

## Stimulation of *trans*-resveratrol biosynthesis in *Vitis vinifera* cv. Kyoho cell suspension cultures by 2, 3-dihydroxypropyl jasmonate elicitation

Xu Shen<sup>1</sup> · Qingshan Du<sup>2</sup> · Yufang Xu<sup>2</sup> · Yanhua Lu<sup>1</sup> ✉

<sup>1</sup> East China University of Science and Technology, State Key Laboratory of Bioreactor Engineering, Shanghai, PR China

<sup>2</sup> East China University of Science and Technology, Institute of Pesticides and Pharmaceuticals, Shanghai Key Laboratory of Chemical Biology, Shanghai, PR China

✉ Corresponding author: luyanhua@ecust.edu.cn

Received March 24, 2012 / Accepted July 4, 2012

Published online: September 15, 2012

© 2012 by Pontificia Universidad Católica de Valparaíso, Chile

### Abstract

**Background:** Plant cell suspension culture of *Vitis vinifera* is a promising technology for investigating different factors that are able to induce and/or modify stilbenes biosynthesis. Jasmonates have been reported to play an important role in a signal transduction pathway that regulates defence responses as well as the production of secondary metabolites. In this study, 2, 3-dihydroxypropyl jasmonate (DHPJA) was used to investigate its effect on stimulating *trans*-resveratrol (*t*-R) accumulation and the plant defence responses in *Vitis vinifera* cv. Kyoho cell suspension cultures for the first time. **Results:** It demonstrated that DHPJA had superior effects on stilbenoids accumulation over methyl jasmonate (MeJA). The optimal condition was 150  $\mu$ M DHPJA added on day 15 of cultivation period, with the highest level of *t*-R accumulation which was increased 1.8-fold and 1.3-fold compared with the control and 150  $\mu$ M MeJA respectively. DHPJA induced stronger plant defence responses, including oxidative burst and activation of L-phenylalanine ammonia lyase (PAL) than MeJA. H<sub>2</sub>O<sub>2</sub> generation induced by DHPJA played a significant role in enhancing *t*-R accumulation. Adding a specific inhibitor of H<sub>2</sub>O<sub>2</sub> signalling pathway inhibited DHPJA-induced *t*-R accumulation, but had no effects on DHPJA-induced other metabolites accumulation, which resulted in regulations of product diversity. **Conclusions:** This study demonstrated that DHPJA was an efficient elicitor to enhance *t*-R accumulation by activating stronger oxidative burst, and H<sub>2</sub>O<sub>2</sub> signalling pathway could regulate product diversity in DHPJA-induced *V. vinifera* cv. Kyoho cell suspension cultures.

**Keywords:** 2, 3-dihydroxypropyl jasmonate, H<sub>2</sub>O<sub>2</sub>, *trans*-resveratrol, *Vitis vinifera* cell suspension cultures

### INTRODUCTION

*Trans*-resveratrol (*t*-R, 3, 5, 4'-trihydroxy-*trans*-stilbene), belonging to the stilbenoids family, possesses numerous important bioactivities, including anti-inflammatory, antioxidant, anti-aggregatory functions, and modulation of lipoprotein metabolism (Shankar et al. 2007). And it has been shown to possess chemo preventive properties against certain cancers and cardiovascular diseases and to have positive effects on age longevity (Aggarwal et al. 2004; Shankar et al. 2007). Now the demand for *t*-R is growing every day. However, supplying from extracts of intact plant is insufficient. Extensive effort has been prompted to develop alternate sources and means for *t*-R production.

Plant cell culture is a useful tool for producing active secondary metabolites under controlled conditions. Due to the high level of stilbenes accumulation in grape, some authors have undertaken studies on grapecell cultures to investigate different factors that are able to induce and/or modify

stilbenes biosynthesis, regulation and metabolism (Krisa et al. 1999; Commun et al. 2003). New strategies based on the use of *Vitis vinifera* cell cultures have been used to increase *t*-R production (Donnez et al. 2009; Kiselev, 2011). Different elicitors have been used, such as fungal cell wall fragments (Liswidowati et al. 1991), cyclodextrins (CDs) (Bru and Pedreño, 2003; Bru et al. 2006), Na-orthovanadate, laminarin (Aziz et al. 2003), chitin (Yang et al. 2010), jasmonic acid (JA) and its more active derivative, methyl jasmonate (MeJA) (Tassoni et al. 2005; Vezzulli et al. 2007; Belhadj et al. 2008; Lijavetzky et al. 2008; Donnez et al. 2011).

Jasmonates have been reported to play an important role in a signal transduction pathway that regulates expression of defence genes in plants. In these cases, the production of secondary metabolites changed dramatically in response to elicitation (Qian et al. 2004; Hu et al. 2006). JA and MeJA have been proved to be effective in stimulating stilbenoids biosynthesis in different cell lines of *Vitis* spp. (Tassoni et al. 2005; Belhadj et al. 2008; Donnez et al. 2011, Kiselev, 2011). MeJA up-regulated the gene expression of defence-related proteins (PR protein) as well as key enzymes in the phenylpropanoid pathway via which stilbenoids are synthesized. However, it was unclear whether jasmonates elicited *t*-R biosynthesis via a specific signal transduction pathway.

Compared to MeJA, chemically synthesized 2, 3-dihydroxypropyl jasmonate (DHPJA) was demonstrated to have superior effects on taxane, hypericins and hyperforin production (Qian et al. 2004; Liu et al. 2007). It acted as a powerful signal molecular to activate stronger plant defence responses, including oxidative burst and activation of PAL in *Taxus chinensis* suspension cultures (Qian et al. 2004). But in *Panax notoginseng* cell cultures, oxidative burst might not be involved in the jasmonates-elicited signal transduction pathway. MeJA and 2-hydroxyethyl jasmonate (HeJA) induced the ginsenoside biosynthesis via induction of endogenous JA biosynthesis and key enzymes, such as UDPG-ginsenoside Rd glu-cosyltransferase (UGRdGT), in the ginsenoside biosynthetic pathway (Wang et al. 2006). However, the application of DHPJA in other plant cell cultures is rare. Its effect and mechanism of enhancing stilbenoids synthesis has not been studied. And it's uncertain whether oxidative burst is involved in the DHPJA-elicited signal transduction pathway in *V. vinifera* cell cultures.

