

## Article

# Expression Analysis of *DgD14*, *DgBRC1* and *DgLsL* in the Process of Chrysanthemum Lateral Bud Formation

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**Abstract:** The growth of lateral bud can greatly affect the development of apical bud and reduce the quality of single-flower cut chrysanthemum. However, the wide use of artificial bud removal in production leads to the increase on production cost. Therefore, it is important to study the lateral bud development mechanism in chrysanthemum for plant type regulation and genetic improvement. Auxin (IAA), cytokinins (CKs) and strigolactones (SLs) have direct or indirect effects on the formation of lateral buds. *D14*, *BRC1* and *LsL* are key factors regulating the signal pathways of hormones, but their regulation mechanisms on the development of lateral buds in chrysanthemum are still unclear. In this study, single-flower cut chrysanthemum ‘Jinba’ and spray cut chrysanthemum ‘Fenyan’ were used as experimental materials. Quantitative real-time PCR was used to observe the effects of apical bud removal and exogenous hormones on the growth of lateral buds and the expression levels of *DgD14*, *DgBRC1* and *DgLsL*, so as to clarify the expression characteristics of three genes in the process of lateral bud formation. The results showed that GA was effective in promoting the growth of lateral buds, whereas IAA and ABA had little effects on lateral bud growth or even inhibited. Removing apical dominance can significantly affect the expression levels of three genes, which regulated the formation and elongation of lateral buds. Additionally, the three genes showed different responses to different hormone treatments. *DgD14* had a significant response to GA, but a gentle response to ABA. The expression levels of *DgBRC1* varied in different trends, and it responded to IAA in a more dramatic way. The levels of *DgLsL* reached the peaks quickly before decreased in most experimental groups, and its response to GA was extraordinary severe.



**Citation:** Luo, C.; Wang, X.-J.; Ran, A.-N.; Song, J.-J.; Li, X.; Ma, Q.-Q.; Pan, Y.-Z.; Liu, Q.-L.; Jiang, B.-B. Expression Analysis of *DgD14*, *DgBRC1* and *DgLsL* in the Process of Chrysanthemum Lateral Bud Formation. *Agriculture* **2021**, *11*, 1221. <https://doi.org/10.3390/agriculture11121221>

Academic Editor: Pietro Gramazio

Received: 28 October 2021

Accepted: 30 November 2021

Published: 3 December 2021

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**Keywords:** lateral bud; exogenous hormone; quantitative real-time PCR; *DgD14*; *DgBRC1*; *DgLsL*

## 1. Introduction

Chrysanthemum is one of the top ten famous flowers in China and top four cut flowers in the world. Due to its unique ornamental value and high practical value, it is also an important species of cut flowers exported from China [1]. Single-flower chrysanthemum and spray chrysanthemum are two cultivars in cut chrysanthemum [2]. Among them, *Chrysanthemum morifolium* ‘Jinba’ is a popular single-flower cut chrysanthemum cultivar in the international market [3], but its lateral bud occurs seriously [4]. Single-flower chrysanthemums require artificial bud removing whereas spray chrysanthemum requires pinching to improve branching in the production, which results in a significant increase in the production costs of cut chrysanthemum [5].

Branching is a common phenomenon in plant growth and plays an important role in plant morphogenesis. The development of lateral branches mainly consists of two stages: the axillary meristem differentiation to form lateral buds, and the lateral bud elongation to form lateral branches [6]. However, apical dominance exists in most plants. Previous studies have found that after the apical bud of ‘Jinba’ was removed, the elongation of upper

lateral buds was obvious, whereas that of lower lateral buds was inconspicuous. It indicated that apical dominance had an inhibitory effect on the lateral bud of chrysanthemum [7,8].

The development of lateral bud requires the participation and mediation of varieties of plant hormones [9] affected by complex hormone regulatory networks [10,11]. A series of classical experiments have proved that auxins, CKs and SLs are the three most important hormones regulating the lateral bud growth [12,13]. CKs promote lateral bud formation, whereas auxins and SLs inhibit [14,15], and ABA is considered to be an inhibitor of lateral bud growth. Auxins transport downward from the top of the stem to inhibit the growth of lateral bud, forming the apical dominance [16]. The higher the content of auxins in the apical bud of cut chrysanthemum, the stronger the inhibitory effects on the growth of lateral bud [17]. CKs can induce the synthesis [18] and upward transport of auxins in roots, stems and leaves [19], indicating that CKs play a very important role in lateral bud growth [20–22]. Therefore, by regulating the levels of plant hormones, we may be able to regulate lateral bud development and improve plant phenotypes [23,24].

Many genes are involved in the regulation of lateral bud formation, development and growth [25]. SLs play a unique role in lateral bud regulation and lateral root development [26]. So far, one downstream gene and six key genes [27,28] involved in SLs pathway of synthesising and transporting have been found in 'Jinba'. D14 is a key gene in SLs transport pathway, which can exert the physiological activity of SLs and inhibit branch development [28,29]. Wen et al. [30] isolated *DgD14* and found it being induced by auxins and having feedback regulation [31]. The expression of *DgD14* can be downregulated by apical bud removal and restored by exogenous auxins.

BRC1 is the only known inhibitory gene that directly acts on the downstream of SLs pathway to regulate lateral bud elongation. Chen et al. [6] isolated *DgBRC1* from 'Jinba', finding that apical bud removal could downregulate its expression, whereas high planting densities could be upregulated, and it could respond to the application of apical auxins [32].

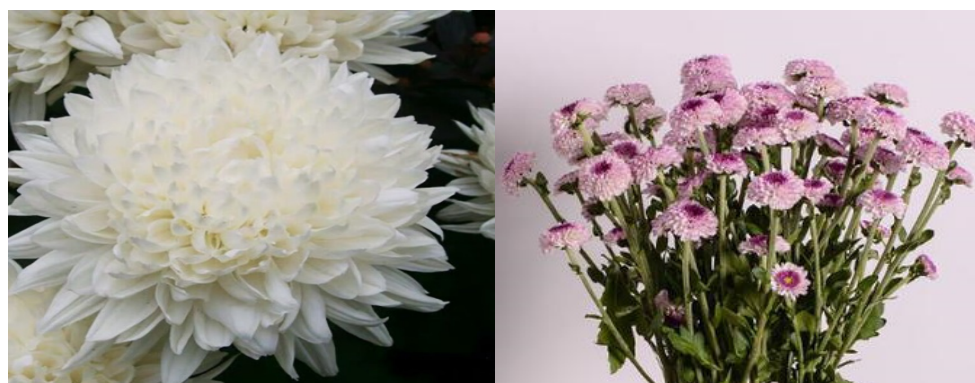
Moreover, *DgLsL* is also related to chrysanthemum branching. Ls is one of the first genes to affect axillary meristem formation [33], and Ls protein is a negative regulator in GA signaling pathway, so GA plays a certain role in axillary meristem formation [34]. *DgLsL*, belongs to GRAS protein family with Ls [35], is an upstream transcriptional regulatory factor with little expression in chrysanthemum which expresses in all organs but concentrates in where lateral branch is formed, regulating the formation of collateral meristem through gene interaction [36]. Previous studies revealed that *DgLsL* regulated chrysanthemum branching by influencing the contents of IAA and GA<sub>3</sub> in the apical buds [37].

In this study, *Chrysanthemum morifolium* 'Jinba' and *Chrysanthemum morifolium* 'Fenyan' were used as plant materials. RT-qPCR was used to study the expression patterns of *DgD14*, *DgBRC1* and *DgLsL* in the process of lateral bud formation, and analyze the effects of different exogenous hormones on the growth of lateral bud and gene expression. It further revealed the mechanism of lateral bud formation regulated by three genes, providing an effective gene reserve for the genetic improvement and oriented design of chrysanthemum type. This study can provide a theoretical basis for the molecular regulation of chrysanthemum lateral bud development, which is of great significance for the selection and breeding of new varieties of chrysanthemum [11,38].

