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The *rolB* and *rolC* genes activate synthesis of anthraquinones in *Rubia cordifolia* cells by mechanism independent of octadecanoid signaling pathway

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Abstract

Several studies have recently revealed an interesting ability of the *rolC* and *rolB* oncogenes of *Agrobacterium rhizogenes* to stimulate secondary metabolite production in plant cells. We have studied the role of the octadecanoid signaling pathway in the signal transduction initiated by the *rol* genes in transformed *Rubia cordifolia* cell cultures. None of the octadecanoid pathway inhibitors tested (diethyldithiocarbamate, propyl gallate, salicylhydroxamic acid and piroxicam) inhibited anthraquinone accumulation in the normal and transformed cultures. This result indicates that the octadecanoid pathway is not involved in the *rolB* and *rolC* gene-mediated increase of anthraquinone production. Summarizing the data of previously reported and present investigations, we suggest that the *rolB* and *rolC* genes act downstream of Ca^{2+} signaling, H_2O_2-generating NADPH oxidase, as well as downstream of salicylic acid-mediated and octadecanoid pathways or perturb secondary metabolism by unknown for plants way. An unusual effect of piroxicam, a member of non-steroidal anti-inflammatory drugs on anthraquinone production was detected: this inhibitor stimulated anthraquinone production in a greater extent then methyl jasmonate.

Keywords: *Rubia cordifolia*; Anthraquinone; Octadecanoid pathway; *rolB* and *rolC* genes; Transformed plant culture

1. Introduction

Agrobacterium rhizogenes causes formation of hairy roots in plants, inserting a part of its plasmid DNA (T-DNA) into the plant genome. Numerous investigations showed that hairy roots, being cultured in vitro, produced high levels of secondary metabolites [1]. Two T-DNA genes, the *rolC* and *rolB* oncogenes, are thought to be causal agents of this phenomenon [2–6]. The single *rolC* and *rolB* genes were able to stimulate secondary metabolite production in transgenic plant tissues, e.g. nicotine production in *Nicotiana tabacum* root cultures, indole alkaloid production in *Catharantus roseus* cultures [2,3], ginsenoside production in cultures of *Panax ginseng* [4], tropane alkaloid production in *Atropa belladonna* hairy root cultures [5] and anthraquinone production in callus cultures of *Rubia cordifolia* [6].

Some data revealed that the *rolC* gene could affect activity of defense proteins, such as 1,3-β-D-glucanases. Expression of the *rolC* gene in ginseng cells caused significant increase of 1,3-β-D-glucanase activity by production of a new enzyme isoform. Activities of other glucanases tested, such as 1,6-, 1,4-β-D-glucanases and 1,4-α-D-glucanase, which are known to be unrelated to defense proteins, were unchanged [7].

Thus, it becomes apparent that the *rol* genes generate effects which resemble defense reactions of plants. Such a function of the *rol* genes would establish an interesting parallel between processes of neoplastic transformation of plant cells and events related to defense responses. Interestingly, plant nuclear protooncogene homologs, such as myb and myc have multiple regulatory functions in metabolic pathways not existing in mammalian cells. They are involved not only in the regulation of developmental processes, such

Abbreviations: AQ, anthraquinone; MeJA, methyl jasmonate; DIECA, diethyldithiocarbamic acid; PG, *n*-propyl gallate; SHAM, salicylhydroxamic acid; SA, salicylic acid; NSAIDs, non-steroidal anti-inflammatory drugs

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as trichome differentiation and seed development, but also in secondary metabolite biosynthesis [8].

Little is known about mechanisms by which the *rol* genes perturb secondary metabolism. Studying the transgenic for the *rolB* and *rolC* genes callus cultures of *R. cordifolia*, we have found that anthraquinone production was greatly increased in both transformed cultures compared with the non-transformed culture [6]. The induction of AQ biosynthesis by *rol* genes did not proceed through the activation of the common Ca^{2+}-dependent NADPH oxidase pathway that mediates signal transduction between an elicitor–receptor complex via transcriptional activation of defense genes [9]. Okadaic acid and cantharidin, inhibitors of protein phosphatases 1 and 2A, caused an increase of AQ production in transgenic cultures. Okadaic acid stimulated AQ accumulation in the non-transformed culture, whereas cantharidin had no effect. These results have shown that different phosphatases are involved in AQ synthesis in normal and transgenic cultures of *R. cordifolia* [9].

