24-Epibrassinolide and 2,6-Dichlorobenzonitrile Promoted Celery Petioles and Hypocotyl Elongation by Altering Cellulose Accumulation and Cell Length

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Abstract: BRs (brassinosteroids), an endogenous hormone in plants, regulate cellulose accumulation, cell elongation and plant growth. Propiconazole (PCZ) is an effective inhibitor of BR biosynthesis. DCB (2,6-Dichlorobenzonitrile) can inhibit the synthesis of cellulose and affects the chemical composition of cell walls. Celery is one important leafy vegetable of the Apiaceae family with rich dietary fiber (including cellulose). The petioles length, leaf blades number and cellulose content determine the yield and quality of celery. The family members of AgCESAs are related to cellulose biosynthesis in higher plants. To investigate the effects of BRs, PCZ and DCB on the growth of celery, celery cv. ‘Jinnan Shiqin’ plants were treated with 24-epibrassinolide (24-EBL, most active form of BRs), PCZ and DCB, respectively. The results showed that exogenous application of BRs up-regulated the expression of AgCESAs genes and accumulated more cellulose in celery. The length of petioles and number of leaf blades in celery plants applied with exogenous BRs (1.24 × 10^{-6} mol/L 24-EBL) were increased 2.16 and 1.37 times of that in the control. The addition of PCZ inhibited the effects of exogenous BRs application. The lengths of hypocotyl and hypocotyl cells of celery plants treated with BRs were longer than that of the control. Under DCB treatments, the expression levels of AgCESAs genes in celery petioles and leaf blades were down-regulated compared with the control, and the celery plants showed decreased cellulose content, shorter petiole length and fewer leaf blades. The length of hypocotyl and hypocotyl cells of celery treated with DCB were shorter than that of the control. This study provided a reference for the functions of BRs and DCB on the growth and development of celery.

Keywords: celery; brassinosteroids; 2,6-Dichlorobenzonitrile; morphological; cell elongation; cellulose accumulation

1. Introduction

Celery (Apium graveolens L.), a biennial herb of the Apiaceae family, is widely cultivated and consumed worldwide [1,2]. The proper amount of dietary fiber (including cellulose) is important for the quality and nutrients of leafy vegetables. Celery leaves (leaf blades and
petioles) with rich dietary fiber, which have demonstrated promising regulatory effects on the gut and important implications for gastrointestinal disorders [3,4]. Dietary fiber is divided into soluble and insoluble, and it also improves glycaemia, insulin resistance and weight loss [5]. The dietary fiber in celery is mainly insoluble cellulose. In plants, the biosynthesis of cellulose is controlled by cellulose synthase (CESA) [6]. Cellulose is the most abundant polysaccharide in nature and the main component of plant cell walls. In the process of cell expansion and elongation, a large amount of cellulose deposition is required to form new cell wall polymers. The lack of cellulose will affect cell growth and cell wall integrity, and then affect the growth and development of plants and their resistance to stress [6–10].

Brassinosteroids (BRs) represent a class of steroid hormones that are widely presented in the plant and play a crucial role in modulating plant growth and development, as well as affect crop yield [11,12]. In *Arabidopsis*, BRs contributed to cell elongation and plant growth through promoting CESA genes expression and cellulose accumulation [13]. The BR signaling activated the transcription of BES1 that could bind to the E-box in CESA1, CESA3 and CESA6 promoters, enhancing these target gene expressions during the development of the primary cell wall; BES1 could also regulate the expression of CESA4 and CESA8 in the secondary cell wall [13]. Transgenic poplar plants overexpressing BR biosynthesis pathway gene *PtoDET2* significantly increased plant growth rate and biomass yield by promoting xylem development and cell wall polymer deposition [14]. In carrot, application of exogenous BRs can promote the accumulation of cellulose, cell length and petiole elongation, and overexpression of *DcBAS1* (a carrot BRs catabolism gene) reduced the level of endogenous BRs and inhibited the biosynthesis of cellulose in transgenic *Arabidopsis* [15,16]. In addition to the characterization of BR deficient mutants, specific BR biosynthesis inhibitors played an essential role in the elucidation of BR function in plants. Propiconazole (PCZ) is a potent and specific inhibitor of BR biosynthesis and an alternative to brassinazole (Brz, a BRs inhibitor). The reduced cost and increased availability of PCZ, compared to Brz, opens new possibilities to study BR function in larger crop species [17].