## 2. Materials and Methods

### 2.1. Plant Materials

Single-flower cut chrysanthemum 'Jinba' and spray cut chrysanthemum 'Fenyan' are shown in Figure 1, and their seedlings which grew to 12 cm tall (same batch, consistent growth) were transplanted in plastic pots of the same size (upper diameter of the flowerpot = 7 cm, lower diameter = 5 cm, height = 10 cm). The seedling substrate was a mixture of perlite and peat soil (1:3), and the soil quality was consistent for each pot. These transplanted chrysanthemum seedlings were incubated in a light incubator (Jiangnan Instrument Factory, Ningbo, China) with a light/dark photoperiod of 16/8 h at 23 °C for one week to ensure their healthy growth before their apical dominance were removed.



**Figure 1.** Single-flower cut chrysanthemum ‘Jinba’ and spray cut chrysanthemum ‘Fenyan’.

### 2.2. Apical Bud Removal and Exogenous Hormone Treatment

After incubating, the apical buds of chrysanthemum seedlings with 3–4 leaflets were removed, and 50 mg/L IAA, GA, ABA and distilled water were sprayed separately three times a day. There were 90 strains in each group and 360 strains in total. Chrysanthemums sprayed with exogenous hormones belonged to experimental groups and those sprayed with distilled water belonged to the control group.

### 2.3. Sampling

After 0, 3 and 6 days, a 1 cm stem segment with the first lateral bud was collected from the uppermost part of each chrysanthemum from where the apical bud had been removed, and the lengths of those lateral buds were separately observed under a microscope (LGX-30A, Shanghai Liguang Precise Instrument Co.,Ltd., Shanghai, China), recorded with a camera and measured with a vernier caliper afterwards. Three lateral buds were taken from each experimental group and control group every time, repeating for three times in total. Then, SPSS 21.0 was used to analyze the differences in lateral bud lengths between GA, IAA and ABA groups on day 0, 3 and 6.

Meanwhile, after 0, 24, 72 and 96 h, a 1 cm stem segment with the first lateral bud from the top of each chrysanthemum from where the apical bud had been removed was collected, frozen in the liquid nitrogen immediately and stored in the refrigerator at  $-80\text{ }^{\circ}\text{C}$  for RNA extraction. Three lateral buds were selected from each group every time, which was repeated four times.

### 2.4. Expression Analysis

RT-qPCR was used to test the expression levels of *DgD14*, *DgBRC1* and *DgLsL* in the first lateral bud of ‘Jinba’ and ‘Fenyan’ after decapitating, spraying IAA, GA, ABA and distilled water. Their variations were collated and analyzed afterwards.

Total RNA was extracted using trizol method (Vazyme Biotech Co., Ltd., Nanjing, China) and was reverse transcribed using the Transcript<sup>®</sup> II All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (TransGen Biotech, Beijing, China) according to the user’s manual. RT-qPCR was performed with three biological repeats using TransStart<sup>®</sup> Top Green qPCR SuperMix (TransGen Biotech, Beijing, China). The PCR amplification program was  $95\text{ }^{\circ}\text{C}$  for 2 min and 45 cycles ( $95\text{ }^{\circ}\text{C}$  for 10 s,  $60\text{ }^{\circ}\text{C}$  for 20 s and  $72\text{ }^{\circ}\text{C}$  for 20 s), the dissolution curve was  $95\text{ }^{\circ}\text{C}$  for 30 s,  $65\text{ }^{\circ}\text{C}$  for 30 s and  $0.6\text{ }^{\circ}\text{C}$  to  $97\text{ }^{\circ}\text{C}$ , followed by the terminated reaction, which was  $42\text{ }^{\circ}\text{C}$  for 2 min using an ABI 7500 Fast Real-Time PCR system with SYBR Green Master Mix (Bio-Rad, CFX Connect, Shanghai, China). The  $2^{\text{Ct (reference gene)} - \text{Ct (target gene)}}$  (Ct means cycle threshold) method was used to evaluate the relative expression level of each gene. The  $\beta$ -Actin gene was used as the internal control. The primers used for RT-qPCR are listed in Table 1.

**Table 1.** Primers of *DgD14*, *DgBRC1*, *DgLsL* and  $\beta$ -actin (as the internal control gene) used for RT-qPCR.

Gene ID	Forward Primers (5'-3')	Reverse Primers (5'-3')
DgD14	TACGAGGCATGGGTGTGTGGATC	GCACGGCGCCTTCACTAACCCCT
DgBRC1	CCCTTTTGGAGAGCATCAAG	AGACGTCGCGGATGAAGTAT
DgLsL	TTTACGCTTTACGGTGGTGGTGAG	GTGGCGGCGGAATCTGTATCTTC
$\beta$ -Actin	TGGCATTGTGTTGGATTCTGG	CCATCCAATCATAGACGGCT

### 3. Results

#### 3.1. Effects of Apical Bud Removal and Exogenous Hormones on the Growth of Lateral Buds

At 0, 3 and 6 days after apical bud removal and different exogenous hormone treatments, the growth rates of lateral buds of different chrysanthemums were separately observed and measured. The results in Tables 2 and 3 showed that the lateral buds of *Chrysanthemum morifolium* 'Jinba' and *Chrysanthemum morifolium* 'Fenyan' elongated obviously in 6 days. Moreover, comparing the measured data, it could be found that the average lengths of lateral buds at day 6 that were sprayed with GA were significantly greater than those sprayed with other exogenous hormones, but the average lengths of lateral buds in IAA and ABA groups were lower than those in the control groups.

**Table 2.** Lengths of 'Jinba' lateral buds treated with exogenous hormones after apical bud removal.

Hormone	0 Days after Treatment (mm)	3 Days after Treatment (mm)	6 Days after Treatment (mm)
Control	1.26 $\pm$ 0.12 a	1.96 $\pm$ 0.15 b	2.33 $\pm$ 0.23 b
GA	1.23 $\pm$ 0.11 a	2.05 $\pm$ 0.18 a	2.40 $\pm$ 0.28 a
ABA	1.24 $\pm$ 0.13 a	1.93 $\pm$ 0.12 c	2.25 $\pm$ 0.23 c
IAA	1.24 $\pm$ 0.12 a	1.91 $\pm$ 0.15 c	2.22 $\pm$ 0.18 c

The statistical analysis of the data is based on one-way analysis of variance in SPSS 21.0. Lowercase letters a, b, c and d represent significant differences in lateral bud length after Duncan's test ( $p < 0.05$ ) between GA, ABA, IAA and control groups 0, 3 and 6 days after treatments. The data are expressed as mean  $\pm$  standard deviation (SD).