Octadecanoid signaling pathway plays a key role in synthesis of phytoalexin-type secondary metabolites during plant-microbe interaction [10–12]. To test whether stimulation of anthraquinone phytoalexins by the *rol* genes is mediated by the octadecanoid pathway, we used a pharmacological approach. Inhibitors of jasmonate biosynthesis are useful for determining a direct involvement of the octadecanoid pathway in response to external stimuli [13]. Several researchers successfully applied inhibitors, such as PG, DIECA and SHAM, to show necessity of the functional octadecanoid pathway in the stimulation of phytoalexins and defense proteins [14,15]. Our results indicate that octadecanoid signaling pathway is not involved in the *rol*-gene-mediated increase of AQ accumulation in the transformed *R. cordifolia* calluses.

2. Materials and methods

2.1. Plant culture

Transgenic cultures Rc-rolC3 and Rc-rolB were established from leaves of a 3-week-old axenic *R. cordifolia* plant [6]. Plasmid vectors pPCV002-CaMVBT and pPCV002-CaMVC [16] were used to obtain the transformed callus cultures. The non-transformed Rc callus culture was established from the leaves of the same plant and cultivated in the identical conditions as the transformed cultures. All cultures were cultivated in 100 ml Erlenmeyer flasks on $W_{B/A}$ solid medium [17], supplemented with 0.5 mg l^{-1} 6-benzylaminopurine and 2.0 mg l^{-1} α-naphthaleneacetic acid, in the dark, at 25 °C with 30-day subculture intervals.

2.2. Inhibitor treatments

Reagents for tissue culture and MeJA were obtained from Sigma, the others were from ICN Pharmaceuticals. MeJA was dissolved in dimethyl sulfoxide, PG (*n*-propyl gallate) and SHAM (salicylhydroxamic acid) were dissolved in ethanol. DIECA (diethyldithiocarbamic acid, trihydrate, sodium salt) was used as an aqueous solution. The NSAID piroxicam (4-hydroxy-2-methyl-3-[pyrid-2-yl-carbamoyl]-2H-1,2-benzothiazine 1,1-dioxide) was solubilized in hot ethanol, cooled and added to the medium. Sterile solutions of the inhibitors were added to the autoclaved media aseptically in desired concentrations. Equal volumes of the appropriate solvents were added to the control flasks.

2.3. Estimation of the anthraquinone content

Purpurin and munjistin were previously isolated from *R. cordifolia* calluses and analysed as described by Mischenko et al. [17]. Anthraquinone content was determined in 30-days cultures photometrically [17]. Briefly, 100 mg of the dried powdered calluses with 0.3 ml 5 N HCl added were extracted with 3 ml ethanol at room temperature and absorption spectra were recorded. The purpurin content of the ethanol extract was determined by absorption at 515 nm. The content of munjistin was quantified by absorption at 421 nm, subtracting the absorption due to purpurin at this wavelength. All cultures produced only two major AQs, with munjistin and purpurin representing 90% of the total AQ yield [6].

3. Results

3.1. Stimulatory effect of MeJA on AQ production

Anthraquinone content was reported to be 0.2% DW for *R. cordifolia* wild-growing roots and 0.62–1.22% DW for non-transformed callus cultures, derived from different organs of *R. cordifolia* [17]. The AQ levels in Rc culture ranged from 0.4 to 1.2% DW, whereas those of the Rc-rolC3 and Rc-rolB cultures was 1.4–2.3 and 1.9–3.2% DW, respectively [9]. The highest level of biosynthetic ability of *R. cordifolia* calluses, which we detected in different elicitor experiments, was 5.0–5.2% DW [7,9].

We studied effect of MeJA on the *rolB* calluses for ten month. Within this period the Rc-rolB culture accumulated 1.53–4.47% of AQs. Fig. 1 shows a relationship between initial content of AQs in MeJA-treated *rolB* calluses and stimulatory effect of MeJA. High negative correlation dependence ($r = -0.97$) was found between these parameters: the more initial level of AQs, the less stimulatory effect of MeJA. Thus, when the *rolB* culture produced some ‘saturating’ level of AQs, MeJa no longer activated AQ synthesis. Such dependence was not found for the Rc and Rc-rolC cultures ($r = -0.09$ and -0.37, respectively), probably because they produced less AQs.

