



The effect of auxin and strigolactone on *ATP/ADP isopentenyltransferase* expression and the regulation of apical dominance in peach

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Abstract

Key message We confirmed the roles of auxin, CK, and strigolactones in apical dominance in peach and established a model of plant hormonal control of apical dominance in peach.

Abstract Auxin, cytokinin, and strigolactone play important roles in apical dominance. In this study, we analyzed the effect of auxin and strigolactone on the expression of *ATP/ADP isopentenyltransferase (IPT)* genes (key cytokinin biosynthesis genes) and the regulation of apical dominance in peach. After decapitation, the expression levels of *PpIPT1*, *PpIPT3*, and *PpIPT5a* in nodal stems sharply increased. This observation is consistent with the changes in tZ-type and iP-type cytokinin levels in nodal stems and axillary buds observed after treatment; these changes are required to promote the outgrowth of axillary buds in peach. These results suggest that *ATP/ADP PpIPT* genes in nodal stems are key genes for cytokinin biosynthesis, as they promote the outgrowth of axillary buds. We also found that auxin and strigolactone inhibited the outgrowth of axillary buds. After decapitation, IAA treatment inhibited the expression of *ATP/ADP PpIPTs* in nodal stems to impede the increase in cytokinin levels. By contrast, after GR24 (GR24 strigolactone) treatment, the expression of *ATP/ADP IPT* genes and cytokinin levels still increased markedly, but the rate of increase in gene expression was markedly lower than that observed after decapitation in the absence of IAA (indole-3-acetic acid) treatment. In addition, GR24 inhibited basipetal auxin transport at the nodes (by limiting the expression of *PpPIN1a* in nodal stems), thereby inhibiting *ATP/ADP PpIPT* expression in nodal stems. Therefore, strigolactone inhibits the outgrowth of axillary buds in peach only when terminal buds are present.

Keywords Apical dominance · Cytokinin · Auxin · Strigolactone · Isopentenyltransferase · Peach

Abbreviations

GR24	(3a <i>R</i> *,8b <i>S</i> *, <i>E</i>)-3-(((<i>R</i> *)-4-methyl-5-oxo-2,5-dihydrofuran-2-yloxy)methylene)-3,3a,4,8b-tetrahydro-2 <i>H</i> -indeno[1,2- <i>b</i>]furan-2-one, a synthetic strigolactone
iP	Isopentenyladenine
iPR	iP riboside
IAA	Indole-3-acetic acid
SL	Strigolactone

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PpIPT	Peach gene adenosine phosphate isopentenyltransferase
tZ	Trans-zeatin
tZR	Trans-zeatin riboside

Introduction

In many plants, the growing shoot apex inhibits the outgrowth of axillary buds. This phenomenon is referred to as apical dominance. Decapitation leads to the release of dormant axillary buds below the growing shoot apex to enable branch formation (Cline 1991; Leyser 2003). Apical dominance allows plants to focus resources into the main axis of growth, while activation of dormant buds allows for recovery after damage or loss of the main shoot (Muller and Leyser 2011). Early studies have showed that auxin and cytokinins play antagonistic roles in regulating shoot branching. More recently, SLs, a newly identified group of plant hormones, were shown to play a pivotal role in inhibiting shoot branching.

Auxin, which is derived from the shoot apex, inhibits the growth of axillary buds, while cytokinin, thought to be derived from roots (recent studies have revealed the importance of local cytokinin biosynthesis) (Ongaro and Leyser 2008; Muller and Leyser 2011; Yang et al. 2017), and promotes the growth of axillary buds (Cline 1991; Leyser 2003; Shimizu-Sato and Mori 2001; Tanaka et al. 2006; Liu et al. 2011). These observations have been confirmed in many plant species. There is widespread agreement that the outgrowth of axillary buds depends on the ratio of these two hormones rather than their absolute levels (Emery et al. 1998; Shimizu-Sato and Mori 2001; Wang et al. 2006; Liu et al. 2011). Applying auxin to decapitated stumps at the shoot apex can replace the role of the growing apex in restoring branch inhibition (Thimann and Skoog 1933; Cline 1991), and externally applied auxin inhibits the outgrowth of tiller buds (Harrison and Kaufman 1982). By contrast, externally applied cytokinins stimulate tiller bud growth, even in the presence of an intact shoot apex (Langer et al. 1973; Panigrahi and Audus 1966; Sachs and Thimann 1964, 1967; Miguel et al. 1998). Moreover, the concentrations of cytokinins in axillary buds increase significantly after decapitation (Bangerth 1994; Turnbull et al. 1997; Emery et al. 1998). The outgrowth of axillary buds correlates well with cytokinin levels in the axillary buds. Studies of transgenic plants have clearly demonstrated that auxin and cytokinins are involved in apical dominance (Ward and Leyser 2004; Zubko et al. 2002; Medford et al. 1989; Romano et al. 1991). In addition, SLs, a group of sesquiterpene lactones derived from carotenoids (or a derivative thereof) were recently shown to play a pivotal role in inhibiting shoot branching (Ongaro and Leyser 2008; Muller and Leyser 2011). This

observation has been confirmed in many studies of branching mutants (Umehara et al. 2008). These studies have mainly focused on the separate functions of these hormones. However, elucidating the relationships and links between auxin, cytokinins, and SLs is highly important for elucidating the molecular mechanisms underlying apical dominance.

Since cytokinin is the key factor promoting the outgrowth of axillary buds, it is important to examine the effects of auxin and SL on cytokinin synthesis. ATP/ADP IPT (isopentenyltransferase, IPT) is the key enzyme involved in cytokinin de novo biosynthesis as well as an important rate-limiting enzyme (Blackwell and Horgan 1994; Takei et al. 2001). Most studies have shown that *ATP/ADP IPT* genes play a key role in regulating cytokinin levels (Golovko et al. 2002; Tanaka et al. 2006; Sakano et al. 2004; Takei et al. 2002). Experiments in *Pisum sativum* have revealed that the cytokinin biosynthetic genes *PsIPT1* and *PsIPT2* are differentially expressed in the nodal regions of stems before and after decapitation (Tanaka et al. 2006), suggesting that auxin controls local cytokinin biosynthesis in the nodal stems under apical dominance in pea. The relationship between cytokinin and SL remains unclear. Currently, there are two main hypotheses about the molecular mechanism underlying the role of SLs. One hypothesis is that SL acts as a second messenger for auxin action and that this messenger directly suppresses bud outgrowth (Beveridge et al. 1997, 2000, 2006; Brewer and Beveridge 2009; Hayward et al. 2009). The other hypothesis is that SL controls shoot branching by regulating basipetal auxin transport (Bennett et al. 2006; Mouchel and Leyser 2007; Ongaro and Leyser 2008). Studies employing different species have produced different results. Although the main points of these two hypotheses differ, they both suggest that the role of SL is related to auxin. Overall, our knowledge about the effect of auxin and SL on the expression of *ATP/ADP IPTs* in the control of shoots branching remains both unclear and controversial.