**Table 3.** Lengths of 'Fenyan' lateral buds treated with exogenous hormones after apical bud removal.

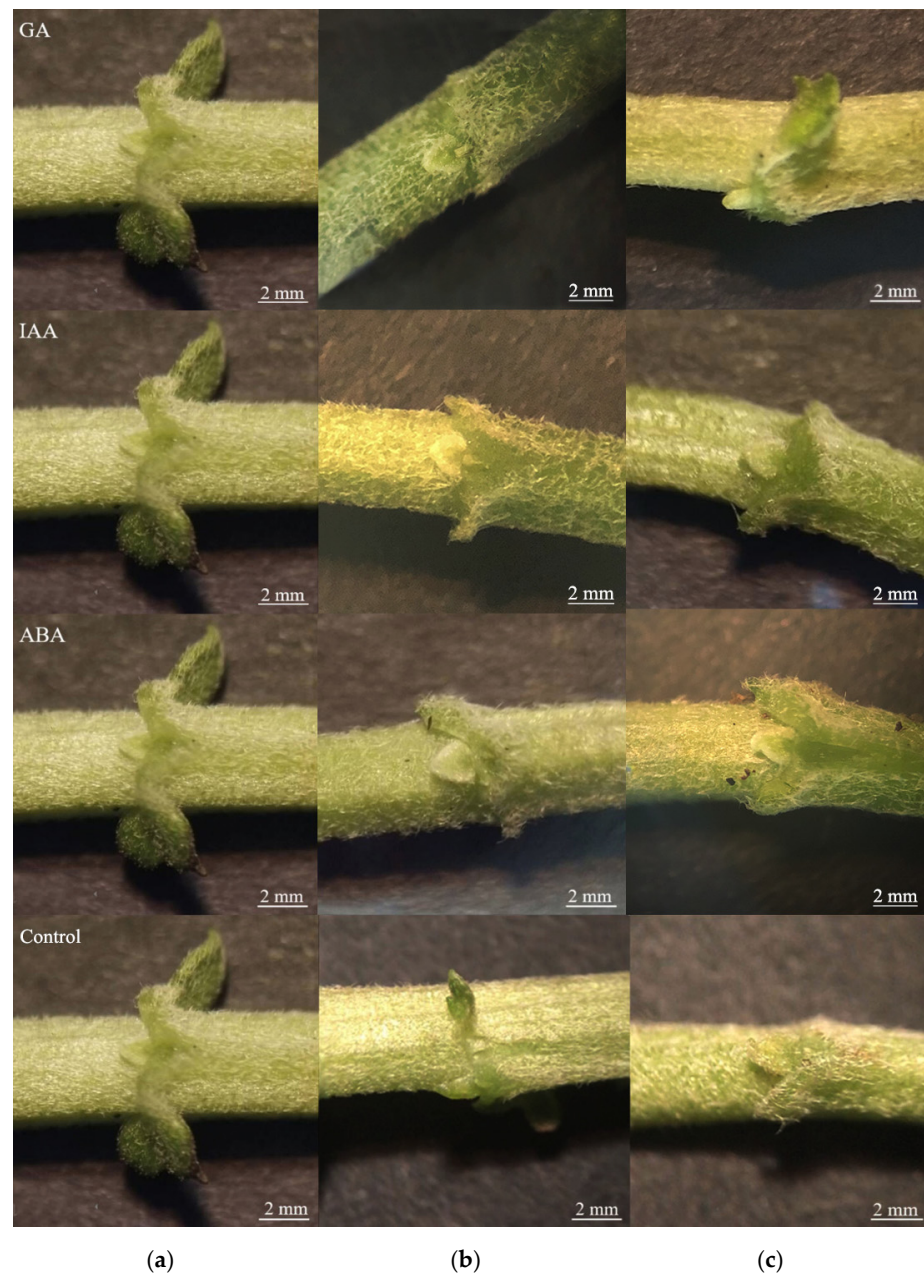
Hormone	0 Days after Treatment (mm)	3 Days after Treatment (mm)	6 Days after Treatment (mm)
Control	1.22 $\pm$ 0.08 a	1.99 $\pm$ 0.21 b	2.26 $\pm$ 0.14 b
GA	1.23 $\pm$ 0.08 a	2.11 $\pm$ 0.23 a	2.45 $\pm$ 0.33 a
ABA	1.23 $\pm$ 0.08 a	1.89 $\pm$ 0.17 d	2.20 $\pm$ 0.20 c
IAA	1.22 $\pm$ 0.07 a	1.94 $\pm$ 0.15 c	2.19 $\pm$ 0.16 c

The statistical analysis of the data is based on one-way analysis of variance in SPSS 21.0. Lowercase letters a, b, c and d represent significant differences in lateral bud length after Duncan's test ( $p < 0.05$ ) between GA, ABA, IAA and control groups 0, 3 and 6 days after treatments. The data are expressed as mean  $\pm$  standard deviation (SD).

In conclusion, according to the differences between the lengths of lateral buds before and after treatments, GA promoted the growth of lateral buds compared with the control group, whereas IAA and ABA inhibited the growth of lateral buds.

Meanwhile, the growth phenotypes of chrysanthemum lateral buds which were under different hormone treatments were separately photographed and recorded. As can be seen from Figures 2 and 3, the lateral buds of *Chrysanthemum morifolium* 'Jinba' and *Chrysanthemum morifolium* 'Fenyan' continued to elongate within 6 days. The lateral bud elongation in IAA and ABA groups was slightly smaller than that in the control group treated with distilled water, but the difference was not significant.

In that way, the results in Figures 2 and 3 further confirmed the accuracy of the measured data to some extent.

