RESEARCH PAPER

A role of brassinosteroids in early fruit development in cucumber

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Abstract

Brassinosteroids (BRs) are essential for many biological processes in plants, however, little is known about their roles in early fruit development. To address this, BR levels were manipulated through the application of exogenous BRs (24-epibrassinolide, EBR) or a BR biosynthesis inhibitor (brassinazole, Brz) and their effects on early fruit development, cell division, and expression of cyclin and cyclin-dependent kinases (CDKs) genes were examined in two cucumber cultivars that differ in parthenocarpic capacity. The application of EBR induced parthenocarpic growth accompanied by active cell division in Jinchun No. 4, a cultivar without parthenocarpic capacity, whereas Brz treatment inhibited fruit set and, subsequently, fruit growth in Jinchun No. 2, a cultivar with natural parthenocarpic capacity, and this inhibitory effect could be rescued by the application of EBR. RT-PCR analysis showed both pollination and EBR induced expression of cell cycle-related genes (CycA, CycB, CycD3;1, CycD3;2, and CDKB) after anthesis. cDNA sequences for CsCycD3;1 and CsCycD3;2 were isolated through PCR amplification. Both CsCycD3;1 and CsCycD3;2 transcripts were up-regulated by EBR treatment and pollination but strongly repressed by Brz treatment. Meanwhile, BR6ox1 and SMT transcripts, two genes involved in BR synthesis, exhibited feedback regulation. These results strongly suggest that BRs play an important role during early fruit development in cucumber.

Key words: Brassinosteroids, cell division, Cucumis sativus, cyclin, flow cytometry, parthenocarpy.

Introduction

Plant ovaries undergo cell division, cell expansion, and ripening stages to form fleshy fruits. In most plants, early fruit development can be divided into three phases: development of the ovary, cell division, and subsequent cell expansion. It is well known that the transfer of the first phase to the second phase is triggered by pollination/fertilization (Gillaspy et al., 1993), a process associated with the synthesis of phytohormones such as auxins, GAs, and cytokinins. Previous studies have shown that there are significant increases in the accumulation of auxins, GAs, and cytokinins after fertilization (Gillaspy et al., 1993). Some species or varieties have natural parthenocarpic capacity and can start fruit development in the absence of pollination/fertilization (Gillaspy et al., 1993; Gorguet et al., 2005). Subsequent studies have shown that these varieties usually have higher levels of auxins, GAs, or cytokinins in the ovaries before anthesis than other varieties without such parthenocarpic capacity, indicating that phytohormones are of prime importance for early fruit development (Nitsch, 1970; Gillaspy et al., 1993). Meanwhile, parthenocarpy could be artificially induced by the application of exogenous growth substances (Schwabe and Mills, 1981; García-Martínez and Hedden, 1997; Vivian-Smith and Kolunow, 1999; Li et al., 2003), or by overexpressing auxin synthesis genes (Rotino et al., 2003).
Cell division is regulated by hormones such as auxins and cytokinins (Himanen et al., 2002). The progression through different stages of the cell mitotic cycle, especially through the two principal control points of the late G1 phase and the G2/M boundary, is regulated by heterodimeric complexes of cyclin and cyclin-dependent kinase (CDK) (Mironov et al., 1999; Inzé and De Veylder, 2006). Five distinct classes of CDKs, CDKA to CDKE, have so far been identified (Joubès et al., 2000a).

The expression and translation patterns of CDKAs are constitutive during the cell cycle while CDKBs have a specific role in regulation of the G2/M boundary. However, the functions of other CDKs remain unclear (Mironov et al., 1999). Among the five groups of cyclins identified, both the A- and B-type cyclins accumulate during the G2 and early M phases; A-type cyclins also accumulate during the S phase of the cell cycle (Mironov et al., 1999). D-type cyclins, also known as G1 proteins, control the progression through the G1 phase in response to growth factors and nutrients and, therefore, represent key regulators of cell commitment to division (Fuerst et al., 1996; Gutierrrez et al., 2002; Shen, 2002; Trimarchi and Lees, 2002). It has been observed that cytokinins and brassinosteroids (BRs) could increase cell division by increasing CycD3 transcript levels in Arabidopsis cell suspension (Riou-Khamlichi et al., 1999; Hu et al., 2000). Differential expression patterns of cyclin and CDK genes have also been investigated during tomato fruit development (Joubès et al., 1999, 2000b). Our previous work has shown that pollination and cytokinin application enhanced CycD3 expression and activated cell division in the ovaries of white-flower gourd, a relative of cucumber (Li et al., 2003).