In this study, we utilized the economically important fruit tree peach (*Prunus persica*). Peach is the third most important temperate fruit crop according to its cultivated surface area. Additionally, peach is a member of the economically important Rosaceae family, which also includes important crops such as apples, pears, cherries, plums, apricots, strawberries, almonds, and roses (Immanen et al. 2013). Peach is a model plant for the family Rosaceae due to its small genome size (~230 Mb). The genome sequence for peach was released to the Genome Database for Rosaceae by the International Peach Genome Initiative on April 1, 2010 (<http://www.rosaceae.org/peach/genome>) (Arús et al. 2012; Verde et al. 2013). However, no previous study has examined apical dominance in Peach. A better understanding of the relationship between auxin, SL, and cytokinin in peach is important for elucidating the growth characteristics of this crop and devising strategies for yield improvement.

In this study, to better understand the molecular mechanisms of plant hormonal control of apical dominance in peach, we focused on the expression of the key cytokinin biosynthesis genes *ATP/ADP IPTs*. The goal of this study was to analyze the effect of auxin and SL on *ATP/ADP IPT* gene expression to uncover the relationship and possible links between auxin, SL, and cytokinin in the control of shoot branching in peach.

Materials and methods

Experimental site and plant material

The experimental site is located in the Hongmiao peach-planting base in Tai'an Shandong province, China, at an altitude of 150 m. The exact location coordinates are E 117°08', N 36°11'. This area has a warm temperate continental monsoon climate. The average annual temperature is 12.8 °C, the highest temperature is 40 °C, the extreme minimum temperature is −22 °C, and the annual precipitation is 600–800 mm. The study was conducted in May and June 2014. The experimental material was 2-year-old yellow peach trees (*P. persica*) planted at a spacing of 2 m × 5 m. Current-year shoots from these peach trees, which have about 20 nodes and 50–60 cm, were employed. The shoot cuttings used in this study were also isolated from these peach trees. The isolated shoots were cultured in an incubator at 25 °C with humidity > 70% under a 16 h light (360 μmol/m²/s) photoperiod. Different treatments were applied to these shoots: decapitation (shoots were decapitated 1 cm above the axillary bud, the fourth or fifth axillary bud below the apical bud was selected), IAA (indole-3-acetic acid, sigma) treatment [applying 1% (w/v) IAA in lanolin at the top after decapitation], Lovastatin treatment [applying 1% (w/v) Lovastatin in lanolin at both the top and the axillary bud after decapitation], Lovastatin + cytokinins treatment [applying 1% (w/v) Lovastatin together with 1% (w/v) tZ in lanolin at both the top and the axillary bud after decapitation], GR24 (Beijing Daqin Science and Technology Co., Ltd.) treatment with decapitation [applying 10 μM, GR24 (a synthetic SL) in lanolin at both the top and the axillary bud after decapitation] (Akiyama et al. 2005), GR24 treatment without decapitation (applying 10 μM GR24 to axillary buds), and the control (normally grown shoots without decapitation; there was no difference in the gene expression between the lanolin treatment and the control, so only one control was shown in this article.). For treatments without decapitation the length of the tenth axillary bud below the apical bud was measured, and for treatments with decapitation the length of the bud at the same position was measured. Same treatments were also repeated using isolated shoots cultured in the incubator. Treatments were carried out at

8 am. The shoots were observed daily and sampled at 1, 2, 3, 5, 7, 9, 12, 24, 72, and 240 h after treatment. Nodal stem segments (1 cm long) and axillary buds were sampled. The samples used for RNA isolation and cDNA synthesis were frozen immediately in liquid nitrogen and stored at −80 °C for subsequent use.

Gene isolation

IPT, *PIN* (encoding key auxin transport protein), and *CCD7/8* (encoding plastidic carotenoids cleavage dioxygenase, key gene in SL biosynthesis) in peach were identified by searching the *P. persica* genome sequence, version 1, using databases available via The Genome Portal of the Department of Energy Joint Genome Institute (http://genome.jgipsf.org/Poptr1_1/Poptr1_1.home.html) and Phytozome portal (http://www.phytozome.net/search.php?org=Org_Ptrichocarpa_v3.0; http://www.phytozome.net/search.php?method=Org_Persica) (Song et al. 2012). According to the *P. persica* genome sequence and the predicted gene transcripts, we directly found and then verified that there are 4 members in *ATP/ADP PpIPT* gene family, 6 members in *PpPIN* gene family, 1 *PpCCD7* gene, and 1 *PpCCD8* gene. Their transcript IDs and phylogenetic trees can be found in Additional files: Table S1, Figs. S1, S2, and S3.

RNA isolation and cDNA synthesis

Total RNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. RNase-free DNase I (TaKaRa, Dalian, China) was added to remove any remaining genomic DNA. RNA yield and purity were checked using a NanoDrop 2000 spectrophotometer (Thermo, USA), and RNA integrity was verified by electrophoresis on 1.5% agarose gels. First-strand cDNA was synthesized from total RNA using an oligo (dT) primer and the PrimeScript RT reagent kit (TaKaRa) according to the manufacturer's instructions.

