

# Effects of Phenylcarboxylic Acids on Mitosis, Endoreduplication and Expression of Cell Cycle-Related Genes in Roots of Cucumber (*Cucumis sativus* L.)

Yun Zhang · Min Gu · Xiaojian Xia · Kai Shi ·  
Yanhong Zhou · Jingquan Yu

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**Abstract** Several benzoic and cinnamic acid derivatives were identified from cucumber root exudates. The effects of these phenylcarboxylic acids on root growth and cell cycle progression were examined in germinated seeds of cucumber. All 12 phenylcarboxylic acids (0.25 mM) tested significantly inhibited cucumber radicle growth, and cinnamic acid exerted a dose-dependent inhibitory effect. At 6 h after exposure to the acids, transcript levels of the cell cycle-related genes, including two cyclin-dependent kinases (CDKs) and four cyclins were reduced. Among them, transcript of *CycB*, a marker gene for mitosis showed a remarkable reduction. The temporal analysis showed that expression of mitotic genes (*CDKB*, *CycA*, and *CycB*) were reduced throughout the experiment, while the reduction of the other genes (*CDKA*, *CycD3;1*, and *CycD3;2*) were observed only at earlier time points. At 48 h after treatment with benzoic and cinnamic acids, an enhancement of endoreduplication was observed. Further time course analysis indicated that endoreduplication started as early as 6 h after exposure to cinnamic acid. These results provide evidence that exposure to benzoic and cinnamic acids can induce rapid and dramatic down-regulation of cell cycle-related genes, thus leading to root growth inhibition. Meanwhile, the block of mitosis caused by phenylcarboxylic acids also induced an increased level of endoreduplication.

**Keywords** Allelopathy · Phenylcarboxylic acid · Cell cycle · Cyclin · CDKs · Endoreduplication

## Introduction

Plants synthesize an array of chemicals that are involved in a variety of plant-plant, plant-microbe, and plant-herbivore interactions (Inderjit and Duke 2003; Weir et al. 2004). The allelochemicals that are responsible for plant-plant allelopathy are delivered into the rhizosphere mainly through leaching, volatilization, decomposition, and root exudation (Rice 1984; Singh et al. 1999). Allelopathy is usually interspecific (Weidenhamer et al. 1989; Callaway and Aschehoug 2000), but also may occur within the same species, which is called autotoxicity (Singh et al. 1999, Yu et al. 2000). The problem of autotoxicity is common in croplands, and is one of the major reasons for growth reduction in monocropping (Singh et al. 1999; Yu et al. 2000). Although autotoxicity has long been recognized, little is known about its exact action mode.

Investigation of the chemical composition of allelopathic plants reveals that a great number of secondary metabolites serve as allelochemicals. Among them, benzoic and cinnamic acid derivatives are frequently identified from soils or root exudates (Yu and Matsui 1994; Blum 1996; Inderjit and Duke 2003). Root growth inhibition by benzoic and cinnamic acids has been widely observed (Chon et al. 2002; Iqbal et al. 2004; Hiradate et al. 2005; Rudrappa et al. 2007; Batish et al. 2008). Furthermore, exogenous addition of allelochemicals can influence physiological and biochemical reactions, such as photosynthesis, respiration, water and nutrient uptake, and generation of reactive oxygen species (Hejl et al. 1993, Hejl and Koster 2004; Gonzalez et al. 1997; Yu and Matsui 1997; Ding et al.

Y. Zhang · M. Gu · X. Xia · K. Shi · Y. Zhou · J. Yu (✉)  
Department of Horticulture, Huajiachi Campus,  
Zhejiang University,  
Kaixuan Road 268,  
Hangzhou 310029, People's Republic of China  
e-mail: jqyu@zju.edu.cn

J. Yu  
Key Laboratory of Horticultural Plants Growth,  
Development and Biotechnology, Agricultural Ministry of China,  
Hangzhou, People's Republic of China

2007), as well as induce changes in gene expression (Golisz et al. 2008). All such physiological, biochemical, and transcriptional changes are implicated directly or indirectly in plant growth inhibition.

Plant growth ultimately is driven by the process of cell division coupled with the subsequent expansion and differentiation of the resulting cells (Beemster et al. 2003; Jakoby and Schnittger 2004). Cell division plays a role both in the developmental processes that create plant architecture and in the modulation of plant growth rate in response to the environment (Cockcroft et al. 2000; Beemster et al. 2002). The normal cell cycle mode is characterized by a round of DNA replication (S phase) followed by mitosis and cytokinesis (M phase), and separated by two gap phases ( $G_1$  and  $G_2$ ) (Inzé 2005). Cyclin-dependent kinases (CDKs) and their cyclin partners regulate the  $G_1/S$ - and  $G_2/M$ -phase transitions as well as progression through and exit from the cell cycle (Menges et al. 2002; Beemster et al. 2005; Inzé and De Veylder 2006). Studies have shown that regulation of cell cycle-related gene expression in different phases is an important mechanism for control of progression through the cell cycle (Menges et al. 2002; Beemster et al. 2005). At present, little is known about the relationship between allelopathy and cell division. Cell cycle progression has been proposed as a target (Burgos et al. 2004; Nishida et al. 2005; Sánchez-Moreiras et al. 2008). However, no data are available concerning the influence of allelochemicals such as phenylcarboxylic acids on the regulation of cell cycle progression by investigation of cell cycle-related gene expression.

