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Enhanced Resveratrol Production in *Vitis vinifera* Cell Suspension Cultures by Heavy Metals Without Loss of Cell Viability

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Abstract The effects of heavy metal ions (Co^{2+}, Ag^{+}, Cd^{2+}) on cell viability and secondary metabolite production, particularly anthocyanins and phenolic acids in *Vitis vinifera* cell suspension cultures, were investigated. Of these, Co at all three used concentrations (5.0, 25, and 50 μM), Ag, and Cd at low concentration (5.0 μM) were most effective to stimulate the phenolic acid production, increasing the 3-*O*-glucosyl-resveratrol up to 1.6-fold of the control level (250.5 versus 152.4 μmol/g), 4 h after the treatments. Meanwhile, the elicitors at effective concentrations did not suppress cell growth, while the cell viability maintained. In contrast, Ag and Cd at high concentrations (25 and 50 μM) remarkably reduced the cell viability, decreasing the cell viability up to about 15 % of the control level, 24 h after the treatments. The heavy metal ions did not affect the anthocyanin production. These observations show how, in a single system, different groups of secondary products can show distinct differences in their responses to potential elicitors. The 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, peroxidase activity, medium pH value, and conductivity were only slightly elevated by the heavy metal ions. The results suggest that some of the secondary metabolites production was stimulated by the used elicitors, but there was not a stress response of the cells.

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Introduction

Several strategies have been developed to increase the productivity of secondary metabolite production in plant cell cultures: media optimization [1], selection of a suitable plant tissue culture [2], elicitation [3, 4], and addition of precursors [5]. The use of biotic or abiotic elicitors to stimulate product formation has become an important progress strategy and has been very useful in reducing the process time required to attain high product concentrations and increased volumetric productivity.

Heavy metals have become one of the main abiotic stress agents for living organisms because of their increasing use in the developing fields of industry and agrotechnics, and high bioaccumulation and toxicity. The effect of their toxic influence on plants is largely a strong and fast inhibition of growth processes of the above- and underground parts, as well as the activity decrease of the photosynthetic apparatus, often correlated with progressing senescence processes [6]. Growth inhibition and senescence stimulation, caused by heavy metals in excess, are intriguing effects, as the knowledge of their mechanisms can have a great significance in ecophysiology and medicine. Recently, heavy metal ions were studied to reveal their effects on plant secondary metabolite production, e.g., silver [4, 7–9], cobalt [8, 10], cadmium [4, 8], copper [11], and nickel [12]. Ag^{+} was employed as an abiotic elicitor to stimulate the secondary metabolite production in hairy root culture of *Salvia miltiorrhiza*, resulting in more than twofold increase in the yields of tanshinones [7], inducing tanshinone production and isoprenoid pathways [9]. In cell cultures of *S. miltiorrhiza*, Ag^{+} and Cd^{2+} increased the total tanshinones content by more than tenfold [8]. The treatment of root cultures of *Datura stramonium* with cadmium salts at external concentrations of approximately l mM has been found to induce the rapid accumulation of high levels of sesquiterpenoid defensive compounds, notably lubimin and 3-hydroxylubimin, but not alkaloid [11].

In this study, the effects of three heavy metal elicitors (Co^{2+}, Ag^{+}, and Cd^{2+}) on cell viability and production of anthocyanins and phenolic acids on grape wine cell suspension culture in early phase of growth stage were investigated. In our previous studied, the *Vitis vinifera* cell suspension cultures were most sensitive to elicitors at early phase [13]. Antioxidant activity determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and peroxidase (POD) activity were monitored to examine the relationship between the production of desired secondary metabolites and the defense mechanisms of the plant. Here, we investigate heavy metal-induced elicitation responses in *V. vinifera* cell suspension cultures. In particular, we report on the level of accumulation of anthocyanins and phenolic acids.

