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ORIGINAL ARTICLE

Elevated gibberellin altered morphology, anatomical structure, and transcriptional regulatory networks of hormones in celery leaves

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Abstract

Gibberellins (GAs), as one of the important hormones in regulating the growth and development of higher plants, can significantly promote cell elongation and expansion. Celery is a widely grown leafy vegetable crop with rich nutritional value. However, the effect of gibberellins on celery leaves is unclear. In this paper, the celery variety "Jinnan Shiqin" plants were treated with gibberellic acid (GA_3) and paclobutrazol (PBZ, a gibberellin inhibitor). Our results showed that GA_3 treatment promoted the growth of celery leaves and caused lignification of celery leaf tissue. In addition, the transcript levels of genes associated with gibberellins, auxin, cytokinins, ethylene, jasmonic acid, abscisic acid, and brassinolide were altered in response to increased or decreased exogenous gibberellins or inhibitor. GA_3 may regulate celery growth by interacting with other hormones through crosstalk mechanisms. This study provided a reference for further study of the regulation mechanism of gibberellins metabolism, and exerted effects on understanding the role of gibberellins in the growth and development of celery.

Keywords Gibberellin · Anatomic · Hormone interaction · Development · Leaves · *Apium graveolens* L

Abbreviations

CPS	*ent*-copalyl diphosphate synthase
GA	Gibberellin
GA_3	Gibberellic acid
GA20ox	GA20-oxidase
GA2ox	GA2-oxidase
GA3ox	GA3-oxidase
GGPP	Geranylgeranyl diphosphate
GID1	Gibberellin insensitive dwarf1
IPP	Isopentenyl pyrophosphate
KAO	*ent*-kaurenoic acid oxidase
KO	*ent*-kaurene oxidase
KS	*ent*-kaurene synthase
PBZ	Paclobutrazol
RT-qPCR	Quantitative real-time polymerase chain reaction
UV	Ultraviolet
SHI	Short internode
SLY1	Sleepy1
SPY	Spindly

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Introduction

Plant hormones play an important role in plant growth and stress response. Among them, gibberellin is a kind of biguanide compound, which is one of the essential hormones for plant growth and development (Achard et al. 2009; Ubeda-Tomás et al. 2009; Gao et al. 2011; Wang et al. 2015a; Zhuang et al. 2015). Up to now, more than 130 different types of gibberellins have been discovered and identified from different plants, but only a few have physiological activity (Silverstone and Sun 2000). The regulation of exogenous gibberellins and its mechanism provide a theoretical basis for crop yield increase and quality improvement.

Gibberellins synthesis and metabolic pathways have been extensively studied. In higher plants, isopentenyl pyrophosphate (IPP) synthesizes the precursor of gibberellin (GA) biosynthesis geranylgeranyl diphosphate (GGPP) (Hedden and Phillips 2000). The GA synthesis

process is mainly divided into three phases, completing in different subcellular structures. The first stage is completed in the plastid, and GGPP is catalyzed by *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS) to the *ent*-kaurene, the precursor of gibberellin. In the second stage, *ent*-kaurene synthesizes GA_{12}-aldehyde under the action of *ent*-kaurene oxidase (KO) and *ent*-kaurenoic acid oxidase (KAO). In the final stage, GA_{12}-aldehyde was converted to other GA species by the action of GA20-oxidase (GA20ox), GA3-oxidase (GA3ox), and GA2-oxidase (GA2ox) (Hedden 2001; Wang, et al. 2015b). Commercially available GA, which is widely used in agricultural production and horticulture, is mainly a mixture of GA_3 and GA_7 isolated from gibberella.

The CPS and KS of *Arabidopsis thaliana* are encoded by a single copy of the *GA_1* and *GA_2* genes, and the KO is encoded by the *GA_3* gene. GA deletion mutant results in a severely dwarfed phenotype of *Arabidopsis* (Helliwell et al. 2001). GA3ox catalyzes GA_9 and GA_{20} into biologically active GA_4 and GA_1, respectively (Hedden and Phillips 2000). Paclobutrazol (PBZ) is a type of GA biosynthesis inhibitor that primarily delays the activity of KO and some monooxygenases. As one of the important hormones regulating higher plants, GA is affected by many factors and is strictly controlled in time and space (Ross and O'Neill 2002; Wolbang et al. 2004).

Previous studies have shown that other hormones are also involved in the regulation of GA biosynthesis. Auxin was reported to regulate the growth and development of plants by regulating the biosynthesis of GA (Olszewski et al. 2002). Studies in peas and tobacco have found that treatment with exogenous auxin transport inhibitors reduces the amount of GA_1 in stems (Wolbang and Ross 2001; Ross et al. 2010). Exogenous GA treatment can partially restore the height phenotype of ethylene mutant *ctr1* plant (Achard et al. 2006). GA promotes cell elongation required BR (brassinosteroid) signaling and BR-activated BRASSINAZOLE RESISTANT1 (BZR1). BR mutant lacks intranuclear BZR1; GA-induced DELLA degradation does not increase BZR1 activity and thus does not promote cell elongation (Gallego-Bartolomé et al. 2012; Bai et al. 2012). BR also plays a positive role in the regulation of *GA20ox1* expression level at special stage of plant growth and development.

Celery is a biennial herb of the Apiaceae family, which is a nutrient-rich leafy vegetable (Li et al. 2018). The molecular research of celery is lagging, and there are few reports on the expression of genes related to gibberellins metabolism and signal transduction. In this study, morphological, anatomical characteristics, and hormonal interactions were analyzed to fully elucidate the effects of applied GA_3 on leaf growth. This study helped further elucidate the roles of GA in celery growth.

Materials and methods

Plant material and GA_3 and PBZ treatments

The seeds of celery "Jinnan Shiqin" were planted in the climate-controlled chamber at the state key laboratory of crop genetics and germplasm enhancement in Nanjing Agricultural University (32° 04′ N, 118° 85′ E). The climate chamber was set at 25 °C for 16 h during the day and at 18 °C for 8 h during the night. The celery plants were planted in a plastic pot (18 × 18 × 16 cm) mixed with vermiculite, organic soil, and perlite compounds (volume ratio of 2:2:1). After 70 DAS (days after sowing), 100 mL of GA_3 (150 mg L^{-1}), PBZ (20 mg L^{-1}), or a mixture of the both was sprayed onto the plants respectively. Plants sprayed with aqueous solution used as the control. The treatments were performed every 4 days for a total of four treatments. Three biological replicates were tested and samples were collected and stored at − 80 °C.

