The effect of methyl jasmonate on production of antioxidant compounds in shoot cultures of *Salvia officinalis* L.

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Summary

The shoots of *Salvia officinalis* growing in MS liquid medium supplemented with IAA 0.1 mg l^{-1}) and BAP (0.45 mg l^{-1}) were treated with methyl jasmonate (MeJA) to increase production of compounds with antioxidant activity (carnosic acid, carnosol and rosmarinic acid). The increase in metabolite production depended on MeJA concentration, the period of exposure to elicitor and type of compound. The MeJA action was observed 24 h after elicitation. It was found that the maximum level of diterpenoids, calculated as the sum of CA and Car (about 8 mg g^{-1} dry wt) was achieved at 3 days after elicitation with 20 μM methyl jasmonate. The highest amount of rosmarinic acid (about 41 mg g^{-1} dry wt) was achieved with 50 or 100 μM methyl jasmonate on the 5th day after elicitation. It was almost 2-fold higher compared to the control (cultures treated with only ethanol).

Key words: *diterpenoids, methyl jasmonate, rosmarinic acid, Salvia officinalis L.*

INTRODUCTION

Salvia officinalis L. has been used in cosmetology, food industry and medicine. Many European pharmacopoeias have listed sage leaves. They have anti-inflammatory, antioxidant, antibacterial, antiviral and antihydrotic activity [1-4]. Sage biological activity is a result of the presence of essential oil, di- and triterpenes, phenolic acids, flavonoids and tannins. Among them rosmarinic acid (RA) and two diterpenoids: carnosic acid (CA) and carnosol (Car) have been identified as the most active compounds with antioxidant properties. These natural antioxidants

reduce damage from cellular components, such as lipids, proteins and DNA as well as help to prevent mutagenesis, carcinogenesis and aging due to their radical scavenging activities [3]. The antioxidative effect of rosmarinic acid measuring the radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (EC_{50}=2.7 μg/ml) was comparable to that of ascorbic acid [5]. It has been reported that carnosic acid and carnosol inhibit cytochrome P450 activation of carcinogenesis in human cells *in vitro* [6] and enhance the activity of conjugating enzymes involved in carcinogen detoxification pathways *in vivo* [7]. These diterpenoids were also shown to inhibit superoxide anion production in the xanthone oxidase system [3]. According to Richheimer et al. [8] their radical scavenging activity follows a mechanism analogous to α-tocopherol.

Our previous reports described the production of antioxidant compounds, i.e. carnosic acid, carnosol and rosmarinic acid in the various *in vitro* cultures of *S. officinalis* and as well as in shoots and roots of *in vitro* regenerated plants [9]. Elicitation is a widely known method which aims to enhance the production of secondary metabolites and has been described for many plant species [10-14]. The stimulus is received by receptors which then result in the activation of secondary messengers. These transmit the signals into the cell leading to gene expression and biochemical changes.

In this work the influence of methyl jasmonate (MeJA) on the biosynthesis of antioxidant compounds in sage shoot culture is being reported. The MeJA signaling pathway includes a wide variety of plant secondary products such as terpenoids, flavonoids, alkaloids and phenylopropanoids [10-14]. Hitherto, the effect of MeJA on diterpenoids and rosmarinic acid accumulation in sage shoot culture has not been studied.

MATERIAL AND METHODS

Plant material

Experiments were carried out with *S. officinalis* liquid shoot culture. The shoots were cultured in Magenta vessels (77x77x97 mm) containing 25 ml of liquid MS [15] medium supplemented with IAA (0.1 mg l^{-1}) and BAP (0.45 mg l^{-1}) as described previously [16].

Treatment with MeJA.

Three concentrations (20, 50 and 100 μM) of MeJA (in 96% ethanol) were added to the 14-day-old shoot culture. The shoots were harvested 1 (15 days in culture), 3 (17 days in culture), 5 (19 days in culture) and 7 (21 days in culture) days after elicitation. In control cultures, 96% ethanol (20 μl per Magenta vessel) was added on the 14th day of culture and shoots were harvested at the same days as MeJA treated shoots. All cultures were maintained at 26±2°C under a 16 h photoperiod provided by cool white fluorescent lamps (approximately 40 μM m^{-2} s^{-1}). In MeJA treated shoots and corresponding control cultures, the growth (fresh and dry weight) as well as contents of diterpenoids and rosmarinic acid were determined.