Materials and Methods

Plant Cells and Culture Conditions

The cell culture of *V. vinifera* L. cv. Gamay Fréaux was originally obtained from Francois Cormier (Food Research and Development Centre, Agriculture Canada) and has since been continuously maintained at the Department of Food Biotechnology and Process Engineering, Technical University Berlin. The cell culture was cultivated in the B5 medium [14].

Callus cultures were transferred every 28 days to fresh solidified sterile medium. Red pigmented cell aggregates were selected preferably. Cell suspension cultures were established by transferring cell aggregates into 50 ml of liquid B5 medium in 200-ml Erlenmeyer flasks and continuously agitating the flasks on a rotary shaker at 110 rpm. The cell cultures were transferred to new media every 3 weeks. Cultures were maintained at 25±2 °C under fluorescent light (approx. 3,000 lx). The inoculum size was about 100 g fresh weight l^{-1} medium.

Treatment Procedure

Suspension cultures at the end of the log-growth phase of *V. vinifera* cells were filtered under sterile conditions, and the medium passed the filter dropwise (15 min). From this pool, 5 g of fresh cells was transferred to 100-ml flasks containing 25 ml sterile B5 medium. Three heavy metals (Co^{2+}, Ag^{+}, and Cd^{2+}) were tested, each at three concentrations (5, 25, and 50 μM), in the initial elicitation experiments. Cell cultures were treated with one of three different heavy metals 1 day after sub-cultivation. The elicitors were cobalt chloride, silver nitrate, and cadmium chloride. All the used elicitors were purchased from Sigma-Aldrich (Germany). All elicitors were prepared as a concentrated stock solution in distilled water and added to culture medium to the desired concentration, adjusted to pH 5.5, and autoclaved at 121 °C for 15 min. All the treatments were done in triplicate. Untreated cultures were used as the control group. Sampling was performed in 4 and 24 h after treatments.

Determination of Medium pH Value and Conductivity

The pH values of the growth media were determined by a pH meter (CG811, Schott Geräte GmbH, Hofheim, Germany). The conductivities of the growth media were measured using a conductivity meter (WTW LF323, Weilheim, Germany).

Cell Viability Assay

Viability of the suspended cells (percentage of viable cells in a cell population) was estimated by the 2,3,5-triphenyltetrazolium chloride reduction method adapted as described by Towill and Mazur [15]. Triphenylfonnazan reduced by viable cells was measured at 485 nm of its maximum absorption wavelength. The cell viability was expressed in percentage terms by taking the absorbance (at 485 nm) of a sample without treatment as a control, i.e., 100 % cell viability.

Phenolic Acid Extraction and Determination

The cells were harvested and frozen in liquid nitrogen to prevent volatilization of phenolic compounds. Afterwards, the samples were lyophilized. After the freeze-drying process, the material was ground by flint mill (20,000 rpm, 2 min). Forty milligrams of ground samples was extracted for 15 min using 750 μl 70 % methanol (*v*/*v*, pH 4, phosphoric acid) in an ultrasonic water bath on ice. Samples were centrifuged for 5 min at 6,000 rpm. The supernatants were collected and the pellets were re-extracted twice with 500 μl 70 % methanol. Forty microliters of 3 mM *p*-coumaric acid was added as an internal standard to the first extraction (the recovery of *p*-coumaric acid was above 90 %). The combined supernatants from each sample were reduced until dry in a centrifugation evaporator (SPD 111V Speed Vac. Concentrator, Thermo Scientific, USA; CVC 3000V, Vacuubrand GmbH, Wertheim, Germany) at 25 °C. Samples were then re-dissolved in 1 ml high-performance

liquid chromatography (HPLC)-grade water. The samples were filtered using 0.22 μm filter (SPIN-X centrifuge tube filter) and then analyzed with HPLC.