2,6-Dichlorobenzonitrile (DCB) was served as an effective inhibitor of cellulose synthesis in higher plants [18]. It inhibited the polymerization of UDP-Glc into β-1,4-linked glucan and may also affect β-1,4-glucan crystallization at the plasma membrane [19]. In tomato cells, DCB prevented the formation of the cellulose-xyloglucan network in cell walls [20]. In *Pinus sylvestris* Zucc, the inhibition of cellulose synthesis caused by DCB affected the organization of cytoskeleton and vesicle trafficking in pollen tubes and induced the changes of chemical composition in the tube wall [21]. *Arabidopsis* seedlings displayed a dwarf phenotype when treated with 1 mM DCB. Environmental scanning electron microscopy (ESEM) assay found that DCB-treated *Arabidopsis* cotyledon epidermis appeared to cause loss of cell shape uniformity and severely swollen cells [22].

For vegetables with petiole as the main edible part, raising the length of the petiole has a beneficial impact on the yield [23]. Here, we aimed to investigate the effects of 24-epibrassinolide (24-EBL, most active form of BRs), PCZ (BRs inhibitor) and DCB (inhibitor cellulose biosynthesis) on the growth and development of celery. Morphological and anatomical characteristics, cellulose accumulation, and related-gene expression profiles were measured to elucidate the roles of applied BRs, PCZ and DCB in the celery. The results hoped to gain a reference for BRs and DCB functions in cellulose biosynthesis and cell elongation of celery.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Celery cv. ‘Jinnan Shiqin’ was selected in this experiment. The seedlings of celery were grown in pots within a soil/vermiculite mixture in phytotron at the State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University. The phytotron program was 25 °C/18 °C (day/night) for 16 h/8 h. The light intensity was 150 µmol m⁻² s⁻¹ at daytime, with relative humidity of 75%. Additionally, celery seeds
were also sown in Murashige and Skoog (MS) medium before germinating, and then transplanted into MS medium with or without 24-EBL/DCB (control) after germinating. The samples were collected and stored at −80 °C. Three biological replicates were tested.

2.2. Exogenous 24-EBL, PCZ and DCB Treatments

To investigate the effects of 24-EBL/PCZ/DCB (Yuanye, Shanghai, China) on ‘Jinnan Shiqin’ leaf blades and petioles, the celery plants of 30 DAG (day after germinating) were treated with different concentrations of exogenous 24-EBL (0, 8.32 × 10^{-7}, 1.24 × 10^{-6}, 1.66 × 10^{-6} and 2.08 × 10^{-6} mol/L). A combination of exogenous optimum concentration of celery treated with 24-EBL and PCZ (a BRs inhibitor) was used to treat celery at 40 DAG—concentrations of treatments (0, 2.90 × 10^{-3} mol/L PCZ + 1.24 × 10^{-6} mol/L 24-EBL). Exogenous DCB (0, 2.32 × 10^{-5}, 3.49 × 10^{-5}, 4.65 × 10^{-5} and 5.81 × 10^{-5} mol/L) were used to treat celery at 45 DAG, respectively. Each pot with seven to nine plants was sprayed with 100 mL of different concentrations of 24-EBL, PCZ and DCB. Plants sprayed with water were used as control (CK). Treatments were carried out five times in total, and the interval of each treatment is 2 days. Then, the plants were allowed to grow for 10 days and harvested for morphology determination. To investigate the potential effect of 24-EBL and DCB on celery hypocotyl, 0 and 5 DAG ‘Jinnan Shiqin’ were treated. Different concentrations of exogenous 24-EBL (0, 1.04 × 10^{-5}, 2.08 × 10^{-5}, 4.16 × 10^{-5} and 8.32 × 10^{-5} mol/L), exogenous DCB (0, 2.33 × 10^{-4}, 3.49 × 10^{-4}, 4.65 × 10^{-4} and 5.81 × 10^{-4} mol/L) were applied, respectively. Then, the plants were allowed to grow for 8 days and then harvested for morphology determination.