BRs play prominent roles in various physiological processes including induction of stem elongation, pollen tube growth, xylem differentiation, leaf epinasty, ethylene biosynthesis, proton pump activation, gene expression and photosynthesis, and adaptive responses to environmental stress (Clouse and Sasse, 1998; Dhaubhadel et al., 1999; Khripach et al., 2000; Steber and McCourt, 2001; Krishna, 2003; Yu et al., 2004; Vert et al., 2006). Recently, several studies have showed that biosynthesis of BRs is enhanced in the developing seeds or fruits of tomato, pea and Arabidopsis (Shimada et al., 2003; Montoya et al., 2005; Nomura et al., 2007). The application of BRs can also accelerate the ripening of tomato and grape fruits (Vardhini and Rao, 2002; Symons et al., 2006). To our knowledge, however, BRs have not been implicated in the regulation of early development of fruits, although some have observed that exogenous BRs can increase fruit set (Kamuro and Takatsuto, 1999).

Cucumber, a monococious annual cucurbit plant, is a good model plant to study fruit growth since genotypes with different parthenocarpic capacity are available. To understand the role of BRs in early fruit development, an attempt was made to manipulate the BR levels in ovaries of Jinchun No. 4 (a non-parthenocarpic cultivar) and Jinchun No. 2 (a parthenocarpic cultivar) of cucumber (Cucumis sativus L.) through the application of exogenous BRs and a BR biosynthesis inhibitor, brassinazole (Brz) (Asami et al., 2000). The effects of altered BR levels on ovary growth and cell division were subsequently investigated. The effects on the expression of six cell cycle-related genes as well as genes involved in BRs biosynthesis were also examined. These results strongly suggest that BRs play an important role in early fruit development in cucumber.

Materials and methods

Plant materials

Two cucumber (Cucumis sativus L.) cultivars, Jinchun No. 4 and Jinchun No. 2, were used throughout this study. Jinchun No. 4 is a cultivar lacking natural parthenocarpic capacity and Jinchun No. 2 is a cultivar with natural parthenocarpic capacity. Plants of both cultivars were grown in March 2006, in a greenhouse at Zhejiang University as previously described by Yu (1999).

The experiments with cultivar Jinchun No. 4 included five treatments: (i) non-pollination, (ii) pollination, (iii) 0.02 μM EBR (24-epibrassinolide) treatment of unpollinated ovaries, (iv) 0.2 μM EBR treatment of unpollinated ovaries, and (v) 2.0 μM EBR treatment of unpollinated ovaries, respectively. EBR was sprayed on to unpollinated ovaries at anthesis. The experiments with cultivar Jinchun No. 2 included the following seven treatments: (i) non-pollination, (ii) 0.4 μM Brz (brassinazole) treatment, (iii) 0.4 μM Brz+0.2 μM EBR treatment, (iv) 4 μM Brz treatment, (v) 4 μM Brz+0.2 μM EBR treatment, (vi) 40 μM Brz treatment, (vii) 40 μM Brz+0.2 μM EBR treatment, respectively. Brz was sprayed on to the ovaries from the ovary initiation stage to 4 days after anthesis (DAA) while EBR was applied 2 h after Brz treatment each time. In all cases, flowers were bagged before anthesis and then pollinated or treated with Brz and EBR. Each ovary of Jinchun No. 4 was treated with approximately 0.5 ml EBR while the amount of Brz and EBR applied increased from 0.1 ml per ovary at the initiation stage to 0.5 ml on 2 DAA for the ovary of Jinchun No. 2. EBR and Brz were first dissolved in ethanol at concentrations of 0.2 mM and 4 mM, respectively, and then diluted to the designated concentrations with water. Each treatment had 15 female flowers from nodes 12–15 of the main vine from 15 plants with four replicates. Fruit length was measured at designated times, and ovaries or fruits were simultaneously sampled, frozen quickly in liquid nitrogen, and stored at −80 °C for further analysis.