Quantitative reverse-transcription PCR

Quantitative RT-PCR (qRT-PCT) amplifications were performed in triplicate using the gene-specific primers shown in Table 1. The 25 μL PCR reactions contained 100 ng of cDNA, 10 pmol of each primer, and 12.5 μL of 2 × SYBR Green PCR Premix ExTaq (TaKaRa) according to the manufacturer's instructions. PCR was carried out on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, USA) under the following conditions: 30 s at 95 °C, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s. The Actin gene was used for normalization of qRT-PCR data. Relative gene expression was calculated using the comparative 2^{−ΔΔC_T} method (Livak and Schmittgen

Table 1 Sequences of peach gene-specific oligonucleotide primers used for qRT-PCR

Gene ID	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')
<i>PpIPT1</i>	GGCATTTCGTCGGGCTATT	CCATCTTTGAACAGAAACTCCTTT
<i>PpIPT3</i>	GGCAAGGAATCCGATGAA	GTACAGAAACTGACGAACAATC
<i>PpIPT5a</i>	AAGAGGATGGTTGGTAACTTC	GGCATAGATGTGGCAAGAA
<i>PpIPT5b</i>	AAGAGGTGGTCAGAGATATG	CTACTCCTCCAAGAAGCG
<i>PpPIN1a</i>	CCACTCCTCTCCTTCCACTTCATCTC	CCAGACAGCAAGCACAGCAAGAA
<i>PpPIN1b</i>	GCCCAATGCGAAGAAGAAACCCAAT	CGTTGCCATACTCTCCACCTCTGAA
<i>PpPIN3</i>	GGTACACTCTCCTCTCTTCTCTTC	CCAATCTCGGGCTCGGTCTC
<i>PpPIN5</i>	GCTATCGTGTGGCTGACAATTCTTCT	CTACTGCTGCTCATCGTGCTCAT
<i>PpPIN6</i>	CGCCGAGCCATAACGAAGT	TGAGGAGCCAGGGAGTGATGAC
<i>PpPIN8</i>	TATTGTTTCTTTGAGTTCAGGGCTGCTAA	CTTCTTGTTTCATCCTCTGCTTCTCTTTG
<i>PpCCD7</i>	CAATCTCCACACCCAACGAAACCAAT	AACCGAACCATCCACGACTCTGA
<i>PpCCD8</i>	GCGAGTGTGGAAGTGCCATTATACATAAC	CGTACCCTCCCATCCTCATCT

2001). The real-time quantitative PCR results are shown as the means \pm SD of three independent biological replicates.

Measurement of endogenous cytokinins

Extraction and purification of trans-Zeatin (tZ), trans-Zeatin riboside (tZR), isopentenyladenine (iP), and iP riboside (iPA) was performed as described by Dobrev and Kaminek (Dobrev and Kaminek 2002). Determination of tZ, tZR, iP, and iPA levels was performed by HPLC-MS as described by Pilkington et al. (2013). Analyses were carried out on a UHPLC-MS system consisting of a DIONEX Ultimate 3000 high-performance liquid chromatography (HPLC) coupled to a Thermo TSQ Quantum Access Max Mass Spectrometer (Thermo Scientific, USA). The injection volume was 10 μ L for each sample. Electrospray ionization (ESI) analysis was performed in positive modes. The capillary spray voltage was 3 kV in ESI-positive mode; sheath gas flow-rate was 30 L/min; auxiliary gas flow-rate was 2 L/min; capillary temperature was 300 °C. The peak area quantified using the Thermo XCalibur software version 2.1.0.

Measurement of endogenous auxin

The extraction and purification of IAA were performed using high performance liquid chromatography–electrospray–mass spectrometry (HPLC–ESI–MS/MS) described by Bacaicoa et al. (2011).

Results

Exploration of the role of auxin, cytokinin, and strigolactone on the outgrowth of axillary buds in peach

To determine the roles of auxin, cytokinin, and SLs in apical dominance in peach, we first research the outgrowth of axillary buds after various treatments using decapitation, IAA or GR24. According to our experimental results, after 10 days of treatment, the lengths of axillary buds under decapitation treatment were markedly greater than those of the control (Fig. 1; Table 2), and the levels of the tZ, tZR, iP and iPR were markedly higher in both nodal stems and axillary buds after decapitation (Table 3). The outgrowth of axillary buds was markedly inhibited by Lovastatin, an inhibitor of cytokinin synthesis, after decapitation of the shoot apex (Laureys et al. 1998) (Fig. 1; Table 2). And exogenous tZ can eliminate this inhibitory effect (Fig. 1). Therefore, the presence of cytokinins is a necessary condition for promoting the outgrowth of axillary buds. The outgrowth of axillary buds was also markedly inhibited by auxin after decapitation (Fig. 1; Table 2). SL did not inhibit the outgrowth of axillary buds after decapitation, but it markedly inhibited the outgrowth of axillary buds in the absence of decapitation (Fig. 1; Tables 2, 4). SL plays an inhibitory role relies on the presence of an intact shoot apex, it means that SL's function may be associated with auxin. To further investigate the site of cytokinin synthesis, whether it comes from the root, we repeated this experiment using shoot cuttings. The results show that the lengths of axillary buds were markedly higher in shoot cuttings after decapitation (Fig. 1; Table 5).