Anatomical structure analysis

The fresh sample was cut into slices and stored in phosphate buffer solution (pH 7.2) including 2.5% glutaraldehyde at 4 °C. The samples were subjected to safranin-*O*/fast green staining, and the morphological structure of the plant cells was observed, and the lignified parts were stained red (Wang, et al. 2016). The lignified cell wall was autofluorescent under ultraviolet (UV) fluorescence microscopy; the degree of lignification of celery leaves was observed under different treatments. Three independent biological replicates of each celery plant sample were prepared for histochemical staining.

Total RNA isolation and cDNA synthesis

Total RNA from celery leaf blades and petioles was extracted according to the requirements of the RNA extraction kit (Tiangen, Beijing, China). RNA was reverse transcripted into cDNA using a reverse transcription kit (Vazyme Biotech, Nanjing, China), and the cDNA was diluted 15-fold in deionized water for RT-qPCR analysis.

Gene expression analysis by RT-qPCR

Genes involved in GA, auxin, cytokinins, ethylene, jasmonic acid, abscisic acid, and brassinolide pathways were selected from CeleryDB (Feng et al. 2018). Specific primers were designed using Primer Premier 6.0 for RT-qPCR and submitted to Nanjing Genscript Inc. for synthesis. *AgACTIN* was used as a standard analysis of the results of the celery reference gene (Li et al. 2014, 2016). RT-qPCR analysis was performed according to TaKaRa SYBR Premix *Ex Taq* (Takara, Dalian, China) using a 15-μL system with 1.5 μL of diluted cDNA,

0.3 μL of forward and reverse primer pairs, 7.5 μL of SYBR Premix *Ex Taq*, and 5.4 μL of ddH_2O. Each reaction performed in three biological repeats. The operating procedure for RT-qPCR is 95 °C for 30 s, 40 cycles for 5 s at 95 °C, followed by 60 °C for 30 s, and melting curve analysis, increasing 0.5 °C at 5 s intervals. The relative expression of selected gene was calculated by formula $2^{-\Delta\Delta Ct}$ method (Pfaffl 2001). The primer sequences of the genes are shown in Tables 1 and 2.

Statistical analysis

Duncan method was applied to analyze difference at the 0.05 significance level using the SPSS statistics software.

Results

Effects of GA_3 or PBZ treatment on the growth of celery plants

In order to investigate the effects of exogenous GA_3 or PBZ treatment on the growth of celery, the celery leaves treated with GA_3 or PBZ were collected. The phenotypes of "Jinnan Shiqin" under different treatments were shown in Fig. 1. The number of celery leaf blades treated with GA_3 was the highest, which was 1.035-fold than that of the control. The number of celery leaves treated with PBZ was the lowest, which were 1.035- and 0.779-fold than that of the control (Fig. 2). The co-treatment with GA_3 and PBZ caused a phenotype similar to that of the control, which was the intermediate between the GA_3 and the PBZ-treated plants. Compare with other treatments, the area and the number of celery leaf blade treated with GA_3 were the highest, which were 1.028- and 1.035-fold than that of the control, respectively (Fig. 3).

The celery leaves were evaluated by measuring the lengths and fresh weights (Figs. 4 and 5). The length of the petiole between the different treatments was not obviously different. The length of the petiole under GA_3 + PBZ treatment was 1.077-fold than that of the PBZ treatment. Compared with the control, the length of the petiole under GA_3 treatment increased, and the effect was also observed when GA_3 was applied together with PBZ. The length of the petiole under GA_3, PBZ, and the GA_3 + PBZ were 1.013-, 0.991-, and 1.066-fold than that of the control, respectively. Treatment with GA_3 increased the leaf weight of celery, but this

Table 1 Primer sequences for gibberellin biosynthetic and signaling pathway-related genes used for RT-qPCR

Gene	Molecular function	Gene ID in celery	Primer sequences (forward/reverse)
AgKS	*ent*-kaurene synthase	comp30605_c1_seq4	TCTTGCTGTTGTGGTGGATGAC/ TCACATTGCGTGCTTGCCATT
AgKO	*ent*-kaurene oxidase	comp16392_c0_seq1	ACCACCCTCAAATCTCCCTCCT/ CACCACAGCCTCCTTGACAACA
AgKAO	*ent*-kaurenoic acid oxidase	comp34313_c0_seq1	GGCGGGCTAATGGGTGGTTT/ AGGAATCAGGATTGCCGGACTT
AgGA20ox1	Gibberellin 20-oxidase	comp15598_c0_seq2	CCGACAACGACGAGGCATTAGT/ GCACTTGAAGCTGCGGAGGAT
AgGA20ox2	Gibberellin 20-oxidase	comp15387_c0_seq1	CCGACAACGACGAGGCATTAGT/ GCACTTGAAGCTGCGGAGGAT
AgGA3ox1	Gibberellin 3-oxidase	comp19536_c0_seq1	AGACGGAGCTAGTGGTTATGGC/ GCGAGCATGTTGGAGAGGAG AA
AgGA2ox1	Gibberellin 2-oxidase	comp20311_c0_seq2	TCCAGTTCCAGCAGACCAAGAC/ TTTGCCAATACCCTGTGCCTCA
AgGA2ox2	Gibberellin 2-oxidase	comp9471_c0_seq1	TCACAGAACTCCCGCACCATT/ ACTTGAACCCAGGTCCCATCTT
AgGA2ox3	Gibberellin 2-oxidase	comp37697_c0_seq1	TCCAGTTCCAGCAGACCAAGAC/ TTTGCCAATACCCTGTGCCTCA
AgGID1b	Gibberellin receptor GID1B	comp5356_c0_seq2	TACCTTCTGTCGCCGCCTTGT/ TCCTTCCGCTCTTCACCACCAA
AgGID1c	Gibberellin receptor GID1C	comp8136_c0_seq1	ACGAGCCTGCTGACCCGAAT/ TCCGCCGAACATTGGATTGAGT
AgDELLA	DELLA protein GAI	comp870_c0_seq1	TGAACTCAACCCGCAACAACCT/ CCGCAACCGCCTGTAATGGAA
AgSLY1	F-box protein GID2	comp1078_c0_seq2	GGCACCGATTCCGATCATCTCT/ GCGAGTACAACAGAACGAAGCT
AgSPY	UDP-N-acetylglucosamine–peptide N-acetylglucosaminyltransferase SPINDLY	comp10213_c0_seq1	ACTGCGTGTTGACCTTCTTCCT/ TTGTTCCTGAGGCGGCTGCT
AgGAMYB	Transcription factor GAMYB	comp36468_c0_seq1	TGAGGAAGCAACCAGTGAAGGA/ CCCAGCGAAGACGGCAACTT
AgSHI	Short internodes	comp25432_c0_seq1	TGGAGCCACTGCTGGTTCCT/ ATTCCACCGCCTCCTCCTGTAG