Flow cytometry

Relative DNA contents of the nuclei were measured in cucumber ovaries at anthesis and in fruits at 2 DAA and 4 DAA following various treatments. Samples were chopped with a razor blade in 1 ml ice-cold buffer (45 mM MgCl2, 30 mM sodium citrate, 20 mM MOPS, pH 7, and 1% Triton X-100) (Galbraith et al., 1983).
Role of BRs in early fruit growth

Table 1. Primers used for real-time RT-PCR assays

| Gene                        | Encoding protein       | Accession no. | Primer pairs                      |
|-----------------------------|------------------------|---------------|-----------------------------------|
| CycA                        | A-type cyclin          | EW968279      | F: GATTTGTTGCTGCTGCTCAA R: TAATGCGCAAGGAGAATTGG |
| CycB                        | B-type cyclin          | EW968280      | F: GAGAAGCAGAACGACGACACCTCA R: CAATCTTTGTCGCAAAGGAAA |
| CycD3;1                     | D-type cyclin          | EW968283      | F: CATGTGGGAGAAAGCAGA R: TTGGATCTGACACAACTTGC |
| CycD3;2                     | D-type cyclin          | EW968284      | F: CTATCAACCTCACAACACG R: TTGGTCTCTATTGTTTCAG |
| CDKA                       | Cyclin-dependent kinase A | EW968281    | F: CTCCTGGAGCGATCAGGAGAAAGC R: TAATTCGGCAAGGAGAATTGG |
| CDKB                       | Cyclin-dependent kinase B | EW968282    | F: CAATCCCTTATGCTCTTCAG R: GCTTGAGATCACGGTGAAGA |
| SMT                        | S-adenosyl-L-methionine:Δ24-sterol-C-methyltransferase | ABK55702     | F: CTTTCCGGGAGACGCTTTAAG R: RCAAAGGAGTCCTGCTGCTG |
| BR6ox1                      | BR-6-oxidase           | EW968286      | F: ATGAGAGGTGCTCTGCTGCTG R: TAGATGAGCGAGAGCCCATC |
| actin                       |                        | AAZ74666      | F: TGGAGACTCGTATGATGTTA R: CAATGAGGAGTCCTGCTT |

PCR amplification of cyclin gene fragments

3’- and 5’- rapid amplification of cDNA ends (RACE) were used to isolate cucumber cyclin D3 full-length cDNAs. The single- and double-stranded cDNA used as template for 3’- and 5’-RACE were synthesized using the SMART™ cDNA Library Construction Kit (Takara, Japan) according to the manufacturer’s instruction. Based on EST sequences, two specific primers (P1: 5’-TTTGGCCTGCAAGGAAAATC R: TAATGCGCAAGGAGAATTGG) were designed for 3’-RACE cDNA cloning. For 5’-RACE, primers specific for CycD3 genes were designed according to the cDNA sequences of the 3’ region obtained from 3’-RACE: P3: 5’-TAG CCT CAA ACA CAT ACT TAG CAT C-3’ for CsCycD3;1 and P4: 5’-TCA ATC ACG CCA TTT GGG CTA TC-3’ for CsCycD3;2. The obtained fragments were cloned into pGEM-T easy vectors (Promega, USA) and sequenced at Sangon company (Shanghai, China).

Northern analysis

Total RNA (10 μg per sample) was subjected to electrophoresis in the form of aldehyde–agarose gels and blotted onto nylon membranes (Hybond N+, Amersham Pharmacia Biotech, Sweden) using standard procedures (Sambrook et al., 1989). Probes for CsCycD3;1 and CsCycD3;2 were prepared by the DNA fragments obtained from 3’-RACE, and labelled by [α-32P]-dCTP (3000 Ci μmol-1) with the Random Primed DNA Labeling Kit (Takara, Japan). Hybridizations were carried out overnight at 60 °C. Membranes were washed according to the standard procedures (Sambrook et al., 1989) and analysed by Typhoon 8600 (Pharmacia, Sweden).

Results

Fruit development, cell division, and endoreduplication

In order to study whether BRs can regulate early fruit growth, fruit set and ovary growth were measured after application of 24-epibrassinolide (EBR) and brassinazole (Brz). For the non-parthenocarpic cultivar Jinchun No. 4, unpollinated ovaries did not grow after anthesis as all ovaries failed to set and damped off eventually (Figs 1A, 2A). Pollination resulted in a gradual growth in ovary to 35 cm in length (Fig. 2C). Interestingly, application of EBR to unpollinated flowers induced parthenocarpic growth and production of fruits similar to those of the pollinated flowers. Among them, 0.2 μM EBR treatment showed the highest parthenocarpy percentage (c. 80.1%), which was not significantly different from the pollination treatment.

For the parthenocarpic cultivar Jinchun No. 2, 61.2% of the unpollinated ovaries set fruits that grew to normal size (Figs 1B, 2B). Application with 0.4 μM Brz had little...
effects on fruit set while Brz at 4 μM and Brz at 40 μM all completely prevented the ovaries from growth (Fig. 1B). Subsequent application of 0.2 μM EBR could rescue the growth inhibition induced by 4 μM Brz (Figs 1B, 2D), but failed to rescue the growth inhibition induced by 40 μM Brz (data not shown).