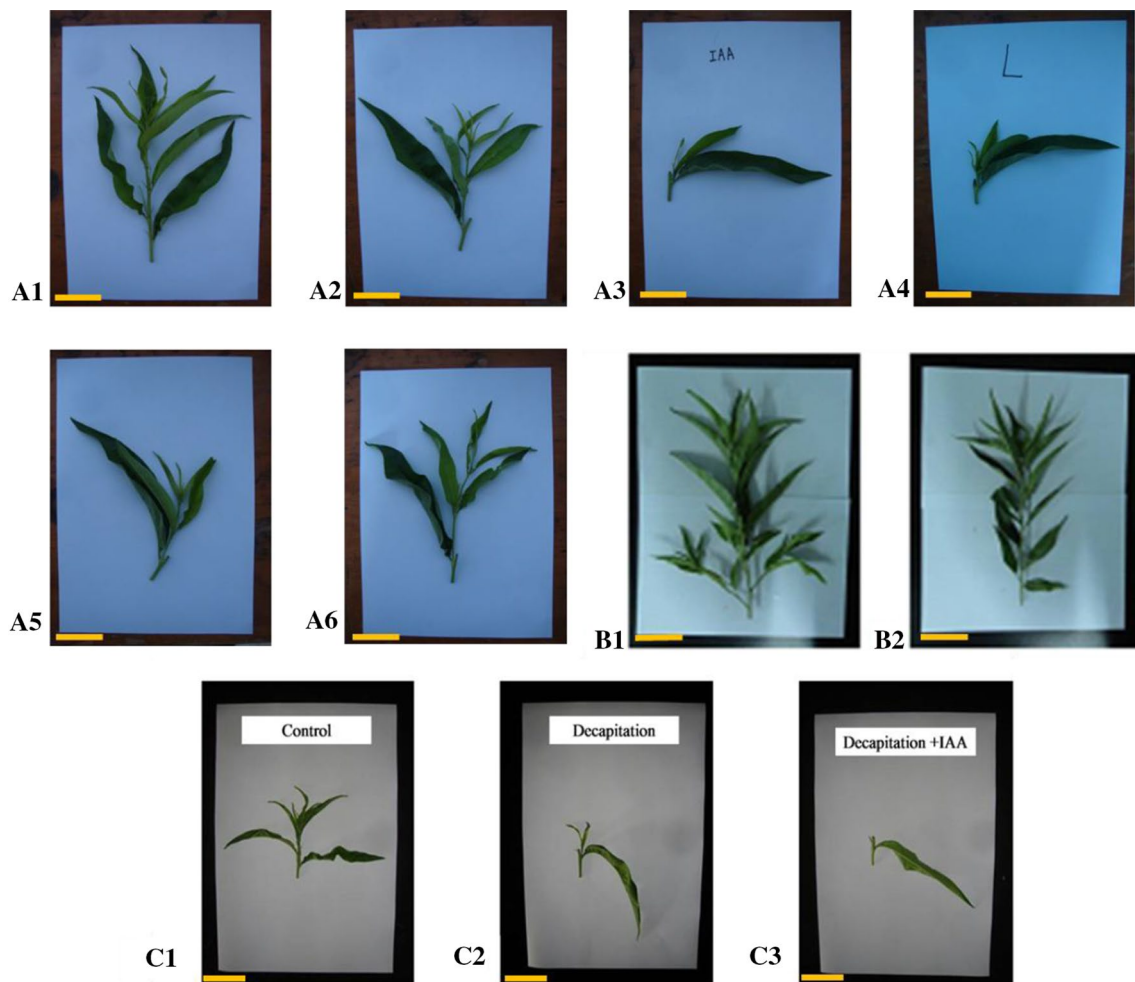


Fig. 1 The outgrowth of axillary buds after various treatments. Representative photographs taken 10 days after different treatments: **a1** control, **a2** decapitation, **a3** IAA treatment, **a4** Lovastatin treatment, **a5** GR24 treatment with decapitation, **a6** Lovastatin + tZ treatment with decapitation, **b1** control, **b2** GR24 treatment without decapita-

tion. **c** Shoot cuttings were used, representative photographs taken 3 days after different treatments: **c1** control, **c2** decapitation, **c3** IAA treatment. Each treatment contains three biological replicates and each replicate consisted of 30 inoculated shoots. Scale bars 5 cm

Table 2 Length of the bud (mm) below the cut after various treatments with decapitation

Treatments	Time after treatments (days)	
	0	10
Control	4.75 ± 1.12	5.73 ± 1.04a
Decapitation		107.67 ± 7.20d
Decapitation + IAA		22.93 ± 5.38b
Decapitation + GR24		98.39 ± 4.80c
Decapitation + Lovastatin		29.10 ± 3.84b

Measured on day 0 and 10 following various treatments. The average of three biological replicates is shown ± SD, and each replicate consisted of 30 inoculated shoots. Mean values with the same letters are not significantly different ($P < 0.05$)

Identification of ATP/ADP PpIPT genes expressed in the stem after decapitation

To determine whether decapitation affected the expression of ATP/ADP PpIPTs in nodes and axillary buds, we examined the expression patterns of ATP/ADP PpIPTs in the nodal stems and axillary buds before and after decapitation (Fig. 2). After decapitation, the expression levels of PpIPT1, PpIPT3, and PpIPT5a in nodal stems increased markedly. The expression levels of PpIPT1 and PpIPT5a reached a maximum level by 5 h after decapitation. In contrast to the nodal stems results, the expression of the ATP/ADP PpIPT genes in the axillary buds located at the nodal stems did not markedly change, or was even reduced, after decapitation (Fig. 2).

Table 3 Cytokinin contents of nodal stems and axillary buds after various treatments

Cytokinins (pmol/g FW)	Treatments	Stem			Axillary bud		
		0 h	3 h	6 h	0 h	3 h	6 h
tZ	Control	0.29 ± 0.06	0.32 ± 0.04a	0.29 ± 0.04a	0.48 ± 0.12	0.55 ± 0.09a	0.51 ± 0.10a
	Decapitation		0.59 ± 0.10b	1.51 ± 0.18b		1.42 ± 0.33b	6.15 ± 1.17c
	Decapitation + IAA		0.31 ± 0.03a	0.27 ± 0.03a		0.49 ± 0.07a	0.39 ± 0.09b
	Decapitation + SL		0.56 ± 0.08b	1.38 ± 0.15b		1.38 ± 0.36b	5.37 ± 1.10c
tZR	Control	0.40 ± 0.05	0.38 ± 0.08a	0.39 ± 0.09a	0.52 ± 0.09	0.54 ± 0.08a	0.47 ± 0.09a
	Decapitation		2.37 ± 0.46b	9.71 ± 1.04b		5.59 ± 1.14b	17.06 ± 2.85b
	Decapitation + IAA		0.42 ± 0.06a	0.34 ± 0.02a		0.49 ± 0.08a	0.45 ± 0.05a
	Decapitation + SL		2.39 ± 0.48b	7.81 ± 1.28c		5.19 ± 0.97b	13.89 ± 2.35c
iP	Control	0.31 ± 0.04	0.28 ± 0.02a	0.29 ± 0.04a	0.94 ± 0.16	1.02 ± 0.09a	0.92 ± 0.14a
	Decapitation		0.89 ± 0.11c	1.46 ± 0.21b		3.11 ± 1.00b	6.58 ± 1.09b
	Decapitation + IAA		0.33 ± 0.03b	0.27 ± 0.04a		0.93 ± 0.09a	0.81 ± 0.12a
	Decapitation + SL		0.84 ± 0.10c	1.26 ± 0.22b		3.21 ± 0.36b	4.87 ± 0.54c
iPR	Control	0.81 ± 0.15	0.70 ± 0.09a	0.74 ± 0.09a	1.28 ± 0.25	1.32 ± 0.33a	1.41 ± 0.42a
	Decapitation		5.30 ± 0.89b	15.82 ± 2.47b		19.27 ± 3.17b	39.11 ± 6.26b
	Decapitation + IAA		0.76 ± 0.11a	0.79 ± 0.10a		1.35 ± 0.45a	1.19 ± 0.20a
	Decapitation + SL		5.49 ± 0.97b	12.3 ± 2.01c		18.11 ± 2.89b	29.99 ± 5.19c