Table 1
Growth of *R. cordifolia* cultures in the presence of octadecanoid pathway inhibitors

Inhibitor	Callus culture		
	Rc	Rc-rolC3	Rc-rolB
DIECA (1 mM)	56 ± 5	57 ± 15	70 ± 9
PG (1 mM)	32 ± 6	55 ± 5*	70 ± 8*
SHAM (100 μM)	25 ± 1.5	57 ± 9*	61 ± 6*
Piroxicam (100 μM)	15 ± 2	47 ± 4*	48 ± 8*

The values are represented as percentage from values of the cultures growing on inhibitor-free medium. Values are means ± S.E. from two experiments with 10 replicates each.

* $P < 0.05$ vs. Rc culture, Student's *t*-test.

3.2. Effect of DIECA, PG and SHAM on anthraquinone production

To explore the involvement of the octadecanoid pathway in the *rol*-gene-mediated increase of AQ accumulation in *R. cordifolia* cultures, we tested different inhibitors of this pathway. We used those concentrations of inhibitors that suppressed growth of the *R. cordifolia* cultures approximately half-maximally (Table 1). Table 1 shows that both transgenic cultures were more resistant to PG, SHAM and piroxicam than the normal culture.

In plants, DIECA inhibits the octadecanoid signaling pathway downstream of lipoxygenase by shunting 13-hydroperoxylinolenic acid to 13-hydroxylinolenic acid, thereby reducing the precursor pool leading to cyclization and eventual synthesis of jasmonic acid [18]. By suppressing of the octadecanoid pathway, DIECA inhibited ajmalicine synthesis [14] and elicitor-induced terpenoid indole alkaloid biosynthetic gene expression in *C. roseus* [13].

In our experiments, DIECA failed to inhibit AQ synthesis in non-transformed and both transformed cultures (Fig. 2A). When added to the culture media simultaneously with MeJA, DIECA did not prevent stimulatory effect of MeJA (Fig. 2A). Moreother, AQ accumulation was enhanced in the Rc, Rc-rolC and Rc-rolB cultures on 36, 68 and 35%, respectively, under this treatment.

The similar paradoxical effect of DIECA on phytoalexin production was firstly found and explained by Jabs et al. [19]. These authors have shown that DIECA treatment of parsley cells resulted in a steady O_2^- accumulation up to 70% of the oxidative burst maximum, caused by elicitors. Addition of DIECA to the parsley cells in the absence of elicitor prevented rapid degradation of O_2^- and thus stimulated accumulation of furanocoumarin phytoalexins to 18 ± 4% of the elicitor response [19].

PG is often used as an inhibitor of lipoxygenases [20,21]. PG inhibited expression of those genes, which activity is regulated via the octadecanoid pathway, e.g. the thionin gene [15] and acetyl-CoA carboxylase [22]. PG repressed ajmalicine synthesis in *C. roseus* cells [14].

The patterns of AQ inhibition in Rc and Rc-rolC *R. cordifolia* cultures by PG were similar to those observed in the experiment with DIECA (Fig. 2B). Interestingly, the Rc-rolB culture, which in this experiment accumulated high level of AQs (5.3% DW) without any treatment, was not susceptible to PG and MeJA treatments (Fig. 2B). However, when both effectors were applied simultaneously, the rise of AQ production was detected (Fig. 2B). It is noteworthy that the *rolB* culture has accumulated in this experiment 34-fold more AQs (6.8% DW) than wild-growing roots.

PG limited the availability of Fe^{2+} and prevented the generation of hydroxyl radicals thus protecting cells from H_2O_2-induced damage [23]. However, PG induced single strand breaks in DNA at concentrations higher than 0.25 μM, changing the antioxidative and cytoprotective properties to prooxidative and cytotoxic properties, when