Cell division activity during early fruit development of different treatments was characterized by flow cytometric analysis of the nuclear DNA contents in ovaries at 0, 2, and 4 DAA in Jinchun No. 4. Nuclei of ovaries at anthesis were divided almost equally between two major peaks at 2C (50.8%) and 4C (49.2%) DNA levels (Table 2). In unpollinated ovaries, the proportion for the 2C DNA contents increased sharply while the 4C proportion decreased significantly. Both pollination and EBR treatment resulted in a significant decrease of the 2C peaks, but a sharp increase of the 4C peaks at 2 DAA. Based on the comparison of 4C/2C peak ratios at 2 DAA, EBR was more effective in triggering the cell division than pollination. At 4 DAA, a population of 8C cells was observed together with a high level of DNA replication. This indicated that a short initial cell division phase was followed by a high activity of endoreduplication in the ovaries after pollination or EBR treatment.
RT-PCR analysis for the expression of cell cycle-related genes

RT-PCR was used to analyse the relative transcript levels of two cyclin genes (CycA and CycB) and two CDK genes (CDKA and CDKB) in Jinchun No. 4 fruits at 1 DAA and 4 DAA after different treatments (Fig. 3). At 1 DAA, the transcript levels for the two cyclin genes and CDKB were up-regulated by both pollination and, to a greater extent, by EBR treatment. By contrast, the expression of CDKA was slightly down-regulated by both pollination and EBR treatment. A similar expression pattern of the cell cycle-related genes were found at 4 DAA. Meanwhile, it was found that EBR application and pollination had more remarkable effects on the expression of cyclin D3 genes than cyclin A and B (data not shown). Isolation of full-length cDNA and further examination on the transcripts were, therefore, carried out with cyclin D3 genes.

Table 2. Flow-cytometric analysis of nuclei from ovaries at anthesis, 2 DAA and 4 DAA after non-pollination, pollination or 24-epibrassinolide (EBR) treatment in Jinchun No. 4

The data presented are means ± SD. EBR was applied to unpollinated ovaries at a concentration of 0.2 μM.

| Treatments        | Days after treatment (d) | Percentage of area value for different DNA contents (±SD) |
|-------------------|--------------------------|---------------------------------------------------------|
|                   |                          | 2C   | 4C   | 8C   |
| At anthesis       | 0                        | 50.8±1.2 | 49.2±1.2 |     |
| Non-pollination   | 2                        | 52.7±1.2 | 47.3±1.2 |     |
| Pollination       | 4                        | 54.8±0.2 | 45.2±0.2 |     |
| EBR               | 2                        | 46.2±1.4 | 53.8±1.4 |     |
|                   | 4                        | 32.6±0.7 | 52.2±1.9 | 14.8±0.8 |
|                   |                          | 40.8±0.9 | 59.2±0.9 |     |
|                   |                          | 36.9±0.8 | 49.1±1.7 | 14.0±2.4 |

Northern blot analysis of CycD3 genes

Northern analysis showed that expression of CycD3 genes were most abundant at anthesis, and then decreased gradually in unpollinated ovaries in Jinchun No. 4 (Fig. 6A). Pollinated ovaries exhibited much higher CsCycD3;1 and CsCycD3;2 transcript levels than unpollinated ovaries. Importantly, EBR-treated ovaries always exhibited higher transcript levels at 1, 2, 4, and 6 DAA for CsCycD3;1 and at 2 DAA and 4 DAA for CsCycD3;2 than pollinated ovaries. This result was consistent with the higher activity of EBR treatment in promoting cell division than pollination.
CsCycD3;1 transcript decreased gradually after anthesis and became undetectable at 8 DAA in the naturally parthenocarpic fruits (Fig. 6B). Brz treatment substantially suppressed the expression of CsCycD3;1 as only a very weak hybridization signal could be detected throughout the experiment in Brz-treated ovaries. Furthermore, accumulation of CsCycD3;1 transcripts after Brz treatment could be rescued by EBR treatment. A similar trend was also observed with the accumulation of the CsCycD3;2 transcripts although the highest transcript levels were observed at 2 DAA in the naturally parthenocarpic fruits and EBR-treated fruits.