The separation of phenolic compounds was performed on HPLC (UltiMate SR-3000, Dionex, Germany), equipped with LPG-3400SD pump, WPS-3000SL automated sample injector, AcclaimPA C16-column (3 μm, 2.1×150 mm, Dionex), and DAD-3000 diode array detector (Dionex) and software Chromeleon 6.8. The column was operated at a temperature of 35 °C. The mobile phase consisted of 0.1 % (*v*/*v*) phosphoric acid in water (eluent A) and of 40 % (*v*/*v*) acetonitrile (eluent B). A multistep gradient was used for all separations with an initial injection volume of 40 μl and a flow rate of 0.4 ml min^{-1}. The multistep gradient was as follows: 0–1 min, 0.5 % (*v*/*v*) B; 1–10 min, 0.5–40 % B; 10–12 min, 40 % B; 12–18 min, 40–80 % B; 18–20 min, 80 % B; 20–24 min, 80–99 % B; 24–30 min, 99–100 % B; 30–34 min, 100–0.5 % B; 34–39 min, 0.5 % B. Simultaneous monitoring was performed at 290, 330, and 254 nm. Diode array detection was used for the identification of the compounds. Retention times and UV/visible absorption spectra of the peaks were compared with those of the authentic standards. All of the standards were purchased from Sigma-Aldrich (Germany). Compounds were identified by using standards and by performing liquid chromatography–mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) [16]. Phenolic acid quantity was calculated from HPLC peak areas at 290 nm against the internal standard.

Determination of Total Anthocyanin Content

Anthocyanins were extracted from 100 mg of fresh cells, after removing the medium with a suction filter. To each sample, 750 μl of 79 % (*v*/*v*) ethanol with 1 % (*v*/*v*) glacial acetic acid (extraction solvent) was added and samples were incubated in a heat block at 85 °C for 20 min. After centrifugation at 13,000 rpm for 5 min, the supernatants were collected and the pellets re-extracted with 600 μl extraction solvent twice. Supernatants were combined and 50 μl of 37 % (*v*/*v*) hydrochloric acid for stabilizing anthocyanins was added. After 10-min incubation in the dark at room temperature, the sample was diluted to 1:1 (*v*/*v*) with the extraction solvent. Total anthocyanins were determined by the absorbance at 535 nm recorded using ε=98.2 (dilution factor=2). For compound identification, the extracts were analyzed with HPLC [16].

Antioxidant Activity Assay

DPPH radical scavenging activity was used to analyze antioxidant activity [17], with some modifications. The stock reagent solution (1×10^{-3} M) was prepared by dissolving 22 mg of DPPH in 50 ml of methanol and stored at −20 °C until use. Working solutions (6×10^{-5} M) were prepared by mixing 6 ml of stock solution with 94 ml of methanol to obtain an absorbance value of 0.8±0.02 at 515 nm, as measured using a spectrophotometer. Twenty milligrams lyophilized dry weight was mixed with 1 ml distilled water and heated at 90 °C for 15 min. Then, it was centrifuged at 12,000 rpm for 5 min. The supernatant was collected and the pellet was re-extracted with 1 ml distilled water at 90 °C for 10 min. After centrifugation, the collected supernatants were combined. Fifty microliters of the extract was vortexed for 30 s with 1.95 ml DPPH working solution and left to react for 30 min; afterwards, the absorbance at 515 nm was recorded. A control without added extract was also analyzed. Scavenging activity was calculated as follows:

$$\text{DPPH radical scavenging activity } (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where A is the absorbance at 515 nm.

POD Assay

POD activity in enzyme extracts was assayed by monitoring changes in absorbance at 420 nm in mixtures consisting of 0.32 ml 0.01 M KH_2PO_4 buffer (pH 6.5), 0.16 ml 0.5 % (*w*/*v*) H_2O_2, 0.32 ml 5 % (*w*/*v*) pyrogallol, 0.1 ml enzyme extract, and 2.1 ml distilled water, in a total volume of 3 ml. Enzyme activity unit represents the amount of enzyme that caused an increase in absorbance of 0.001 unit/min under assay conditions.