The cytokinin contents were analyzed as described in the “Materials and methods”. Mean value and SD were calculated from three replicate samples. Lower case letters a, b and c represent mean values that are significantly different between different treatment by Duncan’s test ($P < 0.05$, $n = 3$)

Table 4 Length of the tenth axillary bud (mm) below the apical bud after various treatments without decapitation

Treatments	Time after treatments (days)	
	0	10
Control	11.98 ± 1.91	72.37 ± 6.78b
GR24		17.62 ± 2.47a

Measured on day 0 and 10 following various treatments. The average of three biological replicates is shown ± SD, and each replicate consisted of 30 inoculated shoots. Mean values with the same letters are not significantly different ($P < 0.05$)

Table 5 Length of the bud (mm) below the cut after various treatments with decapitation in cutting shoots

Treatments	Time after treatments (days)	
	0	3
Control	4.17 ± 1.21	4.84 ± 0.39a
Decapitation		30.33 ± 2.58b
Decapitation + IAA		4.69 ± 0.42a

Measured on day 0 and 3 following various treatments. The average of three biological replicates is shown ± SD, and each replicate consisted of 30 inoculated shoots. Mean values with the same letters are not significantly different ($P < 0.05$)

Effect of auxin and strigolactone on ATP/ADP PpIPT gene expression in the nodal stems

To examine whether the external application of auxin and SL affected the expression of ATP/ADP PpIPT in nodal stems, we examined the expression levels of PpIPT1, PpIPT3, and PpIPT5a, which were upregulated as a result of decapitation. The results indicate that the application of IAA inhibited the promotive effect of decapitation on the expression of these three genes in nodal stems (Fig. 3); this result is consistent with the cytokinin levels observed at 3 h and 6 h after treatment (Table 3).

By contrast, after SL treatment, the expression of these three genes in nodal stems still increased markedly (Fig. 2). However, the rate of increase in the expression of these genes was markedly lower than that observed after decapitation in the absence of SL treatment. Indeed, the expression levels of PpIPT1 and PpIPT5a did not reach their maximum levels by 5 h after treatment, instead continuing to increase at 9 h after treatment. The rate of increase in cytokinins levels was also reduced. SL does not directly inhibit the expression of ATP/ADP PpIPTs in the nodal stems. The outgrowth of axillary buds shows that SL plays an inhibitory role relies on the presence of an intact shoot apex. Previous studies have shown that the function of SL is closely related to basipetal auxin

Fig. 2 The expression of *ATP/ADP PpIPT* genes after decapitation and GR24 treatment. The expression levels of *PpIPT1*, *PpIPT3* and *PpIPT5a* in nodal stems (**a1–a3**) or axillary buds (**b1–b3**) were determined after decapitation. Values are the means of relative mRNA levels (in fold changes) detected using qRT-PCR, with at least three technical replicates for each of three biological replicates. Error bars represent the SD calculated for the combined technical and biological replicates. The β -actin gene was used as an internal control. Each treatment contains three biological replicates and each replicate consisted of 30 inoculated shoots at each time point. Shoots were decapitated 1 cm above the axillary bud, and the fourth or fifth axillary bud below the apical bud was selected. For GR24 treatment, 10 μ M GR24 in lanolin was applied at both the top and the axillary bud after decapitation. Intact shoots as Control