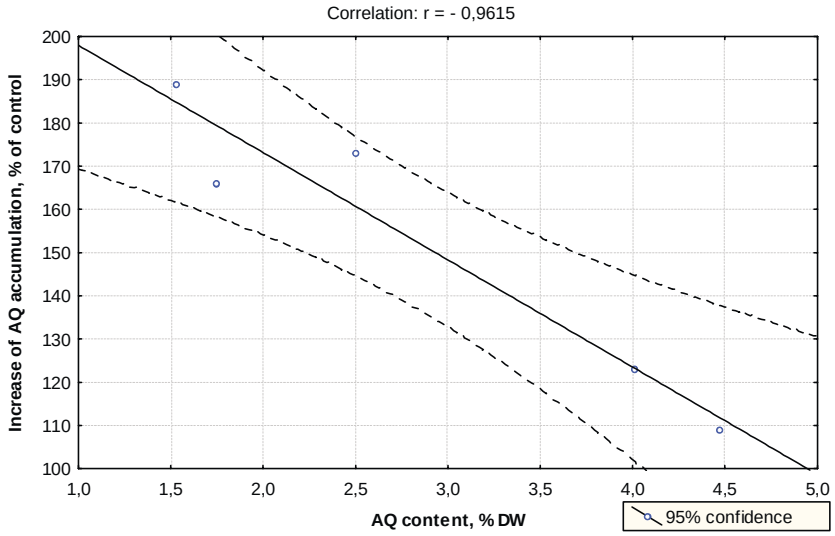

Fig. 1. Effect of MeJA (10 μM) on AQ accumulation in Rc-rolB calluses in dependence of background AQ levels. Cultures were grown for 4 weeks on $W_{B/A}$ medium.

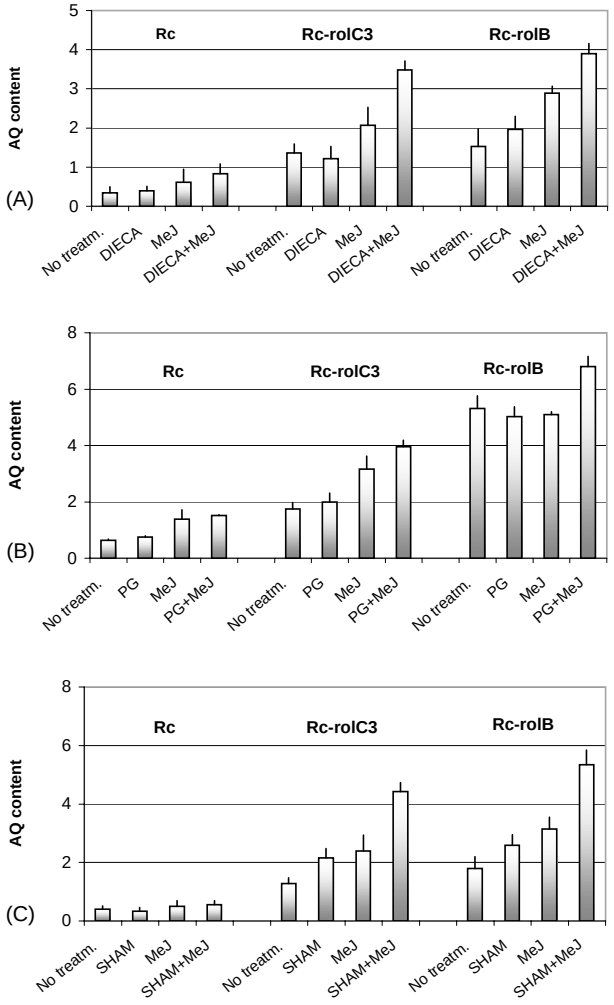

Fig. 2. Effect of DIECA (A), PG (B) and SHAM (C) on AQ content (% DW) in *R. cordifolia* non-transformed (Rc) and transformed (Rc-rolC3 and Rc-rolB) callus cultures. Values are means from two experiments with ten replicates each. Vertical bars represent standard errors.

it was combined with copper [24]. Copper sulfate is a component of the $W_{B/A}$ medium, which we use for cultivation of *R. cordifolia* calluses. In a separate experiment, we cultivated Rc, Rc-rolB and Rc-rolC calluses on the media lacking $CuSO_4$. The elimination of copper from the medium did not change growth and biosynthetic properties of all cultures tested (data not shown), suggesting that possible prooxidative effect of PG/Cu^{2+} combination could be excluded from our further considerations.

PG and SHAM are used as lipoxygenase inhibitors possessing very similar pharmacological activity [20]. SHAM like PG did not inhibited AQ production either in non-transgenic or transgenic *R. cordifolia* cultures (Fig. 2C). The synergistic effect of PG/MeJA on AQ accumulation was confirmed in the experiment with SHAM. When grown in the presence of SHAM and MeJA, the Rc, Rc-rolC and Rc-rolB cultures accumulated 11, 84 and 70% more anthraquinones, respectively, than the same cultures grown with MeJA (Fig. 2C). One can speculate that PG and SHAM changed their antioxidative properties to prooxidative ones, being combined with MeJa in our test system. It could lead to the generation of reactive oxygen intermediates, which in concert with MeJA activated AQ production.