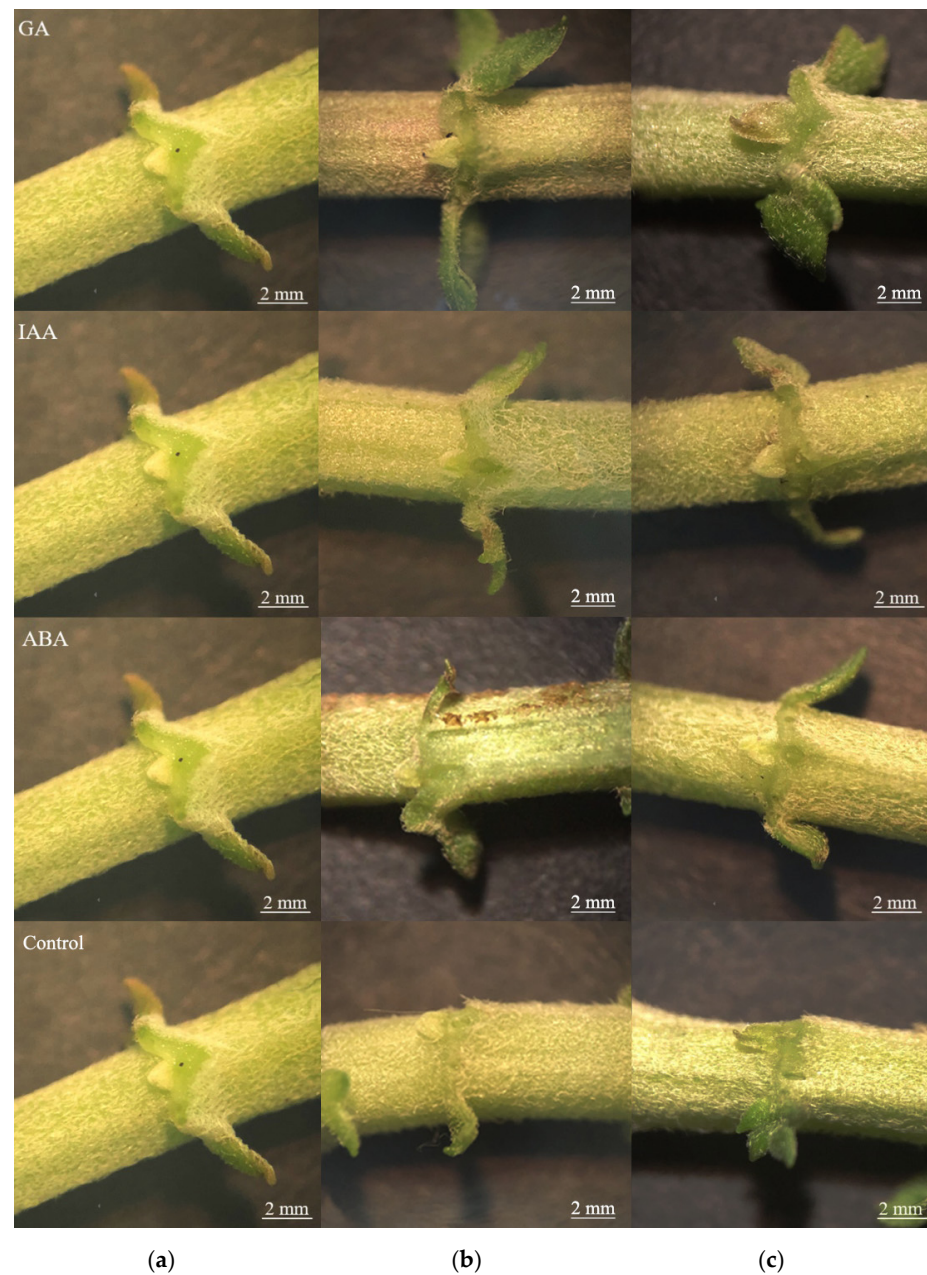


**Figure 2.** Lateral bud phenotypes of ‘Jinba’ that were treated with GA, IAA, ABA and distilled water after apical bud removal. Subfigure (a–c) represent 0, 3 and 6 days after treatments.

### 3.2. Effects of Apical Bud Removal on Gene Expression Characteristics

Chrysanthemum has a strong apical dominance, and the upper lateral buds can form quicker after the apical buds are removed. In order to clarify how genes that regulate lateral bud development during its formation respond to apical bud removal, the expression characteristics of *DgD14*, *DgBRC1* and *DgLsL* in the first lateral buds of ‘Jinba’ and ‘Fenyan’ were analyzed. The results revealed that these three genes in the lateral buds were able to respond quickly after the removal of apical buds, increasing or decreasing rapidly within 96 h. In both cultivars, the expression levels of *DgD14* and *DgLsL* increased to the highest level at 24 h and then gradually decreased, and the variations in the *DgLsL* level were more significant after being removed the apical dominance. Both expression levels of *DgBRC1* declined within 96 h, which became more significant during 24–72 h. Comparing these two cultivars, the expression levels of *DgBRC1* and *DgLsL* in the lateral buds of ‘Jinba’

were always lower than those of 'Fenyan', whereas the level of *DgD14* was always higher in 'Jinba'.



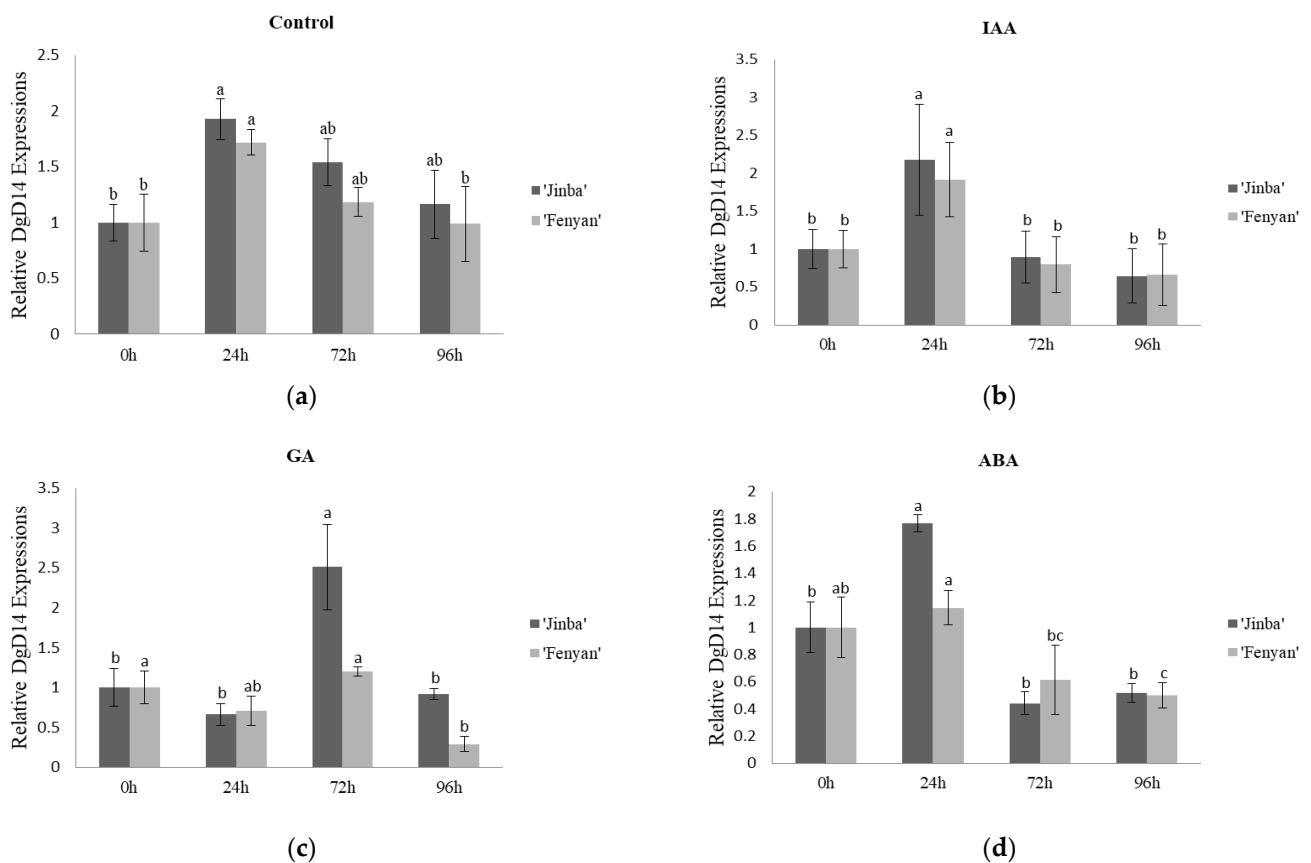
**Figure 3.** Lateral bud phenotypes of 'Fenyan' that were treated with GA, IAA, ABA and distilled water after apical bud removal. Subfigure (a–c) represent 0, 3 and 6 days after treatments.

The results above suggested that removing apical dominance can significantly affect the expression characteristics of three genes to regulate lateral bud formation and elongation. Different genes involved in the regulation of lateral buds responded to the sudden loss of auxin source in different ways after apical bud removal, which may be due to the fact that they were regulated by different plant hormones, reflecting the different changing methods of hormone concentration in the lateral buds.

### 3.3. Effects of Exogenous Hormones on Gene Expression Characteristics

#### 3.3.1. Effects on the Expression Characteristics of DgD14

The results shown in Figure 4 indicated that after removing apical buds and spraying hormones, the responses of *DgD14* to various exogenous hormones were on time and different in the lateral buds of two cultivars of chrysanthemum. However, all of the responses experienced the process of being promoted and then inhibited, which were lower than the level before treatments at 96 h. The expression level in IAA group was slightly higher than that in control group at 24 h, whereas the levels in GA and ABA group were always lower. This suggested that spraying GA and ABA inhibited the expression of *DgD14*, whereas spraying IAA promoted the expression of *DgD14*.

