Effect of salicylic acid and yeast extract on curcuminoids biosynthesis gene expression and curcumin accumulation in cells of *Curcuma zedoaria*

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Abstract The aim of this study is to evaluate the effect of yeast extract (YE) and salicylic acid (SA) on the expression of curcuminoid-biosynthesis genes (*CzDCS* and *CURS1-3*), and accumulation of curcumin in *Curcuma zedoaria* cell cultures. The results showed that, in cells treated with YE or SA, the expression levels of curcuminoid genes were 1.14- to 3.64-fold higher than the control (untreated cells), in which the YE exhibited a stronger effect in comparison with SA. Curcumin accumulation also tended to be similar to gene expression, curcumin contents in YE- or SA-treated cells were 1.61- to 2.53-fold higher than the control. The SA treatment at the fifth day of culture stimulated the curcumin accumulation and expression in all four genes compared to that at the beginning. While the YE treatments gave different results, the *CzCURS1* and *CzCURS3* genes were expressed strongly in cells that were treated at the beginning. However, the *CzDCS* and *CzCURS2* genes showed the opposite expression pattern, they were activated strongly in the treatments at day five of the culture. However, the content of curcumin reached its maximum value on the fifth day of culture in all investigations.

Keywords *Curcuma zedoaria*, curcuminoid genes, elicitor, salicylic acid, yeast extract

Introduction

Zedoary (*Curcuma zedoaria* Rosc.) is a perennial herb that belongs to the Zingiberaceae family found in tropical countries such as India, Japan, Thailand (Lobo et al. 2009), and Vietnam (Loi 2004). The rhizome of zedoary has been used in traditional and folk medicine for the treatment of menstrual disorders, dyspepsia, cough, vomiting, inflammations, hepatitis and diarrhoea (Azam et al. 2017).

Curcuminoid is a mixture of curcumin and its derivatives such as bisdemethoxycurcumin and demethoxycurcumin isolated from the rhizome of zedoary (Lobo et al. 2009) and other *Curcuma* species (Katsuyama et al. 2009; Lee et al. 2014; Gilani et al. 2015; Behar et al. 2016). Previous studies suggested that curcuminoids, especially curcumin, have valuable biological activities such as antioxidant, antitumor, anti-inflammatory, anti-acidogenic, radioprotective and neuroprotective properties (Hayakawa et al. 2011; Amalraj et al. 2017).

Genes involved in the pathway of curcuminoid metabolism in *Curcuma longa* included two type III polyketide synthase genes, *DCS* (diketide-CoA synthase) gene and *CURS1* (curcumin synthase 1), *CURS2* and *CURS3* genes, and their expression levels were reported by Katsuyama et al. (2009a, b). Brand et al. (2006) characterized another type III polyketide synthase gene, chalcone synthase (*CHS*) gene, in *Wachendorfia thyrsiflora*. This gene is also involved in the biosynthesis of curcuminoids. Behar et al. (2016) analysed expression of genes which participate in curcuminoid biosynthesis in *C. caesia* including *DCS*, *CURS*, *CURS2*, *CURS3* and *CHS1*. All the genes exhibited a higher expression level in the rhizome compared to leaves. Sandeep et al.

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(2017) evaluated expression of *CURS* gene in *C. longa* at different experimental areas and harvesting phases towards cultivation for high curcumin yield. In a recent study, we also found four similar curcuminoid genes (named *CzDCS*, *CzCURS1*, *CzCURS2* and *CzCURS3* with the corresponding accession numbers are MF663785, MF402846, MF402847, and MF987835) in zedoary (*C. zedoaria*, *Cz*) and their expression in rhizome and callus (Lan et al. 2018).

Elicitors are the chemical compounds which have been used to modify the pathway of secondary metabolism in order to enhance of the biosynthesis of pharmaceutically significant metabolites or phytopharmaceuticals in plant cell cultures (Jeong and Park 2005; Namdeo 2007). According to Abraham et al. (2011), yeast extract (YE) has been applied in plant tissue culture due to its ability to stimulate the defence mechanism, which leads to increase production of secondary metabolites. Salicylic acid (SA) is recognized as one of important signals in plant defense response and are also widely used for secondary metabolite production in plant cell cultures (Badrhadad et al. 2013). The present work therefore investigated the effect of elicitors such as YE and SA on activation of curcuminoid genes in callus cells of zedoary. Our results provided the first evidence for role of SA and YE as positive regulators of elicitor-induced gene expression in this plant species.