Table 2 Primer sequences implicated in auxin, cytokinins, ethylene, jasmonic acid, abscisic acid, and brassinolide biosynthesis

Gene	Molecular function	Gene ID in celery	Primer sequences (forward/reverse)
AgABAH1	Cytochrome P450 707A1	comp18056_c0_seq1	ACCAAGGACCTTACCATGCCAA/ TTGTGTCACGGGCTGCGAAA
AgTSB	Tryptophan synthase beta chain 2	comp33547_c0_seq1	GCCTTCTCCTCCACAACTTCCA/ GAACACCAAGACCCGACCCATT
AgDWF7	Cytochrome P450 90B1	comp16112_c0_seq2	GCCGCATGTCATAGCACTCTTC/ TGCCAAACATCCAGTCCATCCA
AgLOG1	Cytokinin riboside 5′-monophosphate phosphoribohydrolase	comp26836_c2_seq2	ATTGCCTTGCCAGGTGGTTATG/ GCTGCTCTGTCTCCCAGTTCAA
AgLOG3	Cytokinin riboside 5′-monophosphate phosphoribohydrolase	comp26708_c1_seq1	AACAAGAAGCAGCAGCAGCAAT/ ACCTCCTCCATACACCAAGTCG
AgSAMS2	S-adenosylmethionine synthase	comp24580_c1_seq2	GAGAGGTGGCAACGGAAGGTT/ GGCTGAGAGCAGGCAATACC AA
AgJMT	Jasmonic acid carboxyl methyltransferase	comp10632_c0_seq1	TCTGGAGGATGTGCTGTGCTTA/ CCTAACCGCCATTGCCACTG

phenomenon was alleviated when GA_3 and PBZ were applied simultaneously. The root weights of the GA_3 + PBZ-treated plants were the intermediate between the GA_3 and the PBZ-treated plants, which were 0.381- and 1.305-fold than that of GA_3 and PBZ, respectively. In general, treatment with GA_3 stimulated the leaf growth of celery.

Anatomical structure analysis of celery under GA_3 treatments

In order to clarify the effect of exogenous GA_3 or PBZ treatment on the development of celery leaf tissue, the anatomical structure of celery leaves was visualized by safranin-*O*/fast green staining and observed by microscopic technique (Fig. 6). Among them, the distribution of epidermal cells, palisade tissue, sponge tissue, and vascular bundles were clearly observed. Lignin is mainly distributed in the epidermis, collenchyma, and vascular bundles. The palisade tissue and sponge tissue composed the tissue structure of celery leaves. In this study, the degrees of lignification in celery leaves were distinct under different treatment conditions. The accumulation of lignin in vascular bundles was most obvious under GA_3 treatment, especially in petiole, showing a distinct purple-red color. Sponge tissue (St) and palisade tissue (Pt) were also arranged more closely, and these results were consistent with observations under UV fluorescence (Fig. 7). Anatomical structure analysis showed that the applied GA_3 resulted in significant lignification of the xylem of celery leaves.

Effects of GA_3 treatment on the expression levels of GA biosynthetic genes

AgKS, *AgKO*, *AgKAO*, *AgGA20ox1*, *AgGA20ox2*, *AgGA3ox1*, *AgGA2ox1*, *AgGA2ox2*, and *AgGA2ox3* genes were annotated as GA biosynthetic pathway-related genes based on celery genome database. To illustrate the effects of applied GA_3 on GA biosynthesis, we selected these genes and

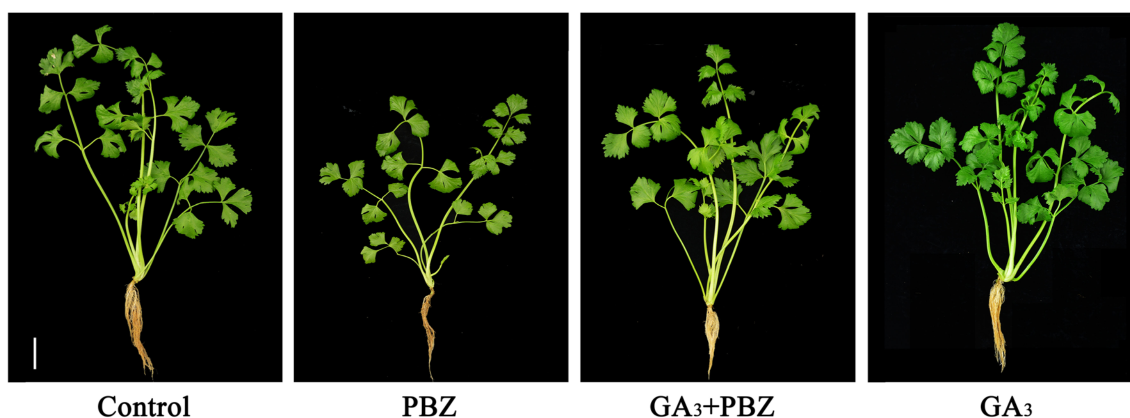

Fig. 1 Effects of applied GA_3, PBZ or GA_3 + PBZ on celery growth. White line in the lower left corner of plant represents 5 cm in that pixel

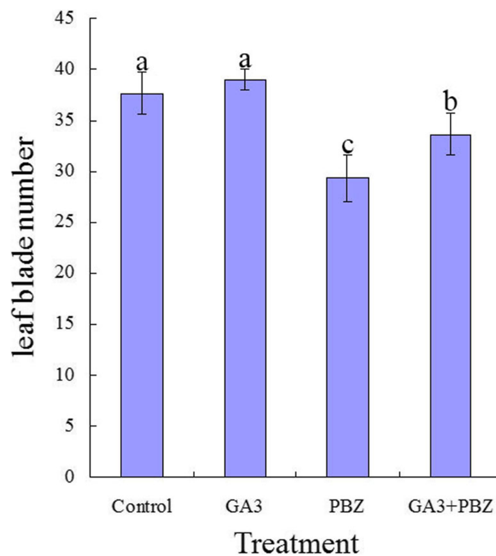

Fig. 2 Effect of applied GA_3, PBZ, or GA_3 + PBZ on number of leaf blades in celery. Bars represent the mean values of three biological replicates ± standard deviation. Different letters in columns indicate statistically significant differences ($P < 0.05$)

