CARYOLOGIA Vol. 60, no. 1-2: 169-171, 2007

Resveratrol production in *Vitis vinifera* cell suspensions treated with several elicitors

Righetti Laura, Marina Franceschetti, Maura Ferri, Annalisa Tassoni and Nello Bagni*

Department of Biology E.S., University of Bologna, Via Irnerio 42, 40126 Bologna, Italy.

Abstract — We investigated the effect of several elicitors on the production of resveratrol in *Vitis vinifera* cv. Barbera cell suspension cultures. Salicylic acid, Na-orthovanadate, jasmonates, chitosan and the monomers D-glucosamine and N-acetyl-D-glucosamine, ampicillin and rifampicin differently elicitated the accumulation of the *trans* and *cis* resveratrol isomers, both in the cells and in the culture media. Proteomic analyses also showed that methyl-jasmonate and chitosan stimulated the *ex-novo* synthesis of the stilbene synthase protein.

Key words: Elicitor, Resveratrol, Stilbene synthase, *Vitis vinifera*.

INTRODUCTION

Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenolic phytoalexin with pharmacological potential as antioxidant and in the prevention of cancer, cardiovascular and neurodegenerative diseases. This molecule occurs in *trans* and *cis* isomeric forms and it is synthesized by many plants including grape, which is the mayor source for human diet (Jeandet *et al*. 2002). Due to its high levels in the grape skin, variable amounts of resveratrol are present in the commercial wines, especially the red ones (Musiani *et al*. 2006). For this reason *Vitis vinifera* is a good model system to investigate the factors able to influence resveratrol metabolism. One of our main targets is to enhance resveratrol production and its release in the culture medium in cell suspensions obtained from cv. Barbera petioles.

We tested the effect on cell growth, viability and accumulation of *trans*- and *cis*-resveratrol of methyl-jasmonate, jasmonic acid, salicylic acid, chitosan, D-glucosamine, N-acetyl-D-glucosamine, ampicillin, rifampicin and Na-orthovanadate, the only abiotic elicitor.

MATERIALS AND METHODS

Establishment of callus cultures, cell suspension cultures, elicitor treatments, quantification of resveratrol by HPLC analyses and protein gel analyses, were performed as previously reported in Tassoni *et al.* (2005) and Musiani *et al.* (2006).

RESULTS AND DISCUSSION

All the tested elicitors seemed to be able to promote resveratrol accumulation in the cells and some of them also stimulated the released in the culture medium (Table 1) without significantly affecting cell growth and viability (data not shown). Resveratrol accumulation always reached the maximum level during the first week of culture and most frequently at day 2 or 4.

Jasmonates represented suitable elicitors for enhancing the production of *cis*- and *trans*-resveratrol, while Na-orthovanadate slightly induced the synthesis and/or intracellular accumulation only at the highest concentration. Salicylic acid was a good elicitor, showing about the same effect at the two different concentrations tested. On the whole chitosan seemed more effective than the monomers D-glucosamine and N-acetyl-D-glucosamine. The addition of 25 µg/ml rifampicin caused a significant intracellular accumulation and the highest excretion in the culture medium. This concentration was the most effective in preventing or reducing microbial contaminations and increased the intracellular and the released amount of both isomers, mimicking the *in vivo* effect of the plant-pathogen interaction (Ferri *et al.* 2007).

* Corresponding author: phone +39 051 2091280; fax: +39 051 242576; e-mail: bagninel@alma.unibo.it

Table 1 — Elicitor effect on resveratrol accumulation in *Vitis vinifera* (cv. Barbera) cell cultures. Data refer to the day of maximum accumulation of total resveratrol (*cis* plus *trans*) respect to the specific control: (a) 0.1% (v/v) ethanol, (b) water, (c) 0.01% (v/v) acetic acid, (d) 0.1% (v/v) methanol.

Elicitor	Intracellular resveratrol		Released resveratrol	
	Day	Ratio elicitor/control	Day	Ratio elicitor/control
Methyl-jasmonate (10 µM) [a]	2	4.8	2	1.4
Jasmonic acid (10 µM) [a]	7	3.0	4	4.1
Na-orthovanadate (100 µM) [b]	2	1.2	2	0.3
Na-orthovanadate (1 mM) [b]	4	2.9	2	0.4
Salicylic acid (20 µM) [b]	0	3.1	0	2.1
Salicylic acid (100 µM) [b]	0	3.3	4	1.8
Chitosan (50 µg/ml) [c]	4	3.2	4	5.0
D-glucosamine (50 µg/ml) [b]	2	10.1	4	0.1
N-acetyl-D-glucosamine (50 µg/ml) [b]	2	2.5	2	0.3
Ampicillin (100 µg/ml) [b]	3	2.2	4	2.5
Rifampicin (25 ng/ml) [d]	2	2.3	3	4.3
Rifampicin (25 µg/ml) [d]	2	4.7	2	10.3