Materials and Methods

Cell culture and elicitation

Buds (1.5 cm) from the rhizome of zedoary (*C. zedoaria* Roscoe) distributed in Thua Thien Hue province (Vietnam) were sterilized and cultured on the MS (Murashige and Skoog, 1962) medium including 2% (w/v) sucrose and 0.8% (w/v) agar, supplemented with 20% (v/v) CW (coconut water), 3 mg/L BAP (benzylaminopurine), 0.5 mg/L IBA (indolebutyric acid) and 1.5 mg/L AgNO₃ for shoot multiplication. Single shoots (3–4 cm) were isolated from the shoot clusters to develop into plantlets on the MS medium supplemented with 20% (v/v) CW, 2 mg/L NAA (naphthaleneacetic acid) and 1.5 mg/L AgNO₃. Leaf-bases (0.5×0.5 cm) of plantlet were cultured on the MS medium supplemented with 3 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid) and 3 mg/L BAP for callus induction, callus was then subcultured on the MS medium with 1 mg/L 2,4-D and 1 mg/L kinetin for proliferation.

Cell culture of zedoary was established by agitating 3 g of callus in 250 mL Erlenmeyer flask containing 50 mL

Fig. 1 *In vitro* cultures of zedoary. A: plantlet, B: callus, C: suspension cells, D: fresh cell biomass.

of liquid MS medium including 2% (v/v) of sucrose, supplemented with 3 mg/L of 2,4-D and 3 mg/L of BAP until a suspension of free cells formed. The suspension was then cultured on a rotary shaker at a speed of 150 rpm for 12 days to produce biomass (Fig. 1).

Three gram of 12 day old cells (at near the end of the growth phase) were subcultured on the same medium but supplemented with 100 μM of SA or 1 g/L of YE at various culture times to investigate changes in expression of curcuminoid genes related to curcumin biosynthesis.

Salicylic acid solution was prepared as a stock solution by completely dissolved 0.138 g SA into 5 mL of 100% ethanol. Five milliliters of water was added to solution to a final concentration of 100 mM and filtrated through Millipore™ Membrane Filter (0.22 μm pore size). After then, 50 μL of stock solution were added to 50 mL liquid medium to a concentration of 100 μM. Yeast extract was directly added to medium to a concentration of 1 g/L before autoclaving.

The cell culture was incubated at 25±2°C with a photoperiod of 10 h daylight at an light intensity of approximately 6.75 μmol/s/m².

Gene expression

Expression level of curcuminoid genes (*CzDCS*, *CzCURS1*, *CzCURS2* and *CzCURS3*) in various zedoary samples was analysed by semi-quantitative RT-PCR (reverse transcription-polymerase chain reaction) with the primers designed

Gene	Primer name	Primer sequence (5'- 3')	Amplified length (bp)	Annealing temperature (°C)
<i>CzDCS</i>	ID-F	TGCTCCGAGGTCACCGTGC	272	55
	ID-R	GGTCAGCCCAATTTTCGCGG		
<i>CzCURS1</i>	IC1-F	CCGCTGGAAGGAATTGAAAAA	286	55
	IC1-R	GAGCTTGTCCGGGCTCAGCTG		
<i>CzCURS2</i>	IC2-F	CCACCTCCGCGAGGTGGGGCT	211	55
	IC2-R	GCGGTGGCCAGCTTGCTCTGT		
<i>CzCURS3</i>	IC3-F	CACCTGAGGGAAATCGGCTGG	202	50
	IC3-R	GCGAGCTTCCCCTGTTCCAGC		

CzDCS CCTCGCGGAGAAACAACCGGGCGCGCGGTGCTCGCGCTCTCTCGCGAGTTCACCGTGCT 711

CzCURS1 CCTCGCGGAGAAACAACAGGGGCGCGCGGTGCTGGTGGTCTCGCTGCGAGATCACCGTGCT 657

CzCURS2 CATCGCGGAGAAACAACCGTCCGCTCGCTCTCTCGTCTGCGCTCGGAGATCACCGTGCT 705

CzCURS3 CCTCGCGGAGAAACAACGGGGCGCGCGCTCTGGCCGTCTCTGCGAAATCACCGTGTT 679

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CzDCS CTCTGCTACCGCGGCGCCCAACCCCGGCCACATCGAGAGCGCTCTTCTGCTCAAGCTCTGTGTTGG 771