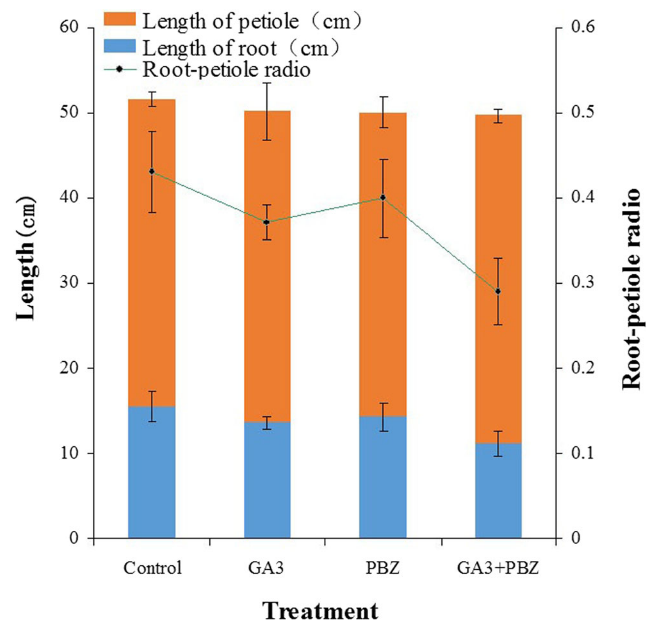

Fig. 4 Characteristics of length of root (blue columns) and petiole (red columns) and root-petiole ratio (black round) in celery. Bars represent the mean values of three biological replicates ± standard deviation. Different letters in columns indicate statistically significant differences ($P < 0.05$)

detected the changes in the expression profiles of these genes by RT-qPCR (Table 1; Fig. 8). Compared with the control, exogenous GA_3 treatment promoted the transcription levels of *AgKO*, *AgKAO*, *AgGA20ox1*, *AgGA20ox2*, *AgGA3ox1*, *AgGA2ox1*, and *AgGA2ox3* in leaf blades, but decreased the transcription levels of *AgKS* and *AgGA2ox2*. Similarly, under the treatment of GA_3 + PBZ, the expression levels of *AgKS* decreased, and the expression levels of *AgKAO*, *AgGA20ox1*, *AgGA20ox2*, *AgGA3ox1*, *AgGA2ox1*, *AgGA2ox2*, and *AgGA2ox3* increased. Under PBZ treatment, the expression levels of *AgKS*, *AgKO*, *AgGA3ox1*, and *AgGA2ox2* decreased. Among them, *AgKO* and *AgGA3ox1* showed upregulation under GA_3 treatment and reached the highest expression

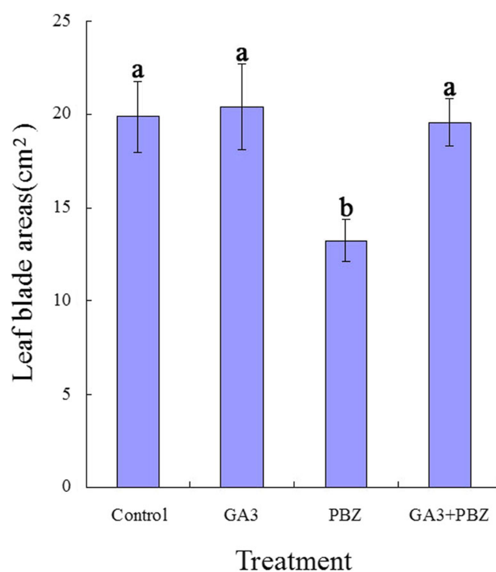

Fig. 3 Effect of applied GA_3, PBZ, or GA_3 + PBZ on leaf blade areas in celery. Bars represent the mean values of three biological replicates ± standard deviation. Different letters in columns indicate statistically significant differences ($P < 0.05$)

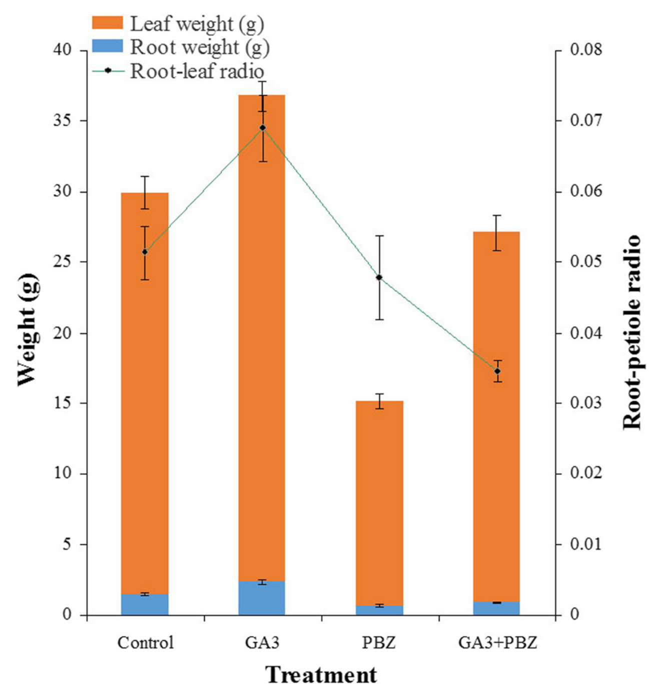

Fig. 5 Characteristics of fresh weight of root (blue columns) and leaf (red columns) and root-leaf ratio (black round) in celery. Bars represent the mean values of three biological replicates ± standard deviation. Different letters in columns indicate statistically significant differences ($P < 0.05$)

