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Regulatory Mechanisms of Strigolactones on the Development of Lateral Branches in Cucumber

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Abstract. Cucumber (*Cucumis sativus* L.) belongs to the cucumber genus of the Cucurbitaceae family, and the selection of cultivars with minimal or no lateral branches can enhance the cultivation management efficiency. The growth of lateral branches is inhibited by strigolactone. To investigate the regulatory mechanism of strigolactone on the lateral branch development in cucumber, the cultivar LZ1 exhibiting multiple lateral branches was selected as the experimental material. The axillae of the plants were infiltrated with 1, 5, and 10 μmol·L^{-1} germination releaser 24 (GR24) at the four- to five-leaf stage. It was identified that 1 μmol·L^{-1} GR24 exhibited the most potent inhibitory effect on cucumber lateral branches. Additionally, exogenous strigolactone decreased the auxin content in the apical bud and axillae and increased the auxin content in the stem. This inhibited polar auxin transport in the axillary bud and promoted polar auxin transport in the apical bud. The content of strigolactone in the axilla region of cucumbers was elevated, whereas the synthesis and expression of cytokinin in the same area were reduced. A low concentration of GR24 induced the expression of cucumber *branched 1* (*csbrc1*), whereas a high concentration of GR24 downregulated the expression of cucumber *lateral suppressor* (*cscls*) and *blind* (*csblind*), which inhibited the growth of cucumber lateral branches.

The lateral branches of plants are a key feature of their architectural structure and are regulated by axillary meristems (Du et al. 2008). Environmental factors (Wang et al. 2019), sugars (Barbier et al. 2015), plant hormones (Domagalska and Leyser 2011; Gomez-Roldan et al. 2008; González-Grandío et al. 2017; Müller et al. 2015; Peng 2020), and nutrients (Le Moigne et al. 2018; Minakuchi et al. 2010; Takeda et al. 2003; Xi et al. 2015) affect the development of lateral branches in plants. Cucumber (*Cucumis sativus* L.) is an important cultivated vegetable crop in China and is classified in the family Cucurbitaceae. To optimize cucumber cultivation and management, it is essential to select cultivars with minimal lateral branches or control their growth. This approach eliminates the need for artificial pruning of branches, thereby saving labor and time while boosting work efficiency. Furthermore, it enhances ventilation, reduces the incidence of diseases, and ultimately increases production.

The regulatory effect of auxins, specifically indole-3-acetic acid (IAA), on plant branching is primarily achieved through two distinct pathways: polar transport and second messenger mechanisms (Bhoi et al. 2021). Apical dominance, which controls bud activity, is crucial in this process. When auxin levels in the main stem reach saturation through polar transport, auxin export from axillary buds is inhibited, leading to indirect arrest of axillary bud growth (Domagalska and Leyser 2011).

The second messenger plays a crucial role in regulating branching through the control of two hormones: strigolactone (SL) and cytokinin (CTK). SL is a carotenoid-derived molecule that is synthesized in roots and stems and is transported from the morphological lower end to the morphological upper end to axillary buds, where it regulates the growth of lateral branches (Bhoi et al. 2021). GR24, a synthetic analog of SL, has been used to inhibit branch development in non-bearing Chinese cabbage (*Brassica campestris* L.), tomato (*Solanum lycopersicum* L.), tobacco (*Nicotiana tabacum* L.), Mongolian oak (*Quercus mongolia* Fisch. ex Ledeb.), and other plants (Cui et al. 2016; Fang 2021; Han et al. 2021; Sun 2020). CTK, on the other hand, is a downstream signal of IAA. Exogenous CTK promoted the growth of axillary buds and opposed the effects of IAA and SL in regulating the development of lateral branches in plants. In the shoot apex, IAA induces the expression of the CTK oxidation/dehydrogenase gene *cytpkinin oxidase/dehydrogenase* (*ckx*) to facilitate CTK degradation and suppresses the expression of the CTK synthesis gene *isopentenyltransferase* (*ipt*) to inhibit branch growth (Shimizu-Sato et al. 2009). After application of GR24, the expression of *osckx9*, a CTK degradation gene in rice (*Oryza sativa* L.), was upregulated, whereas the expression of *type A response regulator 5* (*osarr5*) was downregulated, leading to inhibition of tillering in rice (Duan et al. 2019).

