EFFECT OF METHYL JASMONATE ON THE PRODUCTION OF SESQUITERPENE LACTONES IN THE HAIRY ROOT CULTURE OF *LACTUCA VIROSA* L.

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ABSTRACT

Hairy roots of *Lactuca virosa* L. transformed with *Agrobacterium rhizogenes* and treated with methyl jasmonate, are capable of producing sesquiterpene lactones – crepidiaside B and lactuside A in amounts higher than in untreated roots. An HPLC analysis showed the highest content of crepidiaside B after 24 hours of methyl jasmonate treatment (60% above the control). The increase in the content of lactuside A was relatively small and reached 27% after 48 hours of the treatment, in comparison with the control.

KEY WORDS: *Lactuca virosa* L., Asteraceae, hairy root culture, *Agrobacterium rhizogenes*, methyl jasmonate, sesquiterpene lactones, lactuside A, crepidiaside B.

INTRODUCTION

Jasmonic acid (JA) and methyl jasmonate (MeJA) are plant hormone-like compounds showing activities similar to those of abscisic acid (Sembdaner et al. 1993). Gundlach et al. (1992) have proved that these compounds play an essential role as transducers of signals in the defense system of plants activated upon a pathogen and herbivore attack. The signal transduction process leads to defence gene activation and, ultimately, results in the production both of high and low molecular weight defense compounds (Rickauer et al. 1992; Gundlach et al. 1992).

The synthesis of defense secondary metabolites in plant cell cultures induced by elicitors, e.g. fungal cell wall fragments, can be correlated with an increase in JA and MeJA levels. Exogenously supplied MeJA is also capable of inducing production of these compounds, as elicitors do (Ignatov et al. 1996; Mizukami et al. 1993). The experiments of Parchmann et al. (1997) showed that 12-oxo-phytodienoic acid (12-oxo-PDA), a JA precursor, often shows a considerably higher biological activity than that of JA. Both compounds control the same biosynthetic pathways and their secondary metabolite inducing activity was confirmed in many different plant cell culture species (Gundlach and Zenk 1998).

Sesquiterpene lactones are constitutively accumulated secondary metabolites in plants of the Asteraceae. The compounds are considered to play a natural role in the defense of the plants against pathogens and also against herbivores because of their antifeedant activity (Rees and Harborne 1985; Grayer and Harborne 1994). We have studied an effect of exogenous MeJA on the production of some sesquiterpene lactones in a hairy root culture of *Lactuca virosa* L., trans-

formed with *Agrobacterium rhizogenes* LBA 9402. The culture was established in our laboratory and was found to possess the ability to produce sesquiterpene lactones, e.g. crepidiaside B and lactuside A (Fig. 1), characteristic for the roots of the intact plant collected in the first year of its vegetative period (Kisiel et al. 1995; Kisiel and Barszcz 1997).

MATERIALS AND METHODS

Plant material

Seeds of *Lactuca virosa* L., obtained from the Botanical Garden in Oberholz (Germany) were sterilized for 30 s in 70% ethanol and then in 15% solution of Dimanin C (Bayer). The sterilized seeds were rinsed four times with sterile water and afterwards inoculated on a modified MS agar medium (Murashige and Skoog, 1962) without hormones, containing one-fourth of micro- and macroelements. Germination was conducted at 25°C and under continuous illumination (40 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Hairy roots of *L. virosa*, transformed with *Agrobacterium rhizogenes* LBA 9402, were induced on leaf explants from sterile grown seedlings and cultivated in a hormone-free MS liquid medium containing macroelements reduced to half concentration and 3% sucrose, on a gyrotory shaker (110 r.p.m.) in the dark.

Addition of methyl jasmonate

In the course of the experiment, MeJA (Serva) was dissolved in ethanol and added (on day 6 after the last inoculation) to the hormone-free MS liquid medium mentioned above (100 μM MeJA/l of the medium). The medium without MeJA was used as control.

For phytochemical analysis, the transformed roots were harvested at 24, 48 and 72 hours after MeJA addition.

Phytochemical analysis

Lyophilized hairy roots (0.25 g) were extracted twice with 12.5 ml of ethanol at room temperature. The extracts were filtered and concentrated *in vacuo*. The residue was dissolved in 1 ml of methanol and centrifuged (20 000 r.p.m., 4°C, 5 min). The supernatant (5 µl) was injected into the Waters HPLC system. Analysis was performed on a µ-Bondapak C₁₈, 21 mm x 300 mm column (Waters, No 86609), coupled with a UV photodiode array detector, using methanol-water (35: 65) mixture as mobile phase at 0.5 ml/min flow rate. Each extraction was repeated three times, and each sample was injected three times.

