliana* root development by disturbing polar auxin transport

#### 3.3.1. The responses of auxin carrier mutants to weisiensin B

Based on the changes in auxin distribution induced by weisiensin B in the root tips, we speculated that polar auxin transport may be involved in the response of *A. thaliana* roots to weisiensin B. Polar auxin transport is mediated by auxin influx carriers from the AUX/LAX protein family and by auxin efflux proteins from the PIN family and the ABCB/PGP family (Kerr and Bennett, 2007; Wiśniewska et al., 2006). Thus, we examined the effects of weisiensin B on primary root growth and lateral root formation in seven auxin carrier protein mutants (*aux1-7*, *pin1*, *pin2*, *pin3*, *pin4*, *pin2,3,4* and *pin3,4,7*) with impaired auxin transport (Kerr and Bennett, 2007; Wiśniewska et al., 2006; Muller et al., 1998). As shown in Fig. 4, the *A. thaliana* auxin carrier mutants responded differently to weisiensin B. The mutants *aux1-7*, *pin2,3,4* and *pin3,4,7* showed a lower level of sensitivity to weisiensin B in terms of the inhibition of root elongation and lateral root formation compared with the WT seedlings, demonstrating that the auxin carriers *aux1*, *pin2*, *pin3*, *pin4*, and *pin7* may be involved in the effect of weisiensin B on primary root and lateral root development. However, root elongation and lateral root formation in the single mutants *pin1*, *pin2*, *pin3*, *pin4*, and *pin7* in response to weisiensin B was similar to the WT seedlings,



**Fig. 3.** Weisiensin B altered the content and distribution of auxin in the root tips. (A) Increase in *DR5::GUS* activity in a dose-dependent manner in weisiensin B-treated roots. (B) Increase in *DR5::GFP* expression in a dose-dependent manner in the weisiensin B-treated roots. (C) and (D) Relative GFP expression of plants treated as in B. The plants were treated with weisiensin B (10 and 20 µM) or NPA (0.3 µM). ( $P \leq 0.05$ ).

suggesting that the mutants did not contribute to the inhibition of weisiensin B on primary root elongation and lateral root development. The differential response to weisiensin B from the single mutants (*pin1*, *pin2*, *pin3*, *pin4*, and *pin7*) and triple mutants (*pin2,3,4* and *pin3,4,7*) is attributed to gene compensation in the single mutant seedlings.

### 3.3.2. Weisiensin B modulates the expression of auxin transport genes in *A. thaliana* roots

In order to evaluate the experimental results from the auxin carrier protein mutant, we examined the effects of weisiensin B on the expression of *PIN* and *AUX* genes in *A. thaliana* roots by visualizing the *PIN::GUS* reporter. The inhibitory effect of weisiensin B on the seedling roots was observed at 24–36 h, and thus *PIN::GUS* activity was detected at 12 and 36 h after the *PIN::GUS* seedlings were transferred to weisiensin B-containing medium. As shown in Fig. 5, *PIN2*, *PIN3*, *PIN4*, *PIN7*, and *AUX1* promoter activity was obviously reduced in the *PIN3::GUS*, *PIN4::GUS*, *PIN7::GUS*, and *AUX1::GUS* seedling roots following weisiensin B treatment, while the *PIN1* reporter was slightly stimulated in *PIN1::GUS*. The above results indicated that the expression of auxin transport carriers *PIN1*, *PIN2*, *PIN3*, *PIN4*, *PIN7*, and *AUX1* at the transcriptional level was altered by weisiensin B.