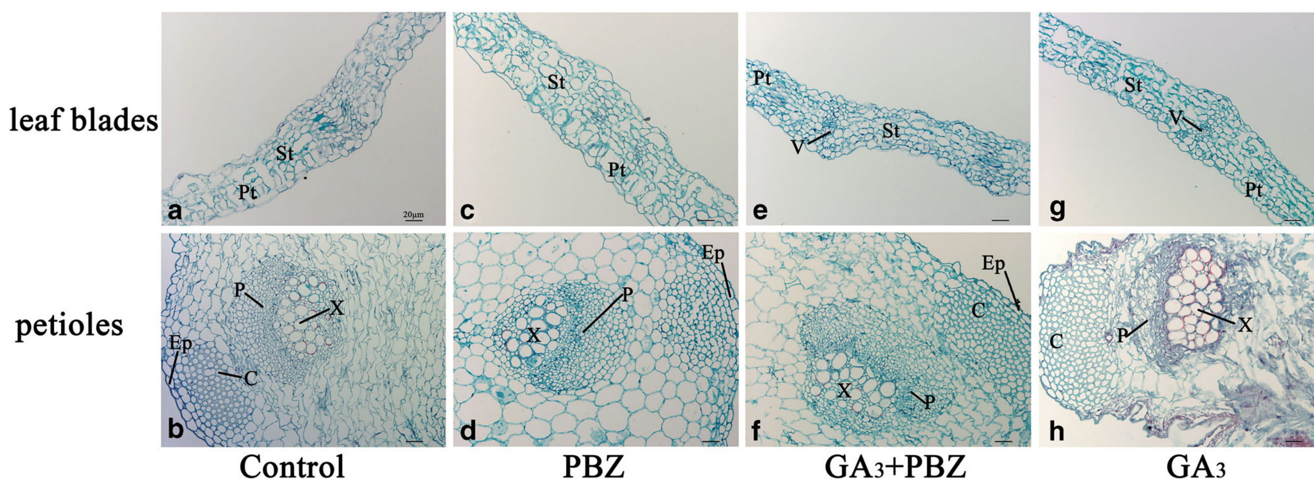

Fig. 6 Effects of applied GA_3, PBZ or GA_3 + PBZ on the anatomical structure in celery leaves (leaf blades and petioles). C, collenchyma; Ep, epidermis; X, xylem; V, vascular bundles; Pt, palisade tissue; St, spongy tissue. Black lines in the lower right corner of the figure **a**–**h** panels are 20 μm in length

levels, which were 2.532- and 2.182-fold than that of the GA_3 + PBZ, respectively.

In the petiole, exogenous GA_3 upregulated the expression levels of *AgGA20ox1*, *AgGA20ox2*, *AgGA2ox*, and *AgGA2ox3* and downregulated the mRNA abundance of *AgKS*, *AgKO*, *AgKAO*, *AgGA3ox1*, and *AgGA2ox1*. Under GA_3 + PBZ treatment, the expression levels of *AgGA20ox2* in leaf blade and petiole markedly increased to the highest value, which was 11.844- and 16.495-fold than that of the control, respectively. In addition, the expression levels of the *AgGA20ox1* and *AgGA20ox2* were high and peaked at the treatment of GA_3 + PBZ treatment, which were 7.989- and 9.940-fold than that of the GA_3, respectively. Under different treatments, the expression levels of *AgGA20ox1* and *AgGA20ox2* in leaves showed consistent characteristics. Overall, for most GA biosynthetic genes, exogenous GA_3 treatment upregulated its expression in leaves, and on the other hand, treatment with GA_3 + PBZ promoted its expression in the petiole.

Effects of GA_3 or PBZ treatment on the expression levels of GA response genes

There is a molecular network of GA signal transduction in plants. When the gibberellin receptor senses the GA signal, it activates the signal transduction pathway and regulates the expression of downstream genes, thereby affecting plant growth and morphogenesis. The genes encoding gibberellin receptors and important components of signal transduction, such as *AgGID1b*, *AgGID1c*, *AgDELLA*, *AgSLY1*, *AgSPY*, *AgGAMYB*, and *AgSHI*, were examined in the celery. These genes were used to initially analyze the effect of GA_3 or PBZ treatment on the response of GA signals (Table 1; Fig. 9). In the leaf blade, exogenous GA_3 downregulated the expression

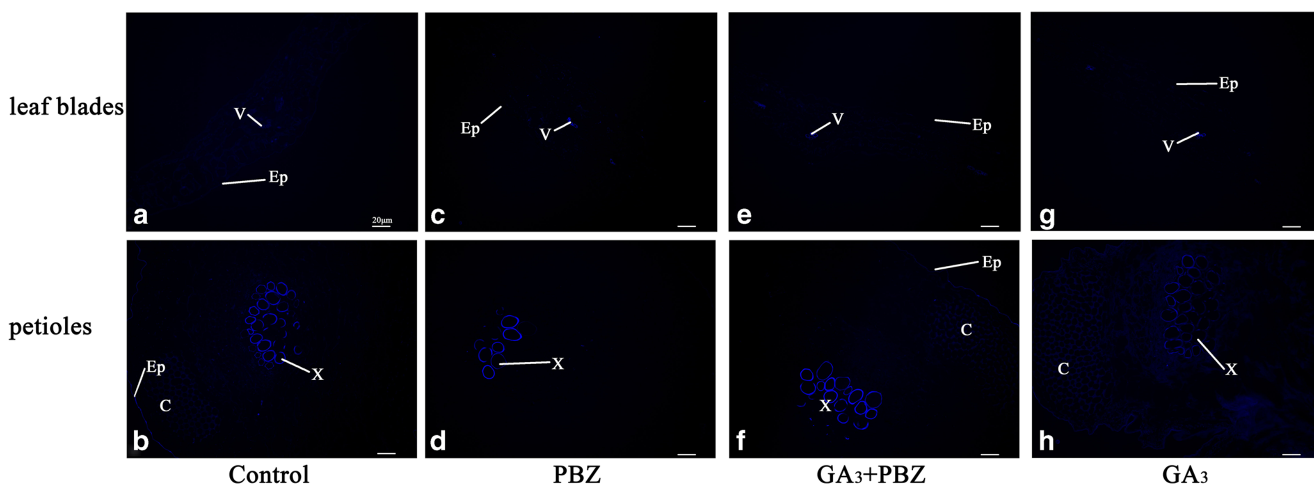

Fig. 7 Fluorescence micrographs of transverse sections of celery leaves (leaf blades and petioles) treated with GA_3, PBZ or GA_3 + PBZ. C, collenchyma; Ep, epidermis; X, xylem; V, vascular bundles; Pt, palisade tissue; St, spongy tissue. Scale bars in the figure are 20 μm in length. White lines in the lower right corner of the figure **a**–**h** panels are 20 μm in length