Branched 1 (*brc1*), a gene belonging to the TCP family, plays a crucial role in the integration of signals that regulate branching in axillary buds (Su et al. 2021). Cucumber *csbrc1* can directly bind to the auxin efflux carrier, *pin-formed 3* (*cspin3*), which prevents the transport of IAA in axillary buds and subsequently inhibits the growth of axillary buds. In addition, *Csbrc1* has been shown to promote the growth of lateral branches using RNAi technology (Shen et al. 2019). Moreover, expression levels can be enhanced by the application of GR24 and reduced by the presence of CTK (Braun et al. 2012). According to a study by Zhao et al. (2018), RNAi technology interfering with *Leafy* (*Cslfy*) disrupts cucumber leaf development and inhibits axillary bud growth (Braun et al. 2012). The *lateral suppressor* (*cscls*) is a member of the GRAS transcription factor family and has been shown to be expressed in axillae at various stages of development

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through RNA in situ hybridization. This suggests that *cls* plays a crucial role in the formation and branching of cucumber axillary tissues (Yuan et al. 2010). *Blind* is a member of the R2R3 class of transcription factors and has been identified as a MYB gene. In the *blind* mutant of tomato, the normal growth and development of all lateral branches and inflorescence meristems were inhibited, suggesting that *blind* plays a positive regulatory role in the growth and development of tomato lateral branches (Schmitz et al. 2002).

Materials and Methods

Plant material and growth conditions. The test material used was the Yunnan local multibranched cucumber material 'LZ1', sourced from and cultivated in the greenhouse of the Yunnan Agricultural University Yunnan-Taiwan Engineering Research Center for Characteristic Agriculture Industrialization. Seeds were selected and soaked in warm water at 55 °C for 10 min. The seeds were subsequently placed in petri dishes covered with filter paper, and an appropriate quantity of distilled water was added. The seeds were incubated at 27 °C until they turned white, after which they were planted in a cavern with imported substrate. Upon reaching two true leaves, seedlings were transplanted into nonwoven bags. Daytime temperature was ∼22 ± 2 °C, and nighttime temperature was ∼15 ± 2 °C. Three replicates were set for each treatment, with six plants allocated to each treatment. Throughout this period, water and fertilizer management, as well as pest control measures, intensified.

Effects of varying GR24 concentrations on cucumber plants. To dissolve the GR24 (Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China) powder, 1 mL of acetone (Tianjin Fengboat Chemical Reagent Technology Co., Ltd., Tianjin, China) was added to 1 mg of the powder, and distilled water was added to 10 mL to prepare the mother liquor at a concentration of 0.1 mg·mL^{-1}. Working solutions with concentrations of 1, 5, and 10 μmol·L^{-1} were prepared. After the plants had grown to the four leaves and one heart stage, they were treated with the working solutions. The axillae without axillary buds were wrapped in cotton in advance, and the working solution was applied daily using a dropper at 18:00 until the cotton was saturated and no longer dripped. The untreated control group was used as the blank control (CK). The treatment was administered once per day, for a total of three times, with the third treatment being recorded on day 0.

Collection of agronomic characteristic data. The initial placement of the first true leaf was considered as the first node, and measurements of the lateral branches of the eight nodes were recorded using a steel tape measure on day 28 following treatment. On day 30, the height of the plant, length and width of the leaf, and leaf area of the cucumber plant were determined using a steel tape measure (cucumber leaf area = leaf length × leaf width × 0.743). Additionally, the stem diameter of the cucumber plants, 2 cm from the ground, was measured using a vernier caliper. After the cucumber roots were cleaned, each component was placed in an oven (LDO-9053A; Shanghai Longyue Instrument Equipment Co., Ltd., Shanghai, China) and oven-dried at 105 °C for 15 min, followed by drying at 80 °C for 12 h until a constant weight was reached. The dry weight of each component was measured using an analytical balance (ME204TE/02; Mettler Toledo Instrument Co., Ltd., Zurich, Switzerland) (Xu et al. 2022). Each process was repeated three times.

Hormone extraction and quantification. After administering varying concentrations of GR24 for an interval of 12 h, the apical buds, stems, roots, and axillary structures were promptly immersed in liquid nitrogen and subsequently cryogenically preserved at –80 °C (EXF32086VGP-ULTS; Thermo Fisher Scientific Inc., Waltham, MA, USA). First, the samples stored at an ultra-low temperature were removed from the storage and meticulously ground into a powder using a grinder (MM400; Verder Retsch Shanghai Trading Co., Ltd., Arzberg, Germany). Ground samples (50 mg) were weighed and added to a new centrifuge tube. Subsequently, 10 μL of an internal standard mixture with a concentration of 100 ng·mL^{-1} and 1 mL of a methanol (Merck; Darmstadt, Hesse, Germany)/water/formic acid (Sigma-Aldrich; St. Louis, MO, USA) (15:4:1) extractant were added and mixed thoroughly. The resulting mixture was vortexed for 10 min and centrifuged at 13,400 ×*g* for 5 min at 4 °C to obtain the supernatant. The supernatant was transferred to a new centrifuge tube and concentrated using a vacuum concentrator. Finally, the concentrated sample was redissolved in 100 μL of 80% methanol/aqueous solution and filtered through a 0.22-μm filter membrane. The resulting sample was then placed in a sample bottle for liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis (QTRAP 6500+; SCIEX, Boston, MA, USA). Each process was repeated three times.