### 3.3.3. Weisiensin B alters the cellular localization or abundance of auxin transport carriers in *A. thaliana* roots

The *A. thaliana* lines *PINs::PINs-GFP/AUX1::AUX1-YFP* provide a useful tool for studying the expression of *A. thaliana* auxin transport carriers at the protein level. To test whether weisiensin B affects the localization or abundance of auxin transport proteins in *A. thaliana* roots, six fusion protein lines (*PIN1::PIN1-GFP*, *PIN2::PIN2-GFP*, *PIN3::PIN3-GFP*, *PIN4::PIN4-GFP*, *PIN7::PIN7-GFP*, and *AUX1::AUX1-YFP*) were used to monitor the changes in auxin carriers in the weisiensin B-treated seedlings. As shown in Fig. 6, the localization of *PIN1*, *PIN2*, *PIN3*, *PIN4*, *PIN7*, and *AUX1* in the control seedlings was consistent with previous reports (Araniti et al., 2017; Giehl and Joni, 2012; Swarup et al., 2001), but the distribution or abundance of six fusion proteins in the weisiensin B-treated root tips differed. *PIN1* is generally located at the bottom of the stelar cells and endodermal cells in *A. thaliana* roots. The abundance of *PIN1* exhibited a slight increase in the weisiensin B-treated roots of the *PIN1::PIN1-GFP* seedlings at 12 and 36 h (Fig. 6A and B, a1–a3). The *PIN2* protein, which is located at the cortex, epidermis, and lateral root cap, was dissociated from the plasma membrane and was observed in the cytoplasm after treatment with weisiensin B for 12 or 36 h (Fig. 6A and B, b1–b3), although the