Expression of BR metabolic genes

RT-PCR analysis was performed to quantify mRNA levels of genes involved in BR biosynthesis (BR6ox1 and SMT) in cucumber ovaries. As shown in Fig. 7, the transcript level of BR6ox1 was reduced by EBR or pollination treatment in the fruits of Jinchun No. 4. The transcript level of BR6ox1 for parthenocarpic fruits in Jinchun No. 2 was similar to that of the pollinated ones in Jinchun No. 4 (Fig. 7A). Meanwhile, the transcript level of BR6ox1 was increased by Brz treatment and this Brz-induced expression was completely blocked by treatment with EBR in Jinchun No. 2 (Fig. 7B). SMT, a gene encoding S-adenosyl-l-methionine:Δ24-sterol-C-methyltransferase exhibited a similar expression pattern.
as BR6ox1 at 0–2 DAA. The transcript level of SMT was increased by pollination and EBR treatment, but decreased by Brz treatment at 6 DAA (Fig. 7C, D).

**Discussion**

In plants, the decision to set fruit is dependent on the successful completion of pollination and fertilization. It has been found previously that parthenocarpic growth could be induced by auxins, GAs or cytokinins (Schwabe and Mills, 1981; García-Martínez and Hedden, 1997; Li *et al.*, 2003). Meanwhile, BRs have been found to play a role in fruit maturity (Vardhini and Rao, 2002; Symons *et al.*, 2006). However, there has been no report about the role of BRs in early fruit development. It is reported here that parthenocarpic growth in a non-parthenocarpic cultivar
Jinchun No. 4 could be induced by EBR treatment (Figs 1, 2). In addition, the application of Brz, an inhibitor of BR biosynthesis, reduced fruit-set and the final size of the parthenocarpic fruits in Jinchun No. 2, and this inhibitory effect could be fully rescued by EBR application (Figs 1, 2). These results indicate that BRs play an important role in early fruit growth both in non-parthenocarpic and natural parthenocarpic cultivars. The induction of parthenocarpic growth by BRs in cucumber provides strong evidence for a critical role of BRs in the early growth of fruits.

It is generally accepted that fruit set and associated cell division occur as a result of the co-ordinated action of phytohormones produced in the ovary after pollination and/or fertilization (Gillaspy et al., 1993; Gorguet et al., 2005). In this study, EBR treatment and pollination triggered cell division, which was observed by flow cytometric analysis in the very young ovaries at 2 DAA. Endoreduplication occurred at 4 DAA accompanied by the appearance of 8C peak in the pollinated and EBR-treated fruits. By contrast, cell division was not activated in unpollinated ovaries based on the increased ratio of 2C to 4C DNA levels relative to that in the ovaries at anthesis (Table 2). These results suggest that BRs are involved in the triggering of cell division and subsequent endoreduplication in ovaries after anthesis.

It is known that cell division temporarily ceased after anthesis, and could be reactivated by both pollination and growth substances such as auxins and cytokinins (Gorguet et al., 2005). Until now, there are no reports about the triggering of cell division in unpollinated fruits by BRs, although several studies have shown stimulatory effects of BRs on the division of cultured cells (Hu et al., 2000; Nakaya et al., 2002). Several cyclins are involved in the regulation of cell cycle progress (Inzé and De Veylder, 2006). Among them, A- and B-type cyclins are preferentially expressed in the S-G2-M phase while D-type cyclins are important regulators of G1 progress and G1 to S phase transition. During the G1 to S phase transition, the CDK activity can be induced after stimulation by growth factors and CycD3 transduce extracellular signals to trigger cell division (Fuerst et al., 1996; Mironov et al., 1999; Gutierrez et al., 2002; Shen, 2002; Trimarchi and Lees, 2002). In the present study, it was found that the transcript levels for a number of cyclins and CDKs genes increased in ovaries after pollination or EBR treatment (Fig. 3). It has been reported that the expression of genes encoding cyclins and CDKs increases at the early stage of tomato fruit development (Joubès et al., 1999, 2000a). So far, there is little evidence about the role of BRs in regulating the cell cycle-related genes, except the enhancement of CDKB1;1 and CycD3 genes by BRs in Arabidopsis (Yoshizumi et al., 1999; Hu et al., 2000). The effects of EBR on the expression of cell cycle-related genes in this study indicate that cell division, as well as fruit development, induced by EBR may be achieved through the up-regulation of the transcript levels of the cell cycle-related genes.

