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The *rolB* gene-induced overproduction of resveratrol in *Vitis amurensis* transformed cells

K.V. Kiselev [a,c,*], A.S. Dubrovina [a,c], M.V. Veselova [b], V.P. Bulgakov [a], S.A. Fedoreyev [b], Y.N. Zhuravlev [a]

[a] *Institute of Biology and Soil Science, Far East Branch of Russian Academy of Sciences, 159 Stoletija Str., Vladivostok 690022, Russia*
[b] *Pacific Institute of Bioorganic Chemistry, Far East Branch of Russian Academy of Sciences, Vladivostok 690022, Russia*
[c] *Far Eastern State University, 690090 Vladivostok, Russia*

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Abstract

Resveratrol is a stilbene, which prevents carcinogenesis at stages of tumor initiation, promotion and progression. In the present investigation, we developed cell cultures of wild-growing grape (*Vitis amurensis* Rupr.). The cultures produced low levels of resveratrol, up to 0.026% dry wt., i.e., comparable to levels reported for other plant cell cultures previously established. Different methods commonly used to increase secondary metabolite production (cell selection, elicitor treatments and addition of a biosynthetic precursor) only slightly enhanced cell productivity. Transformation of *V. amurensis* V2 callus culture by the *rolB* gene of *Agrobacterium rhizogenes* resulted in more than a 100-fold increase in resveratrol production in transformed calli. The *rolB*-transformed calli are capable of producing up to 3.15% dry wt. of resveratrol. We show that the capability to resveratrol biosynthesis is tightly correlated with the abundance of *rolB* mRNA transcripts. Tyrosine phosphatase inhibitors abolished the *rolB*-gene-mediated stimulatory effect, thus documenting for the first time the involvement of tyrosine phosphorylation in plant secondary metabolism.

Keywords: *Vitis amurensis*; Resveratrol; *rolB* gene; Callus culture

1. Introduction

Stilbenes play an important role in protecting plants against fungal infections (Dixon and Harrison, 1990) and constitute the main group of phytoalexins within the *Vitaceae* (Jeandet et al., 2002). In recent years, much attention has been emphasized to the effects of stilbenes, especially resveratrol, on human health. Although resveratrol synthesis has been reported in

Abbreviations: MeJA, methyl jasmonate; PAO, phenylarsine oxide; Phe, phenylalanine; PFP, *p*-fluoro-DL-phenylalanine; SA, salicylic acid; SNP, sodium nitroprusside; BAP, 6-benzylaminopurine; NAA, α-naphthaleneacetic acid; IAA, indoleacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid

* Corresponding author at: Institute of Biology and Soil Science, Far East Branch of Russian Academy of Sciences, 159 Stoletija Str., Vladivostok 690022, Russia. Tel.: +7 4232 312129; fax: +7 4232 310193.
E-mail address: kkv5@mail.ru (K.V. Kiselev).

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several plants such as peanut, lily, mulberries, eucalyptus, spruce and pine, grapevine is the main source of this compound (Pervaiz, 2003). Multiple lines of compelling evidence indicate its beneficial effects on neurological, hepatic, and cardiovascular systems. One of the most striking biological activities of resveratrol investigated during the late years has been its cancer chemopreventive potential (Pervaiz, 2003). More recent data provide interesting insights into the effect of this compound on the lifespan of yeast and flies, implicating the potential of resveratrol as an anti-aging agent in treating age-related human diseases (Lastra and Villegas, 2005).

Only small amounts of resveratrol (less than 0.01% DW) have been reported for plant cell cultures of different species (Ku et al., 2005; Tassoni et al., 2005). Therefore, efforts have been made to overcome the inability of cultured plant cells to synthesize high levels of resveratrol. *Vitis vinifera* suspension cultures responded to MeJA and sodium orthovanadate by a five- to six-fold increase in resveratrol production (Tassoni et al., 2005). Likewise, cell cultures of *Arachis hypogaea* responded to ultraviolet (UV) irradiation with a 40–50-fold increase in resveratrol production, but the effect disappeared after 24 h of the treatment (Ku et al., 2005). After these treatments, the resulting levels of resveratrol in plant cell cultures did not exceed 0.02–0.03% cell dry wt. (Ku et al., 2005; Tassoni et al., 2005).

Genetic transformation by single Agrobacterium *rol* genes has emerged as a powerful tool for secondary metabolite manipulation in cultured plant cells (Bulgakov et al., 2002, 2003). One of the *rol* genes, *rolB*, is essential for plant (Altamura et al., 1994) and animal (Bulgakov et al., 2006) cell growth and development. In *Rubia cordifolia* transformed cells, the *rolB* gene was shown to be a more powerful inductor of anthraquinone biosynthesis than the *rolC* gene (Bulgakov et al., 2002). The development of additional *rolB*-transformed plant model systems is important to exploit this new biotechnological tool for genetic engineering of plant secondary metabolism. The RolB protein was shown to exhibit tyrosine phosphatase activity (Filippini et al., 1996) and to interact with 14-3-3 proteins (Moriuchi et al., 2004). Protein tyrosine phosphatases or 14-3-3 proteins have not been considered as components of signaling pathways regulating secondary metabolism in plants. However, taking into account the more pronounced stimulatory effect of the *rolB* gene on anthraquinone biosynthesis than that of MeJA or SA, one can suppose a potential significance of these proteins in plant secondary metabolism.

In the present study, we tested different approaches to increase resveratrol production in cultured *Vitaceae* cells. Wild-growing plants of *V. amurensis* were used as a source of explants, because this species was previously characterized as a rich source of stilbenes (Huang et al., 2001). We show that genetic transformation of *V. amurensis* cells with the *rolB* gene was efficient for high resveratrol production while aggregate cell selection, metabolite selection, precursor addition and elicitor treatments were not effective.

2. Materials and methods

2.1. Plant material and cell cultures

Wild-growing plants of *Vitis amurensis* Rupr. (*Vitaceae*) were collected from the southern Primorsky Region of the Russian Far East and identified in the Botany Department of the Institute of Biology and Soil Science. The V1, V2 and V3 callus cultures were established in 2002 from young stems of the mature plants. Cultures were cultivated in 100 ml Erlenmeyer flasks using $W_{B/A}$ medium (Bulgakov et al., 2002) supplemented with 0.5 mg/l 6-benzylaminopurine and 2.0 mg/l α-naphthaleneacetic acid in the dark, at 25 °C. The inoculum mass for all cultures was 0.2 g. The calli were grown for 35–40 days.

2.2. Elicitor and effector treatments

Sterile solutions of methyl jasmonate (MeJA), and salicylic acid (SA) were added to the culture medium aseptically in desired concentrations as described (Bulgakov et al., 2002). Phenylalanine and sodium nitroprusside were dissolved in sterile hot water. Stock solutions of phenylalanine and sodium nitroprusside (100 and 40 mg/ml, respectively) were used. Phenylarsine oxide (PAO) was dissolved in dimethyl sulfoxide (10 mM stock solution) and supplied at the final concentrations indicated in the figure legends. All reagents were added to the medium after autoclaving. The chemicals were obtained from Sigma and ICN Pharmaceuticals.