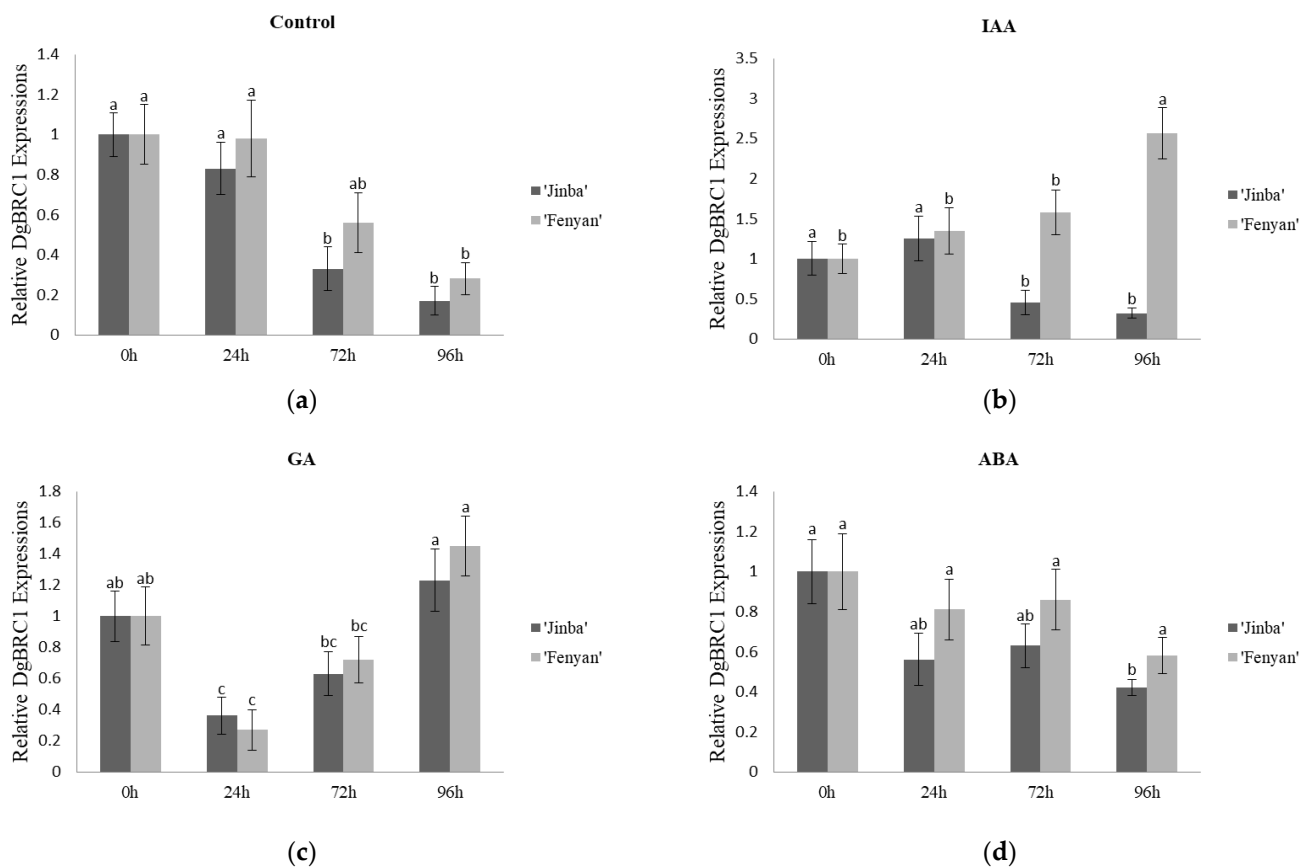


**Figure 4.** Effects of hormones on *DgD14* expression characteristics after apical bud removal. Based on one-way analysis of variance in SPSS 21.0, lowercase letters in subfigure (a–d) represent significant differences in *DgD14* levels after Duncan's test ( $p < 0.05$ ) between control, IAA, GA and ABA groups 0, 24, 72 and 96 h after treatments in 'Jinba' and 'Fenyan'.

In terms of genetic variation, *DgD14* in the lateral buds was strongly responsive to GA, but slightly responsive to ABA after the apical buds were removed. In IAA, ABA and GA groups, the expression levels of *DgD14* in the lateral buds of 'Jinba' were basically higher than those in the lateral buds of 'Fenyan', and the variation ranges were larger. In particular, the expression level of *DgD14* in the lateral buds of 'Jinba' that were sprayed with GA reached about 2.5 times as high as the original value at 72 h.

#### 3.3.2. Effects on the Expression Characteristics of DgBRC1

As shown in Figure 5, the expression levels of *DgBRC1* dramatically decreased in the control groups where lateral buds were sprayed with distilled water. In the experimental groups, *DgBRC1* showed the strongest response to IAA and the gentlest response to ABA.



**Figure 5.** Effects of hormones on *DgBRC1* expression characteristics after apical bud removal. Based on one-way analysis of variance in SPSS 21.0, lowercase letters in subfigure (a–d) represent significant differences in *DgBRC1* levels after Duncan's test ( $p < 0.05$ ) between control, IAA, GA and ABA groups 0, 24, 72 and 96 h after treatments in 'Jinba' and 'Fenyan'.

After spraying GA, the expression levels of *DgBRC1* in two cultivars both decreased from 0 h to 24 h and gradually increased after 24 h, reaching the highest at 96 h. After spraying IAA, the expression level of *DgBRC1* in the lateral buds of 'Jinba' peaked at 24 h and then began to decrease, whereas that in the lateral buds of 'Fenyan' always showed an increasing trend. After spraying ABA, the levels of *DgBRC1* in two cultivars both showed a general declining trend within 4 days but a slight increase during 24–72 h. Moreover, in the three experimental groups, the expression levels of *DgBRC1* in the lateral buds of 'Jinba' were basically lower than those in the lateral buds of 'Fenyan', but the variation range of *DgBRC1* expression level in 'Jinba' was smaller in IAA and GA group and larger in ABA group.

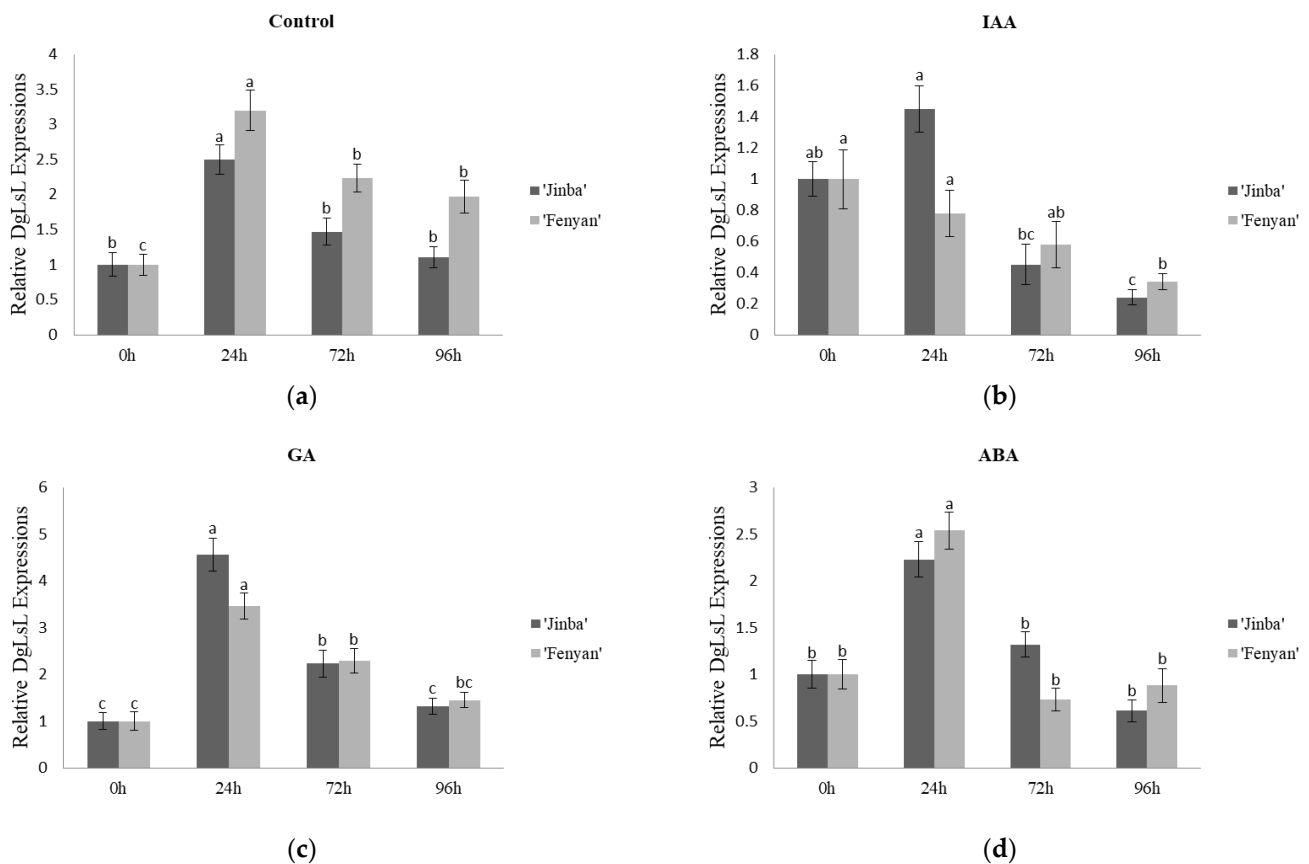
### 3.3.3. Effects on the Expression Characteristics of *DgLsL*