Real-time quantitative expression analysis of collateral-related genes. Following a 12 h exogenous treatment with varying concentrations of GR24, RNA extraction (RNA Prep Pure Polysaccharide Polyphenol Plant Total RNA Extraction Kit (DP441); Tiangen Biotech Beijing Co., Ltd., Beijing, China) and cDNA synthesis (All-In-One 5X RT MasterMix; Applied Biological Materials Inc., Viking Way Richmond, Canada) were performed on the apical buds, leaves, stems, roots, and axillaries of cucumber plants. *β-Actin* was employed as the internal reference gene. The reaction volume was 20 μL, and the reaction was performed on a Bio-Rad CFX96 PCR instrument (CFX96 Touch and CFX384 Touch Real-Time PCR Detection Systems; Bio-Rad Laboratories Inc., Hercules, CA, USA). The program consisted of 95 °C for 3 min, followed by 40 cycles at 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 30 s. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Notably, △Ct = Ct target gene-Ct internal reference gene, △△Ct = △Ct experimental group-△Ct control group, and a total of three replicates were set.

Data analysis. Microsoft Excel (Microsoft Excel 2010; Microsoft, Redmond, WA, USA) was employed for statistical calculations, and SPSS (IBM SPSS Statistics version 20.0.0; IBM Corp., Armonk, NY, USA) was used for advanced statistical analysis and differential expression analysis. One-way analysis of variance and least significant differences were used to evaluate the dissimilarities between the various datasets. The results are presented as mean ± *SE*. GraphPad Prism (GraphPad Prism 5; GraphPad Software, San Diego, CA, USA) was used for graphical representation.

Results

Lateral branch growth of cucumbers. The effect of GR24 treatment on lateral branch growth of cucumbers was studied. The results showed that the growth of the lateral branches from nodes 1 to 5 of the cucumber plants treated with 1 μmol·L^{-1} GR24 was inhibited compared with the CK (Fig. 1A), with lateral

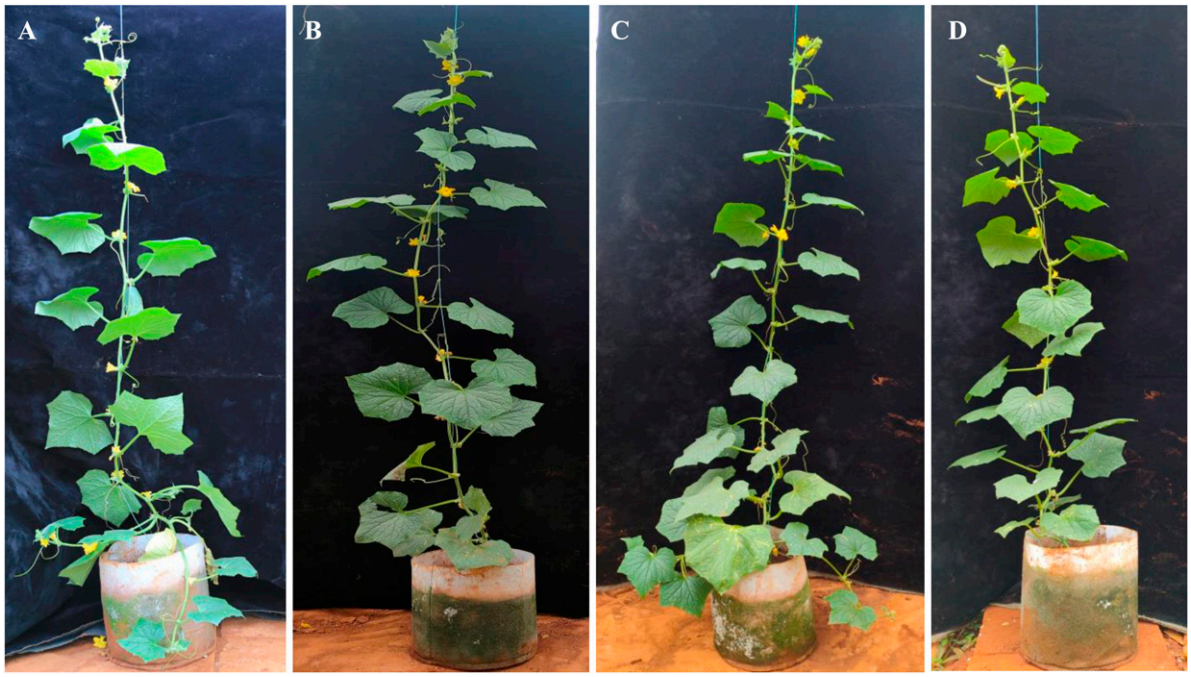

Fig. 1. (**A–D**) Growth of cucumber lateral branches after treatments of water (0 μmol·L^{-1}, blank control), 1, 5, and 10 μmol·L^{-1} germination releaser 24. Note in panel **A**, the control, that growth of lateral branches was normal, and the growth at base nodes was more vigorous than that at upper nodes.