CzCURS1 CAGCTTTCGCGGGCCGAACAGAGGCGCACTTCGAGGCGCTTCGCGGGGCGAGCCGCGCTTCGG 717

CzCURS2 CAGCTTTCGCGGGCCCGAGCGAGCGCACTTCAGGCGCTTGC CGGGGCGAGCCGCGCTTCGG 765

CzCURS3 CAGCTTTCGCGGCTCCGACGCGGCGCACTTCGAGGCCCTCGCGTGTCTAGGCGCGCTTCGG 739

* * * * *

CzDCS CGACGCGCTGCGCGCTCGTGGTCTGGGTCCGACCCCGTCGATGGCGTCGAGCGCCCCAT 831

CzCURS1 CGACGCGCGGGGGCGCTGCTGCTCGTGGGGCGCAGCCGCTGGAAGGAATTGAAATACCAAT 777

CzCURS2 CGACGCGCGCGGGCGGATGATCGTGGGGCGCAGCCCGCTCTCGGCGCTCGAGCGGGCGCT 825

CzCURS3 CGACGCTGCCGCTGCGCTGCTGCTGGGGCGCAGCCCCCTCCGGGGCGTCGAGAGGCCAT 799

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CzDCS CTTTCAAAATCGCTCGGCATCCCAAGTAATCGTGTCTCCAGTCGAAATTAACAAAATTC 891