Results shown in Figure 6 revealed that the expression characteristics of *DgLsL* in two cultivars showed differences under different exogenous hormone treatments. In the control group, the expression of *DgLsL* in the lateral buds of 'Jinba' was less than that in the lateral buds of 'Fenyan', and its rangeability was also smaller. At 24 h after spraying IAA and GA, the levels of *DgLsL* in the lateral buds of 'Jinba' were higher than those in the lateral buds of 'Fenyan', but then they decreased, being slightly lower. In addition, the variation range of *DgLsL* level in 'Jinba' that was sprayed with ABA was always smaller than that of 'Fenyan'.

Additionally, there were similarities between the expression levels of *DgLsL* in two cultivars under different exogenous hormones. The levels of *DgLsL* in different groups showed gradual downward trends after peaking at 24 h, except the level in the IAA group, which decreased all of the time. Four days later, the expression levels of *DgLsL* in the GA and control groups were all higher than the original values, whereas those in the IAA and



ABA groups were all less. Moreover, *DgLsL* had the strongest response to GA and the gentlest response to IAA in both cultivars.



**Figure 6.** Effects of hormones on *DgLsL* expression characteristics after apical bud removal. Based on one-way analysis of variance in SPSS 21.0, lowercase letters in subfigure (a–d) represent significant differences in *DgLsL* levels after Duncan's test ( $p < 0.05$ ) between control, IAA, GA and ABA groups 0, 24, 72 and 96 h after treatments in 'Jinba' and 'Fenyan'.

#### 4. Discussion

In recent years, the molecular biology of lateral bud development in chrysanthemums has been progressing rapidly [39]. Auxin can increase the expression of genes in the SLs pathway and locally inhibit the expression of genes in the CKs pathway [40]. CKs and SLs can directly enter the lateral bud to regulate its development [41], and they also have antagonistic effects on the regulation of lateral bud elongation. Therefore, auxin, CKs and SLs can form a large feedback regulation loop to make a stable balance of lateral bud regulation [42]. Besides *DgCCD7* [43], *DgCCD8* [27], *DgBRC1* [7], *DgD14* [8], *DgD27* [44], *DgMAX2* and *DgMAX1*, which belong to the SLs pathway, genes associated with chrysanthemum branching including *DgSZFP*, *CmPIN1* and *DgLsL* were also studied [44].

Spraying GA was the most effective treatment in promoting the growth of lateral buds after removing apical buds, whereas spraying IAA, ABA and distilled water made little difference to the growth of lateral buds. However, to some extent, IAA and ABA could also inhibit the growth of lateral buds compared with distilled water. Removing apical dominance could make *DgD14*, *DgBRC1* and *DgLsL* in the lateral buds respond quickly and affect their expression characteristics, so as to regulate lateral bud formation and elongation. After the removal of apical buds, the expression levels of *DgD14* and *DgLsL* all reached the highest and then decreased gradually, and the level of *DgLsL* varied more significantly. Meanwhile, the level of *DgBRC1* always showed a gradual decline, proving that *DgBRC1* could be downregulated by apical bud removal, and it could be restored and increased after the application of exogenous regulators [32]. It showed that different genes involved

in the regulation of lateral buds responded to the sudden loss of auxin source in different ways after apical bud removal.

*DgD14*, *DgBRC1* and *DgLsL* could respond to different exogenous hormones after the removal of apical dominance, indicating that these three genes were the response regulators of hormones regulating the growth of lateral buds in chrysanthemum. *DgD14* showed a strong response to GA, but a gentle response to ABA. At 24 h after spraying IAA, the expression level of *DgD14* in the lateral bud of chrysanthemum increased, revealing that *DgD14* could be induced by auxin that enhanced the apical dominance and inhibited the growth of lateral buds. It was consistent with the conclusion of previous studies in that it was induced by auxin and regulated by feedback, responding to exogenous auxin quickly [2] and complementing the phenotype of *Arabidopsis thaliana* mutant D14-1 [31]. Meanwhile, spraying GA and ABA further weakened apical dominance and accelerated the growth of lateral buds, which inhibited the expression of *DgD14*.

*DgBRC1* showed the strongest response to IAA and the gentlest response to ABA, and the expression levels of *DgLsL* experienced the increase that followed by decrease under different treatments. The expressions of *DgBRC1* were inhibited in GA groups and promoted in IAA and ABA groups to some extent, which were contrary to those of *DgLsL*, proving that the growth of lateral buds in chrysanthemum was negatively correlated with *DgBRC1* expression, but positively correlated with *DgLsL* expression. Moreover, in the IAA group, the expression level of *DgBRC1* in the lateral bud of 'Fenyan' gradually increased and reached a peak within a certain number of days, which was contrary to the conclusion of previous studies that BRC1 was a suppressive gene directly acting on the lateral bud. This may be because that high planting densities could upregulate the expression of *DgBRC1*, which was able to respond to the application of apical auxin [32].

Comparing the two chrysanthemum cultivars, the expression levels of three genes in the lateral buds of 'Jinba' and 'Fenyan' were different. The expression level of *DgD14* in 'Jinba' in each group was higher than that in 'Fenyan', but the levels of *DgBRC1* and *DgLsL* in 'Jinba' in most groups were lower than those in 'Fenyan', which may be due to the differences in the branching characteristics of different chrysanthemum cultivars. As a spray chrysanthemum cultivar, 'Fenyan' was more branched, and its expression levels of genes which promoted lateral bud formation were higher. In contrast, the apical dominance was more obvious in single-flower chrysanthemum cultivars, thus the genes which regulated lateral bud formation were inhibited in 'Jinba'.