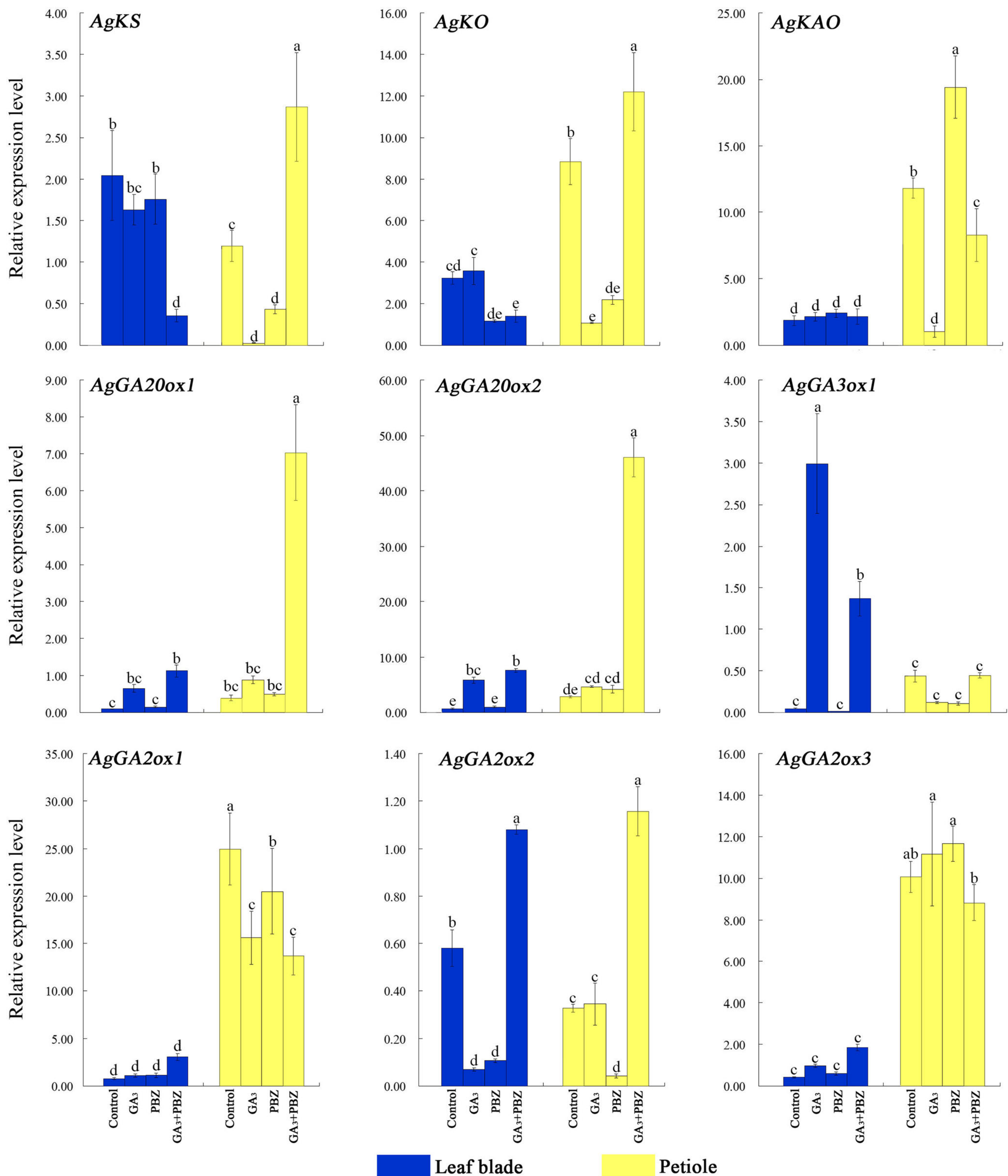

Fig. 8 Effects of GA_3, PBZ, or GA_3 + PBZ on the expression levels of GA pathway-related genes. Bars represent the mean values of three biological replicates ± standard deviation. Different letters in columns indicate statistically significant differences ($P < 0.05$)

levels of *AgGID1b*, *AgGID1c*, *AgSPY*, and *AgGAMYB* and upregulated the expression levels of *AgDELLA*, *AgSHI*, and *AgSLY1*. In the petiole, the expression levels of *AgGID1b*, *AgGID1c*, *AgSPY*, and *AgGAMYB* under GA_3 treatment decreased, and the expressions levels of *AgDELLA*, *AgSLY1*, *AgGAMYB*, *AgSPY*, and *AgSHI* reached the highest value