Poor plant growth in monocropping of cucumber is supposedly related to autointoxication partly arising from root exudates (Yu et al. 2000). Phenylcarboxylic acids including benzoic and cinnamic acids have been identified from root exudates of cucumber plants, and they had detrimental effects on ion uptake and enhanced the incidence of *Fusarium* wilt by triggering oxidative stress in cucumber (Yu and Matsui 1994, 1997; Ye et al. 2004, 2006; Ding et al. 2007). This study was performed to investigate the influences of benzoic and cinnamic acids on cucumber root growth and cell cycle progression in radicle tips.

## Methods and Materials

**Plant Material** Cucumber (*Cucumis sativus* L. cv. Jinyan No.4) seeds were germinated at 25°C in darkness. Fifty seeds were imbibed in petri dishes (diameter=15 cm) on two layers of Whatman filter paper saturated with 15 ml distilled water. When the radicle had begun to emerge, germinating seeds were transferred to another petri dish with different solutions of derivatives of benzoic and cinnamic acid

(benzoic, vanillic, *p*-hydroxybenzoic, *o*-hydroxybenzoic, 3,4-dihydroxybenzoic, gallic, cinnamic, 3-phenylpropionic, caffeic, *p*-coumaric, ferulic, and sinapic acids) at 0.25 mM for another 48 h. Distilled water served as the control. To study concentration effect of cinnamic acid, germinating seeds were transferred to petri dishes containing cinnamic acid at 0.05, 0.1, 0.25, and 0.5 mM for another 48 h. Radicle length was determined at 48 h after exposure to different phenylcarboxylic acids, and inhibition rates of radicle length were calculated. Each experiment was carried out with three independent replicates.

**Nuclei DNA Content Analysis** We analyzed the relative nuclei DNA content of cucumber radicle tips (0–2 mm) at 48 h after treatments with different kinds of derivatives of benzoic and cinnamic acids (0.25 mM). Afterwards, changes in nuclei DNA content with different concentrations of cinnamic acid (0.05, 0.1, 0.25, and 0.5 mM) were investigated in the radicle tips of cucumber seeds at 48 h after treatment. Additionally, temporal changes in nuclei DNA content of radicle tips were determined at 6, 24, and 48 h after treated with cinnamic acid (0.25 mM). Samples were chopped with a razor blade in 1 ml of ice-cold buffer (45 mM  $MgCl_2$ , 30 mM sodium citrate, 20 mM MOPS, pH=7, and 1% Triton X-100) (Galbraith et al. 1983), filtered over a 45- $\mu$ m nylon mesh, and stained with 4,6-diamidino-2-phenylindole. Stained nuclei were analyzed by a FACSCalibur flow cytometer (Becton, Dickinson and Company, USA). Calibration of C-values was made with nuclei from young leaves of cucumber.

Data were analyzed with CellQuest software to determine the ploidy distribution. Mean C-value (MCV) was calculated as the sum of the number of nuclei of each ploidy level multiplied by its endoreduplication cycle and divided by the total number of nuclei (Barow and Meister 2003), where C-value represents the DNA content of the monoploid chromosome set of an organism (Greilhuber et al. 2005; Greilhuber 2008).

**RNA Extraction** Total RNA was extracted from 0–2 mm radicle tips of cucumber with different treatments with TRIZOL reagent (Sangon, China) according to the manufacturer's instructions at various time after treatments. After extraction, total RNA was dissolved in diethyl pyrocarbonate-treated water.

**Transcript Level Estimation with qRT-PCR** Quantitative real-time PCR (qRT-PCR) assays were performed to determine the relative transcript level of each cDNA. Gene-specific primers for qRT-PCR were designed based on EST sequences for six cell cycle-related genes, *CDKA* (cyclin-dependent kinase A), *CDKB* (cyclin-dependent kinase B), *CycA* (A-type cyclin), *CycB* (B-type cyclin),

*CycD3;1* (D-type cyclin), and *CycD3;2* (D-type cyclin). The specific sets of primers used for the amplification of each cDNA are summarized in Table 1. The first-strand cDNA used as template for qRT-PCR was synthesized by using a RevertAid™ first strand cDNA Synthesis Kit (Fermentas) from 2 µg of total RNA purified by using a RNeasy Mini Kit (Qiagen).

qRT-PCR was performed with an iCycler iQ Multicolor Real-time PCR Detection System (Bio-Rad, Hercules, CA, USA). Each reaction (20 µl total volume) consisted of 10 µl iQ SYBR Green Supermix, 1 µl of diluted cDNA, and 0.1 µM of forward and reverse primers. PCR cycling conditions were as follows: 95°C for 3 min, 40 cycles of 95°C for 10 s, and 58°C for 45 s. Fluorescence data were collected during the 58°C step. The cucumber *actin* gene was used as an internal control. Relative gene expression was calculated as described by Livak and Schmittgen (2001).