Statistical Analysis

All analyses were performed with OriginPro7 software for Windows (OriginLab Corporation, MA, USA). Data were reported as mean±standard deviation. Differences were assessed using one-way analysis of variance. A p value <0.05 was considered statistically significant by Tukey's post hoc test.

Results

Effect of Heavy Metals on Cell Viability of *V. vinifera* Cell Culture

Cell viability of Co-elicited cell culture was not significantly different from that of control (p value>0.05, Fig. 1). Ag had a significant inhibition effect of cell viability and had a concentration-dependent manner. Ag at concentration of 50 μM severely inhibited the cell growth; the cell viability remained only 27.4 and 15.5 % of the control level, 4 and 24 h after the treatment, respectively. Cd had also a negative effect on cell growth, but not in a concentration-dependent manner. In contrast to Ag, Cd at the highest concentration was the most preferable.

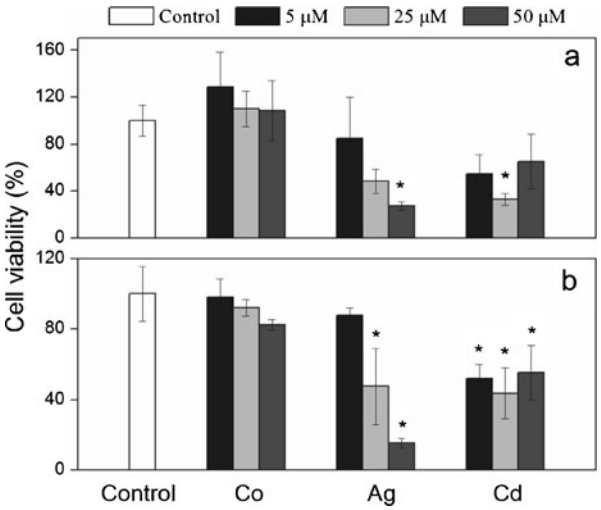

Fig. 1 Effects of heavy metals at three concentrations on cell viability of *V. vinifera* culture harvested 4 h (**a**) and 24 h (**b**) after the treatments; *error bars* for standard deviations, n=3, *p<0.05 indicates significant difference compared to the corresponding control

Effect of Heavy Metals on Anthocyanin and Phenolic Acid Production in *V. vinifera* Cell Culture

The anthocyanin and phenolic compounds present in *V. vinifera* cell culture were identified in LC-MS and NMR, as described in our previous publication [16]. The major anthocyanin monoglucosides were cyanidin 3-*O*-glucoside and peonidin 3-*O*-glucoside, and the major cinnamoyl derivatives were cyanidin 3-*O*-*p*-coumarylglucoside and peonidin 3-*O*-*p*-coumarylglucoside. Three minor anthocyanin compounds were found: delphinidin 3-*O*-glucoside, petunidin 3-*O*-glucoside, and delphinidin 3-*O*-*p*-coumarylglucoside. The major phenolic acids were 3-*O*-glucosyl-resveratrol and 4-(3,5-dihydroxy-phenyl)-phenol.

The treatments of heavy metal elicitors at different concentrations had no significant effects on total anthocyanin production from *V. vinifera* cell cultures during the 24 h after treatments (Fig. 2). The highest amount of total anthocyanins was produced by 5.0 μM Cd-treated cells 24 h after treatments, which was 2.85 mg g^{-1}DW. The production was inhibited by 50 μM Ag treatment 24 h after treatments.