This study compared the effects of MeJA and DHPJA on inducing *t*-R accumulation in *V. vinifera* cell suspension cultures, and investigated the role of H<sub>2</sub>O<sub>2</sub> in enhancing stilbenoids synthesis and activating of PAL. Meanwhile, using a specific inhibitor of H<sub>2</sub>O<sub>2</sub> signalling pathway to regulate product diversity was firstly investigated in DHPJA-induced *V. vinifera* cv. Kyoho cell cultures.

## MATERIALS AND METHODS

### Grape callus induction and cell cultures

The callus, induced from the peel of mature berries of healthy *Vitis vinifera* cv. Kyoho in later ripening stage, were cultivated on Murashige and Skoog (MS) medium containing 6 mg/L 1-naphthylacetic acid (NAA), 0.6 mg/L 6-benzyladenine (BA), 30 g/L sucrose and 0.9% (w/v) agar at 25 ± 2°C under dark. Cell suspension cultures were maintained in liquid MS medium containing 30 g/L sucrose and the same growth regulator combination in 50 mL Erlenmeyer flasks on an orbital shaker at 110 rpm at 25 ± 2°C under dark. Each of the cultures flasks was filled with 10 mL medium and inoculated with 1 g fresh cells from the solid cultures.

### Elicitation experiments

MeJA and DHPJA were supplied by Institute of Pesticides and Pharmaceuticals, East China University of Science and Technology. They were dissolved in ethanol at the concentration of 0.04 mM for stock.

To determine the optimal eliciting time, 150 µM of DHPJA (final concentration) was added on different days (Figure 1). To determine the optimal concentrations of elicitors, different concentrations of MeJA and DHPJA (Figure 2) were added on day 15, and samples were harvested on day 20.

To inhibit the induction of H<sub>2</sub>O<sub>2</sub> generation, 10 µM NADPH oxidase inhibitor (DPI, diphenyleneiodonium chloride, Sigma) was added 30 min before elicitation.

Control received no treatment. It was confirmed that 1% (v/v) ethanol had no side effects on cell growth, *t*-R accumulation, H<sub>2</sub>O<sub>2</sub> production, or PAL activity (data not shown).

### Determination of cell mass and *trans*-resveratrol accumulation

The cell suspension was filtered under vacuum. The biomass was dried at 45°C to constant weight. And the dry weight (DW) was determined.

For the extraction of intracellular *t*-R, 100 mg dried cells were powdered and dissolved in 1 mL methanol, and the sample was extracted by ultrasonic for 2 times, 30 min per time. Subsequently, the extract was filtered through a 0.22 μm nylon filter and analyzed in a HPLC-DAD system using a reversed phase C18 column (4.6 mm x 250 mm, 5 μm, Agilent ZORBAX Eclipse XDB-C18) at 25°C. The mobile phase was acetonitrile: water (30:70, v/v) with flow rate of 1 mL/min and the injection volume was 20 μL. The content of *t*-R was given on a standard curve made with *trans*-resveratrol standard (Sigma). The samples were also identified by electrospray ionisation mass spectra (ESI/MS) at the Center of Analysis, Shanghai Jiao Tong University. A mass spectrometer (Waters, USA) was used with sheath gas flow rate of 50 arb, sweep gas flow rate of 20 arb, spray voltage of 3.0 kV and capillary temperature of 300°C. The spectrum was scanned at 30 eV from *m/z* 50 to 600.

### Assay of oxidative burst and PAL activity

H<sub>2</sub>O<sub>2</sub> generated in the cells and released into medium was determined according to Sergiev et al. (1997). Briefly, 0.5 mL sample medium was added to 0.5 mL 10 mM potassium phosphate buffer (pH 7) and 1 mL 1 M KI directly. The reaction mixture was incubated for 1 hr at 25°C under dark. The absorbance of the mixture was read at 390 nm before and after incubation. The content of H<sub>2</sub>O<sub>2</sub> was given on a standard curve.

To determine the activity of PAL, 1 g fresh cells were extracted with 2 mL 50 mM pH 8.8 Tris-HCl buffer containing 4 mM of β-mercaptoethanol and 10 mM EDTA in an ice-cooled mortar. The homogenate was centrifuged at 10000 rpm for 15 min at 4°C, and the supernatant was collected for enzyme assay. PAL activity was determined based on the rate of cinnamic acid production as described by Ochoa-Alejo and Gómez-Peralta (1993). One unit of PAL activity is equal to 1 μmol of cinnamic acid produced per minute.

### Statistical analyses

Each experiment was carried out in triplicate, and three different sets of experiments reproduced the same result. Data are presented as mean ± standard deviations (S.D.) Statistical significance was determined using the paired Student's *t* test.

## RESULTS

### Effects of elicitation timing and dosage on cell growth and *t*-R production

Figure 1 showed that day 15 after inoculation was the optimal eliciting time. When the treatment was done on that day, the highest production of *t*-R was observed on day 20 (5 days after the treatment), which was increased 1.96-fold compared with the control. Cell growth was strongly suppressed by 65–84%, and the *t*-R production couldn't be stimulated when elicited in earlier phases. Eliciting in a later phase was also less efficient, although the *t*-R production was increased 1.54-fold compared with the control. Figure 2 showed that 150 μM was the optimal eliciting concentration for both DHPJA and MeJA. Adding an excessive dose depressed cell growth by 47% (Figure 2a), while adding lower doses lowered the elicitation effect (Figure 2b and 2c). It also showed that DHPJA was more efficient in inducing *t*-R accumulation than MeJA. When the adding concentration was 150 μM, the *t*-R production induced by DHPJA increased 1.3-fold compared with MeJA.

### Effects of DHPJA on plant cell defence responses

Figure 3 showed that the H<sub>2</sub>O<sub>2</sub> level changed markedly when treated with DHPJA or MeJA. The H<sub>2</sub>O<sub>2</sub> level reached its maximum at 45 min after exposure to DHPJA (5.04 ± 0.36 µM), which was higher than exposure to MeJA (3.94 ± 0.49 µM). Figure 4 showed that PAL activity in elicited cultures increased significantly at 4 hrs after elicitation, and increased to approximately 4.6-fold at 6 hrs after elicitation, which was also higher than MeJA-elicited cultures.