**Statistical Analysis** Seeds were arranged in three randomized blocks with three replicates per treatment. All results were subject to analysis of variance (Statistica 6.0, Microsoft Corp., 1995), and the means were compared by the Tukey' HSD test at a significance level of  $P \leq 0.05$ .

## Results

**Effects of Benzoic and Cinnamic Acids on Radicle Elongation of Cucumber** The effects of benzoic acids (benzoic, vanillic, *p*-hydroxybenzoic, *o*-hydroxybenzoic, 3,4-dihydroxybenzoic, and gallic acids) and cinnamic acids (cinnamic, 3-phenylpropionic, caffeic, *p*-coumaric, ferulic, and sinapic acids) at 0.25 mM on cucumber radicle elongation were examined at 48 h after treatments (Table 2).

Exposure to these acids all resulted in a reduction in radicle length. The inhibition rates ranged from 20.69 to 59.43%, as compared to the control treated with distilled water. Among the 12 acids assayed, cinnamic acid had the greatest inhibitory effect while benzoic acid had the lowest. Comparatively, benzoic acids showed less inhibitory effects (20.7–36.0%) than cinnamic acids (34.8–59.4%). Additionally, the concentration effect of cinnamic acid, an important autotoxin of cucumber, on cucumber radicle growth was investigated. Cinnamic acid at 0.05 mM had only slight inhibitory effects on radicle elongation while cinnamic acid at 0.1 mM significantly inhibited radicle length (Fig. 1). Exposure to cinnamic acid at 0.25 mM and 0.5 mM resulted in a more marked reduction of radicle length by 52.4% and 69.3%, respectively.

**Effects of Benzoic and Cinnamic Acids on Relative Nuclei DNA Content in Cucumber Radicle Tips** To examine the effects of benzoic and cinnamic acids on mitosis and endoreduplication in cucumber radicle tips, nuclei DNA content was measured by flow cytometric analysis in cucumber radicle sections 2 mm apart in the radicle tip, the zone with most active cell division in the radicle. The first peak of nuclei observed in the histogram for cucumber was defined as 2C, and the following peaks were 4C, 8C, and so on according to Gilissen et al. (1993). Nuclei with 2, 4, and 8C were all found in control radicle tips, and the MCV was relatively constant (3.83–3.99) (Tables 3, 4, and 5). As shown in Table 3, all tested benzoic and cinnamic acids significantly decreased the proportion of 2C but increased the proportion of 8C. Among them, radicles exposed to sinapic, caffeic, and cinnamic acids showed higher 8C values than those exposed to other acids. However, these acids had little effect on the proportion of 4C. The MCV increased from 3.96 for the control to

**Table 1** Primers used for quantitative real-time PCR assays

Gene	Encoding protein	Accession No.	Primer pairs
<i>CycA</i>	A-type cyclin	EW968279	F: GATTTGTTTCGTGCTGCTCAA R: TAATTCGGCAAGGAAGTTGG
<i>CycB</i>	B-type cyclin	EW968280	F: GAGAACGAGAGCAGACCTCA R: CAATCTTTGTGCGCAAGGAAA
<i>CycD3;1</i>	D-type cyclin	EW968283	F: CATGTGGAGGAGAAAGCAGA R: TTGATGCTGACACAATCGTC
<i>CycD3;2</i>	D-type cyclin	EW968284	F: CTATCAACCCTCACAAACGC R: TGTTGCTCTAAATGCCCAAG
<i>CDKA</i>	cyclin-dependent kinase A	EW968281	F: TCTTCGTGGCATTGCATATT R: TCTTGCCAGTCCAAAGTCAG
<i>CDKB</i>	cyclin-dependent kinase B	EW968282	F: CAATCCCTCTATGTCGTTTCG R: GCTTGAGATCACGGTGAAGA
<i>actin</i>		DQ641117	F: AAAGATGACGCAGATAAT R: GAGAGATGGCTGGAATAG

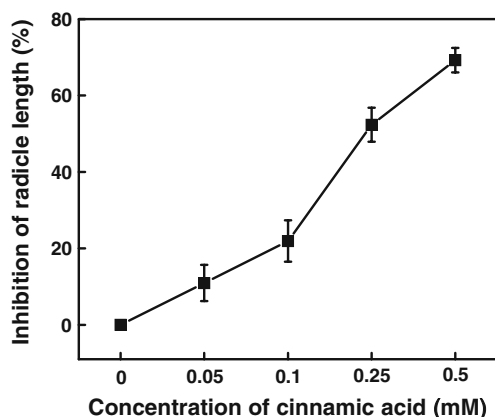
**Table 2** Inhibition of radicle elongation of cucumber plants exposed to 0.25 mM phenylcarboxylic acids for 48 h