branch lengths measuring 0.73, 1.50, 1.60, 1.67, and 1.53 cm (Table 1; Fig. 1B). Compared with CK, the inhibition rates of the lateral branches were 95.28%, 95.39%, 92.79%, 72.49%, and 20.73%, respectively, decreasing with increasing node position. However, the development of lateral branches from nodes six to eight was comparable to that in CK. The growth of lateral branches of cucumber at eight nodes under the 5 μmol·L^{-1} treatment was similar to that of CK (Table 1; Fig. 1C). The lateral branches of cucumber treated with 10 μmol·L^{-1} showed varying degrees of inhibition after treatment. Specifically, the dimensions of the treated branches were 0.53, 0.86, 0.53, 1.60, 1.70, 0.60, 1.27, and 0.80 cm (Table 1; Fig. 1D), with inhibition rates of 96.57%, 97.36%, 97.61%, 73.64%, 11.92%, 61.78%, 11.19%, and 59.39%, respectively. Notably, the inhibition rates of lateral branches generally decreased as the node position increased.

Growth of cucumber plants. The effects of GR24 treatment on the growth of cucumber plants were also investigated. The average plant height of 'LZ1' under 1 μmol·L^{-1} and 5 μmol·L^{-1} GR24 treatments were 223.47 cm and 195.00 cm (Fig. 2A), which were 19.55% and 4.32% higher than those of CK, respectively. Additionally, the average stem diameter under the same treatments were 9.24 and 8.97 mm (Fig. 2B), which were 14.93% and 11.57% coarser than those of CK, respectively. However, the average plant height in the 10 μmol·L^{-1} treatment was 124.50 cm (Fig. 2A), which was significantly lower than that of CK (33.40% lower), and the average stem diameter was 6.68 mm (Fig. 2B), which was 16.92% thinner than that of CK. The average leaf area of functional leaves in the 1, 5, and 10 μmol·L^{-1} GR24 treatments were 282.32, 190.01, and 145.70 cm^2 (Fig. 2C), respectively. Notably, the leaf area in the 1 μmol·L^{-1} treatment was significantly larger than that in the CK group. However, the leaf area in the 5 μmol·L^{-1} and 10 μmol·L^{-1} treatments was less than that in the CK group.

Stem, leaf, and root dry weights all decreased with an increase in GR24 concentration. The stem dry weight treated with 10 μmol·L^{-1} GR24 was 5.83 g, reflecting a 42.84% decrease compared with CK. The leaf dry weight treated with 1 μmol·L^{-1} and 5 μmol·L^{-1} GR24 were 33.63 and 26.03 g, respectively, increase of 30.42% and 10.10% compared with the CK. Furthermore, the leaf dry weight treated with 10 μmol·L^{-1} GR24 was 18.10 g, illustrating a 22.65% decrease compared with CK. The lateral branch dry weights following treatment with 1 and 10 μmol·L^{-1} of GR24 were 0.60 and 0.17 g, respectively. This represented a decrease of 94.81% and 98.53%, respectively, compared with CK, and these differences were statistically significant. The root dry weight following treatment with 1, 5, and

Table 1. Growth of cucumber lateral branches after GR24 treatment (units: cm) on Day 28.

Treatment, μmol·L^{-1}	First position	Second position	Third position	Fourth position	Fifth position	Sixth position	Seventh position	Eighth position
	(Mean ± *SE*)							
0	15.47 ± 1.27 a	32.57 ± 1.01 a	22.20 ± 0.75 a	6.07 ± 0.59 a	1.93 ± 0.38 ab	1.57 ± 0.21 ab	1.43 ± 0.47 a	1.97 ± 0.25 a
1	0.73 ± 0.21 a	1.50 ± 0.44 b	1.60 ± 0.10 a	1.67 ± 0.35 a	1.53 ± 0.21 b	2.03 ± 0.21 a	1.53 ± 0.25 a	2.37 ± 0.35 a
5	15.23 ± 2.12 a	33.27 ± 2.66 a	11.83 ± 1.24 a	9.00 ± 1.06 a	5.67 ± 0.12 a	1.27 ± 0.15 ab	1.00 ± 0.20 a	2.93 ± 0.31 a
10	0.53 ± 0.15 a	0.80 ± 0.10 b	0.53 ± 0.15 a	1.60 ± 0.26 a	1.70 ± 0.62 b	0.60 ± 0.10 b	1.27 ± 0.31 a	0.80 ± 0.00 a

Different lowercase letters indicate significant differences ($P < 0.05$).