3.3. Strong induction of AQs by piroxicam

Piroxicam, a member of non-steroidal anti-inflammatory drugs (NSAIDs), effectively inhibited lipoxygenase activity in extracts from elicited tomato leaves [25]. Piroxicam not only did not inhibited AQ accumulation in the RC, Rc-rolC and Rc-rolB cultures, but caused 2.6-fold, 2.1-fold and 1.6-fold increase of AQs in these cultures, respectively (Fig. 3). The stimulatory effect of piroxicam on AQ production in *R. cordifolia* cultures was even more pronounced than that of MeJA (Fig. 3).

To our knowledge, the effect of piroxicam on production of secondary metabolites was first examined in this

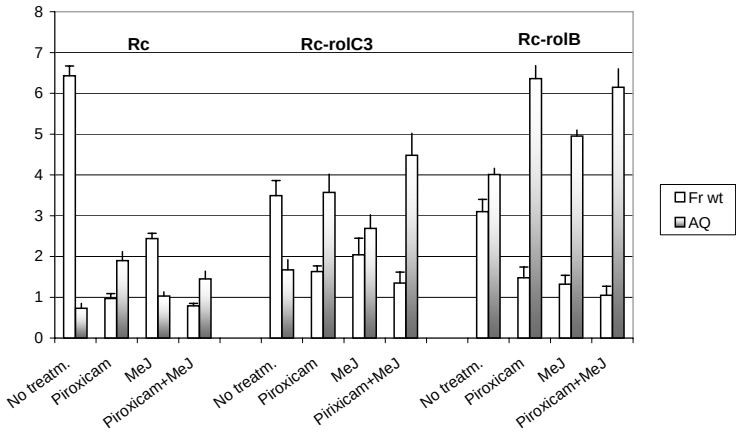

Fig. 3. Effect of piroxicam on fresh biomass accumulation (g) after 4 weeks of culture and AQ content (% DW) in *R. cordifolia* non-transformed (Rc) and transformed (Rc-rolC3 and Rc-rolB) callus cultures. Values are means from two experiments with ten replicates each. Vertical bars represent standard errors.

study. It is intriguing that piroxicam caused the strong induction of AQs. In previous years, most investigations with NSAIDs were carried out on animals, because NSAIDs possess a strong inhibitor activity against mammalian cyclooxygenases and lipoxygenases and exert preventive effect against carcinogenesis [26,27]. In human carcinoma cells, NSAIDs stimulated the three families of MAPK, extracellular regulated kinases, c-Jun N-terminal kinases and p38 MAPK [26]. It is unknown whether or not similar mechanism operates in plant cells. Protein kinases ultimately change the activity of plant transcription factors that regulate the expression of defense genes through the recognition of specific sequences in the promoter region (e.g. [13]). Downstream of the octadecanoid pathway, one or more protein kinases are involved in transducing the jasmonate signal [13]. When piroxicam was used simultaneously with MeJA, no additive effect on AQ accumulation was observed (Fig. 3), suggesting that MeJA and piroxicam might compete for the same activator site.

4. Discussion

Because synthesis of phytoalexin-type secondary metabolites is a part of the defense reaction of plants, the question arises whether or not *rol*-gene signals interfere with general plant defense pathways. It is now generally accepted, that during plant-microbe interaction, the signal is amplified via increased intracellular Ca^{2+} due to the opening of Ca^{2+} channels [28]. The increase of intracellular Ca^{2+} concentration activates a Ca^{2+} dependent protein kinase(s) which, in turn, activates NADPH oxidase [28,29]. The involvement of the oxidative burst generated by NADPH oxidase in the process of phytoalexin stimulation is well known [19,30].

The octadecanoid pathway intermediates, including jasmonic acid and methyl jasmonate, also play a crucial role in activation of synthesis of phytoalexin-type secondary metabolites derived from different biosynthetic pathways [10–12]. This is a general rule, although some data revealed existence of jasmonate-independent signal transduction pathways, leading to activation of secondary metabolites [31,32]. SA- and ethylene-dependent pathways, in concert with MeJA or independently, could also stimulate phytoalexin production in affected by pathogens plants [33].