Among the phenolic acids detected in *V. vinifera* cell culture, 3-*O*-glucosyl-resveratrol was predominant. The effects of heavy metal elicitors on the accumulation of 3-*O*-glucosyl-resveratrol and 4-(3,5-dihydroxy-phenyl)-phenol are presented in Tables 1, 2, and 3. The treatments of Co at three concentrations had the same considerable improving effect on phenolic acid production (Table 1). The production of 3-*O*-glucosyl-resveratrol was significantly stimulated by Co, with 1.4-fold that of the control level, 4 h after the treatments. The production of 4-(3,5-dihydroxy-phenyl)-phenol was stimulated with 1.3-fold that of the control level, 4 h after the treatments. However, after 24 h, the stimulation effects were not obvious. There was no impact on the production of both two compounds. Differently from that of Co, the treatments of Ag at three concentrations had different effect on phenolic acid production (Table 2). Four hours after the treatments, the production of 3-*O*-glucosyl-resveratrol and 4-(3,5-dihydroxy-phenyl)-phenol was stimulated to 1.5- and 1.2-fold of that

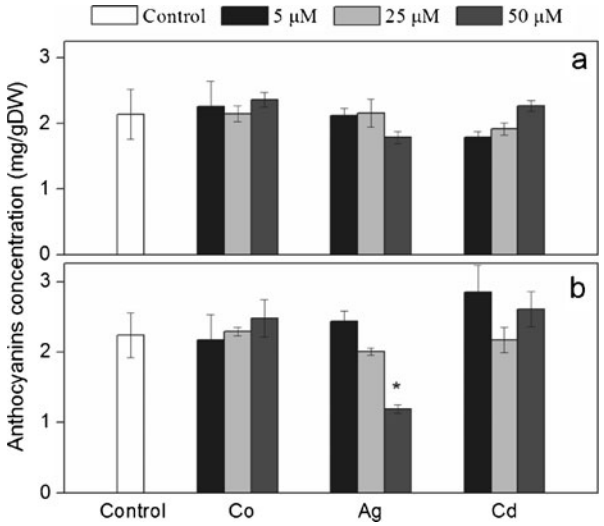

Fig. 2 Effects of heavy metals at three concentrations on total anthocyanins content of *V. vinifera* culture harvested 4 h (**a**) and 24 h (**b**) after the treatments; *error bars* for standard deviations, n=3, *p<0.05 indicates significant difference compared to the corresponding control

Table 1 Effects of cobalt on the accumulation of 3-*O*-glucosyl-resveratrol and 4-(3,5-dihydroxy-phenyl)-phenol in *V. vinifera* cell culture

Time after treatments	Phenolic acids accumulation		Control	Co		
				5.0 μM	25 μM	50 μM
4 h	3-*O*-Glucosyl -resveratrol	Content (μmol/g)	152.4±47.9	216.8±12.8	206.0±3.9	212.2±9.3
		Fold of control	–	1.4	1.4	1.4
	4-(3,5-Dihydroxy -phenyl)-phenol	Content (μmol/g)	97.4±11.7	127.2±1.8	124.6±6.3	125.5±6.1
		Fold of control	–	1.3	1.3	1.3
24 h	3-*O*-Glucosyl -resveratrol	Content (μmol/g)	238.6±4.0	234.2±8.1	248.1 ±5.9	260.3±12.3
		Fold of control	–	1.0	1.0	1.1
	4-(3,5-Dihydroxy -phenyl)-phenol	Content (μmol/g)	117.3±5.6	133.5±11.5	135.7±2.1	140.3±7.3
		Fold of control	–	1.1	1.2	1.2

of the control level, respectively. Twenty-four hours after the treatments, there was no stimulation, but inhibition effect by Ag at concentration of 50 μM. Similar to Ag, the effect of Cd on production of phenolic acids was positive 4 h after the treatments, but negative 24 h after the treatments (Table 3).

Antioxidant Activity and POD Activity Changes Induced by Heavy Metals

To better understand the influence of the used heavy metal elicitors on plant defense and cell culture secondary metabolism, antioxidant activity as well as POD activity were investigated. Figure 3 shows the changes of antioxidant activity and POD activity in *V. vinifera* cell culture after elicitation.

DPPH activity is a proper indicator for investigating the free radical scavenging activities of phenolic compounds [18]. The antioxidant activity of plant extracts is primarily carried out by phenolic compounds [19]. In our study, DPPH radical scavenging activity remained unaffected by heavy metal elicitors, except of Ag at concentration of 50 μM, which significantly reduced the antioxidant activity 24 h after the treatment (Fig. 3b).