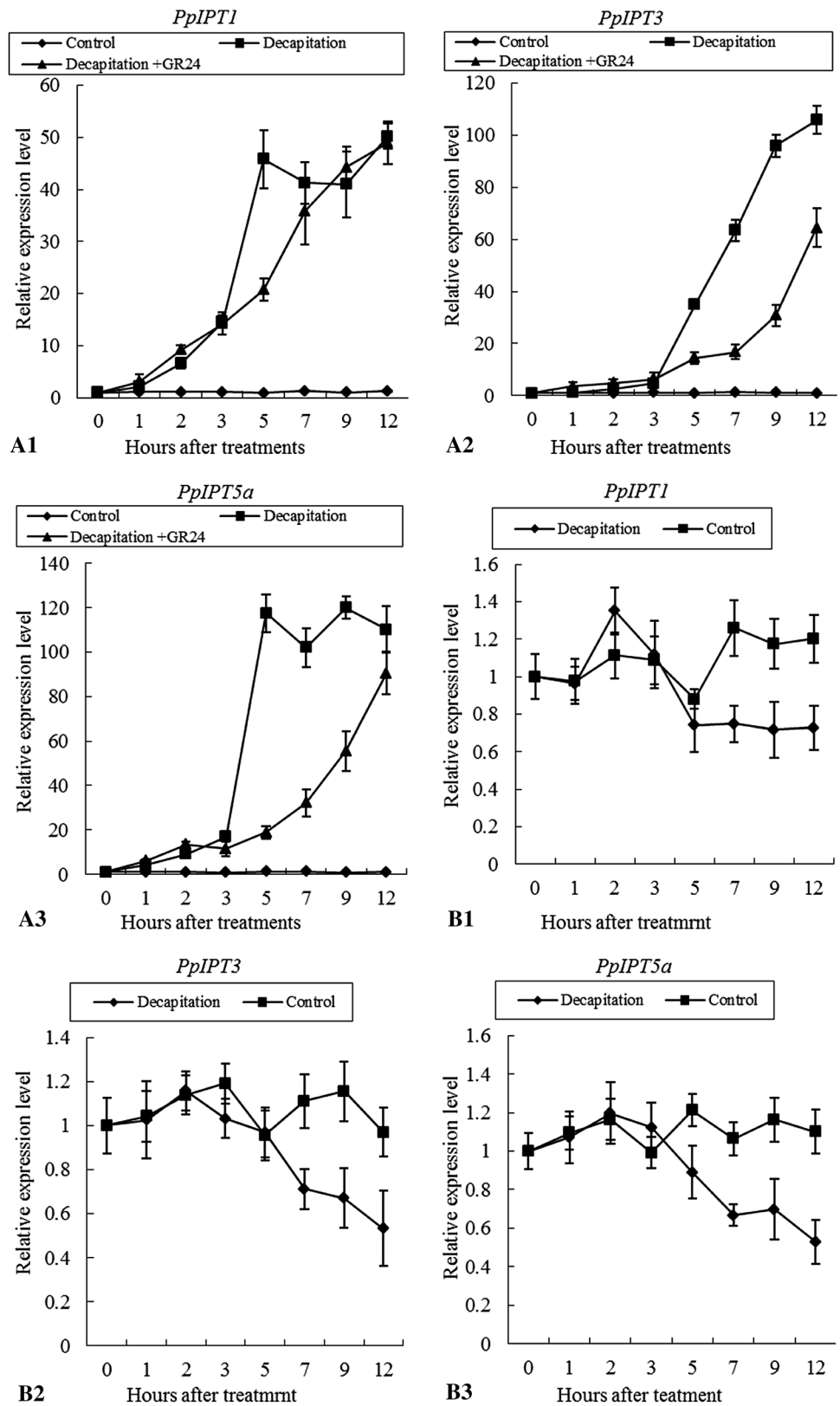
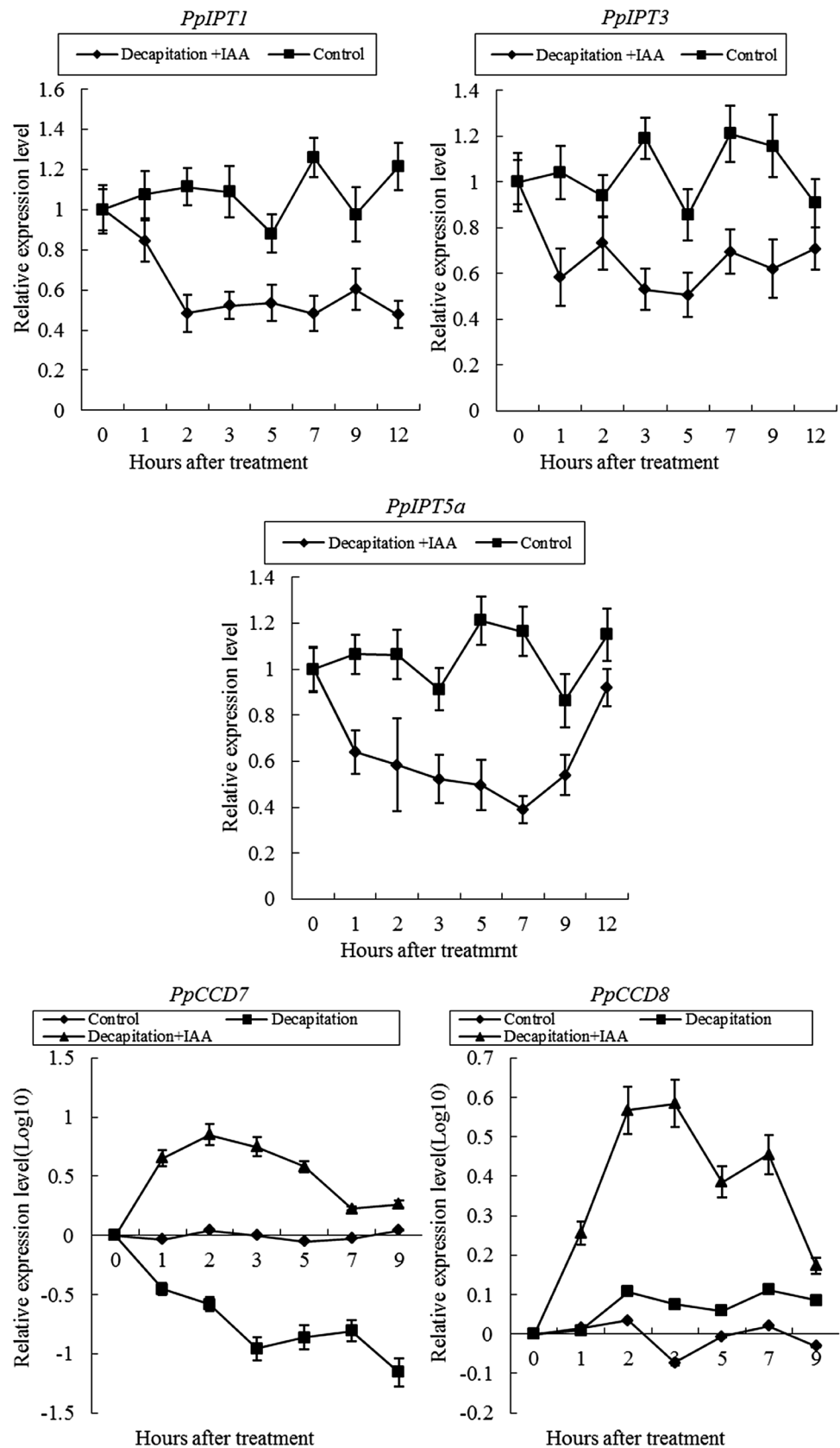


Fig. 3 Effects of exogenous IAA on ATP/ADP *PpIPTs* and *PpCCD7/8* expression in nodal stems after decapitation. The expression levels of *PpIPT1*, *PpIPT3*, *PpIPT5a*, *PpCCD7* and *PpCCD8* in nodal stems after different treatments were determined. Each treatment contains three biological replicates and each replicate consisted of 30 inoculated shoots at each time point. Shoots were decapitated 1 cm above the axillary bud, and the fourth or fifth axillary bud below the apical bud was selected. For IAA treatment, 1% IAA in lanolin was applied at the top after decapitation. The results of *PpCCD7* and *PpCCD8* were given after make a Log10 scale. Intact shoots as Control



transport (Bennett and Leyser 2014; Leyser 2008). Our results also demonstrate that SL can inhibit the outgrowth of axillary buds only when terminal buds are present. Therefore, SL may function by affecting basipetal auxin transport.

Effect of strigolactone on *PpPIN1a* gene expression

To better understand the mechanism underlying how SL inhibits the outgrowth of axillary buds, we examined the expression levels of *PpPIN1a* after SL treatment. The results show that the application of SL markedly inhibited the expression of *PpPIN1a* genes in the nodal stems but not in axillary buds (Fig. 4). The level of IAA in the nodal stems also showed the same result. After decapitation, IAA level in the nodal stems after GR24 treatment is significantly higher than that without GR24 treatment at 6 h after treatment (Table 6). Therefore, SL treatment inhibited basipetal auxin transport at the node, thereby inhibiting the expression of *ATP/ADP PpIPT* genes in nodal stems.