Phenylcarboxylic Acids	Inhibition Rate of Radicle Length (%)
Control	0.0 g
Benzoic Acid	20.7 f
Vanillic Acid	35.2 d
<i>p</i> -Hydroxybenzoic Acid	30.4 e
<i>o</i> -Hydroxybenzoic Acid	36.0 d
3,4-Dihydroxybenzoic Acid	33.2 de
Gallic Acid	33.0 de
Cinnamic Acid	59.4 a
3-Phenylpropionic Acid	48.6 b
Caffeic Acid	43.4 c
<i>p</i> -Coumaric Acid	34.8 d
Ferulic Acid	35.5 d
Sinapic Acid	48.2 b

Numbers with different letters refer to significant differences among the treatments ( $P \leq 0.05$ ). The concentration of each phenylcarboxylic acid was 0.25 mM. Each treatment was carried out using three independent biological replicates.

4.43 and 4.66 after exposure to benzoic and cinnamic acids, respectively. Accordingly, exposure to all these acids resulted in an increased level of endoreduplication in radicle tips.

Table 4 shows that cinnamic acid at 0.05 mM had no significant influence on the MCV but significantly decreased the proportion of 2C. When the concentration of cinnamic acid reached  $\geq 0.1$  mM, the proportion of 2C decreased and the proportion of 8C increased. The MCV increased by 14.7%, 17.8%, and 23.5% in the 0.10, 0.25, and 0.5 mM cinnamic acid treatments, respectively, as compared to the control. A time course study showed that exposure to cinnamic acid at 0.25 mM resulted in a decrease in 2C proportion and an increase in 8C proportion from 6 h after treatment, leading to a significant increase in MCV. Compared with the control, the MCV index increased by 6.0, 11.5, and 10.2% at 6, 24, and 48 h after treatment with 0.25 mM cinnamic acid, respectively (Table 5).

**Fig. 1** Effects of different concentrations (0, 0.05, 0.1, 0.25, and 0.5 mM) of cinnamic acid on inhibition of cucumber radicle elongation (%) at 48 h after treatment

**Effects of Benzoic and Cinnamic Acids on Cell Cycle-related Genes Expression** To gain insight into the mechanism by which these acids regulate the cell cycle, the expression of six cell cycle-related genes, *CDKA*, *CDKB*, *CycA*, *CycB*, *CycD3;1*, and *CycD3;2* were examined in cucumber radicle tips (2 mm) after treatments. Transcript levels of all examined genes were reduced in radicles after exposure to 0.25 mM benzoic, vanillic, *p*-hydroxybenzoic, *o*-hydroxybenzoic, cinnamic, 3-phenylpropionic, and *p*-coumaric acids. Exposure to 3,4-dihydroxybenzoic and gallic acids reduced the transcript level of *CycB* but had negligible effects on transcript levels of other genes (Fig. 2). Caffeic acid had little effect on transcript levels of *CDKA*, *CycA*, and *CycD3;1* but greatly reduced transcript levels of *CDKB*, *CycB*, and *CycD3;2*. Similarly, ferulic and sinapic acids reduced the transcript levels of *CDKA*, *CDKB*, *CycB*, *CycD3;1*, and *CycD3;2* but had negligible effects on transcript levels of *CycA* (Fig. 2).

We further analyzed the changes in transcript levels of cell cycle-related genes in cucumber radicles in response to different concentrations of cinnamic acid at 6 h after treatment, as well as at various time after treatment with 0.25 mM cinnamic acid. As shown in Fig. 3, transcript levels of all six cell cycle-related genes decreased with the increase in the concentration of cinnamic acid. Transcript levels of *CDKA*, *CDKB*, *CycA*, *CycB*, *CycD3;1*, and *CycD3;2* decreased to 61.2, 57.7, 62.2, 20.5, 61.5, and 52.71% of the control, respectively, in radicles after 6 h-exposure to 0.25 mM cinnamic acid (Fig. 3). The inhibition in the expression of six cell cycle-related genes was detectable earlier (at 3 h) after exposure to 0.25 mM cinnamic acid (Fig. 4). The expression of *CDKA* gene was repressed at 3 h after exposure to cinnamic acid, and a more dramatic decrease was observed at 6 h after treatment. Transcript levels of *CDKB*, *CycA*, and *CycB* were down-regulated throughout the treatment time. *CycD3;1* and

**Table 3** Effects of benzoic and cinnamic acids on relative nuclei DNA content and mean c-value in cucumber radicle tips at 48 h after treatment