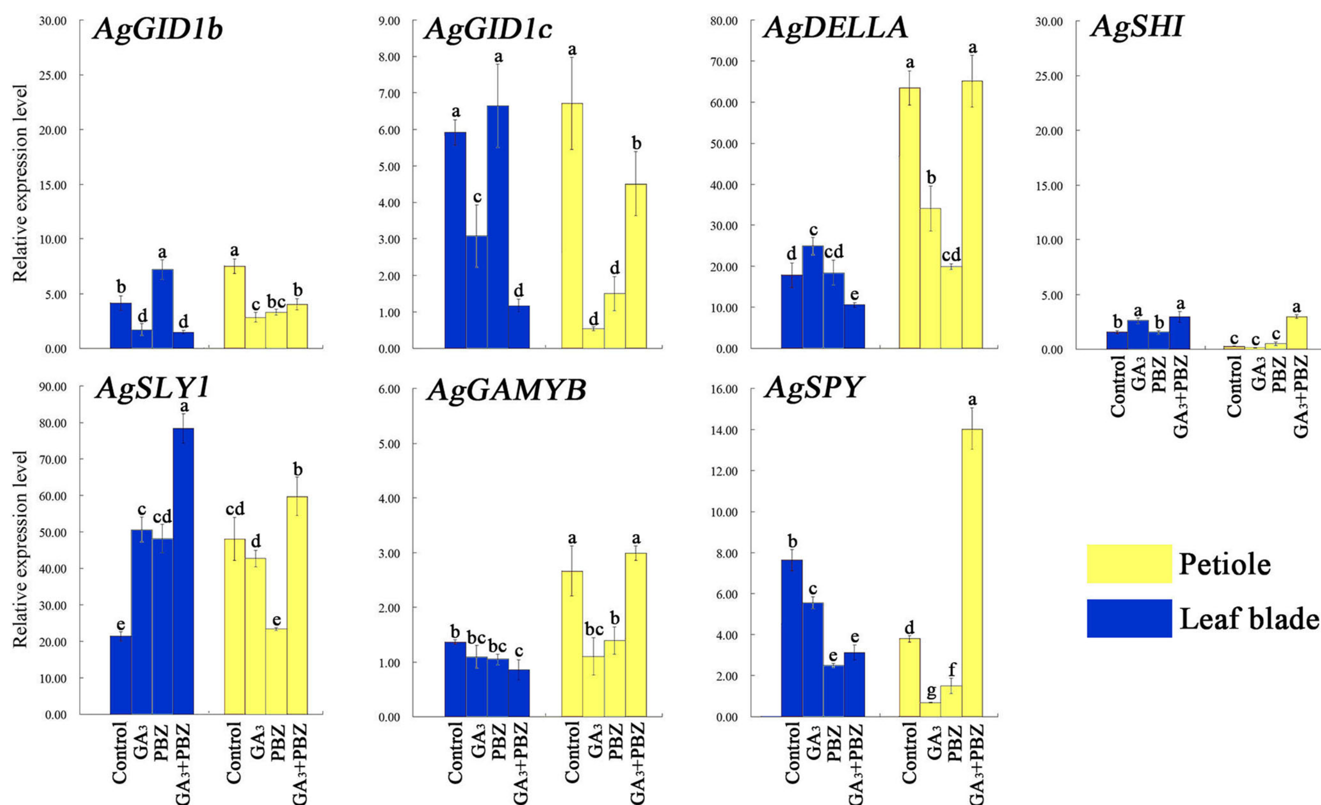

Fig. 9 Effects of GA_3, PBZ, or GA_3 + PBZ on the expression levels of genes involved in GA signaling. Bars represent the mean values of three biological replicates ± standard deviation. Different letters in columns indicate statistically significant differences ($P < 0.05$)

under GA_3 + PBZ treatment. The expression levels of *AgDELLA* and *AgSLY1* under PBZ treatment were significantly reduced, and GA_3 treatment downregulated the expression of GA response genes in petiole at different degrees. The expression levels of different GA response genes in celery leaves were distinct under gibberellin treatment. In some aspects, the expression of these genes can be used to analyze the effect of GA_3 treatment on signal transduction.

Effects of GA_3 or PBZ treatment on the expression levels of genes implicated in other hormone pathway

In order to verify the effects of other plant hormone regulation on gibberellin synthesis and metabolism, we selected seven related genes *AgABAH1*, *AgTSB*, *AgDWF7*, *AgLOG1*, *AgLOG3*, *AgSAMS2*, and *AgJM* from the auxin, cytokinins, ethylene, jasmonic acid, abscisic acid, and brassinolide biosynthesis synthetic pathways and analyzed their response to GA_3 or PBZ treatment (Table 2; Fig. 10). Under GA_3 treatment, the expression levels of seven selected genes in the leaf blades were downregulated. In the leaf blades, under the PBZ treatment, the expression profiles of *AgDWF7* and *LOG1* were significantly upregulated, and transcription was inhibited when GA_3 was applied, which was ameliorated by application of GA_3 + PBZ treatment. Similarly, *ABAH1* and *AgSAMS2* in the petiole were alleviated under GA_3 + PBZ treatment. The transcription levels of *AgSAMS2* was 1.040-fold than that of the control in the leaf blades under GA_3 + PBZ treatment. In addition, in the petiole, the transcription levels of *AgSAMS2* were 1.046- and 8.940-fold than that of the GA_3 + PBZ and GA_3 respectively under PBZ treatment. Treatment with GA_3 or PBZ resulted in changes in the expression levels of hormone-related genes in celery plants.

Discussion

GAs, one type of plant hormones, have been widely used to regulate plant growth in horticultural and agricultural production. The most obvious physiological effect of GA is promoting stem growth. In addition, it also plays roles in seed germination, male flower formation of some dioecious plants, and induction of aleurone in aleurone (Mikihiro et al. 2003; Achard et al. 2009; Susana et al. 2009; Chen et al. 2016).

Celery is a leafy vegetable crop widely cultivated worldwide (Li et al. 2018). It is important to increase the yield by regulating the growth of celery leaves. In this experiment, GA_3 were found promoting the length of petioles and inhibiting root growth. Previous studies have shown that gibberellin treatment significantly affects the distribution of dry matter in the aboveground and underground parts of plants (Wang et al. 2015a).