CCD7 and *CCD8* are key genes in the synthesis of SL (Booker et al. 2004; Snowden et al. 2005). In our study, the expression level of *PpCCD7* was markedly reduced in the nodal stems after decapitation (Fig. 3), and exogenous IAA upregulated the expression of *PpCCD7* and *PpCCD8* in the nodal stems (Fig. 3).

Table 6 Auxin contents of nodal stems after various treatments

Hours after treatment	IAA (pmol/g FW)		
	Control	Decapitation	Decapitation+SL
0 h	196.3 ± 20.3		
6 h	182.6 ± 17.1a	59.6 ± 4.9c	95.9 ± 8.9b

The IAA contents were analyzed as described in the Materials and methods. Mean value and SD were calculated from three replicate samples. Lower case letters a, b and c represent mean values that are significantly different between different treatment by Duncan's test ($P < 0.05$, $n = 3$)

Discussion

Roles of auxin, cytokinin, and strigolactones in apical dominance in peach

In this study, we first confirmed the roles of auxin, cytokinin, and SLs in apical dominance in peach. According to our experimental results, the lengths of axillary buds after decapitation were significantly greater than those of the control, and the levels of tZ, tZR, iP and iPR markedly increased in both nodal stems and axillary buds after decapitation. The outgrowth of axillary buds was significantly inhibited by the cytokinin synthesis inhibitor Lovastatin after decapitation of the shoot apex, and exogenous cytokinin can eliminate this inhibitory effect (Fig. 1). Therefore, cytokinin is required to promote the outgrowth of axillary buds. In addition, the lengths of axillary buds in shoot cuttings were also

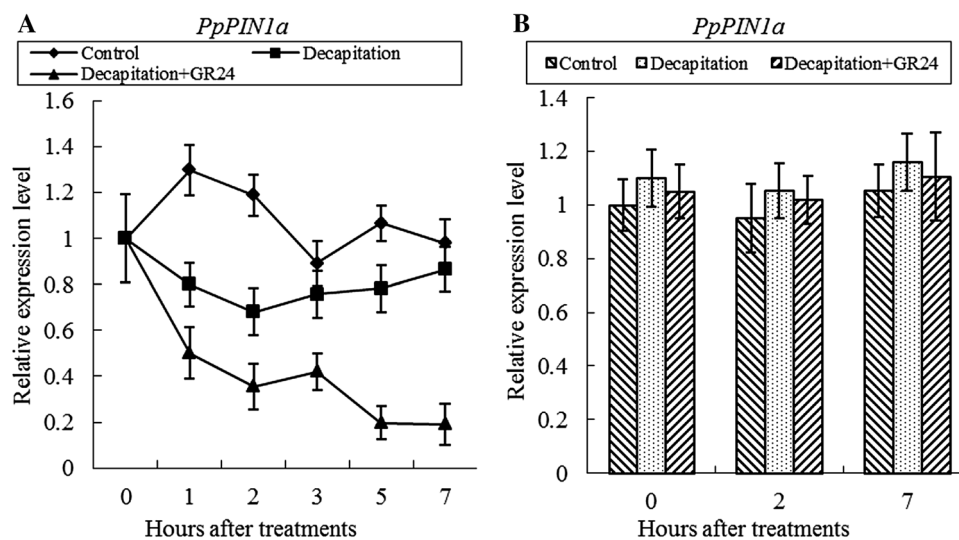


Fig. 4 Effects of exogenous GR24 on *PpPIN1a* expression in nodal stems and axillary buds after decapitation. The expression levels of *PpPIN1a* in nodal stems (a) and axillary buds (b) after GR24 treatment were determined. Each treatment contains three biological replicates and each replicate consisted of 30 inoculated shoots at each

time point. Shoots were decapitated 1 cm above the axillary bud, and the fourth or fifth axillary bud below the apical bud was selected. For GR24 treatment, 10 μ M GR24 in lanolin was applied at both the top and the axillary bud after decapitation. Intact shoots as Control

significantly higher after decapitation, suggesting that the additional cytokinins were biosynthesized in the shoot, not in the root. Moreover, the expression of *ATP/ADP PpIPTs* was significantly upregulated only in stems, not in axillary buds. These results suggest that the additional cytokinins were biosynthesized in the stem, not in axillary buds, and were then transported to the dormant axillary buds. This finding echoes the results of studies in other plant species (Tanaka et al. 2006; Liu et al. 2011).

The outgrowth of axillary buds was significantly inhibited by auxin following decapitation. SL did not inhibit the outgrowth of axillary buds after decapitation, but it significantly inhibited the outgrowth of axillary buds in the absence of decapitation, suggesting that SL does not directly repress bud outgrowth or the point of SL control was rapidly past. SL's function relies on the presence of an intact shoot apex, indicating that its function may be associated with auxin. As we have discussed above, cytokinin is necessary for promoting the outgrowth of axillary buds. While auxin directly represses axillary bud outgrowth, SL can also inhibit the outgrowth of axillary buds, but only when the shoot apex is intact.

ATP/ADP PpIPT genes in nodal stem are key genes for cytokinin biosynthesis by promoting the outgrowth of axillary buds

Adenosine phosphate isopentenyltransferase (IPT) is a key enzyme for cytokinin biosynthesis as well as an important rate-limiting enzyme (Kakimoto 2001; Takei et al. 2001). Most studies have demonstrated that *ATP/ADP IPT* genes play a key role in regulating cytokinin content (Golovko et al. 2002; Tanaka et al. 2006; Sakano et al. 2004; Takei et al. 2002). A number of studies have shown that root is the major organ of cytokinin biosynthesis; roots contain high levels of cytokinins, and cytokinins that are biosynthesized in roots are transported to axillary buds (Bangerth 1994; Bangerth et al. 2000). A study examining the expression pattern of *PsIPT1* and *PsIPT2* showed that cytokinins biosynthesized in nodes are more important for promoting the outgrowth of axillary buds than cytokinins biosynthesized in roots (Tanaka et al. 2006). In the current study, after decapitation, the expression levels of *PpIPT1*, *PpIPT3*, and *PpIPT5a* increased markedly in the nodal stems. And their expression level was well consistent with the content of tZ-type and iP-type cytokinins after decapitation. And the expression of these three genes in the nodal stem also well consistent with the consistence of tZ-type and iP-type cytokinins after IAA and GR24 treatment. In addition, the expression of *ATP/ADP PpIPT* genes in axillary buds located at the nodal stems did not markedly change, or was even reduced, after decapitation. Based on these findings, we conclude that cytokinins, which promote axillary buds

outgrowth, are mainly biosynthesized in the nodes, with little or no contribution from root or buds. In addition, *ATP/ADP PpIPT* genes expressed in the nodal stem are key genes in this process.