CzCURS1 CTACGAGATTCG----- 789

CzCURS2 CTACCAATCAT----- 837

CzCURS3 CTACGAGATTCG----- 811

* * * * *

CzDCS GATCTTTTGATATCGAACGAATTTAAAAATCAAATCTTTGGCACCAGGTTGATGCTTC 951

CzCURS1 -----GGCGGCATCGAGGAGCGGTG 812

CzCURS2 -----GTGCGCGCATCAGACGACGCTGC 860

CzCURS3 -----GGCGGCATCGAGAAACGCTGC 834

* * * * *

CzDCS CGGAGAGCGAAGAGGCGGTAGGCGGGCCACTCCGCGAAATTTGGGTGACCTTCCACCTCA 1011

CzCURS1 CGGAGAGCCAGGGGCGGTGGGGGGCCACTTCGCGGGCTCTCGGCTGACGTTCTACTTCC 872

CzCURS2 CGGAGAGCGAGAAGGCGGTGGGGGGCCACTCCGCGAGTGGGGCTGACCTTCCACTTCT 920

CzCURS3 CGGAGAGCGAGAGGCGGTGGGGGGCCACTTCGAGGAAATCGGCTGGAACCTTCCACTTCT 894

* * * * *

CzDCS AGAGCCAGCTTCCGTCGATCATCGCGAGAACATCGAGCAGAGCCTCAGCATGCTGCT 1071

CzCURS1 TGAACAGCTGCGGGCGATCATCGCCGACAACCTCGGGAGGAGCGCTGAGCGGGCGTGG 932

CzCURS2 TCAACAGCTGCGGGCGATCATCGCCGACAACGCTGGGGAAACAGCTCGCGGAGGCGTTCG 980

CzCURS3 TCAACAGCTGCGGAAGCTGATCGCGGAAACATCGAGGAGCGCTGCGCGGGCGTTC 954

* * * * *

CzDCS CGCCGCTGGGGCTGTCGGAGCTGGAACAGCTGTTCTGGGCGTTACCCCCGGCGCGAG 1131

CzCURS1 CGCCGCTGGGGGTGACGGAAGTGAACAGCAGCTTCTTGGGTGGCGCACCCGGGCACTGGG 992

CzCURS2 AACCAGTCGGGATCAAGGATGGAACAACTCTTCTGGGTGGCGCACCCGGGCACTGGG 1040

CzCURS3 AGCCGCTGGGGATCAGCGAGTGAACAGCAGTGTCTTGGGTGGCGCACCCGGGGAATTGG 1014

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CzDCS CGATCTTGACAGGTTGAGGCGCGCTCGGACTGGAGAAGACCGGCTCGCGCGACGC 1191

CzCURS1 CCATCATGGAGCCATCGAAGCAAGCTCGGCTGAGGCGCGGCAAGCTCAGCAGCCGCC 1052

CzCURS2 CCATCATGGAGCCATCGAAGCAAGCTCGGCTGAGGCTGGAAACAGAGAACTGGGCCAC 1100

CzCURS3 GCATCATGGAGCCATCGAAGCAAGCTGGGGCTGGAACAGGGGAAGCTCGGCACGGCG 1074

* * * * *

CzDCS GGCACGTACTCAGCGAGTACGGCAACATCGAGAGCGCCACCGTGTCTGTTATCTGGAGC 1251

CzCURS1 GCCAGCTCTTACAGAGTACGGCAACATCGAGAGCGCCACCGTGACTCTCGTATGGATG 1112

CzCURS2 GCGACGCTCTTCCGAGTTTCGGCAACATCGAGAGCGCCACCGTCTACTCTGATGGAGC 1160

CzCURS3 GCCACGCTCTCAGCGAGTACGGCAACATCGAGAGCGCCACCGTGACTCTGATGGAGC 1134

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CzDCS AGATCGGGAACCGTTCGGCTGCGGAGGGGCCAGCGCACCCGGCGAGGGGCTCGACTGGG 1311

CzCURS1 AGCTGAGGAACCGTTCGGCGGTGAGAGGGGCGGAGCACCCGGAAGCGGCTTCGAGTGGG 1172

CzCURS2 AGCTCAGGAACCGTTCGGCGCGGAGAACCGGGCGACACCGGCGAGCGGCTTCGGTGG 1220

CzCURS3 AGGTGAGGAACCGTTCGGCGCGGAGGGGCGGGCCACCCGGCGAAGCGCTGAGATGGG 1194

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extracted by Invitrap Spin Plant RNA Mini Kit (STRATEC Biomedical, Germany) and 1st cDNA strand was synthesized by First Strand cDNA Synthesis Kit (Thermo Scientific),

both are under the manufacturer's instructions.

PCR amplification was performed as follows: 2 μ L cDNA, 10 pmol each primer, 6 μ L 2 $\times$ GoTaq® Green Master Mix (Promega), and ddH₂O in a final volume of 12 μ L. The reaction was performed in PCR machine (Veriti 96 Well Thermal Cycler, ABI) with a template denaturation of 95°C for 2 min; followed by 20–30 cycles of 95°C for 30 s, 50–55°C for 30 s (Table 1), and 72°C for 30 s; and a final extension of 72°C for 10 min.

Three microliters of each PCR product was used for electrophoresis on 1% (w/v) agarose gel at 70 V and intensities of DNA bands were calculated using ImageJ program (version: k 1.45).

High performance liquid chromatography

Cell biomass was dried at 50°C to a constant weight, then ground into a fine powder. Two grams of powder was soaked in 20 mL of 70% (v/v) ethanol for 30 min at 30°C and then treated by ultrasound on T700/H machine (Elma, Germany). The sample was filtered with Whatman filter paper (No. 1), the residue was extracted again with the same solvent for several times until the solvent layer became colorless. After filtration with Whatman paper, the filtrates were mixed and the solvent was removed at 70°C by using a vacuum rotary concentrator (Heidolph, Germany). The concentrate was dissolved by 70% (v/v) ethanol to 10 mL (curcumin extract) and filtered through Minisart 0.25 μ m membrane (Sartorius, Germany), the extract was then diluted two times for subjecting HPLC at ambient temperature by using a Hypersil™ MOS C8 LC Column (5 μ m, 4.6 $\times$ 150 mm).

HPLC condition was as follows, flow rate: 1 mL/min, run time: 10 min, detector wavelength: 420 nm, stationary phase: silica gel (reverse phase) and mobile phase: acetonitrile/2% acetic acid (35/65, v/v). Ten microliters of curcumin extract were injected the chromatographic column using Hamilton syringe. HPLC analysis was performed on a Spectra System (Thermo Electron).

All solvents were of analytical grade and were purchased from Sigma and Merck & Co., Inc. The standard curve of curcumin (Sigma) in ethanol at different concentrations from 1–20 μ g/mL is used for determination of the curcumin in the extract. Curcumin content of the extract is calculated based on the peak area with retention time corresponding to the standard curcumin.

Statistical analysis

The experiments were done in triplicate, n=10 for each

replication in *in vitro* culture experiments. The data were analyzed as means followed by one-way ANOVA (Duncan's test, $p<0.05$).

Results and Discussion

Growth of zedoary cells

Zedoary cells in shaking culture were harvested every two days to determine their biomass propagation. Figure 3 shows the growth increased from day 2 to day 14 and then decreased at days 16 and 18. Maximum biomass of approximately 8.3 g of fresh weight (per 50 mL of cell culture volume) is equivalent to 0.73 g of dry weight. Twelve day-old cells were subcultured in the fresh medium for elicitation, cell biomass was then harvested at 14th day of subculture to evaluate expression level of curcuminoid-biosynthesis genes and their curcumin accumulation.