POD (donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7) catalyzes the oxidation reactions using either peroxides or oxygen as hydrogen acceptor. POD activity in plants

Table 2 Effects of silver on the accumulation of 3-*O*-glucosyl-resveratrol and 4-(3,5-dihydroxy-phenyl)-phenol in *V. vinifera* cell culture

Time after treatments	Phenolic acids accumulation		Control	Ag		
				5.0 μM	25 μM	50 μM
4 h	3-*O*-Glucosyl -resveratrol	Content (μmol/g)	152.4±47.9	221.2±3.6	226.5±4.2	233.0±8.1
		Fold of control	–	1.5	1.5	1.5
	4-(3,5-Dihydroxy -phenyl)-phenol	Content (μmol/g)	97.4±11.7	117.0±6.4	119.1±3.6	117.9±2.4
		Fold of control	–	1.2	1.2	1.2
24 h	3-*O*-Glucosyl -resveratrol	Content (μmol/g)	238.6±4.0	251.1±11.5	237.4±8.2	152.9±3.3
		Fold of control	–	1.1	1.0	0.6
	4-(3,5-Dihydroxy -phenyl)-phenol	Content (μmol/g)	117.3±5.6	124.3±0.2	105.3±3.4	50.2±6.5
		Fold of control	–	1.1	0.9	0.4

Table 3 Effects of cadmium on the accumulation of 3-*O*-glucosyl-resveratrol and 4-(3,5-dihydroxy-phenyl)-phenol in *V. vinifera* cell culture

Time after treatments	Phenolic acids accumulation		Control	Cd		
				5.0 μM	25 μM	50 μM
4 h	3-*O*-Glucosyl -resveratrol	Content (μmol/g)	152.4±47.9	238.9±2.8	250.5±1.8	241.4±10.6
		Fold of control	–	1.6	1.6	1.6
	4-(3,5-Dihydroxy -phenyl)-phenol	Content (μmol/g)	97.4±11.7	108.6±4.1	112.8±3.5	100.5±8.9
		Fold of control	–	1.1	1.2	1.0
24 h	3-*O*-Glucosyl -resveratrol	Content (μmol/g)	238.6±4.0	281.8±6.2	256.7±1.6	244.6±2.5
		Fold of control	–	1.2	1.1	1.0
	4-(3,5-Dihydroxy -phenyl)-phenol	Content (μmol/g)	117.3±5.6	121.4±0.7	110.0±4.8	98.7±7.8
		Fold of control	–	1.0	0.9	0.8

increases in response to various biotic and abiotic stresses [20, 21]. Presumably, plant cultures are considered to be grown under stress conditions, particularly oxidative stress, which suggests that plant cell culture is an ideal system for the production of antioxidant enzymes including POD. In this respect, plant cells and hairy roots have been studied for the production of POD [21]. In our study, the POD activity was slightly increased by heavy metal elicitors, except Ag at high concentrations (Fig. 3c, d). Cd at concentration of 50 μM had a remarkable elevation effect of the POD activity, 24 h after the treatments, which was 1.6-fold of that of the control level.