Phenylcarboxylic Acids	2C (%)	4C (%)	8C (%)	Mean C-Value
Control	35.3a	48.1	16.6b	4.0b
Benzoic Acid	25.6b	50.5	23.9a	4.4a
Vanillic Acid	25.6b	50.4	24.0a	4.4a
<i>p</i> -Hydroxybenzoic Acid	26.9b	49.1	24.1a	4.4a
<i>o</i> -Hydroxybenzoic Acid	24.8b	50.4	24.8a	4.5a
3,4-Dihydroxybenzoic Acid	23.8b	49.5	26.7a	4.6a
Gallic Acid	26.7b	48.2	25.2a	4.5a
Cinnamic Acid	25.2b	47.9	27.0a	4.6a
3-Phenylpropionic Acid	27.2b	49.2	23.6a	4.4a
Caffeic Acid	24.1b	48.1	27.8a	4.6a
<i>p</i> -Coumaric Acid	26.2b	48.6	25.2a	4.5a
Ferulic Acid	27.5b	48.1	24.4a	4.4a
Sinapic Acid	23.4b	48.3	28.3a	4.7a

Numbers with different letters in the same column refer to significant differences among the treatments ( $P \leq 0.05$ ). The proportions of 2C, 4C, and 8C nuclei were measured on 10 000 nuclei. The concentration of each phenylcarboxylic acid was 0.25 mM. Each treatment was carried out using three independent biological replicates.

*CycD3;2* genes were down-regulated at 3 and 6 h, and then, their transcript levels increased gradually (Fig. 4).

## Discussion

In the present study, we used benzoic and cinnamic acid derivatives identified from cucumber root exudates (Yu and Matsui 1994; Asao et al. 1999), to investigate their impact on cucumber root growth and cell cycle regulation. Germinated seeds were used since radicles or the young roots usually are the first organs to obtain contact with autotoxic agents. The results showed that all phenylcarboxylic acids caused strong inhibition in cucumber radicle elongation (Table 2), which is consistent with previous studies (Yu and Matsui 1994, 1997; Yu et al. 2003; Dos Santos et al. 2008; Batish et al. 2008; Tharayil et al. 2008). Cinnamic acid, an important autotoxin of cucumber, showed a higher inhibitory effect than other compounds, as has been observed in other plants (Yu and Matsui 1997; Hiradate et al. 2005). It is worth noting that cinnamic acid was applied at reasonable concentrations (0–0.5 mM) in this study since the concentration of autotoxins was

0.1 mM in soils after cucumber cultivation and may reach much higher levels in soils enriched in plant residues (An et al. 2001a, 2001b; Yu et al. unpublished data). The presence of a variety of autotoxins exuded from cucumber roots likely would exhibit synergism (Yu and Matsui 1994; An et al. 2001b). All tested compounds caused significant inhibition in radicle length to different degrees (Table 2), which was possibly due to chemical characteristics such as water solubility (Yu and Matsui 1997; Tharayil et al. 2008).

The elaboration of plant form and function depends on the ability of a cell to divide and differentiate (Inzé 2005). Benzoic and cinnamic acids differently induced down-regulations of two CDKs (*CDKA* and *CDKB*) and four cyclin (*CycA*, *CycB*, *CycD3;1*, and *CycD3;2*) genes at the transcript level (Figs. 2, 3, and 4). Among them, expression of *CycB*, a marker gene for mitosis and specific to the G<sub>2</sub>/M phase of the cell cycle (Hemerly et al. 1992; Ferreira et al. 1994), was the most reduced, suggesting that the transition of G<sub>2</sub> to M was blocked by the phenylcarboxylic acids; thus, the ability of cells to enter cell division was impaired (Cools and De Veylder 2009). This is consistent with a report that benzoxazolin-2(3*H*)-one selectively retards cell cycles at the G<sub>2</sub>/M checkpoint in lettuce seedling root meristems (Sánchez-Moreiras et al. 2008).

**Table 4** Effects of different concentrations of cinnamic acid on relative nuclei DNA content and mean c-value in cucumber radicle tips at 48 h after treatment

Concentrations (mM)	2C (%)	4C (%)	8C (%)	Mean C-Value
0	33.7a	52.4a	13.9c	3.9c
0.05	29.8b	52.4a	16.5c	4.0c
0.1	28.2b	50.0a	23.6b	4.4b
0.25	25.2b	47.9a	27.0a	4.6b
0.5	25.8c	45.7b	30.5a	4.8a

Numbers with different letters in the same column refer to significant differences among the treatments ( $P \leq 0.05$ ). The proportions of 2C, 4C, and 8C nuclei were measured on 10 000 nuclei. Each treatment was carried out using three independent biological replicates.



