Journal of Plant Physiology **166** (2009) 1194—1206

Available online at www.sciencedirect.com

ScienceDirect

JOURNAL OF PLANT PHYSIOLOGY

www.elsevier.de/jplph

Enhanced resveratrol accumulation in *rolB* transgenic cultures of *Vitis amurensis* correlates with unusual changes in *CDPK* gene expression

Alexandra S. Dubrovina[a,b,*], Konstantin V. Kiselev[a,b], Marina V. Veselova[c], Galina A. Isaeva[a,b], Sergey A. Fedoreyev[c], Yuri N. Zhuravlev[a]

[a]*Laboratory of Biotechnology, Institute of Biology and Soil Science, Far Eastern Branch of Russian Academy of Sciences, Vladivostok 690022, Russia*
[b]*Department of Biochemistry and Biotechnology, Far Eastern State University, 690090 Vladivostok, Russia*
[c]*Laboratory of Chemistry of Natural Quinonoid Compounds, Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of Russian Academy of Sciences, Vladivostok 690022, Russia*

Received 7 December 2008; received in revised form 13 January 2009; accepted 13 January 2009

KEYWORDS
Calcium flux;
CDPK;
Resveratrol;
rolB;
Vitis amurensis

Summary

It has been established that transformation of *Vitis amurensis* callus culture with the plant oncogene *rolB* of *Agrobacterium rhizogenes* results in a high level of resveratrol production in the transformed culture. In the present report, we investigated two *rolB* transgenic *V. amurensis* cell cultures with different levels of *rolB* expression and resveratrol production. We examined whether the calcium ion flux and later steps of the calcium-mediated signal transduction pathway play a role in resveratrol biosynthesis in the *rolB* transgenic cultures. It has been shown that the calcium channel blockers, $LaCl_3$, verapamil, and niflumic acid, significantly reduced the accumulation of resveratrol in the *rolB* transgenic cultures. The number of the calcium-dependent protein kinase (CDPK) transcript variants and abundance of some of the transcripts were considerably altered in the *rolB* transgenic cell cultures, as revealed by frequency analysis of RT-PCR products and real-time PCR. Some unusual *CDPK* transcripts with deletions and insertions in the kinase domain were isolated from cDNA probes of *rolB*-transformed cells. These results suggest that active resveratrol biosynthesis in *rolB* transgenic cultures of *V. amurensis* is Ca^{2+} dependent. We propose that the *rolB* gene has an important role in regulation of calcium-dependent transduction pathways in transformed cells.

*Corresponding author at: Laboratory of Biotechnology, Institute of Biology and Soil Science, Far Eastern Branch of Russian Academy of Sciences, Vladivostok 690022, Russia. Tel.: +7 4232 312129; fax: +7 4232 310193.
E-mail address: dubrovina@biosoil.ru (A.S. Dubrovina).

0176-1617/$ - see front matter
doi:10.1016/j.jplph.2009.01.006

Introduction

It has been reported that *rol* oncogenes located in Ri-plasmids of *Agrobacterium rhizogenes* may enhance biosynthesis of certain groups of secondary metabolites in transformed plant cells (Palazon et al., 1997, 1998; Bulgakov et al., 2002). Recently, the *rolB* gene has been shown to stimulate munjistin and purpurin biosynthesis in *Rubia cordifolia* cell cultures, and resveratrol biosynthesis in *Vitis amurensis* cell cultures (Bulgakov et al., 2002; Kiselev et al., 2007). Resveratrol is a promising bioactive natural molecule with potential applications in phytotherapy and pharmacology (Shankar et al., 2007).