According to previously obtained results, ethylene had no effect on AQ production in the normal and transgenic *R. cordifolia* cultures [6]. SA increased AQ accumulation in the transgenic and non-transgenic calluses with very similar dynamics, indicating that the *rol*-gene-activator signals functioned independently from the SA signaling pathway [6]. Ca^{2+} influx is not necessary for activator function of the *rol* genes. Likewise, the NADPH oxidase signaling pathway is not involved in activator effect of these genes [9]. Therefore, if the *rol* genes activated secondary metabolism via octadecanoid pathway, it would establish a link between the *rol*-gene effects and known defense pathways.

However, AQ production was not decreased by all inhibitors of octadecanoid pathway, which was used in the present study (DIECA, PG, SHAM and piroxicam), either in non-transformed or transformed with the *rolC* and *rolB* genes calluses of *R. cordifolia* (Figs. 2 and 3). Based on these results we concluded that (i) the production of AQs in non-transformed cells of *R. cordifolia* does not proceed through the activation of the octadecanoid pathway and (ii) the octadecanoid pathway is not involved in the *rolB* and *rolC* gene-mediated increase of anthraquinone biosynthesis.

Several authors noted that the results obtained with inhibitors of octadecanoid pathway must be interpreted with caution, because most of them possessed additional activities, seemingly unrelated to their possibility to inhibit the octadecanoid pathway [13,14]. Therefore, these results should be considered as preliminary results and additional evidences supporting the inhibitory experiments should be obtained.

We cannot exclude the possibility that, in natural conditions, both SA- and jasmonate-dependent pathways play a role in phytoalexin synthesis during the interaction of pathogenic microorganisms with *R. cordifolia* plants. However, it seems unlikely that these effectors are involved in the increase of AQ production by the *rol* genes.

Summarizing the data of previously reported and present investigations, we suggest two scenarios of the *rolB* and *rolC* genes action on secondary metabolism. According to the first one, they act via known defense mechanisms downstream of the Ca^{2+} signaling, H_2O_2-generating NADPH oxidase complex, as well as downstream of SA- and jasmonate-mediated pathways. According to the second scenario, they perturb secondary metabolism by an unusual way, which is not ubiquitous for plants.

Although processes of ROS generation by mammalian oncogenes, as well as the role of oxidative stress in many aspects of oncology are a subject of unfailing interest of numerous investigations [34,35], such a theme for plant oncogenes has never been considered. Vafa et al. [36] recently demonstrated that in normal human fibroblasts, c-Myc activation could induce DNA damage that correlated with induction of reactive oxygen species. The authors proposed that the oncogene activation could override damage controls, thereby accelerating tumor progression via genetic instability. It is unknown whether the *rol* genes act similarly, but such a possibility cannot be excluded. Studying the effect of the *rolC* gene on ginseng cells, we have found that the gene caused chromosome instability in primary *rolC*-tumors and derived from them teratoma tissues [37]. It will be interesting to determine for future experiments whether the *rol* genes change ROS generation in plant cells. Because the NADPH oxidase signaling pathway thought to be is not involved in the *rolB* and *C* gene-mediated activation of AQ production [9], other known pathways of ROS generation in *rol*-gene-transformed cells would be elucidated, such as ROS generation by cell wall-bound peroxidases and ROS generation by apoplastic amine oxidase-type enzymes [38].