### Effects of H<sub>2</sub>O<sub>2</sub> on DHPJA-induced stilbenoids accumulation

Figure 3 showed that diphenyleneiodonium chloride (DPI) successfully inhibited the DHPJA-induced generation of H<sub>2</sub>O<sub>2</sub> by 90.31%. Correspondingly, DPI inhibited DHPJA-induced *t*-R content by 89.18% (Figure 5 and Figure 6a). Meanwhile, DPI and H<sub>2</sub>O<sub>2</sub> also had impact on the activity of PAL. Data was shown in Figure 4. DPI inhibited the DHPJA-induced activity of PAL by 43.37%. And adding H<sub>2</sub>O<sub>2</sub> significantly stimulated the PAL activity by itself and enhanced the DHPJA elicitation effect. Correspondingly, Figure 5 and Figure 6a showed that adding H<sub>2</sub>O<sub>2</sub> stimulated *t*-R production by itself and also enhanced the DHPJA elicitation effect.

In Figure 6b, peak 1 was demonstrated to be *t*-R. Peak 2 was another compound which was conjectured to be a stilbenoid analogue by profiles of full wavelength scan and mass spectrometer (MS) (Figure 6b and Figure 7). It also could be induced by DHPJA, but the induction could not be inhibited by DPI (Figure 6a).

## DISCUSSION

### Effects of elicitation timing and dosage on cell growth and *t*-R production

Elicitation timing and dosage are the two main variables that affect cell growth and yield of secondary metabolites (Wang et al. 2006). It appears that plant cells responded differently to elicitation timing and dosage, which may be attributed to the differences not only among plant cell species but also among cell lines within species. In micro shoots of *Hypericum perforatum* 100 µM DHPJA enhanced hyperforin production stronger than 50 µM DHPJA, while it was the opposite in micro shoots of *Hypericum sampsonii* (Liu et al. 2007). In our culture system, addition of 150 µM DHPJA on day 15 of cultivation period was found to be optimal. The *t*-R production reached the highest value which was increased 1.8-fold and 1.3-fold compared with the control and 150 µM MeJA respectively.

It is speculated that when a dose lower than optimal is used, the elicitor-interaction sites in cells are not sufficiently utilized for activating the secondary metabolite synthesis, whereas an excessive dose causes cytotoxic effects (Lambert et al. 2009). It also indicated that the response of *t*-R biosynthesis to elicitation timing was closely related to the cellular physiological state. In conclusion, adding excessive doses and/or eliciting in earlier physiological states depressed cell growth, while adding lower doses and/or eliciting in later physiological states lowered the elicitation effect.

### DHPJA-induced defence responses and their effects on *t*-R production

DHPJA activated stronger defence responses including oxidative burst and activity of PAL in *V. vinifera* cell suspension cultures, corresponded well with its superior stimulating activity for *t*-R biosynthesis, compared with MeJA. The results suggested that an oxidative burst occurred in elicited *Vitis vinifera* cell cultures in a manner similar to that seen in other elicited plant cell cultures (Qian et al. 2004). There is an increasing body of evidence that H<sub>2</sub>O<sub>2</sub> can act as a diffusible signal to activate defence genes and the biosynthesis of plant secondary metabolites (Jabs et al. 1997).

Our work indicated that H<sub>2</sub>O<sub>2</sub> played a significant role in *t*-R biosynthesis. However, while the DHPJA-induced *t*-R accumulation was totally inhibited by DPI, the activity of PAL remained higher than control. It suggested that DHPJA also induced the activation of PAL through other molecular pathways to enhance other metabolites synthesis, which might correspond with the accumulation of peak 2. In *Taxus yunnanensis* suspension cells, NO enhanced cerebroside-induced activation of PAL and

accumulation of taxol (Wang et al. 2007). Although PAL is a key enzyme in the phenylpropanoid pathway, the activation induced by DHPJA could not result in *t*-R accumulation without H<sub>2</sub>O<sub>2</sub>. So inducing higher level of H<sub>2</sub>O<sub>2</sub> generation was the basic reason why DHPJA could enhance *t*-R accumulation stronger than MeJA. To our best knowledge, this was the first report to investigate the relationship between H<sub>2</sub>O<sub>2</sub> and stilbenoids synthesis.

In conclusion, our work demonstrated that DHPJA was an efficient elicitor for *t*-R accumulation by activating stronger oxidative burst. It is believed that by taking the place of MeJA, DHPJA might optimize other strategy for *t*-R production. It also indicated that H<sub>2</sub>O<sub>2</sub> signalling pathway could regulate product diversity in DHPJA-induced *V. vinifera* cell suspension cultures.

**Financial support:** This work was supported by the Fundamental Research Funds for the Central Universities (WF1113010), and partially supported by the 863 Project (2011AA090702), Shanghai Leading Academic Discipline Project (B505), and the National Special Fund for State Key Laboratory of Bioreactor Engineering (2060204).