For further examination of the relationship between CycD3 and EBR- or pollination-induced cell division, a complete cDNA sequence has been isolated for CsCycD3;1 and a partial sequence for CsCycD3;2 from cucumber fruits. The predicted amino acid sequence of CsCycD3;1 contains a cyclin box, a LxCxE motif, and possible PEST regions as in other D3 cyclins from Arabidopsis (Soni et al., 1995), alfalfa (Dahl et al., 1995), and tomato (Kvarnheden et al., 2000). High expression levels of CsCycD3;1 and CsCycD3;2 were detected in Jinchun No. 4 and Jinchun No. 2 at anthesis. CsCycD3;1 and CsCycD3;2 transcripts were sharply decreased in unpollinated ovaries in Jinchun No. 4 and were undetectable in Brz-treated ovaries in Jinchun No. 2. By contrast, the levels of CsCycD3;1 and CsCycD3;2 transcripts were increased after pollination in Jinchun No. 4, or EBR application in both Jinchun No. 2 and Jinchun No. 4. Thus, expression of CsCycD3;1 and CsCycD3;2 was closely correlated with cell division in the cucumber ovaries, suggesting that pollination/fertilization and EBR prolonged cell cycle-related gene transcripts to reactivate cell division (Fig. 6). This interpretation is consistent with a previous study that the transcript levels for D3 cyclins coincide with a period of intensive cell division activity (Kvarnheden et al., 2000). While the highest level of CsCycD3;1 transcripts was observed at anthesis, CsCycD3;2 had the highest transcript level at 2 DAA. The differential expression of the two genes suggest that the two cyclins may have distinct roles in the regulation of early fruit development. It has been suggested that CycD3 activates cell division by transducing extracellular signals into the cell-cycle control machinery (Sherr, 1993, 1994; Fuerst et al., 1996; Riou-Khamlichi et al., 1999; Hu et al., 2000). Ectopic D-type cyclin expression in endoreduplicating Arabidopsis trichome cells induced not only DNA replication but also cell division (Schnittger et al., 2002). Dewitte et al. (2003) have shown that leaves of CYCD3;1-overexpressing plants contain a substantially increased number of smaller cells, and cell division replaces cell expansion as the primary mechanism of leaf growth. It has previously been shown that up-regulated expression of LsCycD3;1 and LsCycD3;2 was associated with cytokinin-induced parthenocarpic growth and enhanced the growth rate of white-flower gourd fruits (Li et al., 2003). Here it has been found that EBR-induced parthenocarpic growth in cucumber was also associated with up-regulated cyclin gene transcripts (Fig. 3). In fact, EBR-induced parthenocarpic fruits showed higher CycD3 transcript levels and cell division activity than the pollinated fruits at 1 DAA and 2 DAA (Fig. 6). It is also possible that, even in the pollinated ovaries, the levels of BRs, although increased, have still not reached the optimal levels for activation of gene expression and cell division.
In plants, BRs homeostasis is thought to be maintained through feedback regulation of genes involved in BR metabolism (Tanaka et al., 2005). The expression of genes involved in BR and sterol biosynthesis were increased in BR-deficient mutants and after Brz treatment, but decreased after BR application (Mathur et al., 1998; Noguchi et al., 2000; Asami et al., 2001; Choe et al., 2001; Goda et al., 2002; Tanaka et al., 2005). Likewise, it has been found that inhibition of BR biosynthesis by Brz resulted in enhanced transcript levels of BR6ox1 and SMT, two genes involved in BR biosynthesis while EBR-treated, pollinated and natural parthenocarpic fruits all had reduced expression of these genes (Fig. 7). It is worth noting that EBR and Brz had more significant effects on the transcript of BR6ox1 than SMT, a downstream gene and an upstream gene in the BR biosynthesis pathway, suggesting that BR biosynthesis was enhanced in pollinated and natural parthenocarpic fruits. This is also in agreement with Montoya et al. (2005) who detected increased accumulation of endogenous BRs in early developing tomato fruits. All these results suggested that BRs played a role in the early fruit development.

In summary, the data presented here show that parthenocarpic growth was induced by exogenous BRs but inhibited by the inhibition of BR synthesis. BRs triggered active cell division associated with increased transcripts of cell cycle-related genes, especially that of cyclin D3 genes. These findings indicate that BRs play a regulatory role in early fruit development of cucumber plants. It is worth noting that fruit development is a complex process and BRs could cross-talk with other hormones such as auxins and GAs. It will be interesting to investigate whether the Brz-inhibited fruit growth of the parthenocarpic fruits in Jinchun No. 2 could be rescued by other plant hormones and whether BRs could induce the response of other plant hormones signalling networks.

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