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References

[1] A. Giri, M.L. Narasu, Transgenic hairy roots: recent trends and applications, Biotechnol. Adv. 18 (2000) 1–22.
[2] J. Palazón, R.M. Cusidó, C. Roig, M.T. Piñol, Expression of the *rolC* gene and nicotine production in transgenic roots and their regenerated plants, Plant Cell Rep. 17 (1998) 384–390.
[3] J. Palazón, R.M. Cusidó, J. Gonzalo, M. Bonfill, S. Morales, M.T. Piñol, Relation between the amount the *rolC* gene product and indole alkaloid accumulation in *Catharantus roseus* transformed root cultures, J. Plant Physiol. 153 (1998) 712–718.
[4] V.P. Bulgakov, M.V. Khodakovskaya, N.V. Labetskaya, G.K. Tchernoded, Y.N. Zhuravlev, The impact of plant *rolC* oncogene on ginsenoside production by ginseng hairy root cultures, Phytochemistry 49 (1998) 1929–1934.
[5] V. Bonhomme, D. Laurain Mattar, M.A. Fliniaux, Effects of the *rolC* gene on hairy root: Induction development and tropane alkaloid production by *Atropa belladonna*, J. Nat. Prod. 63 (2000) 1249–1252.
[6] V.P. Bulgakov, G.K. Tchernoded, N.P. Mischenko, M.V. Khodakovskaya, V.P. Glazunov, E.V. Zvereva, S.A. Fedoreyev, Y.N. Zhuravlev, Effect of salicylic acid, methyl jasmonate, ethephon and cantharidin on anthraquinone production by *Rubia cordifolia* callus cultures transformed with the *rolB* and *rolC* genes, J. Biotechnol. 97 (2002) 213–221.
[7] V.P. Bulgakov, M. Kusaykin, G.K. Tchernoded, T.N. Zvyagintseva, Y.N. Zhuravlev, Carbohydrase activities of the *rolC*-gene transformed and non-transformed ginseng cultures, Fitoterapia 73 (2002) 638–643.
[8] A. Loidl, P. Loidl, Oncogene-and tumor-suppressor gene-related proteins in plants and fungi, Crit. Rev. Oncog. 7 (1996) 49–64.
[9] V.P. Bulgakov, G.K. Tchernoded, N.P. Mischenko, Y.N. Shkryl, V.P. Glazunov, S.A. Fedoreyev, Y.N. Zhuravlev, Effects of Ca^{2+} channel blockers and protein kinase/phosphatase inhibitors on growth and anthraquinone production in *Rubia cordifolia* cultures transformed by the *rolB* and *rolC* genes, Planta 217 (2003) 349–355.
[10] H. Gundlach, M.J. Müller, T.M. Kutchan, M.H. Zenk, Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 2389–2393.
[11] M.J. Mueller, W. Brodschelm, E. Spannagl, M.H. Zenk, Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 7490–7494.
[12] S. Blechert, W. Brodschelm, S. Hölder, L. Kammerer, T.M. Kutchan, M.J. Mueller, Z-Q. Xia, M.H. Zenk, The octadecanoic pathway: signal molecules for the regulation of secondary metabolism, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 4099–4105.
[13] F.L.H. Menke, S. Parchmann, M.J. Mueller, J.W. Kijne, J. Memelink, Involvement of the octadecanoid pathway and protein phosphorylation in fungal elicitor-induced expression of terpenoid indole alkaloid biosynthetic genes in *Catharanthus roseus*, Plant Physiol. 119 (1999) 1289–1296.
[14] P. Gantet, N. Imbault, M. Thiersault, P. Doireau, Necessity of a functional octadecanoic pathway for indole alkaloid synthesis by *Catharanthus roseus* cell suspension cultured in an auxin-starved medium, Plant Cell Physiol. 39 (1998) 220–225.
[15] H. Bohlmann, A. Vignutelli, B. Hilpert, O. Miersch, C. Wasternack, K. Apel, Wounding and chemicals induce expression of the *Arabidopsis thaliana* gene *Thi2.1*, encoding a fungal defense thionin, via the octadecanoid pathway, FEBS Lett. 437 (1998) 281–286.
[16] A. Spena, T. Schmülling, C. Koncz, J.S. Schell, Independent and synergistic activity of *rolA*, *B* and *C* loci in stimulating abnormal growth in plants, EMBO J. 6 (1987) 3891–3899.
[17] N.P. Mischenko, S.A. Fedoreyev, V.P. Glazunov, G.K. Tchernoded, V.P. Bulgakov, Y.N. Zhuravlev, Anthraquinone production by callus cultures of *Rubia cordifolia*, Fitoterapia 70 (1999) 552–557.
[18] E.E. Farmer, D. Caldelari, G. Pearce, M.K. Walker-Simmons, C.A. Ryan, Diethyldithiocarbamic acid inhibits the octadecanoid signaling pathway for the wound induction of proteinase inhibitors in tomato leaves, Plant Physiol. 106 (1994) 337–342.
[19] T. Jabs, M. Tschöpe, C. Colling, K. Hahlbrock, D. Scheel, Elicitor-stimulated ion fluxes and O_2^- from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 4800–4805.
[20] A. Vianello, E. Braidot, G. Bassi, F. Macri, Lipoxygenase activity on the plasmalemma of sunflower protoplasts and its modulation, Biochim. Biophys. Acta 1225 (1995) 57–62.
[21] S. Fornaroli, E. Petrussa, E. Braidot, A. Vianello, F. Macri, Purification of a plasma membrane-bound lipoxygenase from soybean cotyledons, Plant Sci. 145 (1999) 1–10.
[22] B. Garcia-Ponce, M. Rocha-Sosa, The octadecanoid pathway is required for pathogen-induced multi-functional acetyl-CoA carboxylase accumulation in common bean (*Phaseolis vulgaris* L.), Plant Sci. 157 (2000) 181–190.
[23] J.R. Reddan, F.J. Giblin, R. Kadry, V.R. Leverenz, J.T. Pena, D.C. Dziedzic, Protection from oxidative insult in glutathione depleted lens epithelial cells, Exp. Eye Res. 68 (1999) 117–127.
[24] H. Jacobi, B. Eicke, I. Witte, DNA strand break induction and enhanced cytotoxicity of propyl gallate in the presence of copper, Free Radical Biol. Med. 24 (1998) 972–978.
[25] T.L. Peever, V.J. Higgins, Electrolyte leakage, lipoxygenase, and lipid peroxidation induced in tomato leaf tissue by specific and non-specific elicitors from *Cladosporium fulvum*, Plant Physiol. 90 (1989) 867–875.
[26] A.M. Lennon, M. Ramauge, M. Pierre, Role of redox status on the activation of mitogen-activated protein kinase cascades by NSAIDs, Biochem. Pharmacol. 63 (2002) 163–170.
[27] G. Liu, W.Y. Ma, A.M. Bode, Y. Zhang, Z. Dong, NS-398 and piroxicam suppress UVB-induced activator protein activity by mechanisms independent of cyclooxygenese-2, J. Biol. Chem. 278 (2003) 2124–2130.
[28] T. Xing, V.J. Higgins, E. Blumwald, Race-specific elicitors of *Cladosporium fulvum* promote translocation of cytosolic components of NADPH oxidase to the plasma membrane of tomato cells, Plant Cell 9 (1997) 249–259.
[29] T. Romeis, P. Piedras, J.D.G. Jones, Resistance gene-dependent activation of a calcium-dependent protein kinase in the plant defense response, Plant Cell 12 (2000) 803–815.
[30] Z.-J. Guo, C. Lamb, R.A. Dixon, Potentiation of the oxidative burst and isoflavonoid phytoalexin accumulation by serine protease inhibitors, Plant Physiol. 118 (1998) 1487–1494.
[31] D. Choi, R.M. Bostok, S. Avdiushko, D.F. Hildebrand, Lipid-derived signals that discriminate wound-and pathogen-responsive isoprenoid pathways in plants: methyl jasmonate and the fungal elicitor arachidonic acid induce different 3-hydroxy-3-methylglutaryl-coensime A reductase genes and antimicrobial isoprenoids in *Solanum tuberosum* L., Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 2329–2333.