## REFERENCES

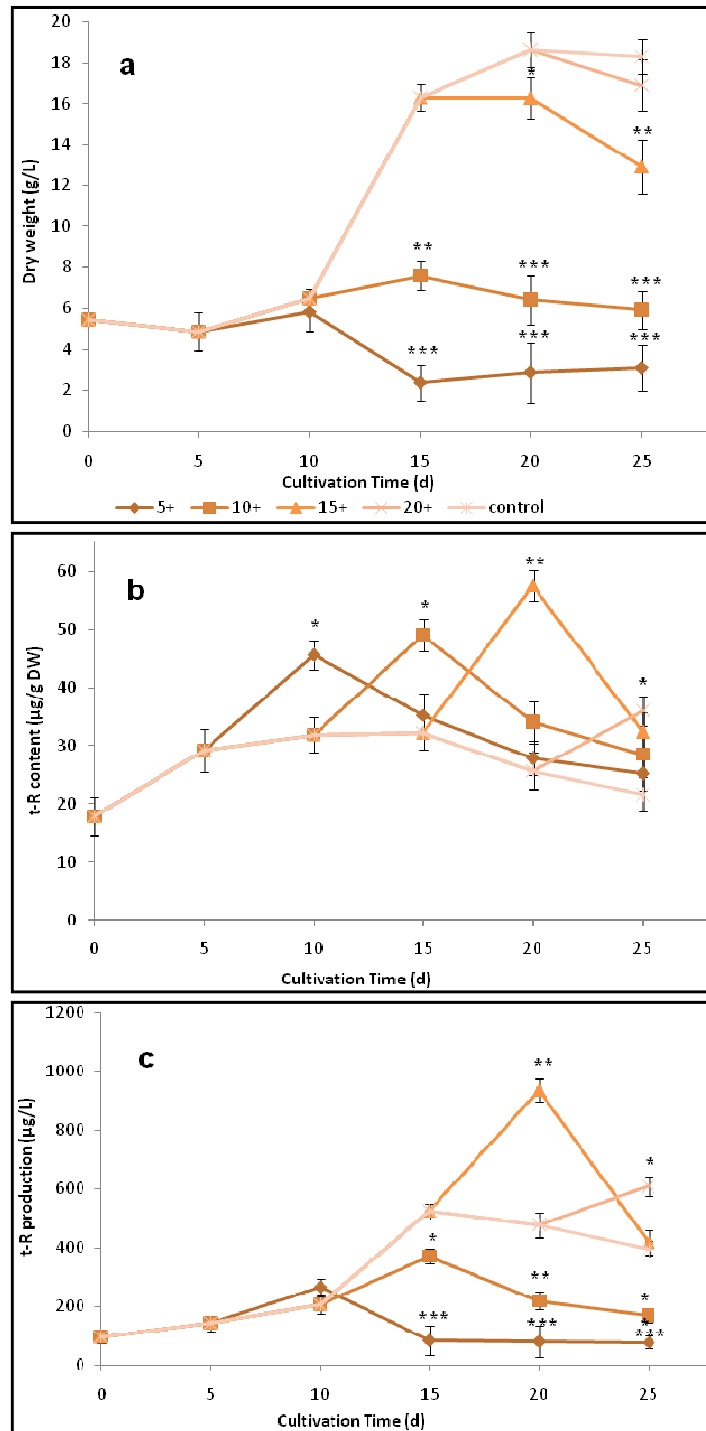
- AGGARWAL, B.B.; BHARDWAJ, A.; AGGARWAL, R.S.; SEERAM, N.P.; SHISHODIA, S. and TAKADA, Y. (2004). Role of resveratrol in prevention and therapy of cancer: Preclinical and clinical studies. *Anticancer Research*, vol. 24, no. 5A, p. 2783-2840.
- AZIZ, A.; POINSSOT, B.; DAIRE, X.; ADRIAN, M.; BÉZIER, A.; LAMBERT, B.; JOUBERT, J.-M. and PUGIN, A. (2003). Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. *Molecular Plant-Microbe Interactions*, vol. 16, no. 12, p. 1118-1128. [\[CrossRef\]](#)
- BELHADJ, A.; TELEF, N.; SAIGNE, C.; CLUZET, S.; BARRIEU, F.; HAMD, S. and MÉRILLON, J.-M. (2008). Effect of methyl jasmonate in combination with carbohydrates on gene expression of PR proteins, stilbene and anthocyanin accumulation in grapevine cell cultures. *Plant Physiology and Biochemistry*, vol. 46, no. 4, p. 493-499. [\[CrossRef\]](#)
- BRU, R. and PEDREÑO, M.A. (2003). Method for the production of resveratrol in cell cultures. PCT Patent Number WO/2003/062406.
- BRU, R.; SELLÉS, S.; CASADO-VELA, J.; BELCHI-NAVARRO, S. and PEDREÑO, M.A. (2006). Modified cyclodextrins are chemically defined glucan inducers of defense responses in grapevine cell cultures. *Journal of Agricultural and Food Chemistry*, vol. 54, no. 1, p. 65-71. [\[CrossRef\]](#)
- COMMUN, K.; MAURO, M.C.; CHUPEAU, Y.; BOULAY, M.; BURRUS, M. and JEANDET, P. (2003). Phytoalexin production in grapevine protoplasts during isolation and culture. *Plant Physiology and Biochemistry*, vol. 41, no. 4, p. 317-323. [\[CrossRef\]](#)
- DONNEZ, D.; JEANDET, P.; CLEMENT, C. and COUROT, E. (2009). Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. *Trends in Biotechnology*, vol. 27, no. 12, p. 706-713. [\[CrossRef\]](#)
- DONNEZ, D.; KIM, K.H.; ANTOINE, S.; CONREUX, A.; DE LUCA, V.; JEANDET, P.; CLÉMENT, C. and COUROT, E. (2011). Bioproduction of resveratrol and viniferins by an elicited grapevine cell culture in a 2 L stirred bioreactor. *Process Biochemistry*, vol. 46, no. 5, p. 1056-1062. [\[CrossRef\]](#)
- HU, F.X.; HUANG, J.H.; XU, Y.F.; QIAN, X.H. and ZHONG, J.J. (2006). Responses of defense signals, biosynthetic gene transcription and taxoid biosynthesis to elicitation by a novel synthetic jasmonate in cell cultures of *Taxus chinensis*. *Biotechnology and Bioengineering*, vol. 94, no. 6, p. 1064-1071. [\[CrossRef\]](#)
- JABS, T.; TSCHÖPE, M.; COLLING, C.; HAHLBROCK, K. and SCHEEL, D. (1997). Elicitor-stimulated ion fluxes and O<sub>2</sub> from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 9, p. 4800-4805. [\[CrossRef\]](#)
- KISELEV, K.V. (2011). Perspectives for production and application of resveratrol. *Applied Microbiology and Biotechnology*, vol. 90, no. 2, p. 417-425. [\[CrossRef\]](#)
- KRISA, S.; LARRONDE, F.; BUDZINSKI, H.; DECENDIT, A.; DEFFIEUX, G. and MÉRILLON, J.M. (1999). Stilbene production by *Vitis vinifera* cell suspension cultures: Methyl jasmonate induction and <sup>13</sup>C biolabeling. *Journal of Natural Products*, vol. 62, no. 12, p. 1688-1690. [\[CrossRef\]](#)
- LAMBERT, C.B.; SPIRE, C.; CLAUDE, N. and GUILLOUZO, A. (2009). Dose- and time-dependent effects of phenobarbital on gene expression profiling in human hepatoma HepaRG cells. *Toxicology and Applied Pharmacology*, vol. 234, no. 3, p. 345-360. [\[CrossRef\]](#)
- LIJAVETZKY, D.; ALMAGRO, L.; BELCHI-NAVARRO, S.; MARTÍNEZ-ZAPATER, J.; BRU, R. and PEDREÑO, M.A. (2008). Synergistic effect of methyljasmonate and cyclodextrin on stilbene biosynthesis pathway gene expression and resveratrol production in Monastrell grapevine cell cultures. *BMC Research Notes*, vol. 1, no. 132. [\[CrossRef\]](#)
- LISWIDOWATI, L.; MELCHIOR, F.; HOHMANN, F.; SCHWER, B. and KINDL, H. (1991). Induction of stilbene synthase by *Botrytis cinerea* in cultured grapevine cells. *Planta*, vol. 183, no. 2, p. 307-314. [\[CrossRef\]](#)
- LIU, X.N.; ZHANG, X.Q. and SUN, J.S. (2007). Effects of cytokinins and elicitors on the production of hypericins and hyperforin metabolites in *Hypericum sampsonii* and *Hypericum perforatum*. *Plant Growth Regulation*, vol. 53, no. 3, p. 207-214. [\[CrossRef\]](#)