**Table 5** Effects of cinnamic acid on relative nuclei DNA content and mean c-value in cucumber radicle tips at different time after treatment

Hours (hr)	Treatments	2C (%)	4C (%)	8C (%)	Mean C-Value
6	Control	39.2a	45.3	15.4b	3.8b
	Cinnamic Acid	33.6b	48.2	18.3a	4.1a
24	Control	36.4a	46.0	17.6b	4.0b
	Cinnamic Acid	26.1b	46.9	27.0a	4.6a
48	Control	35.8a	48.5	15.8b	3.9b
	Cinnamic Acid	28.3b	49.6	22.2a	4.3a

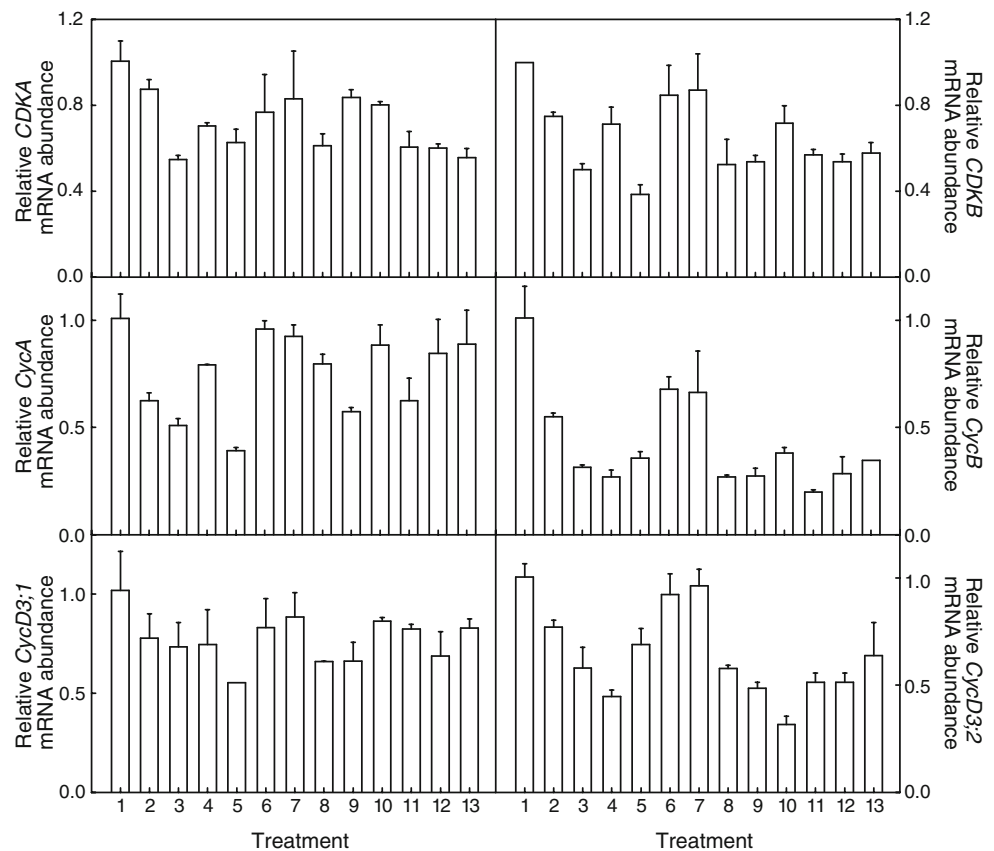
Numbers with different letters in the same column refer to significant differences between the treatments ( $P \leq 0.05$ ). The proportions of 2C, 4C, and 8C nuclei were measured on 10 000 nuclei. The concentration of cinnamic acid is 0.25 mM. Each treatment was carried out using three independent biological replicates.

It also has been demonstrated that salt stress and fungal elicitors also induce the repression of the B-type cyclin gene expression (West et al. 2004; Suzuki et al. 2006). Similar to *CycB* gene, expression of *CycA* gene, an A-type cyclin involved in the control of S-to-M phase, was reduced by the phenylcarboxylic acids. Possibly, cinnamic and other acids induce generation of reactive oxygen species that suppress the transcript level of *CycA*, since the expression of *CycA* gene is sensitive to oxidative stress (Reichheld et al. 1999; Ding et al. 2007). *CDKB* gene is specific to the  $G_2$  and M phases (Magyar et al. 1997; Porceddu et al. 2001) while the *CDKA* gene plays a pivotal role in both the  $G_1$ -to-S and  $G_2$ -to-M transition points, and its activity is rate