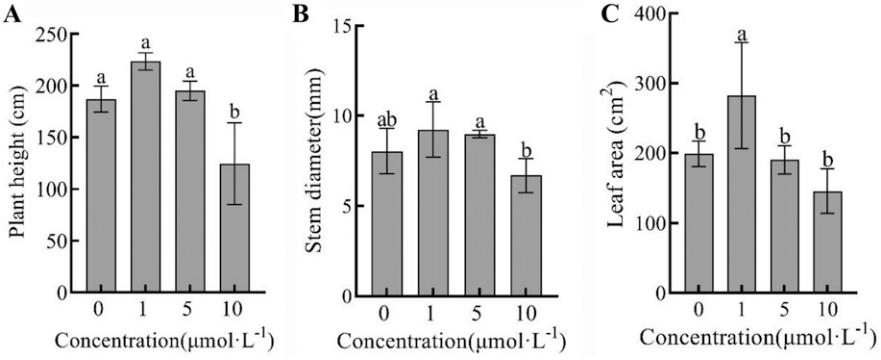

Fig. 2. Changes in cucumber (**A**) plant height, (**B**) stem diameter, and (**C**) leaf area after treatment with water (0 μmol·L^{-1}) and 1, 5, and 10 μmol·L^{-1} germination releaser 24. Different lowercase letters indicate significant differences ($P < 0.05$).

10 μmol·L^{-1} of GR24 was 2.13, 1.43, and 0.87 g, respectively, representing an increase of 176.62%, 85.71%, and 12.99% compared with CK. Only the 1 μmol·L^{-1} GR24–treated groups were significantly different from the CK group (Table 2).

Hormone content analysis. Hormone content in different cucumber parts was analyzed after treatment with GR24 for 12 h at various concentrations (0, 1, 5, and 10 μmol·L^{-1}). The results showed that the IAA content in the apical bud of cucumber was 11.02, 11.99, and 11.98 ng·g^{-1} after treatment with GR24 at the aforementioned concentrations, which was found to be 11.41%, 3.62%, and 3.70% lower than that of the CK and statistically significantly lower than the control at a concentration of 1 μmol·L^{-1} The levels of IAA in the axillary regions were found to be 6.50, 5.73, and 6.53 ng·g^{-1}, which represented decreases of 6.07%, 17.20%, and 5.78% compared with the CK. On the other hand, the IAA content in the stems of the three GR24 concentrations was higher than that of the CK, with values of 8.56, 7.20, and 7.49 ng·g^{-1}, representing increases of 35.66%, 14.10%, and 18.70% compared with the CK.

In the axillae regions, the concentration of CTK in the 1 μmol·L^{-1} treatment group was found to be 3.35 ng·g^{-1}, which was 8.72% lower than that of the CK. In comparison, the 5 μmol·L^{-1} treatment group had a CTK concentration of 3.85 ng·g^{-1}, which was 4.90% higher than that of the CK. Meanwhile, the 10 μmol·L^{-1} treatment group had a CTK concentration of 3.65 ng·g^{-1}, which was 0.54% lower than that of the CK. In the roots, the CTK content of the three treatment groups was lower than that of the CK group, with concentrations of 2.86 ng·g^{-1}, 3.00 ng·g^{-1}, and 2.84 ng·g^{-1}, which were 22.07%, 18.26%, and 22.62% lower than those of the CK, respectively.

The content of SL in the axillae decreased with an increase in GR24 concentration, with values of 3.65 ng·g^{-1}, 3.30 ng·g^{-1}, and 3.27 ng·g^{-1}, respectively. Notably, the SL content at 1 μmol·L^{-1} was the highest compared with that of CK, with an increase of 11.28%. In contrast, the SL content in the roots indicated an opposite trend and increased with an increase in GR24 concentration. The SL content in all treatments was lower than that of the control, with the lowest value observed at 1 μmol·L^{-1} (Table 3).

Expression levels of the genes associated with collateral development. The expression levels of genes associated with collateral development in various parts of the cucumber plants treated with GR24 were investigated. The expression levels of the lateral branch transcription factors *csblind* and *cscls* in the roots of cucumber plants were significantly higher than those in other parts after GR24 treatment. Moreover, the expression levels increased progressively with an increase in the concentration of GR24. Specifically, the expression levels were found to be 26.3, 54.6, and 96.7 times extremely significantly higher than those of CK in the roots of cucumber plants treated with low, medium, and high GR24 concentrations, respectively. Similarly, the expression levels were found to be 1.9, 8.3, and 26.1 times higher than those in the control. The trend of gene expression in the axillae differed from that in the roots, with the exception of the 1 μmol·L^{-1} treatment, which had an extremely significantly higher expression level than the CK, at 3.5 and 4.8 times that of CK, while other concentrations had no extremely significant difference or an extremely significant decrease from the CK (Fig. 3A and B). The expression level of *cslfy*, a gene affecting the plant type of cucumber, was higher in the axillae than in other parts of cucumber and was extremely significant higher significantly higher than that in CK, at 9.4, 10.0, and 5.6 times that in CK (Fig. 3C). *csbrc1* was specifically expressed in the axillae, and its expression decreased with increasing GR24 concentrations. At 1 μmol·L^{-1}, the expression level was extremely significantly higher than CK, at 1.64 times that of CK, whereas at 5 and 10 μmol·L^{-1}, there was no significant difference or extremely significantly lower from CK (Fig. 3D).