- OCHOA-ALEJO, N. and GÓMEZ-PERALTA, J.E. (1993). Activity of enzymes involved in capsaicin biosynthesis in callus tissue and fruits of chili pepper (*Capsicum annuum* L.). *Journal of Plant Physiology*, vol. 141, no. 2, p. 147-152. [\[CrossRef\]](#)
- QIAN, Z.G.; ZHAO, Z.J.; XU, Y.F.; QIAN, X.H. and ZHONG, J.J. (2004). Novel chemically synthesized hydroxyl-containing jasmonates as powerful inducing signals for plant secondary metabolism. *Biotechnology and Bioengineering*, vol. 86, no. 7, p. 809-816. [\[CrossRef\]](#)
- SERGIEV, I.; ALEXIEVA, V. and KARANOV, E. (1997). Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. *Comptes Rendus de Academie Bulgare des Sciences*, vol. 51, no. 3, p. 121-124.
- SHANKAR, S.; SINGH, G. and SRIVASTAVA, R.K. (2007). Chemoprevention by resveratrol: Molecular mechanisms and therapeutic potential. *Frontiers in Bioscience*, vol. 12, p. 4839-4854. [\[CrossRef\]](#)
- TASSONI, A.; FORNALE, S.; FRANCESCHETTI, M.; MUSIANI, F.; MICHAEL, A.J.; PERRY, B. and BAGNI, N. (2005). Jasmonates and Na-orthovanadate promote resveratrol production in *Vitis vinifera* cv. Barberacell cultures. *New Phytologist*, vol. 166, no. 3, p. 895-905. [\[CrossRef\]](#)
- VEZZULLI, S.; CIVARDI, S.; FERRARI, F. and BAVARESCO, L. (2007). Methyl jasmonate treatment as a trigger of resveratrol synthesis in cultivated grapevine. *American Journal of Enology and Viticulture*, vol. 58, no. 4, p. 530-533.
- WANG, W.; ZHAO, Z.J.; XU, Y.; QIAN, X. and ZHONG, J.J. (2006). Efficient induction of ginsenoside biosynthesis and alteration of ginsenoside heterogeneity in cell cultures of *Panax notoginseng* by using chemically synthesized 2-hydroxyethyl jasmonate. *Applied Microbiology and Biotechnology*, vol. 70, no. 3, p. 298-307. [\[CrossRef\]](#)
- WANG, J.W.; ZHENG, L.P. and TAN, R.X. (2007). Involvement of nitric oxide in cerebroside-induced defense responses and taxol production in *Taxus yunnanensis* suspension cells. *Applied Microbiology and Biotechnology*, vol. 75, no. 5, p. 1183-1190. [\[CrossRef\]](#)
- YANG, M.H.; KUO, C.H.; HSIEH, W.C. and KU, K.L. (2010). Investigation of microbial elicitation of *trans*-resveratrol and *trans*-piceatannol in peanut callus led to the application of chitin as a potential elicitor. *Journal of Agricultural and Food Chemistry*, vol. 58, no. 17, p. 9537-9541. [\[CrossRef\]](#)