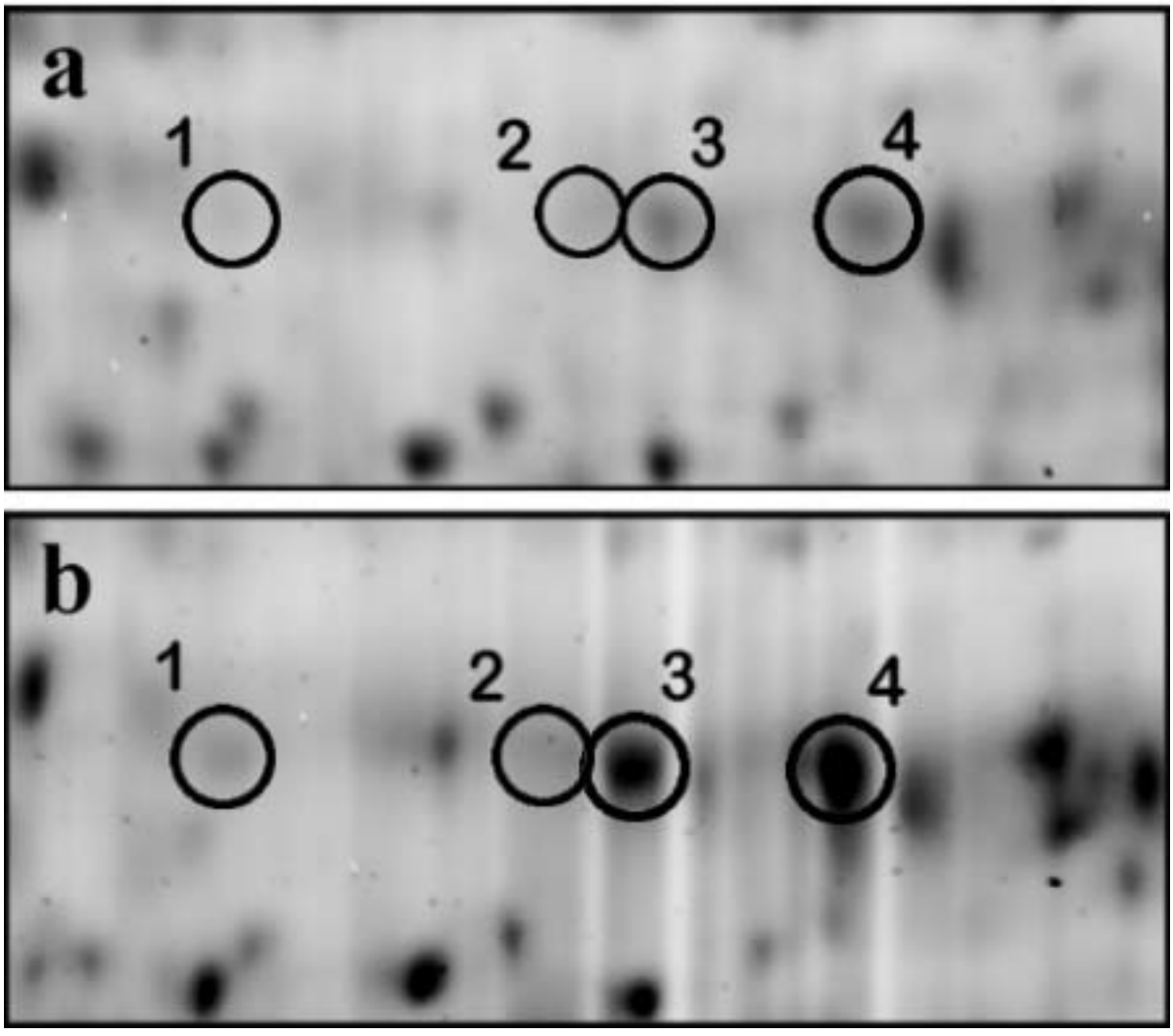

Fig. 1 — Protein identification (MALDI-ToF). 0.1% (v/v) ethanol control (a); 10 µM methyl-jasmonate (b). All the spots correspond to stilbene synthase 1 with 2.4 to 3.1 fold increase) (modified from TASSONI *et al.* 2005).

Proteomic analyses showed that methyl-jasmonate and chitosan induced the *ex-novo* synthesis of the stilbene synthase (Fig. 1) and northern blot analyses demonstrated that chitosan also promote the accumulation of stilbene synthase mRNA (data not shown).

In conclusion methyl-jasmonate, chitosan and rifampicin seem to induce a good elicitation, being useful tools for future biotechnological application.

Acknowledgments — We wish to thank Linda D'Errico, Federica Musiani and Caterina Vacchi Suzzi (Department of Biology E.S., University of Bologna) for collaboration in HPLC analyses. This project was financially supported by PRIN 2005 funds from Ministry of University and Scientific Research of Italy to N.B.

REFERENCES

Ferri M., D'errico A.L., Vacchi Suzzi C., Franceschetti M., Righetti L., Bagni N. and Tassoni A., 2007 — *Use of rifampicin for resveratrol production in Vitis vinifera cell suspensions*. In: P. Jeandet, C. Clément and A. Conreux (eds.), "Macromolecules and Secondary Metabolites of Grapevine and Wine", p. 95-98. Lavoisier Publisher, Paris.

Jeandet P., Douillet-Breuil A.C., Bessis R., Debord S., Sbaghi M. and Adrian M., 2002 — *Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity and metabolism*. Journal of Agricultural and Food Chemistry, 50: 2731-2741.

Musiani F., Bagni N., Mordenti G.L., Franceschetti M. and Tassoni A., 2006 — *Contenuto di resveratrolo in uve e viti romagnoli*. VQ, 5: 51-58.

Tassoni A., Fornalè S., Franceschetti M., Musiani F., Micheal A.J., Perry B. and Bagni N., 2005 — *Jasmonates and Na-orthovanadate promote resveratrol production in Vitis vinifera L. cv. Barbera cell cultures*. New Phytologist, 166: 895-905.

Received November 30th 2006; accepted January 30th 2007