2.3. Anatomical Structure Analysis

The fresh samples, including the petioles of celery plants grown in soil and the hypocotyl of celery seedling grown in MS medium, were cut into slices and stored in phosphate buffer solution (pH 7.2) containing 2.5% glutaraldehyde at 4 °C. The samples were subjected to safranin-O/fast green staining, and the morphological structures of the plant cells were observed. Cellulosic tissues could be stained green via histochemical staining [15]. The photographs of the slices were taken via light microscope, and the cell length was measured by ImageJ (National Institutes of Health, Bethesda, MD, USA).

2.4. Measurement of Cellulose Content

To examine the effects of 24-EBL, PCZ and DCB treatments on cellulose accumulation, celery leaf blades and petioles were sampled after 10 days of exogenous 24-EBL, PCZ and DCB treatments. The samples were ground with liquid nitrogen in a mortar and dried for two days at room temperature. Then, the anthrone–sulphuric acid method as previously described was used to colorimetrically quantify cellulose in the samples [15].

2.5. Total RNA Extraction, cDNA Synthesis

Total RNA of celery was extracted using the total RNA extraction kit (Tiangen, Beijing, China) according to the manufacturer’s instructions. cDNA was obtained by Prime Script RT reagent kit (TaKaRa, Dalian, China) based on the operation instruction.

2.6. RT-qPCR Analysis

The cellulose biosynthesis-related genes (AgCESA1, AgCESA2, AgCESA3, AgCESA4, AgCESA5 and AgCESA8) were retrieved from the celery genome database based on the sequences of homologous proteins from Arabidopsis and other plant species [24,25]. Specific primers were designed using Primer Premier 6.0 software and listed in Table S1. The SYBR Premix Ex Taq (TaKaRa, Dalian, China) and Bio-Rad IQ5 real-time PCR System (Bio-Rad, CA, USA) were used for RT-qPCR reaction. AgTUB gene was used as internal standard [26]. Each reaction set three biological replicates. The relative expression data were analyzed using the 2−ΔΔCt method [27].
2.7. Statistical Analysis

All data in the text were obtained from the average of three biological repeats. Data
significant difference was analyzed using SPSS 24.0 by one-way ANOVA at a 0.05 level.

3. Results

3.1. Effects of Exogenous 24-EBL, PCZ and DCB Treatments on the Growth and Development of Celery

To determine the effects of 24-EBL, PCZ and DCB involved in the regulation of
celery growth, celery plants were treated with 24-EBL or its inhibitor (PCZ), and DCB (inhibitor for cellulose synthesis). Exogenous 24-EBL treatments promoted growth of celery leaves (petioles and leaf blades) and hypocotyl (Figures 1 and S1). With the increase of
24-EBL concentration, celery height increased first and then decreased; among them,
1.24 × 10⁻⁶ mol/L 24-EBL can best promote the growth and development of celery (Figure 1C). The treatment of exogenous application of 1.24 × 10⁻⁶ mol/L 24-EBL + 2.90 × 10⁻³ mol/L PCZ resulted in decreased celery height compared to that of control and separately applied 1.24 × 10⁻⁶ mol/L 24-EBL (Figure 2). The celery height was inhibited under exogenous DCB treatments, and celery seedlings hypocotyl displayed a dwarfed phenotype when treated with DCB (Figures 3 and S2). The increase in celery plant height shared a similar profile with the increase in DCB concentration in treatment groups, and the 2.32 × 10⁻⁵ mol/L DCB treatment showed the best inhibitor of celery (Figure 3B).

Figure 1. Effects of 24-EBL treatments on celery growth, with celery at 50 DAG. (A) Control; (B) 8.32 × 10⁻⁷ mol/L 24-EBL treatment; (C) 1.24 × 10⁻⁶ mol/L 24-EBL treatment; (D) 1.66 × 10⁻⁶ mol/L 24-EBL treatment; (E) 2.08 × 10⁻⁶ mol/L 24-EBL treatment. Scale bars = 5 cm.