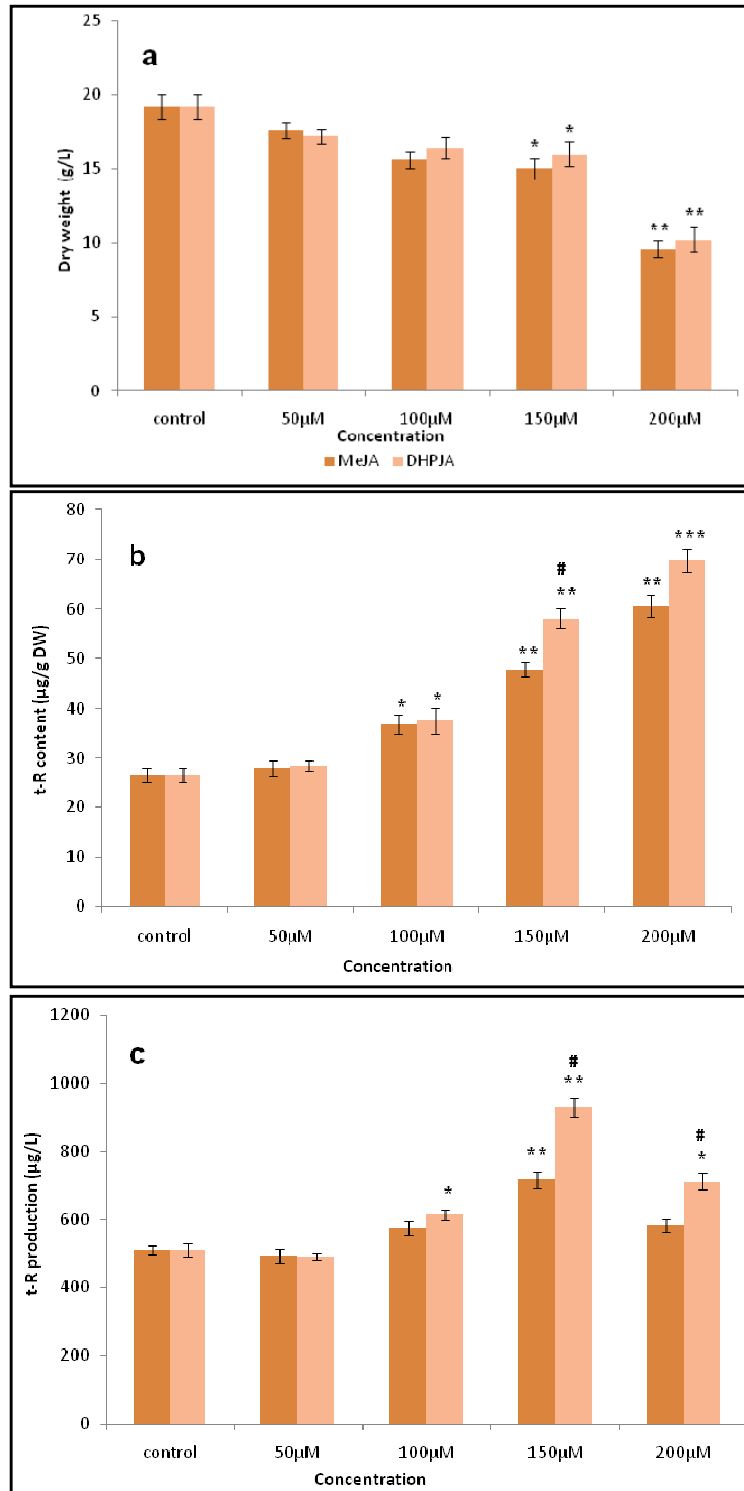
#### How to reference this article:

SHEN, X.; DU, Q.; XU, Y. and LU, Y. (2012). Stimulation of *trans*-resveratrol biosynthesis in *Vitis vinifera* cv. Kyoho cell suspension cultures by 2,3-dihydroxypropyl jasmonate elicitation. *Electronic Journal of Biotechnology*, vol. 15, no. 5. <http://dx.doi.org/10.2225/vol15-issue5-fulltext-4>

## Figures

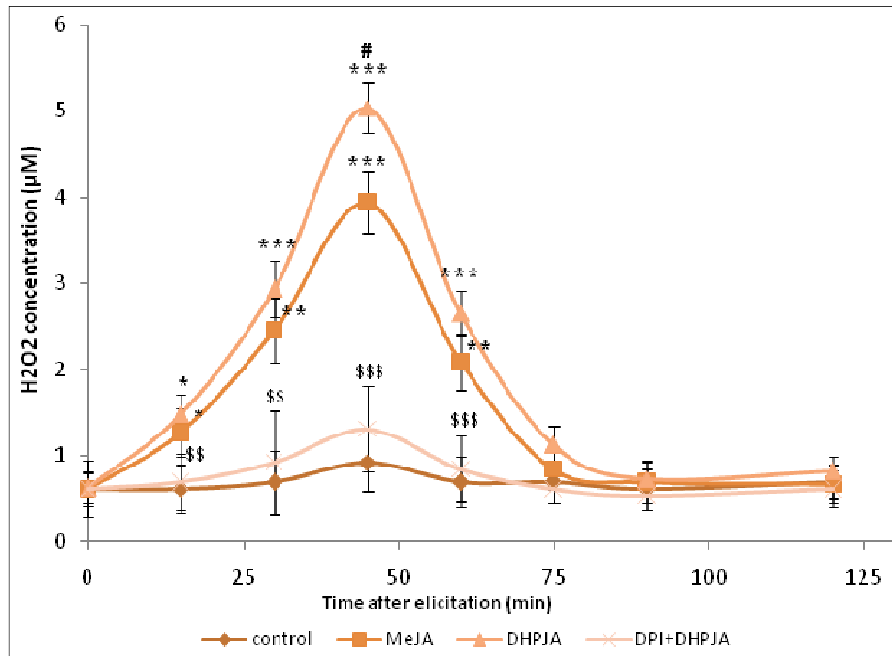


**Fig. 1** Time courses of cell growth and *t*-R accumulation in *V. vinifera* cell suspension cultures elicited with DHPJA on different days: (a) Dry cell weight; (b) *t*-R content; and (c) *t*-R production. In the figure, 5+, 10+, 15+ and 20+ mean that DHPJA (150 µM) was added on day 5, 10, 15 and 20. Data are the means of three flasks and vertical bars show standard deviations. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , significantly different from Control.

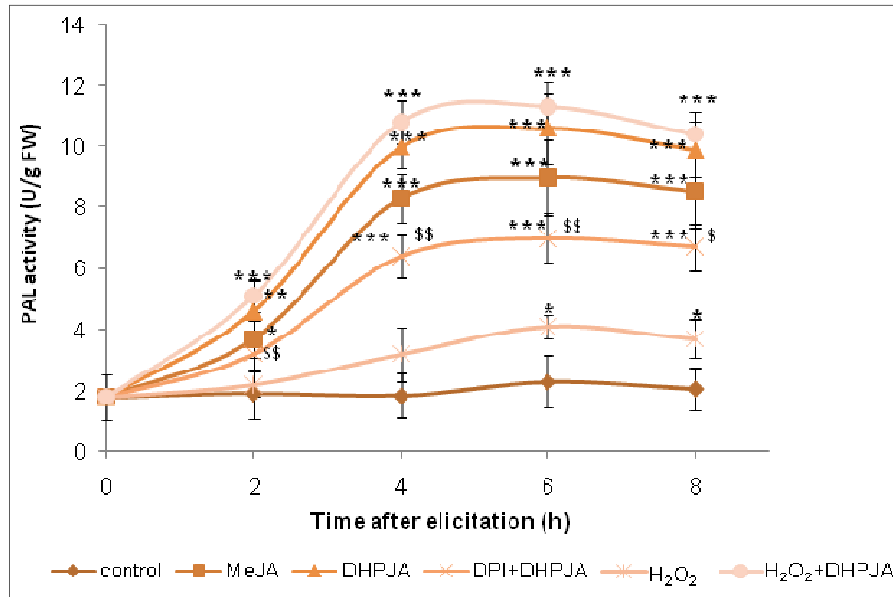


**Fig. 2** Effect of DHPJA and MeJA with various concentrations on cell growth and t-R accumulation in *V. vinifera* cell suspension cultures: (a) Dry cell weight; (b) t-R content; (c) t-R production. Data are the means of three flasks and vertical bars show standard deviations. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , significantly different from Control. #,  $P < 0.05$ , significantly different from MeJA.