Table 2. Effects of germination releaser 24 treatment on cucumber biomass (units: grams).

Treatment, μmol·L^{-1}	Stem DW	Leaf DW	Lateral branch DW	Root DW
	(Mean ± *SE*)			
0	10.20 ± 3.38 a	23.40 ± 9.95 ab	11.57 ± 6.67 a	0.77 ± 0.21 b
1	12.40 ± 0.53 a	33.63 ± 1.50 a	0.60 ± 0.36 b	2.13 ± 1.11 a
5	11.80 ± 3.22 a	26.03 ± 4.24 ab	3.90 ± 1.12 ab	1.43 ± 0.67 ab
10	5.83 ± 4.21 b	18.10 ± 8.46 b	0.17 ± 0.12 b	0.87 ± 0.50 ab

DW = dry weight.
Different lowercase letters indicate significant differences ($P < 0.05$).

Table 3. Effects of germination releaser 24 treatment on endogenous hormones in cucumber (units: $ng \cdot g^{-1}$).

Hormone	Treatment, $\mu mol \cdot L^{-1}$	Apical bud	Axillae	Stem	Root
		(Mean ± *SE*)			
IAA	0	12.44 ± 0.21 a	6.92 ± 0.40 a	6.31 ± 1.36 a	6.78 ± 0.62 a
	1	11.02 ± 0.95 b	6.50 ± 1.84 a	8.56 ± 1.22 a	4.23 ± 0.73 b
	5	11.99 ± 0.47 ab	5.73 ± 0.47 a	7.20 ± 0.72 a	6.44 ± 0.87 a
	10	11.98 ± 0.06 ab	6.52 ± 1.38 a	7.49 ± 1.48 a	7.49 ± 1.06 a
CTK	0	18.20 ± 0.10 b	3.67 ± 0.60 a	4.07 ± 0.69 b	3.67 ± 0.48 a
	1	22.35 ± 2.46 ab	3.35 ± 0.98 a	4.75 ± 0.55 b	2.86 ± 0.85 a
	5	23.28 ± 6.13 ab	3.85 ± 0.79 a	6.57 ± 1.59 a	3.00 ± 0.62 a
	10	24.95 ± 1.29 a	3.65 ± 0.43 a	5.05 ± 0.43 ab	2.84 ± 0.31 a
SL	0	40.82 ± 7.13 c	3.28 ± 0.35 a	2.90 ± 1.52 a	10.57 ± 0.65 a
	1	61.15 ± 2.67 a	3.65 ± 0.53 a	3.00 ± 0.70 a	6.54 ± 1.72 a
	5	44.49 ± 5.34 bc	3.30 ± 0.68 a	1.69 ± 0.62 a	8.62 ± 7.65 a
	10	54.21 ± 8.17 ab	3.27 ± 0.21 a	2.73 ± 0.29 a	10.46 ± 4.59 a

CTK = cytokinin; IAA = indole-3-acetic acid; SL = strigolactone.
Different lowercase letters indicate significant differences ($P < 0.05$).

Discussion

SLs represent a novel class of plant hormones, and GR24 is a widely recognized synthetic analog. The application of exogenous GR24 has been shown to affect the growth and development of lateral branches and tillers significantly in a range of plant species. This phenomenon has been documented in Mongolian oak seedlings (Han 2019), tomatoes (Sun 2020), rice (Zha 2018), cucumbers (Cao 2022), and tobacco (Fang 2021). Notably, Yang et al. (2020) identified the four- to five-leaf stage as a critical phase for lateral branch germination in cucumbers. In this study, the application of varying concentrations of GR24