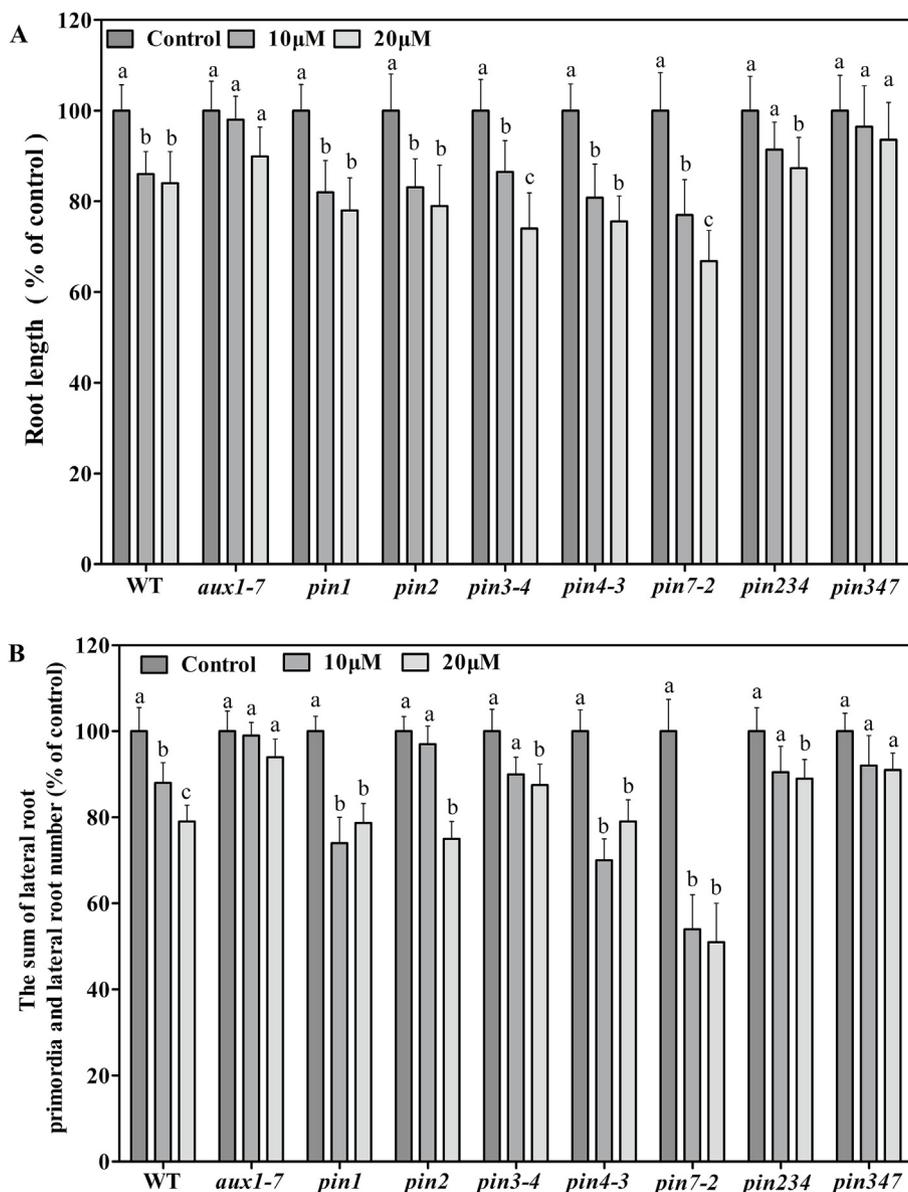


Fig. 4. Effects of weisiensin B on the growth of primary and lateral roots in WT and mutant seedlings. (A) Length of the primary root of the WT and mutant seedlings following weisiensin B treatment. (B) The sum of lateral root primordia and lateral root number of the WT and mutant seedlings following weisiensin B treatment. WT: wild-type *A. thaliana*. ( $P \leq 0.05$ ).

fluorescence intensity was increased in the cytoplasm at 36 h. PIN3 and PIN7 are distributed in the stelar cells and columella cells. The expression of PIN3 and PIN7 was strongly decreased in the columella cells and appeared to be confined to a part of the columella cells as a result of the weisiensin B treatment for 12 or 36 h (Fig. 6A and B, c1–c3 and e1–e3). The abundance of AUX1 was also reduced in the epidermis and lateral root cap cells following weisiensin B treatment for 12 or 36 h (Fig. 6A and B, f1–f3). However, the abundance of PIN4, which basally localizes in the provascular cells and the quiescent center and surrounding cells (Fig. 6A and B, d1–d3), was almost unaffected at 12 h after weisiensin B treatment, but was decreased after 36 h of treatment.

#### 4. Discussion

Secondary metabolites are known to be involved in plant growth and development and may influence the regulation of plant hormones in the stress response by altering auxin synthesis and auxin distribution (Cheng and Cheng, 2015; Vanneste and Friml, 2009). Numerous phenolic compounds, terpenes, and alkaloids have been investigated in

plant allelopathy. Coumarins are highly active allelochemicals that play a key role in plant–plant interactions and communication. They are often found in the roots of higher plants and specifically inhibit seed germination and root growth (Svensson, 1971). Coumarin may modulate auxin distribution through influx or/and efflux proteins (Lupini et al., 2014). 4-Methylumbelliferone (4-MU), a coumarin derivative, was found to regulate lateral root formation by altering auxin redistribution rather than biosynthesis in *A. thaliana* roots (Li et al., 2011). Similarly, narciclasine, an alkaloid isolated from *Narcissus tazetta*, affects plant growth and development by modulating auxin transport gene expression and inhibiting auxin signaling (Hu et al., 2012, 2015; Na et al., 2011). Terpenes are the largest group of secondary metabolites in plants. Numerous terpenoids have been identified as allelochemicals, and their modes of action have been investigated. However, there are few reports on terpenoid-mediated auxin pathways (Araniti et al., 2017; Macias et al., 2008; Cantrell et al., 2007; Moralesflores et al., 2007; Nishida et al., 2005).

Some terpenoids simultaneously display stimulatory and inhibitory effects on seedling growth (Fischer et al., 1994). Our previous