We established two *rolB* transgenic cell cultures of *V. amurensis* with different levels of *rolB* expression and resveratrol production (Kiselev et al., 2007). A high level of *rolB* expression resulted in more than a 100-fold increase in resveratrol production in the transformed culture, compared with both the resveratrol levels registered in control cultures and in other plant cell cultures (Ku et al., 2005; Tassoni et al., 2005). We found that *rolB* transgenic cell cultures of *V. amurensis* cultures also produced low levels of other stilbenes, such as viniferin, ampelopsin and piceatannol (Kiselev et al., 2007). It has been suggested that this significant increase in resveratrol content is a result of *rolB* expression (Kiselev et al., 2007). The present study deals with the mechanism by which resveratrol production is enhanced by *rolB* gene expression in transgenic cell cultures of *V. amurensis*.

The mechanism by which the plant's secondary metabolism is stimulated in *rol*-transformed cell cultures is unknown. A better understanding of this mechanism has both fundamental and applied interest. Not only is there an opportunity to expand our knowledge of general principles of plant secondary metabolism regulation, which could be used to produce biologically active substances by plant cell cultures in an industry setting, it is also possible to obtain new data about the plant oncogenesis process and the mechanisms by which *rolB* influences plant cell metabolism.

At present, little is known about properties and functions of the RolB protein. RolB causes significant morphological and biochemical changes in transgenic plant cells (Spena et al., 1987; Altamura et al., 1994). An important recent finding revealed that RolB has nuclear localization and can interact with protein 14-3-3 and modulate its activity (Moriuchi et al., 2004).

It is known that calcium plays an important role in plant defense reactions, such as phytoalexin biosynthesis (Lecourieux et al., 2006; Ramani and Chelliah, 2007). For example, it has been demonstrated that extracellular calcium availability, calcium influx, and/or intracellular calcium mobilization are necessary for an increase in yield of secondary metabolites (Preisig and Moreau, 1994; Dmitriev et al., 1996; Ramani and Chelliah, 2007). These reports demonstrate that elevated levels of $[Ca^{2+}]_{cyt}$ play an important role in phytoalexin production. Inhibition of Ca^{2+}channels, calcium chelating or blocking of calcium intracellular fluxes, by means of special agents, considerably reduced phytoalexin accumulation. However, contradictory reports have also appeared in the literature, indicating that calcium negatively affected the production of some secondary metabolites (Cacho et al., 1995; Ning et al., 1998; Sanchez-Sampedro et al., 2005).

In the current study, we aimed to investigate whether calcium ion flux and later steps of the calcium-mediated signal transduction pathway play a role in resveratrol biosynthesis in *rolB* transgenic cultures. Therefore, we examined the influence of the calcium channel blockers, $LaCl_3$, verapamil (VER), and niflumic acid (NA), on biomass growth and resveratrol production in control and *rolB* transgenic cell cultures of *V. amurensis*. We also examined calcium-dependent protein kinase (CDPK) gene expression in the cultures. CDPKs are implicated as the major primary sensors of Ca^{2+} flux in plants and play an essential role in plant defense responses (Lecourieux et al., 2006). Treatment of plant cell cultures with the CDPK antagonist W7 or a serine threonine kinase antagonist staurosporine inhibited phytoalexin biosynthesis in *Nicotiana tabacum* and *Catharanthus roseus* cell cultures (Vogeli et al., 1992; Preisig and Moreau, 1994; Ramani and Chelliah, 2007). Cheng et al. (2002) established that CDPKs (specifically, ACPK1) are capable of phosphorylating phenylalanine ammonia-lyase, which is a key enzyme in the production of phytoalexins. Several publications have shown an important regulatory role for CDPKs in the rhizobium-induced nodule formation (Levy et al., 2004; Gargantini et al., 2006). Because the genus *Agrobacterium* belongs to the Rhizobiaceae family, it is likely that *A. rhizogenes* might affect plant cell signaling pathways using CDPK-related mechanisms similar to those that rhizobia utilize. Considering these data, we decided to compare *CDPK* gene expression in the control and *rolB*-expressing cell cultures of *V. amurensis*.

Materials and methods

Chemicals

Reagents for tissue culture were obtained from Sigma (St. Luis, MO, USA). Verapamil (VER), $LaCl_3$,