Figure 2. Effects of 24-EBL and PCZ + 24-EBL treatments on celery growth, with celery at 60 DAG. (A) Control; (B) 1.24 × 10⁻⁶ mol/L 24-EBL treatment; (C) 1.24 × 10⁻⁶ mol/L 24-EBL + 2.90 × 10⁻³ mol/L PCZ treatment. Scale bars = 5 cm.
The leaf blade number, petioles length and petiole cell length of celery treated with 24-EBL were increased, and those treated with PCZ and DCB were decreased compared to that of the control (Figure 4). Under $1.24 \times 10^{-6}$ mol/L 24-EBL treatment, the leaf blade number and petioles length peaked at 50 DAG and were 1.37- and 2.16-fold those of the control, respectively (Figure 4A,B). The largest length of petiole cell was 153.72 μm in the 1.66 × 10⁻⁶ mol/L 24-EBL treatment group and was 1.54-fold that of the control (Figure 4C). The leaf blade number was 13.25 under 2.32 × 10⁻⁵ mol/L DCB treatment group, which was 0.76-fold that of control. The shortest petiole length and petiole cell length were 18.56 cm and 84.05 μm under 3.49 × 10⁻⁵ and 4.65 × 10⁻⁵ mol/L DCB treatment groups, which were 0.65- and 0.57-fold of that in the control group, respectively (Figure 4D–F). Celery treated with $1.24 \times 10^{-6}$ mol/L 24-EBL showed the largest number of leaf blades (17.8), the longest length of petiole (29.35 cm) and longest petiole cells (181.59 μm) at 60 DAG (Figure 4G–I).

3.3. Effects of 24-EBL and DCB Treatments on Length of Hypocotyl and Hypocotyl Cells

The length of hypocotyl and hypocotyl cells of celery treated with 24-EBL were longer and those treated with DCB were shorter than those of the control (Figure 5). The hypocotyl length and hypocotyl cell length of celery treated with $8.32 \times 10^{-5}$ mol/L 24-EBL and $2.08 \times 10^{-5}$ mol/L 24-EBL were the longest (3.82 cm and 105.67 μm), which were 1.62- and 1.68-fold of that in the control, respectively (Figure 5A,B). The lengths of hypocotyl and hypocotyl cells in the control group were 3.09 cm and 150.17 μm, which were longer than those in the DCB treatment groups, respectively (Figure 5C,D). Four concentrations of exogenous 24-EBL/DCB treatments all affected the lengths of hypocotyl and hypocotyl cells, but there was no significant difference in the lengths of hypocotyl and hypocotyl cells between different concentrations of 24-EBL/DCB treatment groups.
Figure 4. Effects of 24-EBL/PCZ/DCB treatments on leaf blade number, petiole length and petiole cell length in celery. (A–C) 24-EBL treatment groups; (D–F) DCB treatment groups; (G–I) PCZ + 24-EBL treatment groups. Column diagrams with different colors represent the data from celery plants in CK, 24-EBL, DCB and PCZ + 24-EBL treatment groups. Data are expressed as the means ± standard deviation (SD) of three replicates. Different letters indicate significant difference at 0.05 level.
The parenchyma cell length of petiole and hypocotyl from celery treated with exogenous DCB treatments was compared with those of the control group and the 1.24 × 10^{-6} mol/L 24-EBL treatment (Figure 6K–M). Compared with the control, the length of petiole and hypocotyl were longer under other concentrations, which were the same as the heights of plants (Figure 6A–E,M). Compared with the control, petiole parenchyma cells of celery treated with 3.49 × 10^{-5} and 4.65 × 10^{-5} mol/L DCB were shorter and slender (Figure 6F–J). The parenchyma cells of celery treated with exogenous 1.24 × 10^{-6} mol/L 24-EBL + PCZ were smaller than those of the control group and the 1.24 × 10^{-6} mol/L 24-EBL treatment (Figure 6K–M).