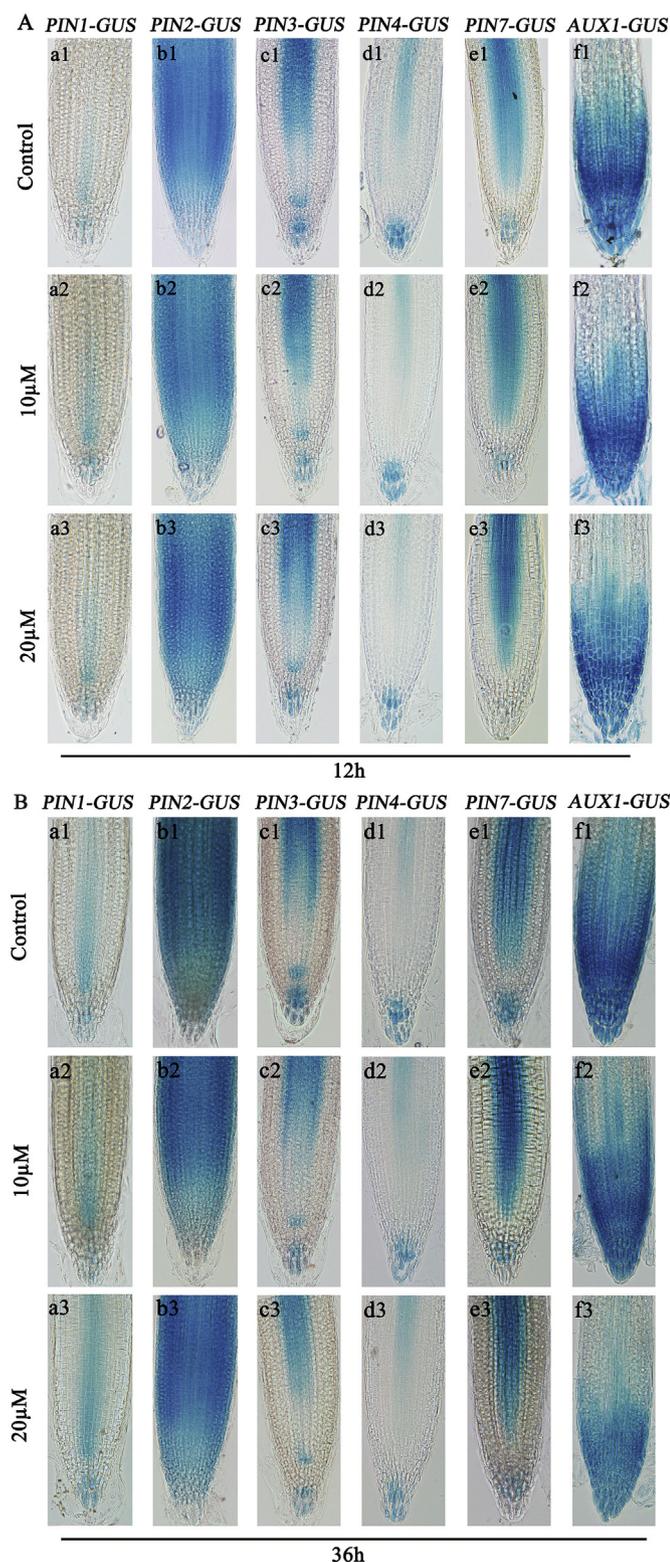


Fig. 5. Weisiensin B modulates the expression of *PIN* and *AUX* genes in *A. thaliana* roots. (A) The expression of *PIN1::GUS*, *PIN2::GUS*, *PIN3::GUS*, *PIN4::GUS*, *PIN7::GUS*, and *AUX1::GUS* in *A. thaliana* roots treated with weisiensin B for 12 h. (B) The expression of *PIN1::GUS*, *PIN2::GUS*, *PIN3::GUS*, *PIN4::GUS*, *PIN7::GUS*, and *AUX1::GUS* in *A. thaliana* roots with weisiensin B for 36 h.

experiments demonstrated that the *ent*-kaurane diterpenoids rabdosin B and epinodosin, purified from *Isodon*, exhibited a biphasic effect (stimulatory and inhibitory) on root growth in lettuce seedlings. The

promoting effect of the two diterpenoids resulted from increased cell length in the mature region and the mitotic index of the meristematic cells in the root tips, and their inhibition was attributed to a reduction in both cell length in the mature region and the division of meristematic cells (Ding et al., 2010a; 2010b). Leukamenin E, an *ent*-kaurene diterpenoid isolated from *Isodon racemosus* (Hemsl) Hara, showed similar inhibitory effects as rabdosin B and epinodosin on the root growth of lettuce and *A. thaliana* seedlings, but no stimulatory effect was observed (Ding et al., 2008; Cheng et al., 2017). Leukamenin E markedly increased the indoleacetic acid (IAA) levels in *A. thaliana* roots, indicating that auxin may play an important role in the regulation of root growth by leukamenin E (Cheng et al., 2017).