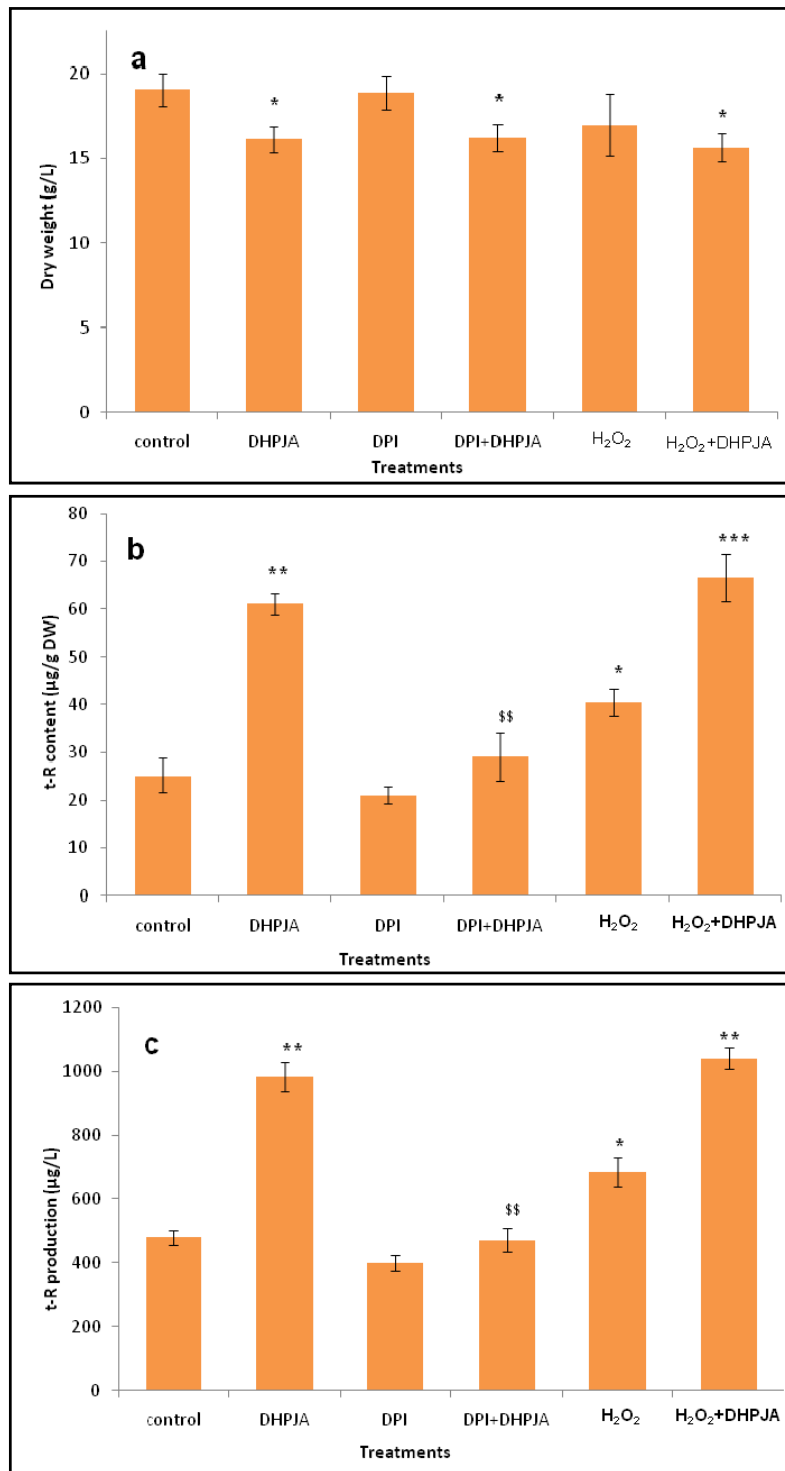




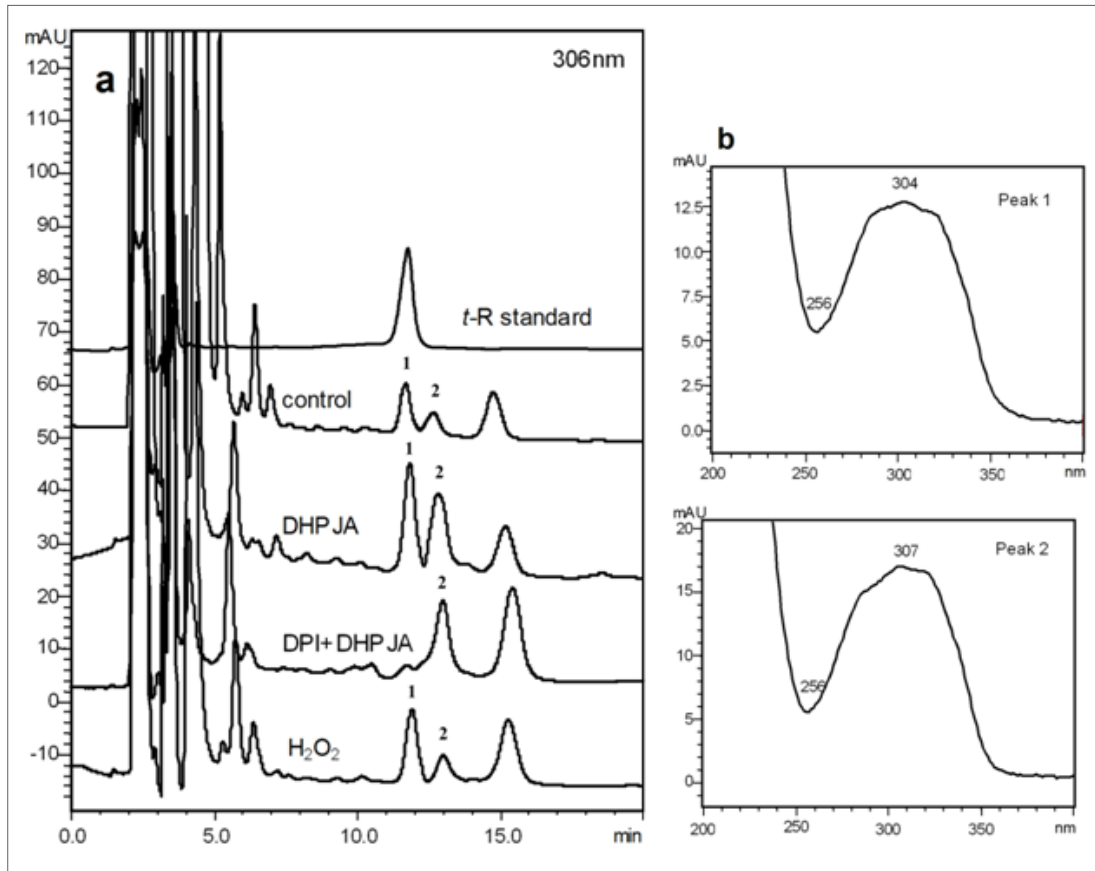
**Fig. 3** The oxidative burst induced by DHPJA and MeJA DHPJA (150 µM) and MeJA (150 µM) were added on day 15. DPI+DHPJA means DPI (10 µM) was added 30 min before DHPJA. Data are the means of three flasks and vertical bars show standard deviations. \*, P < 0.05, \*\*, P < 0.01, \*\*\*, P < 0.001, significantly different from Control. #, P < 0.05, significantly different from MeJA. \$, P < 0.05, \$\$, P < 0.01, \$\$\$, P < 0.001, significantly different from DHPJA.



**Fig. 4 PAL activity in response to different treatments** DHPJA (150  $\mu$ M) and MeJA (150  $\mu$ M) were added on day 15. DPI+DHPJA means DPI (10  $\mu$ M) was added 30 min before DHPJA. H<sub>2</sub>O<sub>2</sub>+DHPJA means 5  $\mu$ M H<sub>2</sub>O<sub>2</sub> was added with DHPJA. Data are the means of three flasks and vertical bars show standard deviations. \*, P < 0.05, \*\*, P < 