Figure 5. Effect of 24-EBL/DCB treatments on the lengths of hypocotyl and hypocotyl cells in celery. (A,B) 24-EBL treatment groups; (C,D) DCB treatment groups. Column diagrams with different colors represent the data from celery plants in CK, 24-EBL and DCB treatment groups. Data are expressed as the means ± standard deviation (SD) of three replicates. Different letters indicate significant difference at 0.05 level.

3.4. Anatomical Structure Analysis of Celery Petiole and Hypocotyl under 24-EBL, PCZ and DCB Treatments

Compared with the control, the length of petiole and hypocotyl were longer under exogenous 24-EBL treatments, and those were shorter under PCZ and DCB treatments. The sections of petiole and hypocotyl were obtained to further analyze the effect of exogenous 24-EBL, PCZ and DCB treatments on the anatomical structure of celery (Figures 6 and 7). The parenchyma cell length of petiole and hypocotyl from celery treated with exogenous 24-EBL were longer than those of the control. Under the treatment of 1.24 × 10^{-6} mol/L 24-EBL, the petiole parenchyma cells at 50 DAG were larger than those treated with other concentrations, which were the same as the heights of plants (Figure 6A–E,M). Compared with the control, petiole parenchyma cells of celery treated with 3.49 × 10^{-5} and 4.65 × 10^{-5} mol/L DCB were shorter and slender (Figure 6F–J). The parenchyma cells of celery treated with exogenous 1.24 × 10^{-6} mol/L 24-EBL + PCZ were smaller than those of the control group and the 1.24 × 10^{-6} mol/L 24-EBL treatment (Figure 6K–M).
Compared with the control, the hypocotyl parenchyma cells of celery were longer after being treated with 24-EBL (Figure 7A–E), and they were shorter and thicker under DCB treatments (Figure 7F–J).

**Figure 6.** Effects of 24-EBL/PCZ/DCB treatments on the anatomical structure of celery petioles. (A–E) The anatomical structure of celery petioles treated with 0, 8.32 × 10\(^{-7}\), 1.24 × 10\(^{-6}\), 1.66 × 10\(^{-6}\) and 2.08 × 10\(^{-6}\) mol/L 24-EBL, respectively. (F–J) The anatomical structure of celery petioles treated with 0, 2.32 × 10\(^{-5}\), 3.49 × 10\(^{-5}\), 4.65 × 10\(^{-5}\) and 5.81 × 10\(^{-5}\) mol/L DCB treatments, respectively. (K–M) The anatomical structure of celery petioles in the control, PCZ + 1.24 × 10\(^{-6}\) mol/L 24-EBL and 1.24 × 10\(^{-6}\) mol/L 24-EBL treatment groups, respectively. (N) The petiole part for slicing. Scale bars in (A–M) = 100 μm, scale bar in (N) = 5 cm.
3.5. Cellulose Content in Celery Leaf Blades and Petioles under 24-EBL, PCZ and DCB Treatments

The effects of exogenous 24-EBL, PCZ and DCB treatments on the accumulation of cellulose in celery petioles and leaf blades were measured (Figure 8). The cellulose content of celery petioles and leaf blades treated with exogenous 24-EBL were significantly higher than those of the control, and with the increase of 24-EBL concentration, the cellulose content increased first and then decreased (Figure 8A,B). The cellulose content in celery petioles and leaf blades treated with exogenous DCB were lower than those of the control (Figure 8C,D). Under 2.32 × 10⁻⁵ mol/L DCB treatment, the cellulose content in celery petioles and leaf blades were the lowest, which were 0.75- and 0.72-fold that of the control group, respectively. The cellulose content of petioles and leaf blades in the 1.24 × 10⁻⁴ and 4.16 × 10⁻⁵ mol/L 24-EBL treatment group was higher than those in PCZ + 1.24 × 10⁻⁶ mol/L 24-EBL treatment, which were 19.87 and 8.19 g/100 g DW (Dry weight), respectively (Figure 8E,F). BRs treatments can promote the accumulation of cellulose, the elongation of petiole and petiole cells and the increase of leaf blade number. PCZ + BRs can inhibit those effects. Compared with the control, the cellulose content, the lengths of petiole and petiole cells and the number of leaf blades all decreased under DCB treatments (Figures 4 and 8). The increase of cellulose content in celery may be involved in the elongation of petiole and petiole cells and increase the number of leaf blades.