In recent years, the model plant *A. thaliana* and its various mutants and transgenic lines have been increasingly used to investigate the effects of natural compounds on plant growth in order to reveal the complex phytohormone pathways mediated by natural compounds. In this study, we evaluated the potential allelopathy of weisiensin B by testing its effects on the root development of WT *Arabidopsis* seedlings. The experimental data indicated that weisiensin B inhibited primary root growth and decreased the number of lateral roots and root hairs. Furthermore, we observed the effects of weisiensin B on the root growth of *A. thaliana* mutants deficient in polar auxin transport carriers in order to explore the correlation between the inhibitory effect of weisiensin B and polar auxin transport. The results showed that the single mutant *aux1-7* and triple mutants *pin2,3,4*, *pin3,4,7* showed a weaker response to weisiensin B compared with the WT seedlings, suggesting that weisiensin B might mediate the physiological process of polar auxin transport. Weisiensin B reduced the promoter activity of the *PIN2*, *PIN3*, *PIN4*, *PIN7*, and *AUX1* genes in the seedling roots of *A. thaliana* and significantly decreased the abundance of *PIN2*, *PIN3*, *PIN4*, *PIN7*, and *AUX1* at their respective locations, simultaneously causing auxin accumulation in the root apex. Auxins are key hormonal signals that control the cellular architecture of the primary roots and the initiation of new lateral root organs in *A. thaliana* (Laskowski et al., 1995). Auxin transport promotes lateral root initiation in *A. thaliana*. Root basipetal and leaf acropetal auxin transport activities are required during the initiation and emergence phases, respectively, of lateral root development (Casimiro et al., 2001). Thus, these results suggested that interference with the expression and cellular location of polar auxin transport proteins may lead to the inhibition of primary root growth and lateral root formation by weisiensin B.

In this work, the auxin transport inhibitor NPA inhibited primary root growth and arrested lateral root development (Fig. 2B and C) and also caused auxin accumulation in the root apex (Fig. 3 A and B). Casimiro et al. (2001) concluded that NPA appears to block basipetal IAA movement from the root tips, thereby causing IAA accumulation in the root apex and concurrently reducing the levels in the basal tissues critical for lateral root initiation (Casimiro et al., 2001). Similarly, the significant inhibition of weisiensin B on the expression of four PIN proteins (*PIN2*, *PIN3*, *PIN4*, and *PIN7*) and *AUX1* may block basipetal auxin transport activities, resulting in IAA accumulation in the root apex and a reduction in IAA levels in the basal tissues critical for lateral root initiation. Auxin is actively transported from cell-to-cell via efflux transporters, known as PIN proteins, from the shoot to the root cap, where it accumulates to form a local maximum and a spatial gradient along the apical-basal axis of the root. Its localization in the root apex promotes cell proliferation and sustains the size of the division zone (Perrot-Rechenmann, 2010). However, excess auxin in the root apex reduces the total length of the roots by reducing that of the elongation zone (Beemster and Baskin, 2000). Thus, we conclude that weisiensin B influenced PIN polar localization and PIN protein abundance, resulting in the accumulation of IAA in the root tips and the inhibition of primary root growth.

Moreover, auxin plays a pivotal role in root hair development (Pitts et al., 1998). Auxin imbalance has been found to lead to developmental defects in the root apex in *A. thaliana*, including a root hair phenotype