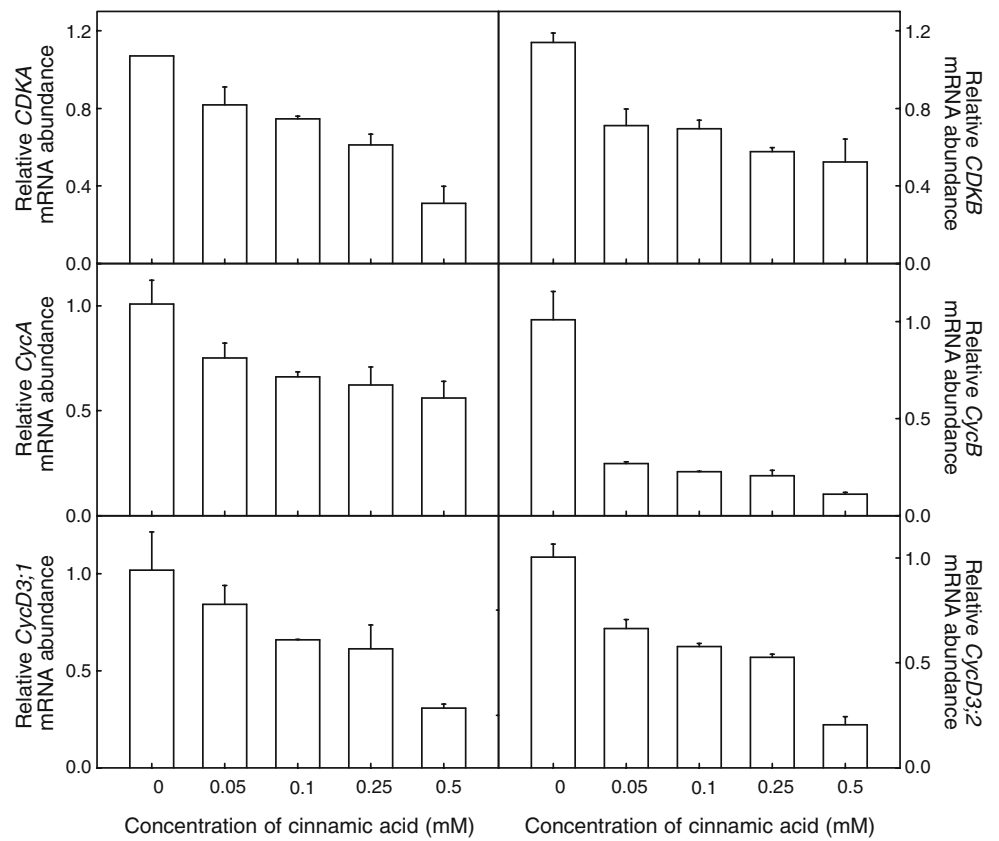
limiting for the plant cell cycle (Hemerly et al. 1995; Inzé 2005). Similar to other studies under salt stress, water stress, and cold night conditions (Setter and Flannigan 2001; West et al. 2004; Rymen et al. 2007), the expression of *CDKA* and *CDKB* genes was down-regulated by the tested phenylcarboxylic acids, especially *CDKB*, which showed a constant down-regulation pattern during the treatment time. This also suggests that mitosis was more seriously inhibited.

D-type cyclins are thought to regulate the  $G_1$ -to-S transition (Menges et al. 2006). The reduced transcript levels of *CycD3;1* and *CycD3;2* in cucumber radicles after exposure to phenylcarboxylic acids suggested that they

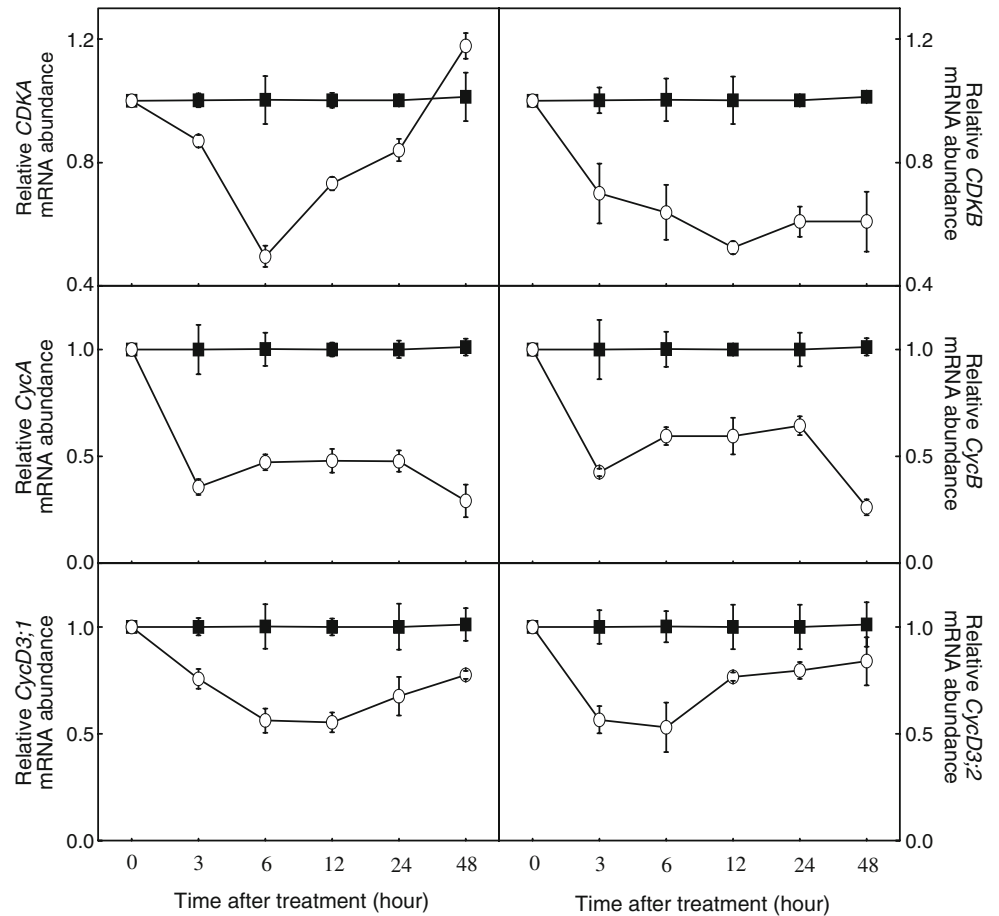
**Fig. 2** Expression of cell cycle-related genes in cucumber radicle tips at 6 h after exposure to 12 phenylcarboxylic acids. The numbers of the 1–13 displaying on the horizontal axis represent different treatments with distilled water, benzoic acid, vanillic acid, *p*-hydroxybenzoic acid, *o*-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, gallic acid, cinnamic acid, 3-phenylpropionic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid, respectively. The concentration of each phenylcarboxylic acid was 0.25 mM. Expression levels by qRT-PCR are expressed as a ratio of the control, which is set as 1