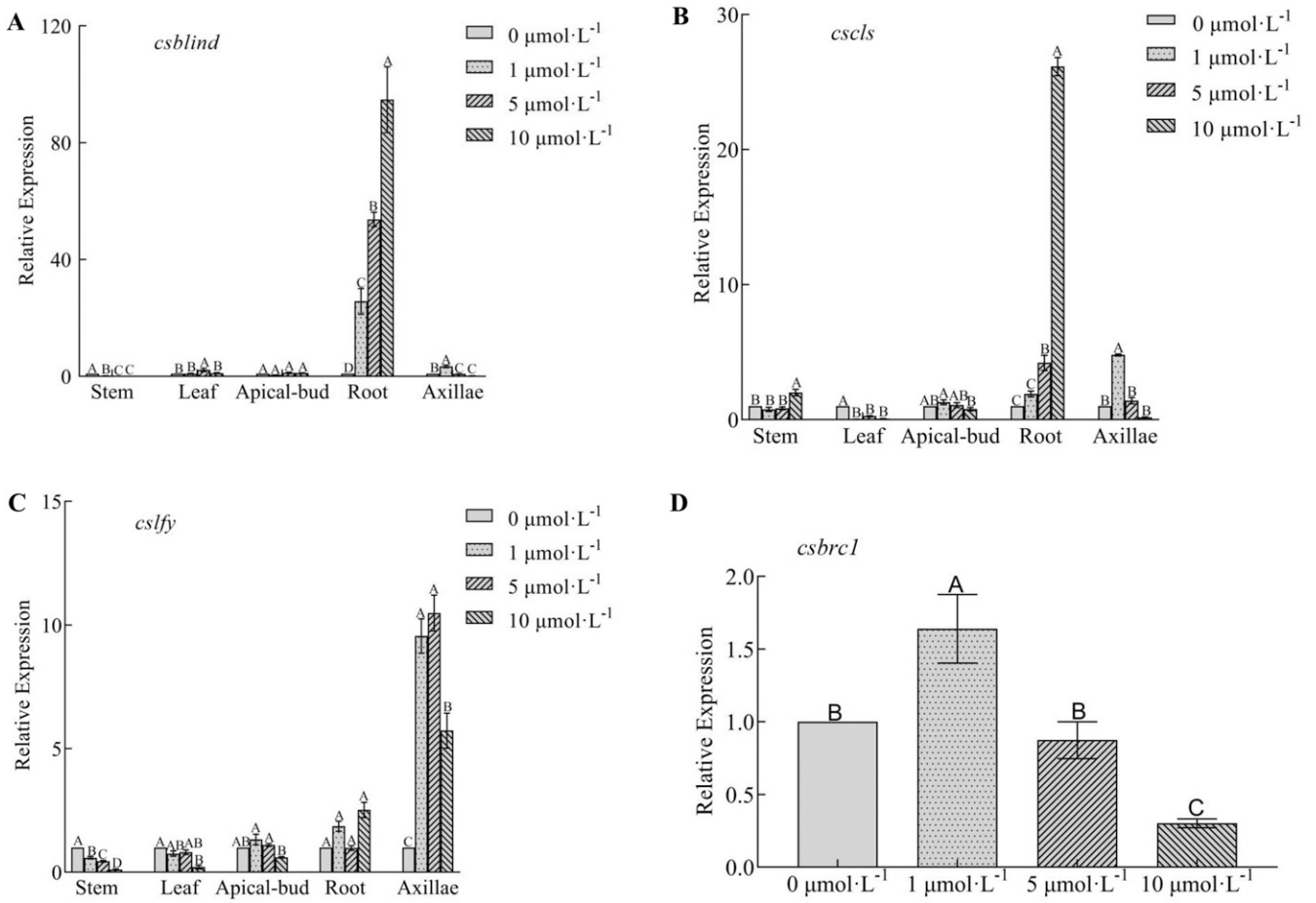

Fig. 3. (**A–C**) Expression of *csblind*, *cscls*, and *cslfy* in cucumber stems, leaves, apical buds, roots, and axillae after water (0 $\mu mol \cdot L^{-1}$) and 1, 5, and 10 $\mu mol \cdot L^{-1}$ germination releaser 24 treatments, respectively. (**D**) Expression of *csbrc1* in cucumber axillae after water (0 $\mu mol \cdot L^{-1}$) and 1, 5, and 10 $\mu mol \cdot L^{-1}$ germination releaser 24 treatments, respectively. Different capital letters indicate extremely significant differences ($P < 0.01$).

(1, 5, and 10 μmol·L^{-1}) to the axillae of cucumbers at the four- to five-leaf stage revealed that the most effective lateral branch inhibition effects were observed in the 1 and 10 μmol·L^{-1} treatments at 1, 5, and 10 μmol·L^{-1} GR24 treatment concentrations. Upon completion of the day 28 treatment regimen, the inhibition rate of low-node lateral branches in cucumbers exceeded 95%. Additionally, the inhibitory effect of lateral branches treated with 5 μmol·L^{-1} was notably lower than that of the other two treatments, exhibiting growth and elongation patterns similar to those of CK. In a study conducted by Han (2019), it was discovered that the application of GR24 at a concentration of 30 μmol·L^{-1} on the axillary buds of Mongolian oak seedlings did not inhibit, but rather accelerated, the lateral branch elongation process, which was similar to the outcome of the 5 μmol·L^{-1} treatment in this study. This suggests that cucumber might exhibit diverse responses to various concentrations of GR24, and that different nodes could possess different sensitivities to GR24, with the lower nodes being the most sensitive.