0.01, \*\*\*, P < 0.001, significantly different from control. #, P < 0.05, significantly different from MeJA. \$, P < 0.05, \$\$, P < 0.01, \$\$\$, P < 0.001, significantly different from DHPJA.



**Fig. 5** Effect of  $H_2O_2$  on cell growth and *t*-R accumulation in *V. vinifera* cell suspension cultures: (a) Dry cell weight; (b) *t*-R content; and (c) *t*-R production. Cell suspension cultures was elicited with different treatments on day 15. And samples were harvested on day 20. Data are the means of three flasks and vertical bars show standard deviations. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ , \*\*\*,  $P < 0.001$ , significantly different from Control. \$\$,  $P < 0.01$ , significantly different from DHPJA.



**Fig. 6** HPLC profiles (a) of cultures with different treatments, and full wavelength scan profiles (b) of peak 1 and peak 2.

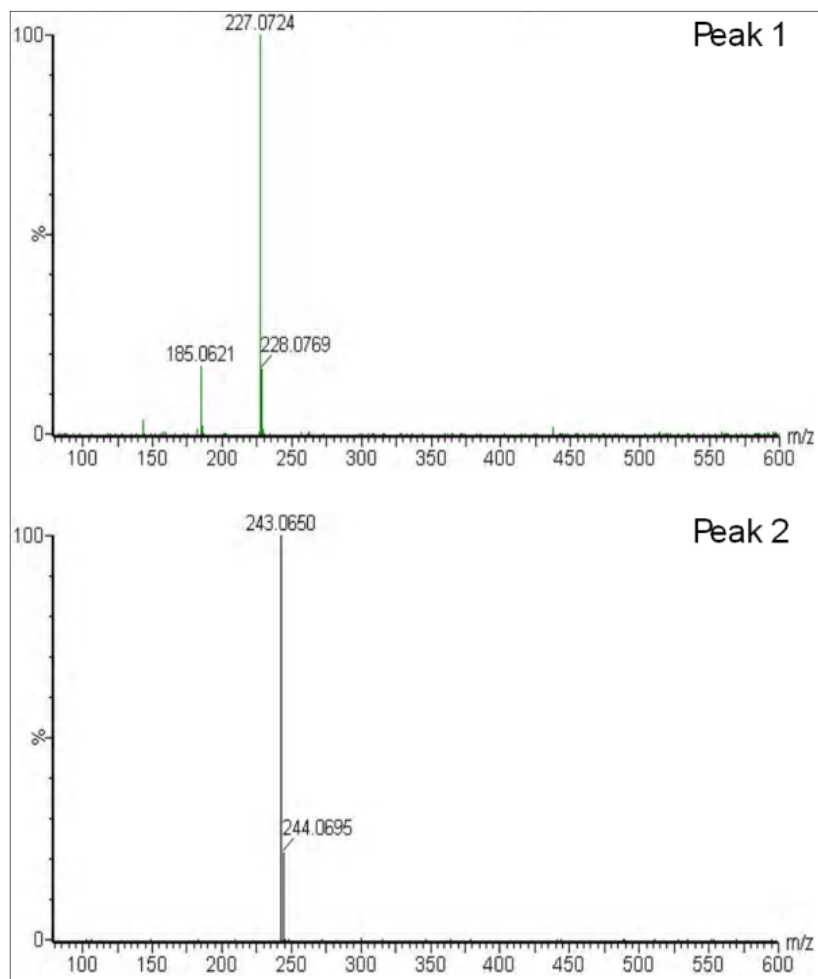


Fig. 7 MS profiles of peak 1 and peak 2.