**Fig. 3** Expression of cell cycle-related genes in cucumber radicle tips at 6 h after exposure to different concentrations of cinnamic acid. The concentrations of cinnamic acid used were 0.05, 0.1, 0.25, and 0.5 mM, respectively. The control was treated with distilled water. Expression levels by qRT-PCR are expressed as a ratio of the control, which is set as 1



**Fig. 4** Expression of cell cycle-related genes in cucumber radicle tips at different hours after exposure to cinnamic acid. The concentration of cinnamic acid was 0.25 mM. Expression levels by qRT-PCR are expressed as a ratio of the control, which is set as 1. The control (filled rectangular), 0.25 mM cinnamic acid (open circle)



altered the G<sub>1</sub>-to-S transition process. However, it is interesting to note that both the expression of *CDKA* and *CycD3* genes increased from 12 h after treated with 0.25 mM cinnamic acid, which was different from the expression patterns of *CDKB*, *CycA*, and *CycB* genes in our study (Fig. 4). It is likely that *CDKA* and *CycD3* genes also play roles in the process of endoreduplication since they are active in the G<sub>1</sub>/S phase (see below).

The existence of 2, 4, and 8C cells in cucumber radicles suggested the occurrence of endoreduplication, as observed in a previous study (Gilissen et al. 1993). Endoreduplication is another mode of the cell cycle. The mitotic cell cycle comprises the duplication and subsequent distribution of chromosomes between two daughter cells, whereas endoreduplication involves repetitive chromosomal DNA replication and marks the exit from the cell division program, leading to an increase in the ploidy level (Joubès and Chevalier 2000; Cools and De Veylder 2009). In this study, we found that all the tested phenylcarboxylic acids induced higher levels of endoreduplication compared with the control. The enhancement of endoreduplication that occurred after phenylcarboxylic acids treatments was possibly a protective and adaptive mechanism to environmental stress as has been observed in cadmium stress (Fusconi et al. 2006; Repetto et al. 2007).

Previous studies have shown that senescence can induce a higher level of endoreduplication, and both benzoic and cinnamic acids can trigger the generation of reactive oxygen species and ultimately cause loss of root viability (Galbraith et al. 1991; Ding et al. 2007). It is possible that the induction of significant amounts of reactive oxygen species by cinnamic acid could consequently result in the process of early senescence and cause a higher level of endoreduplication. Down-regulation of mitotic genes induced by benzoic and cinnamic acids then may directly result in a different distribution of the nuclei class of ploidy in the differentiated roots. *CDKB* and *CycB* genes are actively involved in the cell mitosis phase, whereas reduced expression of these genes is accompanied by the onset of enhanced endoreduplication (Schnittger et al. 2002; Boudolf et al. 2004; Cools and De Veylder 2009). In our study, *CDKB* and *CycB* were significantly and constantly repressed at the transcript levels when exposed to phenylcarboxylic acids (Figs. 2, 3, and 4), indicating a significant inhibition of the mitotic phase. Since the mitotic phase was blocked, the initiation of the endoreduplication phase was enhanced (Tables 3, 4, and 5).

In summary, this study demonstrated that benzoic and cinnamic acid derivatives caused rapid and dramatic down-regulation of cell cycle-related genes in cucumber radicles, leading to radicle growth inhibition. Meanwhile, they induced an increased degree of endoreduplication due to

the blocking of mitosis. This is possibly a protective mechanism against stress.

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