Table 2 shows the growth of *C. zedoaria* cells in all treatments was inhibited significantly, highest biomass per 50 mL of cell culture volume is only approximately 5.2 g fresh weight (0.5 g dry weight) versus about 8.3 g fresh weight (0.73 g dry weight) of the control. In general, the effect of exogenous elicitors on *in vitro* growth depends on the plant species, developmental stage, and the elicitor concentration. Negative effects of SA and YE on the growth have also been observed in different *in vitro* culture systems as in *Sophora flavescens* (Yamamoto et al. 1995), *Dendrobium huoshanense* (Wang et al. 2009), *Salvia miltiorrhiza* (Zhao et al. 2010), a mutant of *Lotus japonicus*, named Ljsym4-2 (Bastianelli et al. 2010), *Centella asiatica* (Loc et al. 2017).

Fig. 3 Time course of *C. zedoaria* cell growth. Data are means $\pm$ SE of three biological replicates

Table 2 Effect of elicitors on the growth and curcumin accumulation of zedoary cells

Elicitation	Cell growth		Curcumin (mg/g)
	Fresh weight (g/50 mL)	Dry weight (g/50 mL)	
YE ⁽¹⁾	5.26 ^b	0.49 ^b	0.92 ^d
YE ⁽²⁾	4.88 ^c	0.42 ^c	1.44 ^a
SA ⁽¹⁾	3.45 ^d	0.31 ^d	1.14 ^c
SA ⁽²⁾	3.16 ^d	0.30 ^d	1.32 ^b
Non-elicited cells	8.26 ^a	0.73 ^a	0.57 ^f
10 month-old rhizome	-	-	0.78 ^e

Elicitors were added in the medium at the beginning of culture (1) and after 5 days of culture (2). Different letters in a column indicate significantly different means (Duncan's test, $p < 0.05$).

Fig. 4 RT-PCR amplification of the specific regions of curcuminoid genes in *C. zedoary*. 1: control (untreated cells), 2 and 4: cells treated with salicylic acid and yeast extracts at the beginning of culture, respectively. 3 and 5: cells treated with salicylic acid and yeast extracts after five days of culture, respectively. M: DNA size marker (1 kb DNA Ladder)

Effect of elicitors on expression of curcuminoid-biosynthesis genes

In the present work, 1 g/L YE or 100 μ M SA was added to the medium at two time-points, at the beginning and the 5th day of culture. The other concentrations and treatment time points of SA and YE were also investigated but curcumin accumulation found in cells is insignificant (data not shown).

Although semi-quantitative RT-PCR will not produce as good quality results as those of quantitative PCR, Lenka et al (2012) and Dewanjee et al (2014) argued that it is still sufficient for differential gene expression analysis. Our results of semi-quantitative RT-PCR in 25 cycles exhibited that expressions of curcuminoid genes in treated cells are higher than that of untreated cells (Fig. 4). Expression levels of genes were evaluated through the intensities of DNA

Fig. 5 Intensities of DNA bands from semi-quantitative RT-PCR of the specific regions of curcuminoid-biosynthesis genes. 0 and 5: elicitors were added in the medium at the beginning and after five days of culture, respectively. Data are means $\pm$ SE of three biological replicates

bands in agarose gel showed that they are 1.14 to 3.64-fold higher than the control (Fig. 5). Expression of curcuminoid genes in treated and untreated cells was not different significantly in 20 and 30 cycles of amplification (data not shown).

In general, YE showed a higher elicitation effect compare to SA, the total intensity of DNA bands reached a maximum value of 71528 versus 23455 (approx. 3-fold) from treatments at day 0 and 57397 versus 31625 (approx. 1.8-fold) from treatments at day 5. Data in Figure 5 also indicated that the SA treatment at 5th day of cell culture stimulated the stronger expression in all four genes, *CzDCS* and *CzCURS* 1–3, compared to that at the beginning. While YE treatments gave different results, the *CzCURS1* and *CzCURS3* genes were expressed strongly in cells which treated at the beginning, but the *CzDCS* and *CzCURS2* genes showed the opposite effect, they were activated strongly in the treatments at day 5 of the culture. Overall, the YE treatment at the beginning resulted in the total intensity of all four genes

is about 1.3-fold higher than that at 5th day of culture.