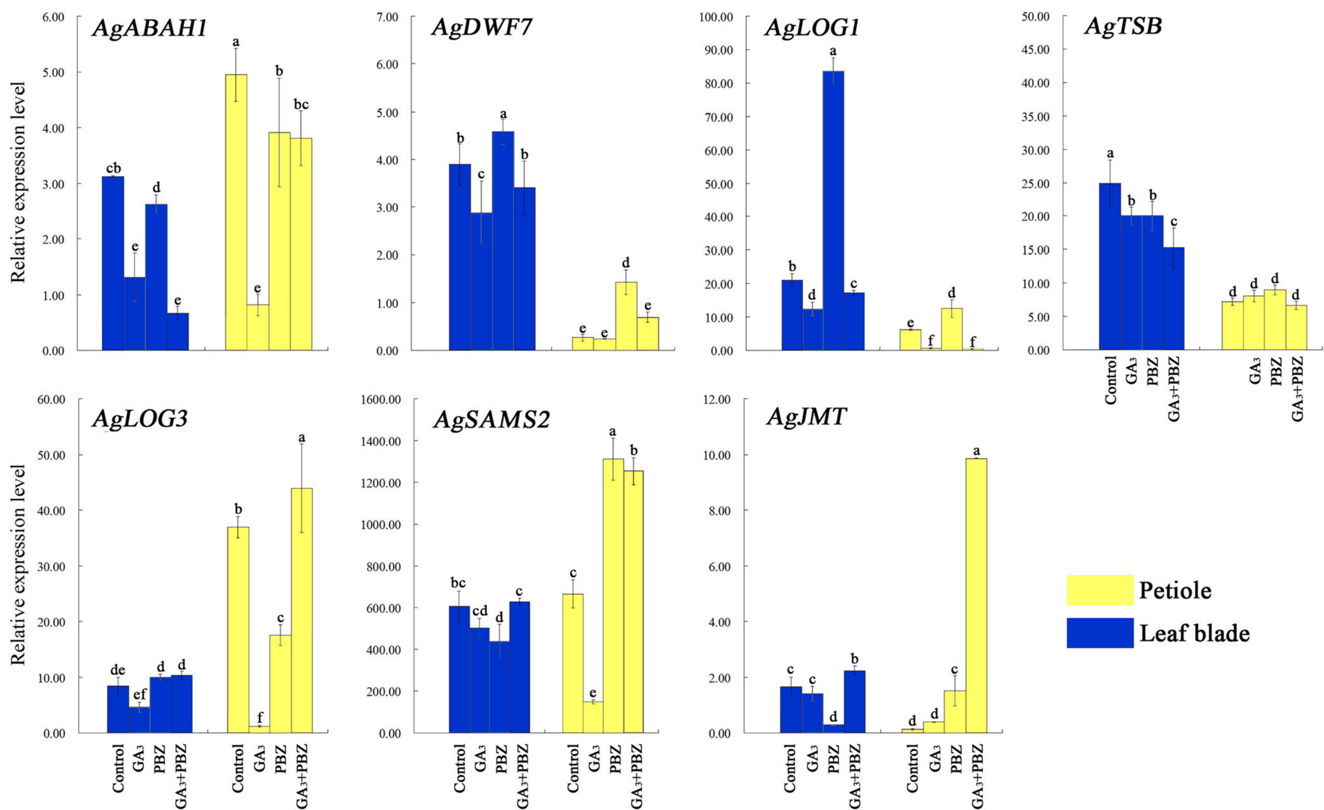

Fig. 10 Effects of GA_3, PBZ, or GA_3 + PBZ on the expression levels of genes involved in auxin, cytokinins, ethylene, jasmonic acid, abscisic acid, and brassinolide biosynthesis synthetic pathways. Bars represent the mean values of three biological replicates ± standard deviation. Different letters in columns indicate statistically significant differences ($P < 0.05$)

GA regulates many key enzymes of the GA synthesis pathway through feedforward or feedback. Transcription level of *AtKAO* gene is negatively regulated by active GA during seed germination (Helliwell et al. 1998, 1999). Overexpression of *GA20ox* can increase the endogenous activity of gibberellin in plants, allowing *Arabidopsis* to flower in advance (Huang et al. 1998). Excessive *GA20ox* can lead to excessive synthesis of GA and significantly promote plant growth (Huang et al. 1998). GA20ox is a key rate-limiting process in GA synthesis, and GA30ox is also a key factor regulating the synthesis of active GA during seed germination (Yukika et al. 2004). The expression levels of *GA20ox* and *GA3ox* are negatively regulated by feedback of GA, while *GA2ox* gene expression is positively regulated by feedforward of GA (Coles et al. 1999; Hedden and Phillips 2000). Previous research have shown that the transcription of *GA2ox1* was promoted when treated with active GA in *Arabidopsis* (Phillips et al. 1995; Thomas et al. 1999).

Our results indicated that the expression of *KAO* gene was positively and negatively regulated by GA_3 in the growth process of celery leaf blade and petiole respectively, showing an obvious tissue-specific. *GA2ox3* was induced under excessive GA treatment, and *GA2ox1* and *GA2ox2* also exhibited tissue specificity. The expression trends of *GA20ox1* and *GA20ox2* were same under different treatments in the same period, and the expression of *GA20ox1* and *GA20ox2* was the highest under the GA_3 + PBZ treatment. Plant GA synthesis and metabolic pathways responding to active GA are carried out in two ways. Steady-state equilibrium of active GA levels in plants is achieved by altering the GA synthesis reaction (upregulating or downregulating the expression levels of the *GA20ox* and *GA3ox* genes) or the GA passivation reaction (downregulating or upregulating the expression level of *GA2ox*).

Gibberellins also have synergistic or antagonistic effects with auxin, abscisic acid, cytokinins, ethylene, and brassinolide. SPINDLY (Spy) is a negative regulator of gibberellin signaling, but the *Arabidopsis spy* mutant also exhibits insensitivity to exogenous cytokinins. In the leaves, exogenous GA_3 suppressed *AgSPY* but increased *DcSPY* in carrot (Wang et al. 2015b). This indicated that there was a difference in the *SPY* expression in different species. Spy mutations in *Arabidopsis* and gibberellin treatment inhibit the typical cytokinins phenotype (Yaarit et al. 2005). And whether cytokinin is involved in the regulation of gibberellin synthesis and signal transduction remains inconclusive (Jasinski et al. 2005; Yaarit et al. 2005; Yanai et al. 2005). Ethylene treatment can delay the degradation of DELLA protein mediated by root tip GA in *Arabidopsis thaliana*, leading to high levels of

DELLA protein accumulation and inhibition of root growth (Achard et al. 2003). We found that exogenous gibberellin treatment significantly reduced the transcriptional abundance of the ethylene signal gene *SAMS2* in the petiole of celery, and decreased the transcript level of *DELLA* significantly, but increased the transcription level of *DELLA* in the leaf blade significantly. ABA and GA have antagonistic effects (Zentella et al. 2007). Similarly, GA_3 treatment reduced the expression level of ABA-related gene *ABAH1* in celery leaves. ABA synthesis-deficient mutant *aba2* passes gibberellin synthesis ability through *GA3ox1* and *GA3ox2* (Eunkyoo et al. 2007). At the seedling stage, ABA treatment can reduce the transcription level of *GA2ox1* gene (Zentella et al. 2007).

The biosynthesis of GA is affected by the regulation of other different hormones, which is a common phenomenon. But the type of specific genes regulated may vary from species to species. By applying exogenous GA_3 or PBZ to change the gibberellin content in plants, it may also change the interaction with other hormones to jointly control the growth of celery (Eunkyoo et al. 2007). The synthesis and metabolism of GA are strictly controlled in time and space. Due to the complexity of hormone action and the limitations of research methods, the understanding of the interaction between these plant hormones is still very limited.

Author contributions Conceived and designed the experiments: ASX AQD. Performed the experiments: AQD KF JXL ZSX. Analyzed the data: AQD KF ASX. Contributed reagents/materials/analysis tools: ASX. Wrote the paper: AQD. Revised the paper: ASX FQ. All authors read and approved the final manuscript.

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Compliance with ethical standards

Competing interests The authors declare that they have no conflict of interest.

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