## 5. Conclusions

In this study, the expression differences of *DgD14*, *DgBRC1* and *DgLsL* in *Chrysanthemum morifolium* 'Jinba' and *Chrysanthemum morifolium* 'Fenyan' which were removed the apical buds and sprayed with different hormones were compared, so as to study the responses of three genes to exogenous hormones and the effects of exogenous hormones on the growth of lateral buds. Spraying GA encouraged the growth of lateral buds after removing apical buds, whereas spraying IAA and ABA slightly inhibited their growth. The removal of apical buds could regulate lateral bud formation and elongation by significantly affect the expression characteristics of three genes that responded to it in different ways. The same gene had different responses to different exogenous hormones. GA and ABA led to the decrease in *DgD14* expression, whereas IAA led to the increase, and under different hormones, lateral bud growth was negatively correlated with *DgBRC1* expression and positively correlated with *DgLsL* expression. Meanwhile, due to the differences in branching characteristics among different chrysanthemum cultivars, genes in different cultivars responded differently to exogenous hormones. The expression levels of *DgD14* in 'Jinba' were higher than that in 'Fenyan', but the levels of *DgBRC1* and *DgLsL* in 'Jinba' were generally lower.

**Author Contributions:** Conceptualization, X.-J.W. and A.-N.R.; methodology, C.L.; software, J.-J.S.; validation, J.-J.S., X.L. and Q.-Q.M.; formal analysis, X.-J.W.; resources, B.-B.J.; data curation, C.L. and A.-N.R.; writing—original draft preparation, C.L.; writing—review and editing, C.L.; visualization,

X.-J.W. and A.-N.R.; supervision, B.-B.J.; project administration, Q.-L.L.; funding acquisition, Y.-Z.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by the National Natural Science Foundation of China grant number 31800601 and Innovation Training Program for College Students of Sichuan Agricultural University grant number 040-2021998185.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** All data are contained within the article.

**Conflicts of Interest:** The authors declare no conflict of interest.

### Abbreviations

The following abbreviations are used in this manuscript:

ABA	Abscisic acid
CKs	Cytokinins
GA	Gibberellic acid
IAA	Indole-3-acetic acid
SLs	Strigolactones

### References

- Zhu, W.Y.; Jiang, J.F.; Chen, S.M.; Wang, L.; Xu, L.L.; Wang, H.B.; Li, P.L.; Guan, Z.Y.; Chen, F.D. Intergeneric hybrid between *Chrysanthemum* × *morifolium* and *Artemisia japonica* achieved via embryo rescue shows salt tolerance. *Euphytica* **2013**, *191*, 109–119. [[CrossRef](#)]
- Leyser, O. The control of shoot branching: An example of plant information processing. *Plant Cell Environ.* **2009**, *32*, 694–703. [[CrossRef](#)]
- Wilkins, H.; Anderson, N.O. *Flower Breeding and Genetics*; Springer: Dordrecht, The Netherlands, 2007; pp. 389–437.
- Dong, L.; Ishak, A.; Yu, J.; Zhao, R.; Zhao, L. Identification and functional analysis of three MAX2 orthologs in *Chrysanthemum*. *J. Integr. Plant Biol.* **2013**, *55*, 434–442. [[CrossRef](#)]
- Kubo, T.; Tsuru, M.; Tsukimori, A.; Shizukawa, Y.; Takemoto, T. Morphological and physiological changes in transgenic *Chrysanthemum morifolium* Ramat. ‘Ogura-nishiki’ with *rolC*. *J. Jpn. Soc. Hort. Sci.* **2006**, *75*, 312–317. [[CrossRef](#)]
- Chen, X.; Zhou, X.; Xi, L.; Zhao, R.; Ma, N.; Zhao, L. Roles of *DgBRCL* in regulation of lateral branching in chrysanthemum (*Dendranthema* × *grandiflora* cv. Jinba). *PLoS ONE* **2013**, *8*, 617–717.
- Mehrnia, M.; Balazadeh, S.; Zanol, M.I.; Mueller-Roeber, B. EBE, an AP2/ERF transcription factor highly expressed in proliferating cells, affects shoot architecture in *Arabidopsis*. *Plant Physiol.* **2013**, *162*, 842–857. [[CrossRef](#)]
- Mizoi, J.; Shinozaki, K.; Yamaguchi, S.K. AP2/ERF family transcription factors in plant abiotic stress responses. *Biochim. Biophys. Acta BBA Gene Regul. Mech.* **2012**, *1819*, 86–96. [[CrossRef](#)] [[PubMed](#)]
- Yang, M.; Jiao, Y. Regulation of axillary meristem initiation by transcription factors and plant hormones. *Front. Plant Sci.* **2016**, *7*, 183. [[CrossRef](#)]
- Wang, Y.H.; Li, J.Y. Branching in rice. *Curr. Opin. Plant Biol.* **2011**, *14*, 94–99. [[CrossRef](#)]
- Beveridge, C.A.; Symons, G.M.; Murfet, I.C. The *rms1* mutant of pea has elevated indole-3-acetic acid levels and reduced root-sap zeatin riboside content but increased branching controlled by graft-transmissible signal(s). *Plant Physiol.* **1997**, *115*, 1251. [[CrossRef](#)]
- Xia, X.; Dong, H.; Yin, Y.; Song, X.; Gu, X.; Sang, K.; Zhou, J.; Shi, K.; Zhou, Y.; Foyer, C.H.; et al. Brassinosteroid signaling integrates multiple pathways to release apical dominance in tomato. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2004384118. [[CrossRef](#)]
- Guan, J.C.; Koch, K.E.; Suzuki, M.; Wu, S.; Latshaw, S.; Petruff, T.; Goulet, C.; Klee, H.J.; McCarty, D.R. Diverse Roles of Strigolactone Signaling in Maize Architecture and the Uncoupling of a Branching-Specific Subnetwork. *Plant Physiol.* **2012**, *160*, 1303–1317. [[CrossRef](#)] [[PubMed](#)]
- Domagalska, M.A.; Leyser, O. Signal integration in the control of shoot branching. *Nat. Rev. Mol. Cell Biol.* **2011**, *12*, 211–221. [[CrossRef](#)] [[PubMed](#)]
- Sorefan, K.; Booker, J.; Haurogne, K.; Goussot, M.; Bainbridge, K.; Foo, E.; Chatfield, S.; Ward, S.; Beveridge, C.; Rameau, C.; et al. *MAX4* and *RMS1* are orthologous dioxygenase-like genes that regulate shoot branching in *Arabidopsis* and pea. *Genes Dev.* **2003**, *17*, 1469–1474. [[CrossRef](#)]
- Dun, E.A.; Ferguson, B.J.; Beveridge, C.A. Apical Dominance and Shoot Branching. Divergent Opinions or Divergent Mechanisms? *Plant Physiol.* **2006**, *142*, 812–819. [[CrossRef](#)]
- Thimann, K.V.; Skoog, F. Studies in the growth hormone of plants.III. The inhibiting action of the growth substance on bud development. *Proc. Natl. Acad. Sci. USA* **1933**, *19*, 714–716. [[CrossRef](#)]