SL impeded the growth of plant lateral branches and affected the overall growth and development of plants. Specifically, when treated with 1 μmol·L^{-1} GR24, cucumber plants showed increased growth and development in terms of plant height, stem diameter, leaf area, and biomass. This treatment also resulted in the inhibition of lateral branch growth, while promoting the growth of the main plant. Application of 5 μmol·L^{-1} GR24 promoted the growth of cucumber plants, including the development of lateral branches. Treatment with 10 μmol·L-1 GR24 inhibited the overall growth of the plants and, consequently, the growth and development of lateral branches. This suggests that high concentrations of GR24 may cause damage to plant growth, similar to the phenomenon observed in summer grape buds treated with 20 μmol·L^{-1} GR24 (Min 2019). Overall, treatment with 1 μmol·L^{-1} GR24 was the most effective at inhibiting the growth of cucumber lateral branches.

IAA, CTK, and SL collaborate to regulate the development of the lateral branches and interact with each other. Three distinct concentrations of GR24 were used to treat cucumber axillae. The results revealed that the IAA content in the apical bud and axillae was lower than that in CK but higher in the stem. This suggests that the exogenous application of GR24 enhanced the polar transport of IAA from the apical bud toward the root. When there was an abundance of IAA transport in the stem from the saturated apical bud, it obstructed the export of IAA from the axillary bud to the main stem through axillae, thereby inhibiting the growth of lateral branches. This phenomenon is crucial for the axillary bud to export IAA because it plays a vital role in the growth of lateral branches (Chabikwa et al. 2019; Prusinkiewicz et al. 2009). The accumulation of excessive IAA in the axillary bud can result in limited bud growth, while simultaneously reducing IAA content in the root owing to the reduced output of IAA from the bud. CTK was a positive regulator of lateral branch development in plants, and its content in roots decreased following treatment with three different concentrations of GR24. This suggests that the exogenous application of GR24 can reduce CTK synthesis. Interestingly, except for the 5 μmol·L^{-1} GR24 treatment, the CTK content in the axillae of the other two treatments was lower than that in CK. This study found that the growth of cucumber lateral branches was significantly inhibited by 1 and 10 μmol·L^{-1} treatments when combined with phenotype identification. These findings suggest that exogenous GR24 affects the synthesis and accumulation of CTK in the axillae, which in turn inhibits the growth of lateral branches in cucumbers. Additionally, the SL content in the axillae of cucumber was higher than that in CK, except for the 10 μmol·L^{-1} GR24 treatment. This suggests that exogenous application of GR24 can increase the content of endogenous SL in cucumber axillae, leading to inhibition of lateral branch growth. The SL in the roots decreased after exogenous application of GR24. This phenomenon may be attributed to the feedback regulation mechanism that operates between the hormones in plants. Plant hormones become active only in their unbound state, thus homeostasis within plants is maintained through a feedback relationship between the bound and unbound states. When exogenous hormones are introduced, endogenous hormones are subsequently converted to the bound state and vice versa. Endogenous hormones are converted back to an unbound state.

After 12 h of treatment, the levels of *cscls*, *csblind*, and *csbrc1* in the axillae decreased with increasing concentrations and were significantly higher than those in CK when treated with 1 μmol·L^{-1}. This suggests that *cscls* and *csblind* are more sensitive to high GR24 concentrations, whereas *csbrc1* is more sensitive to low GR24 concentrations. Additionally, all three *cslfy* concentrations were significantly higher than those in CK. It is possible that the lateral branches of cucumber were inhibited by downregulating and inducing the expression of *cscls*, *csblind*, and *csbrc1*, rather than by regulating *cslfy*, after 12 h of exogenous GR24 treatment.

Conclusion

In this study, 1 μmol·L^{-1} GR24 treatment resulted in the most favorable effect on lateral branch inhibition and cucumber plant growth. Additionally, exogenous SL can modulate IAA, SL, and CTK levels in cucumber plants. Specifically, it promoted the polar transport of IAA in the apical buds and inhibited the polar transport of IAA in axillary buds. Consequently, the SL content in the axillae of cucumber increased, whereas the synthesis and expression of CTK in the axillae decreased, leading to the inhibition of lateral branch growth in cucumber plants. The responses of cucumber collateral regulatory factors to varying concentrations of GR24 were distinct. At a low GR24 concentration, the expression of the cucumber collateral inhibitory factor *csbrc1* was induced, whereas at a high GR24 concentration, the expression of cucumber collateral regulatory factors *cscls* and *csblind* was downregulated, thus inhibiting the growth of cucumber collaterals. However, the responses of different genes to varying concentrations of GR24 in different tissues require further investigation.

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