3.6. Expression Patterns of the Cellulose-Related Genes in Petioles, Leaf Blades and Hypocotyl of Celery under 24-EBL, PCZ and DCB Treatments

AgCESA1, AgCESA2, AgCESA3, AgCESA4, AgCESA5 and AgCESA8 genes involved in the regulation of cellulose biosynthesis were selected to detect the relative expression levels (Figure 9). Under exogenous 24-EBL treatments, the relative expression levels of AgCESA4 and AgCESA8 in petioles and AgCESA2 and AgCESA3 in hypocotyl were increased compared to in the control (Figure 9B,C). Expression levels of AgCESA1, AgCESA2 and AgCESA3 in leaf blades and AgCESA2 in petioles by DCB treatments were lower than those in the control, which were consistent with the change of cellulose accumulation (Figure 9D,E). The relative expression levels of AgCESA1, AgCESA4 and AgCESA5 in celery treated with 1.24 × 10⁻⁶ mol/L 24-EBL for 60 DAG were higher than those of control and PCZ + 1.24 × 10⁻⁶ mol/L 24-EBL treatments, which shared a similar profile with the accumulation of cellulose (Figure 9G,H).
Figure 8. The cellulose content in the petioles and leaf blades of celery under 24-EBL/PCZ/DCB treatments. (A,C,E) The cellulose content of celery leaf blades. (B,D,F) The cellulose content of celery petioles. Column diagrams with different colors represent the data from celery plants in the CK, 24-EBL, DCB and PCZ + 24-EBL treatment groups. Data are expressed as the means ± standard deviation (SD) of three replicates. Different letters indicate significant difference at 0.05 level.

Figure 9. The expression levels of the genes related to cellulose biosynthesis in petioles, leaf blades and hypocotyl of celery under different concentrations of 24-EBL/PCZ/DCB treatments. (A) Celery leaf blades under 24-EBL treatments. (B) Celery petioles under 24-EBL treatments. (C) Celery hypocotyl under 24-EBL treatments. (D) Celery leaf blades under DCB treatments. (E) Celery petioles under DCB treatments. (F) Celery hypocotyl under DCB treatments. (G) Celery leaf blades under PCZ + 1.24 × 10^{-6} mol/L 24-EBL treatments. (H) Celery petioles under PCZ + 1.24 × 10^{-6} mol/L 24-EBL treatments. Column diagrams with different colors represent the expression levels of AgCESA1, AgCESA2, AgCESA3, AgCESA4, AgCESA5 and AgCESA8, respectively. Data are expressed as the means ± standard deviation (SD) of three replicates.
4. Discussion

Celery is one of the important leafy vegetables of the Apiaceae family with rich nutrients [28–30], such as anthocyanin [29], apigenin [31,32], carotenoids [33,34], ascorbic acid [35] and dietary fiber [36]. Celery petioles and leaf blades are the edible part. Regulating the petioles and leaf blades growth and development to increase its quality and yield is important in celery research.

4.1. Effects of BRs/PCZ/DCB on the Expression of Cellulose-Related Genes and Cellulose Accumulation in Celery

In higher plants, cellulose synthesis is controlled by cellulose synthase (CESA) [37–42]. In Arabidopsis, AtCesA1, CesA3 and one of CesA2/5/6/9 are required for synthesis of primary cell wall cellulose, while AtCesA4, CesA7 and CesA8 are responsible for cellulose production in secondary cell walls [8,41]. BRs can promote the expression of CESA genes and increase the accumulation of cellulose [13]. The expression levels of AgCESA4 and AgCESA8, as well as cellulose content in celery petioles, were increased under exogenous BRs treatments. PCZ is a potent and specific inhibitor of BR biosynthesis [17]. Cellulose content and cellulose synthase genes (AgCESA1, AgCESA4 and AgCESA5) expression in celery petioles and leaf blades treated with 24-EBL were higher than those of the PCZ + 24-EBL treatment. Cellulose is composed of linear β-1,4 glucan chains, which in secondary cell walls aggregate into highly crystalline cellulose microfibrils [42–44]. DCB affect cellulose synthesis by inhibiting the polymerization of UDP-Glc into β-1,4-linked glucan [21]. Under DCB treatments, compared with the control, the transcription levels of AgCESA1, AgCESA2 and AgCESA3 in leaf blades and AgCESA2 in petioles were lower, and cellulose content was reduced in the petioles and leaf blades.