[32] M. Rickauer, W. Brodschelm, A. Bottin, C. Véronési, H. Grimal, M.T. Esquerré-Tugayé, The jasmonate pathway is involved differentially in the regulation of different defence responses in tobacco cells, Planta 202 (1997) 155–162.
[33] C. Lamb, R.A. Dixon, The oxidative burst in plant disease resistance, Annu. Rev. Plant Physiol. 48 (1997) 251–275.
[34] P. Kovacic, J.D. Jacintho, Mechanism of carcinogenesis: focus on oxidative stress and electron transfer, Curr. Med. Chem. 8 (2001) 773–796.
[35] J.Q. Yang, G.R. Buettner, F.E. Domann, Q. Li, J.F. Engelhardt, C.D. Weydert, L.W. Oberley, v-Ha-ras mitogenic signaling through superoxide and derived reactive oxygen species, Mol. Carcinog. 33 (2002) 206–218.
[36] O. Vafa, M. Wade, S. Kern, M. Beeche, T.K. Pandita, G.M. Hampton, G.M. Wahl, c-Myc can induce DNA damage, increase reactive oxygen species, Mol. Cell 9 (2002) 1031–1044.
[37] V.P. Bulgakov, L.S. Lauve, G.K. Tchernoded, M.V. Khodakovskaya, Yu.N. Zhuravlev, Chromosome variation in ginseng cells transformed with the *rolC* plant oncogene, Russian J. Genet. 36 (2000) 150–156.
[38] J.J. Grant, B.-W. Yun, G.J. Loake, Oxidative burst and cognate redox signaling reported by luciferase imaging: identification of a signal network that functions independently of ethylene, SA and Me-JA but is dependent on MAPKK activity, Plant J. 24 (2000) 369–582.