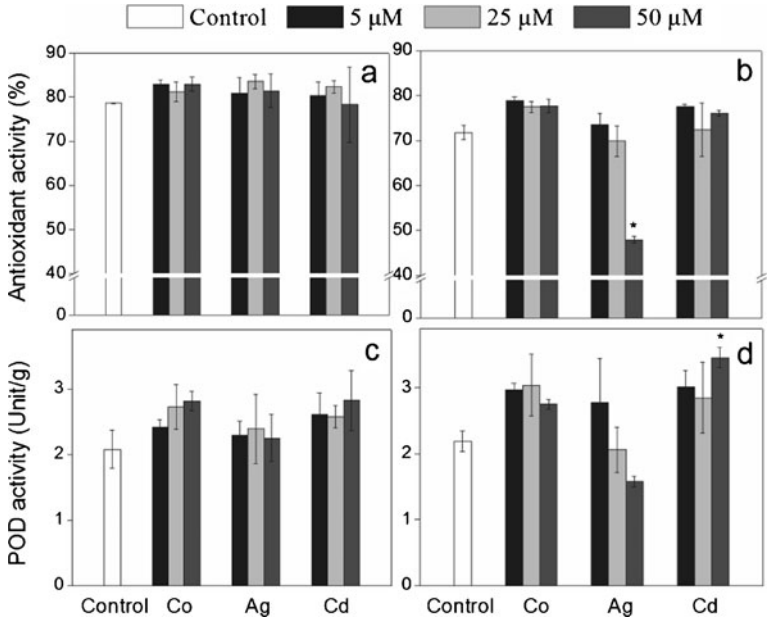

Fig. 3 Effects of heavy metals at three concentrations on antioxidant activity of *V. vinifera* culture harvested 4 h (**a**) and 24 h (**b**) after the treatments; on POD activity harvested 4 h (**c**) and 24 h (**d**) after the treatments; *error bars* for standard deviations, $n=3$, $*p<0.05$ indicates significant difference compared to the corresponding control

Medium pH and Conductivity Changes Induced by Heavy Metals

To better understand the influence of the used heavy metal elicitors on plant cell culture medium, medium pH value and conductivity were investigated. Figure 4 shows the changes of medium pH value and conductivity in *V. vinifera* cell culture after treatment by heavy metal elicitors. The pH value of culture medium decreased from 4 to 24 h after the treatments (Fig. 4a, b). There were difference between the treated cell cultures and control, but the absolute values were only slightly different. The medium conductivity stayed constant for control and Co-treated cells from 4 to 24 h after the treatments, but showed slight increase for Ag- and Cd-treated cells (Fig. 4c, d). The medium conductivity of Ag at 50 μM and Cd at all three concentrations was significantly higher than that of the control level 24 h after the treatments.

Discussion

In this study, we detected a significant effect of some heavy metal elicitation on enhancement of phenolic acid accumulation in *V. vinifera* cell suspension cultures, while they showed no impact on the anthocyanin production. In the phenolic acids detected in *V. vinifera* cell suspension cultures, 3-*O*-glucosyl-resveratrol was more responsive than 4-(3,5-dihydroxy-phenyl)-phenol to the heavy metal elicitation. In transformed root cultures of *D. stramonium*, copper and cadmium ions induced the rapid accumulation of high levels of sesquiterpenoid phytoalexin, but not alkaloids [11]. These observations show how, in a