Effect of auxin and strigolactone on ATP/ADP IPT expression and the control of apical dominance in peach

Apical dominance is one of the classical developmental events believed to be controlled by crosstalk between auxin and cytokinin (Leyser 2003; Tanaka et al. 2006). Auxins can significantly inhibit the outgrowth of lateral buds after decapitation when applied to the stem stump. In the current study, we found that the application of exogenous IAA could inhibit the overexpression of *ATP/ADP PpIPTs* after decapitation. And exogenous IAA inhibited the decapitation-induced increase in cytokinins contents in nodes and axillary buds. During the process of apical dominance, cytokinin biosynthesis in the nodal stem is negatively regulated by auxin through limiting *ATP/ADP PpIPTs* expression. These results are similar to the results of Tanaka et al. (2006), who also determined that the promoter of *PsIPT2* is directly repressed by IAA. Therefore, in peach stems under apical dominance, auxin may directly inhibit the expression of *ATP/ADP PpIPT* genes to impede the increase in cytokinin levels, thereby inhibiting the outgrowth of axillary buds.

Examination of the outgrowth of axillary buds in peach after various treatments indicated that SL alone cannot inhibit the outgrowth of axillary buds; the effect of SL relies on the presence of auxin. Many studies have been done focus on the molecular mechanism underlying the regulation of branching by SLs. Earlier studies have primarily focused on the functions and molecular mechanisms related to auxin, GR24 could decrease PIN1 polar localization at the plasma membrane in shoots (Shinohara et al. 2013). In the current study, we first examined the effect of SLs on the expression of *ATP/ADP PpIPTs*. In contrast to auxin treatment, after SL treatment, the expression of these three genes and the cytokinins level still markedly increased, but the rate of increase in gene expression was markedly lower than that observed after decapitation alone. In addition, the expression levels of *PpIPT1* and *PpIPT5a* did not reach their maximum levels by 5 h after treatment, and they continued to increase at 9 h after treatment. Therefore, SL did not directly inhibit the expression of *ATP/ADP PpIPTs* in nodal stems. Previous studies have shown that the function of SL is closely related to basipetal auxin transport (Bennett et al. 2006; Mouchel and Leyser 2007; Ongaro and Leyser 2008). Therefore, we examined the effect of SLs on basipetal auxin transport. *PIN1*, which generates a unidirectional flow of auxin basipetally from the apex through the stem, is particularly important for polar auxin transport (Okada et al. 1991; Friml et al.

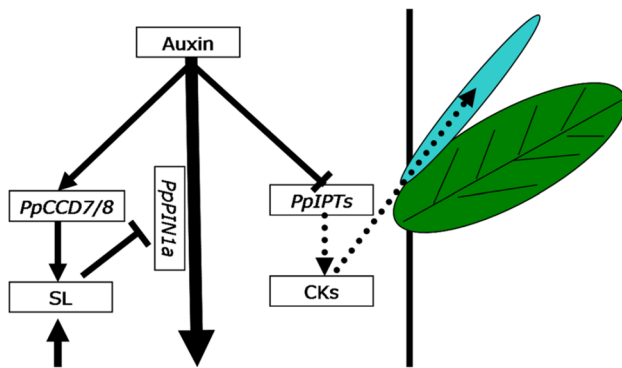


Fig. 5 Model of plant hormonal control of apical dominance in peach. In this model, auxin plays the most important role in apical dominance. Cytokinins and SLs (SLs) can be regarded as second messengers for auxin action. Auxin plays its role via affecting the metabolism of cytokinins and SLs. Cytokinins promote the outgrowth of axillary buds; they are mainly synthesized in the stem, but not in axillary buds. The key genes encoding cytokinin synthetase (ATP/ADP PpIPTs) are under the control of auxin. Auxin directly inhibits the expression of *ATP/ADP PpIPTs* to impede the synthesis of cytokinins in peach shoots, thereby inhibiting the outgrowth of axillary buds. SLs can also inhibit the outgrowth of axillary buds, but their role depends on the presence of auxin. SL inhibits the outgrowth of axillary buds by inhibiting basipetal auxin transport at the node. SL down regulates the expression of *PpPIN1a* in the node. In this way, SL may limit basipetal auxin transport, thereby inhibiting the expression of *ATP/ADP PpIPTs*, and auxin may promote the synthesis of SL in the shoot

2003; Paponov et al. 2005). We found that the application of SL strongly inhibited the expression of *PpPIN1a* in nodal stems, but not in axillary buds. The level of IAA in the nodal stems also showed that the GR24 treatment could reduce the basipetal auxin transport at the node (Table 6). These results suggest that SL treatment inhibits basipetal auxin transport at the node, thereby inhibiting the expressions of *IPT* genes. These results indicate that SL functions by inhibiting the outgrowth of axillary buds through inhibition of basipetal auxin transport at the node.

On the other hand, the expression level of *PpCCD7* was markedly reduced in the nodal stem after decapitation, and exogenous IAA upregulated the expression of *PpCCD7* and *PpCCD8* in the nodal stem. In other words, the auxin may control the synthesis of SL at the node by regulating the expression of *PpCCD7* and *PpCCD8*. Therefore, although SL inhibits basipetal auxin transport, auxin promotes the accumulation of SL in the nodal stems. This result is consistent with the results of studies in *Arabidopsis thaliana* and pea (Hayward et al. 2009). Based on the current results, we established a model of plant hormonal control of apical dominance in peach (Fig. 5). In this model, auxin plays the most important role in apical dominance. Cytokinins and SLs can be regarded as second messengers for auxin action. Auxin plays its role via affecting

the metabolism of cytokinins and SLs. Auxin directly inhibits the expression of *ATP/ADP PpIPTs* to impede the synthesis of cytokinins in peach shoots, thereby inhibiting the outgrowth of axillary buds. SLs can also inhibit the outgrowth of axillary buds by inhibiting basipetal auxin transport at the node.

Author contribution statement FP and ML conceived and designed research. ML and YX conducted experiments. ML and QW analyzed data. ML and FP wrote the manuscript. All authors read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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