4.2. Effects of BRs/PCZ/DCB on Cell Elongation of Celery Petiole and Hypocotyl

Previous studies have demonstrated that BRs contributed to cell elongation through elevated cellulose accumulation and altered gibberellin content [13,15,45–47]. Anatomical analysis of petiole and hypocotyl showed that BRs treatments could promote cell elongation, while PCZ + BRs-treated cells became shorter and smaller. Under 1.24 × 10^{-6} mol/L BRs treatment, the cellulose content of petiole was the highest, while the longest petiole cell length occurred under the 1.66 × 10^{-6} mol/L BRs treatment. In celery, the growth and development of petioles and leaf blades are controlled by other hormones such as auxin, abscisic acid (ABA), GA3 and ethylene [48]. The cellulose synthesis and cell expansion are coordinated, regulated by different mechanisms [44]. DCB can inhibit the synthesis of cellulose and affect the chemical composition of cell walls [19–21]. Compared with the control, parenchyma cells of petiole and hypocotyl of celery treated with DCB were shorter and slender.

4.3. Effects of BRs/PCZ/DCB on the Growth and Development of Celery

Phytohormone is important for plants, and exogenous application of phytohormone with an appropriate concentration has become one of the common methods to promote plant growth [49–51]. Exogenous BRs treatments promoted elongation of petiole and hypocotyl and increased the number of leaf blades. The treatment effect of 1.24 × 10^{-6} mol/L BRs is the best. Added PCZ inhibited the above physiological processes. Arabidopsis seedlings grown on DCB-supplemented agar displayed a dwarfed seedling phenotype [22]. In this study, under DCB treatment, the hypocotyl of celery seedlings showed dwarfed phenotype; petioles length and leaf blade number were also less than those of the control.

4.4. Application of BRs/PCZ/DCB in Celery Production

In the production process of celery, the application of different plant growth regulators (PGRs) could improve the yield and quality of celery [36]. The length of petiole and the number of leaf blades determine the yield of celery. The content of cellulose affects the quality of celery. Our current work showed that BRs/PCZ/DCB treatments changed
the petiole length, leaf blade number and cellulose content of celery. According to the characteristics of PGR, it is recommended to be used in celery facility cultivation.

5. Conclusions

In the present study, BRs/PCZ/DCB can regulate the accumulation of cellulose, the size of cells and celery height. Exogenous application of BRs can increase the expression of celery cellulose synthesis genes (AgCESAs) and cellulose accumulation. Exogenous BR treatment could increase the number of leaf blades, petiole length and cell length. Exogenous spraying of PCZ could inhibit these effects. Compared with the control, cellulose content, petiole length and number of leaf blades in celery were decreased. Exogenous DCB treatment can shorten the hypocotyl length. In the treatment group, $1.24 \times 10^{-6}$ mol/L 24-EBL had the positive effect on petiole elongation and could increase leaf blades and cellulose content. DCB with $2.32 \times 10^{-5}$ mol/L concentration could effectively inhibit the growth and development of celery. The length of petiole, the number of leaf blades and the content of cellulose are related to the yield and quality of celery. This work will provide useful information for the application of BRs/PCZ/DCB in celery production.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy12071670/s1: Figure S1: Effects of 24-EBL treatments on celery hypocotyl growth. Scale bar = 0.5 cm; Figure S2: Effects of DCB treatments on celery hypocotyl growth. Scale bar = 0.5 cm; Table S1: Primers used for real-time quantitative PCR analysis.

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