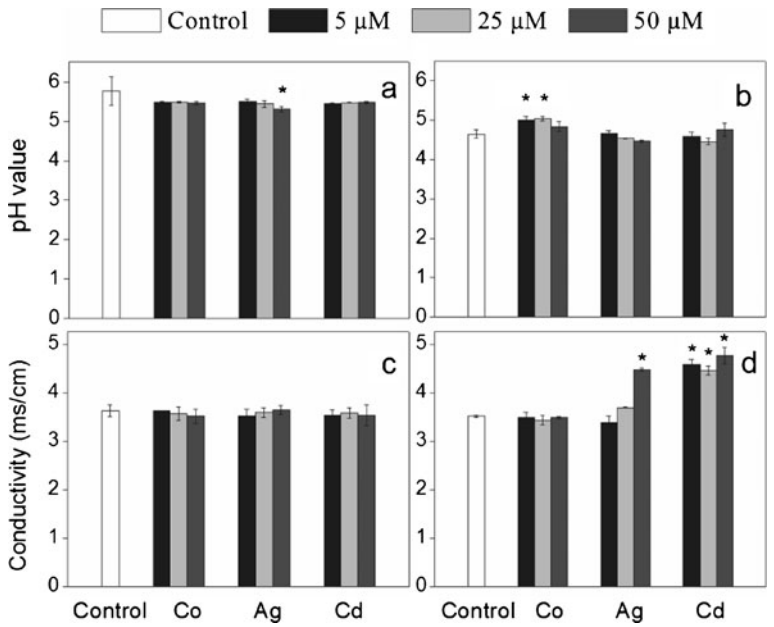

Fig. 4 Effects of heavy metals at three concentrations on medium pH value of *V. vinifera* culture harvested 4 h (**a**) and 24 h (**b**) after the treatments; on medium conductivity harvested 4 h (**c**) and 24 h (**d**) after the treatments; *error bars* for standard deviations, n=3, *p<0.05 indicates significant difference compared to the corresponding control

single system, different groups of secondary products can show distinct differences in their responses to potential elicitors. The stimulation of phenolic acids by elicitors has also been observed in *V. vinifera* cell culture by osmotic stress [22], pulsed electric field [23] and high hydrostatic pressure [24], and in plant in vitro culture of other species, like in *Cocos nucifera* by chitosan [25], in *Daucus carota* by methyl jasmonate [26], and in *Hypericum perforatum* L. by jasmonic acid [27].

In this study, Co did not significantly reduce the cell viability, while the application of Ag and Cd inhibited cell viability remarkably, which is in agreement with some previous observations in cell cultures of *S. miltiorrhiza* [8]. The Ag treatment caused a dose-dependent inhibition of cell viability. Some similar results were observed in *S. miltiorrhiza* hairy root culture [7]. Overall, the effect of elicitors on cell growth is dependent on elicitors, used concentrations or conditions, plant species, the stage when the elicitor is applied, etc. Elevated secondary metabolism is often negatively correlated with cell growth [28], but in this study, the production of phenolic acid was stimulated by Co at all three used concentrations, Ag, and Cd at low concentration (5.0 μM) without loss of cell viability, which is very preferable for obtaining valuable plant secondary metabolites from in vitro cultures.

Secondary metabolites, phenolic compounds in particular, are involved in plant response to biotic and abiotic stresses and provide a significant contribution to the antioxidant activity of plant tissues [29]. High salt strength enhanced DPPH radical scavenging activity in adventitious roots of *Morinda citrifolia*, and a positive correlation was observed between DPPH radical scavenging activity and accumulation of phenolic compounds [30]. In root suspension cultures of *H. perforatum* L., elevated levels of phenolics in the roots grown in a sucrose-rich medium correlated with improved DPPH radical scavenging activity [31]. It was also found that yeast extract stimulated β-thujaplicin accumulation in *Cupressus lusitanica* cell culture, and the culture extract also exhibited higher antioxidant activity [32]. However, in this study, no correlation between elevated phenolic acid production and DPPH radical scavenging activity was found. The POD activity remained unaffected by heavy metal elicitors. But in suspension cultures of sweet potato (*Ipomoea batatas*), POD activity was increased by 50 % by some stress factors like abscisic acid or ethephon [21]. In cell suspension cultures of spruce (*Picea abies* L.), a cell wall elicitor led to a rapid increase of the POD activity to about 1.5-fold [33]. Salt stress increased about 53 % POD activity of cotton grown in nutrient medium [34]. In addition, the medium pH value and conductivity were only slightly elevated by the heavy metal ions. The results suggest that some of the secondary metabolites production was stimulated by the used elicitors, but there was not a stress response of the cells.

Conclusion

In conclusion, the present work demonstrates that heavy metal ions like cobalt, silver, and cadmium at right concentrations are potent elicitors for stimulating phenolic acid production in *V. vinifera* cell culture without loss of biomass, but not anthocyanins. The increase of phenolic acid production induced by elicitors is largely due to 3-*O*-glucosyl-resveratrol. The plant defense system was not triggered by elicitation, evidenced by the unaffected antioxidant activity, POD activity, medium pH value, and conductivity.

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