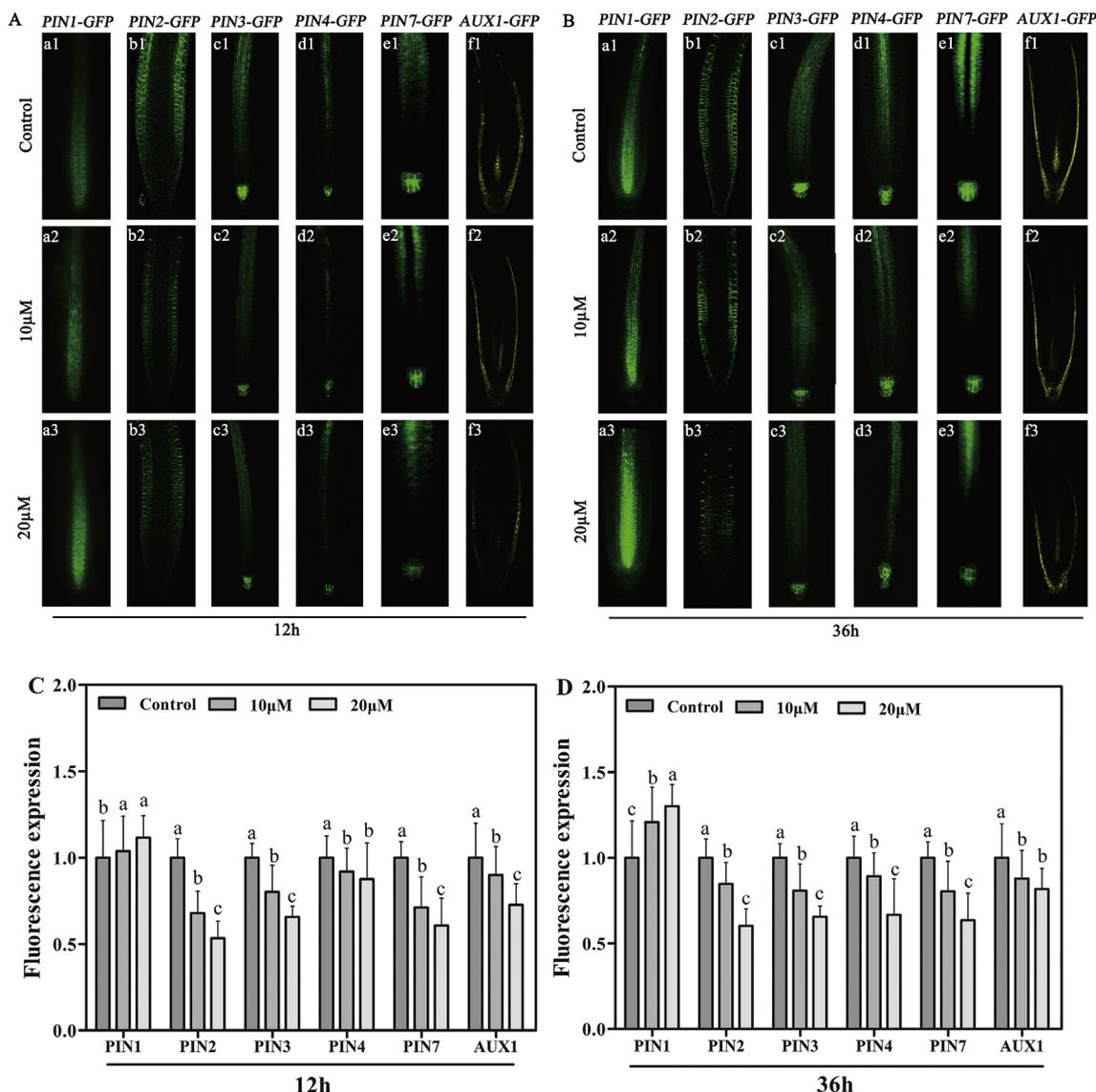


Fig. 6. Weisiensin B alters the localization or abundance of auxin transport carriers in *A. thaliana* roots. (A) and (B) *PIN1*-GFP, *PIN2*-GFP, *PIN3*-GFP, *PIN4*-GFP, *PIN7*-GFP, and *AUX1*-YFP in the roots of *PIN1::PIN1*-GFP, *PIN2::PIN2*-GFP, *PIN3::PIN3*-GFP, *PIN4::PIN4*-GFP, *PIN7::PIN7*-GFP, and *AUX1::AUX1*-YFP seedlings treated without or with weisiensin B for 12 and 36 h. (C) and (D) Relative fluorescence expression of each PIN and treatment with weisiensin B (10 and 20 μM) or without weisiensin B ( $P \leq 0.05$ ).

(Stamatis et al., 2013). Our experimental results suggest that the inhibitory effect of weisiensin B on root hair formation may be attributed to an imbalance in auxin distribution.

**Conflicts of interest**

The authors have no conflicts of interest to declare.

**Authors' contributions**

Lan Ding, Peng Li designed the research. Li Zhang, Peng Li conducted the experiments and analyzed the data. Jing He, Zhaowei Huan conducted the experiments. Lan Ding, Peng Li wrote the text.

Lan Ding: supervised, reviewed, and approved the final version of the manuscript.

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