18. Jones, B.; Gunneras, S.A.; Petersson, S.V.; Tarkowski, P.; Graham, N.; May, S.; Dolezal, K.; Sandberg, G.; Ljung, K. Cytokinin regulation of auxin synthesis in Arabidopsis involves a homeostatic feedback loop regulated via auxin and cytokinin signal transduction. *Plant Cell* **2010**, *22*, 2956–2969. [[CrossRef](#)] [[PubMed](#)]
19. Umehara, M.; Hanada, A.; Yoshida, S.; Akiyama, K.; Arite, T.; Takeda-Kamiya, N.; Magome, H.; Kamiya, Y.; Shirasu, K.; Yoneyama, K.; et al. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **2008**, *455*, 195–200. [[CrossRef](#)] [[PubMed](#)]
20. Tanaka, M.; Takei, K.; Kojima, M.; Sakakibara, H.; Mori, H. Auxin controls local cytokinin biosynthesis in the nodal stem in apical dominance. *Plant J.* **2006**, *45*, 1028–1036. [[CrossRef](#)]
21. Müller, D.; Leyser, O. Auxin, cytokinin and the control of shoot branching. *Ann. Bot.* **2011**, *107*, 1203–1212. [[CrossRef](#)]
22. Kyozuka, J. Control of shoot and root meristem function by cytokinin. *Curr. Opin. Plant Biol.* **2007**, *10*, 442–446. [[CrossRef](#)]
23. McSteen, P. Hormonal regulation of branching in grasses. *Plant Physiol.* **2009**, *149*, 46–55. [[CrossRef](#)]
24. Khodakovskaya, M.; Vankova, R.; Malbeck, J.; Li, A.Z.; Li, Y.; McAvoy, R. Enhancement of flowering and branching phenotype in chrysanthemum by expression of ipt under the control of a 0.821 kb fragment of the LEACOI gene promoter. *Plant Cell Rep.* **2009**, *28*, 1351–1362. [[CrossRef](#)]
25. Schmitz, G.; Theres, K. Genetic control of branching in Arabidopsis and tomato. *Curr. Opin. Plant Biol.* **1999**, *2*, 51–55. [[CrossRef](#)]
26. Kohlen, W.; Charnikhova, T.; Liu, Q.; Bours, R.; Domagalska, M.A.; Beguerie, S.; Verstappen, F.; Leyser, O.; Bouwmeester, H.; Ruyter-Spira, C. Strigolactones are transported through the xylem and play a key role in shoot architectural response to phosphate deficiency in nonarbuscular mycorrhizal host Arabidopsis. *Plant. Physiol.* **2011**, *155*, 974–987. [[CrossRef](#)] [[PubMed](#)]
27. Alder, A.; Jamil, M.; Marzorati, M. The path from  $\beta$ -carotene to carlactone, a strigolactone-like plant hormone. *Science* **2012**, *335*, 1348–1351. [[CrossRef](#)]
28. Zhou, F.; Lin, Q.; Zhu, L. D14-SCF(D3)-dependent degradation of D53 regulates strigolactone signaling. *Nature* **2013**, *504*, 406–410, Corrigendum in **2016**, *532*, 402. [[CrossRef](#)]
29. Yao, R.; Ming, Z.; Yan, L.; Li, S.; Wang, F.; Ma, S.; Yu, C.; Yang, M.; Chen, L.; Chen, L.; et al. DWARF14 is a non-canonical hormone receptor for strigolactone. *J. Nature.* **2016**, *536*, 469–473. [[CrossRef](#)]
30. Wen, C.; Xi, L.; Gao, B.; Wang, K.Y.; Lv, S.H.; Kou, Y.P.; Ma, N.; Zhao, L.J. Roles of DgD14 in regulation of shoot branching in chrysanthemum (*Dendranthema grandiflorum* ‘Jinba’). *Plant Physiol. Biochem.* **2015**, *96*, 241–253. [[CrossRef](#)] [[PubMed](#)]
31. Imai, A.; Takahashi, S.; Nakayama, K.; Satoh, H. The promoter of the carotenoid cleavage dioxygenase 4a-5 gene of Chrysanthemum morifolium (CmCCD4a-5) drives petal-specific transcription of a conjugated gene in the developing flower. *J. Plant Physiol.* **2013**, *170*, 1295–1299. [[CrossRef](#)] [[PubMed](#)]
32. Deruiter, H.A. Development of chrysanthemum cuttings: The influence of age and position of the ancillary buds. *Ann. Bot.* **1996**, *77*, 99–104. [[CrossRef](#)]
33. Schumacher, K.; Schmitt, T.; Rossberg, M.; Schmitz, G.; Theres, K. The lateral suppressor (*Ls*) gene of tomato encodes a new member of the VHIID protein family. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 290–295. [[CrossRef](#)]
34. Tucker, D.J. Endogenous growth regulators in relation to side shoot development in the tomato. *New Phytol.* **1976**, *77*, 561–568. [[CrossRef](#)]
35. Yang, D.H.; Yun, P.Y.; Park, S.Y.; Plaha, P.; Lee, D.S.; Lee, I.S.; Hwang, Y.S.; Kim, Y.A.; Lee, J.S.; Han, B.H. Cloning, characterization and expression of a Lateral suppressor-like gene from chrysanthemum (*Dendranthema grandiflorum* Kitamura). *Plant Physiol. Biochem.* **2005**, *43*, 1044–1051. [[CrossRef](#)] [[PubMed](#)]
36. Bessonov, N.; Morozova, N.; Volpert, V. Modeling of Branching Patterns in Plants. *Bull. Math. Biol.* **2008**, *70*, 868–893. [[CrossRef](#)]
37. Jiang, B.B.; Miao, H.B.; Chen, S.M.; Zhang, S.M.; Chen, F.D.; Fang, W.M. The Lateral Suppressor-Like Gene, DgLsL, Alternated the Axillary Branching in Transgenic Chrysanthemum (*Chrysanthemum*  $\times$  morifolium) by Modulating IAA and GA Content. *Plant Mol. Biol. Rep.* **2010**, *28*, 144. [[CrossRef](#)]
38. Xu, J.; Ding, C.; Ding, Y.; Tang, S.; Zha, M.; Luo, B.; Wang, S. A Proteomic Approach to Analyze Differential Regulation of Proteins During Bud Outgrowth Under Apical Dominance Based on the Auxin Transport Canalization Model in Rice (*Oryza sativa* L.). *J. Plant Growth Regul.* **2015**, *34*, 122–136. [[CrossRef](#)]
39. Brewer, P.B.; Dun, E.A.; Ferguson, B.J.; Rameau, C.; Beveridge, C.A. Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and *Arabidopsis*. *Plant Physiol.* **2009**, *150*, 482–493. [[CrossRef](#)]
40. Johnson, X.; Brcich, T.; Dun, E.A.; Goussot, M.; Haurogné, K.; Beveridge, C.A.; Rameau, C. Branching genes are conserved across species. Genes controlling a novel signal in pea are co-regulated by other long-distance signals. *Plant Physiol.* **2006**, *142*, 1014–1026. [[CrossRef](#)]
41. Liu, W.; Wu, C.; Fu, Y.; Hu, G.; Si, H.; Zhu, L.; Luan, W.; He, Z.; Sun, Z. Identification and characterization of HTD2: A novel gene negatively regulating tiller bud outgrowth in rice. *Planta* **2009**, *230*, 649–658. [[CrossRef](#)] [[PubMed](#)]
42. Gao, C.; Li, P.; Song, A.; Wang, H.; Wang, Y.; Ren, L.; Qi, X.; Chen, F.; Jiang, J.; Chen, S. Isolation and characterization of six AP2/ERF transcription factor genes in Chrysanthemum nankingense. *Int. J. Mol. Sci.* **2015**, *16*, 2052–2065. [[CrossRef](#)] [[PubMed](#)]
43. Xi, L.; Wen, C.; Fang, S.; Chen, X.; Nie, J.; Chu, J.; Yuan, C.; Yan, C.; Ma, N.; Zhao, L. Impacts of strigolactone on shoot branching under phosphate starvation in chrysanthemum (*Dendranthema grandiflorum* cv. Jinba). *Front. Plant Sci.* **2015**, *6*, 694. [[CrossRef](#)] [[PubMed](#)]
44. Beveridge, C.A.; Ross, J.J.; Murfet, I.C. Branching in pea: Action of genes Rms3 and Rms4. *Plant Physiol.* **1996**, *110*, 859–865. [[CrossRef](#)] [[PubMed](#)]