Crepidiaside B and lactuside A (Fig. 1), previously isolated in our laboratory from *Lactuca* species (Kisiel et al. 1995), were used as authentic samples for co-chromatography. UV absorption maxima of crepidiaside B and lactuside A were 254 nm, Rt=9.6 min, and 235 nm, Rt=5.8 min, respectively.

Fig. 1. Chemical structures of the analysed sesquiterpene lactones (Glc=β-D-glucopyranosyl).

RESULTS AND DISCUSSION

Crepidiaside B and lactuside A (Fig. 1) are constitutively accumulated sesquiterpene lactone glycosides in roots of *Lactuca virosa* L. We have studied the influence of exogenous MeJA on the contents of the two compounds in hairy root culture of the plant. The hairy roots were harvested 24, 48 and 72 hours after addition of 100 µM MeJA to the culture medium. As shown in Fig. 2, 24 hours after the treatment with MeJA the crepidiaside B content in extracts from the hairy roots was 60% higher than that of control. During the following 48 hours its level decreased gradually to the control level. In the case of lactuside A, the increase in the content was slower and not so high. The amounts of the compound after 24 and 48 hours were ca. 21% and 27% higher than

Fig 2. Influence of methyl jasmonate on sesquiterpene lactone biosynthesis in hairy root culture of *Lactuca virosa* L., calculated as mg% of dry weight.

those in controls, respectively, and after 72 hours decreased back to the control value.

Biosynthetic studies of lettuce guaianolides (Song et al. 1995) supported a previously proposed biogenesis for sesquiterpene lactones *via* the acetate-mevalonate-germacradiene pathway. The mevalonate synthesis is catalysed by 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR; E.C.1.1.1.34), a key enzyme in the pathway. Maldonado-Mendoza et al. (1994) reported on the induction of some HMGR isogene expression 24 hours after MeJA addition to *Catharanthus roseus* and *Camptotheca acuminata* suspension cultures, producing terpenoid indole and quinoline alkaloids, respectively.

Recently, it has been apparent, that exogenous MeJA can not only induce the *de novo* synthesis of antimicrobial secondary metabolites (phytoalexins), but also stimulate the synthesis of constitutively accumulated defense compounds. It has been reported that treatment with MeJA can enhance the accumulation of alkaloids in a range of plant species (Zabekakis et al. 1999). In *Catharanthus roseus* hairy root culture, for example, concentrations of different alkaloids increased by 60-500% after MeJA treatment (Rijhwani and Shanks 1998). It has also been established that exogenous MeJA stimulates accumulation of phenolic compounds in several plant species (Mizukami et al. 1993; Lee et al. 1997; Miksch and Boland 1996; Szabo et al. 1999). In the latter case, suspension culture of *Coleus blumei* enhanced accumulation of rosmarinic acid threefold. Highest concentration of the compound (3.3% of the cell dry weight) were measured 56 hours after MeJA addition.

The result of our study indicate that exogenous MeJA can also increase the constitutive concentrations of sesquiterpene lactones. This suggests that the compounds are involved in defence response.

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WPLYW JASMONIANU METYLU NA BIOSYNTEZĘ LAKTONÓW SESKWITERPENOWYCH W HODOWLI KORZENI WŁOŚNIKOWATYCH *LACTUCA VIROSA* L.

STRESZCZENIE

Korzenie *Lactuca virosa* L., transformowane *Agrobacterium rhizogenes* i traktowane jasmonianem metylu, syntetyzują laktony seskwiterpenowe – krepidiazyd B i laktuzyd A w ilościach większych niż korzenie nie poddawane wpływowi jasmonian metylu. Analiza HPLC wykazała najwyższy wzrost zawartości krepidiazylu B (60% w porównaniu z kontrolą) po 24 godzinach traktowania jasmonianem metylu. Wzrost zawartości laktuzylu A następował wolniej, osiągając maksimum (27% w porównaniu z kontrolą) po 48 godzinach.

SŁOWA KLUCZOWE: *Lactuca virosa* L., Asteraceae, korzenie włośnikowate, *Agrobacterium rhizogenes*, jasmonian metylu, laktony seskwiterpenowe, laktuzyd A, krepidiazyd B.