One issue with our approach for gene expression analysis was that a proper internal control such as chalcone synthase is not accessible since the such gene sequences in *C. zedoaria* are not yet made available on Genbank. The scarcity of sequence information of *C. zedoaria* also makes it impossible for including pathogenesis related genes in our elicitation experiments to validate that changes in genes expressions were due to the effects of used elicitors. To ensure that the differences observed in gene expression levels are genuine, we included total RNA in our analysis to show that equal amounts of RNA, as well as cDNA were used for the semi-quantitative RT-PCR.

Several previous studies also found an enhanced expression of elicitor-induced genes in plant cell cultures such as *OsWRKY53* gene in rice cells treated with fungal cerebroside (Chujo et al. 2007), defense genes in parsley (*Petroselinum crispum*) cells treated with methyl jasmonate (Ellard-Ivey and Douglas, 1996), or defense genes in cells of two grapevine species (*Vitis rupestris* and *V. vinifera*) treated with Harpin (Qiao et al. 2010). Especially, Park et al. (2016) showed that YE and silver nitrate can induce the higher expression of phenylpropanoid biosynthesis genes and enhance accumulation of rosmarinic acid in *Agastache rugosa* cell culture. In another report, we determined the influence of SA on expression of genes involved in the pathway of phytosterol and triterpene biosynthesis in *in vitro* centella (*Centella asiatica*) cells (Loc et al. 2016).

The effect of the exogenous SA and YE on the gene expression level depends on the plant species, developmental stage, and elicitor concentration. Although, both YE and SA trigger the accumulation of endogenous jasmonic acid that up-regulates the expression of key genes involved in secondary metabolism of plants (Rahimi et al. 2014). Our results indicated that in comparison to abiotic elicitor is SA, YE biotic elicitor is more effective in enhancing the expression of curcuminoid-biosynthesis genes.

Effect of elicitors on curcumin production

Curcumin is one of three components of curcuminoid group found in *Curcuma* species including demethoxycurcumin and bisdemethoxycurcumin. In the curcuminoid pathway in *C. longa* which started by phenylalanine, CzDCS metabolizes feruloyl-CoA from p-coumaroyl-CoA into feruloyldiketide-CoA, then CzCURS1-3 will convert feruloyldiketide-CoA into curcumin. Besides, CzCURS3 can produce demethoxylcurcumin from feruloyldiketide-CoA. Simultaneously, CzDCS also participate the transformation of p-coumaroyl-

CoA into p-coumaroyldiketide-CoA, and then CzCURS1-3 or single CzCURS3 will metabolize this compound into demethoxycurcumin and bisdemethoxycurcumin (Katsuyama et al. 2009).

The correlation between expression of curcuminoid genes and curcumin biosynthesis in *C. zedoaria* cells was found through RT-PCR amplification and HPLC analysis. The effect of YE treatment on curcumin production is higher than that of SA and the control. Curcumin content from cells treated with SA and YE at 5th day of culture is approximate from 1.2- to 1.6-fold higher than that treated at the beginning, 1.32 vs 1.14 mg/g and 1.44 vs 0.92 mg/g (Table 2). In the YE treatment, the results on the curcumin contents did not appear to correspond to expression levels of curcuminoid genes. Total intensity of PCR products obtained from treated cells at the beginning of culture is stronger than that at 5th day (Fig. 5). The cause may be the curcuminoid genes contributed to not only in the biosynthesis of curcumin but also demethoxycurcumin and bisdemethoxycurcumin.

Generally, our results are also similar to several previous studies. The accumulation of major products of secondary metabolism in plant cell cultures has been correlated with the expression of coordinate genes under the effect of suitable elicitors. For example, activation of expression of phenylpropanoid genes and accumulation of furanocoumarin in *Petroselinum crispum* cells treated with methyl jasmonate (Ellard-Ivey and Douglas, 1996) or rosmarinic acid in *Agastache rugosa* cells treated with YE and silver nitrate (Park et al. 2016).

In conclusion, expression levels of curcuminoid-biosynthesis genes in YE- and SA-treated *C. zedoaria* cells were 1.14 to 3.64-fold higher than those in untreated cells, in which YE at 1 g/L showed a higher stimulation effect for gene expression than SA at 100 μ M, 1.8- to 3-fold after 9 and 14 days of treatment, respectively. Curcumin accumulation in treated cells was 1.61- to 2.53-fold higher than the control.

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Conflict of interest

We declare that there is no conflict of interest regarding the publication of this article.

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