Quantitative analysis and statistics

Identification of compounds was achieved by comparison of retention times and UV spectra in range of 200–400 nm with commercial standards. Diterpenoids and RA were determined by HPLC on a Waters Symetry ® C18 column, as described previously [9]. RA was detected at λ=330 nm, and diterpenoids – 230 nm. All experiments were performed in triplicate and the results shown in figure 1 are mean values ± standard error. Significant differences between treatments were determined with the U Mann-Whitney test at the 0.05 probability level.

RESULTS AND DISCUSSION

In this study, various concentrations of MeJA (20, 50 and 100μM) and elicitation periods (1, 3, 5 and 7 days) were tested to determine the effect of the elicitor on accumulation of rosmarinic acid and diterpenoids (carnosol and carnosic acid) in liquid sage shoot culture. Metabolite contents were determined by HPLC and results were shown in figure 1.

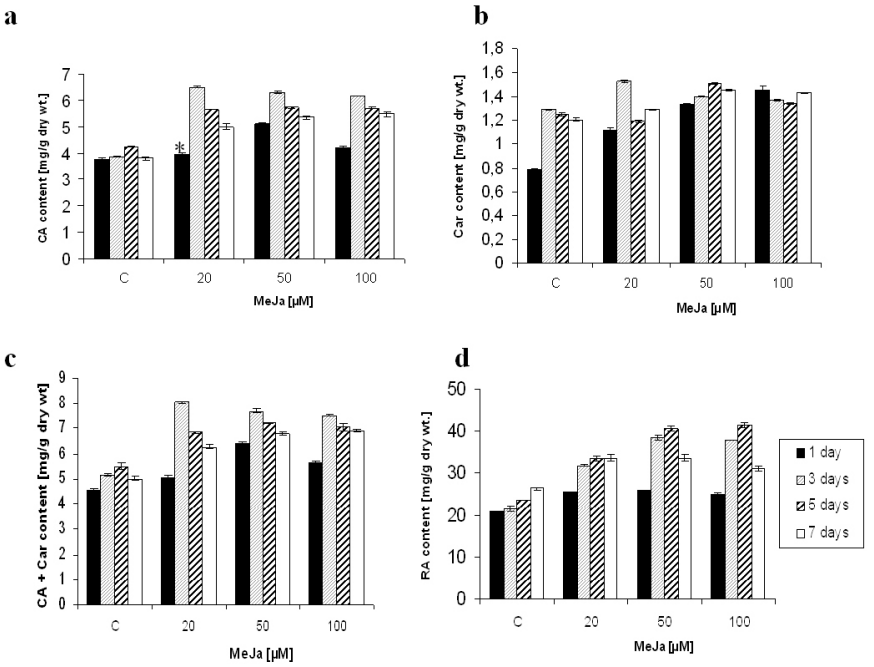

Figure 1. Carnosic acid (a), carnosol (b), carnosic acid + carnosol (c), rosmarinic acid (d) content in sage shoot culture after 1, 3, 5 and 7 days of MeJA elicitation. Shoot grown in MS liquid medium supplemented with IAA (0.1 mg l-1) and BAP (0.45 mg l-1). Elicitor was added on the 14th day after inoculation. C – control, shoot culture treated after 14 days only ethanol (20 μl). The values are the means ± SE * – the mean do not differ statistically from control for the same day at p=0.05 (the U Mann-Whitney test)

The data in figure 1 indicated that exposure of sage shoots to MeJA resulted in a significantly higher level of both tested diterpenoids (CA and Car), but optimal elicitation parameters for these compounds (concentration of elicitor, time exposure to MeJA) were different. For Car accumulation, the optimum concentration of MeJA was 100 μM at the time of exposure of 24 h (fig. 1b). Under these conditions the diterpenoid level was 1.45 mg g^{-1} dry weight, almost twice higher compared with control (0.79 mg g^{-1} dry wt). The highest increase in CA content was observed when 14-day-old shoot culture was treated with 20 μM MeJA at exposure time of 72 hours (fig. 1a). The shoots accumulated 6.5 mg g^{-1} dry wt of CA i.e. 70% more than untreated control shoot culture (fig. 1a). Exposure of the shoot culture to 20 μM MeJA for 72 h resulted also in the maximum total ditepenoid content (calculated as the sum of CA and Car) (fig. 1c). The amount was roughly higher than that in untreated control shoots (8.1 mg g^{-1} dry wt *versus* 5.2 mg g^{-1} dry wt). The higher concentrations of MeJA (50–100 μM) and longer exposure periods to elicitor (5 or 7 days) resulted in a lower total diterpenoid content although the values were always significantly higher compared to controls (fig. 1c).

MeJA elicitation affected also biosynthesis of rosmarinic acid. The optimum concentration of MeJA for the production of the compound in sage shoot culture was found to be 50 or 100 μM at 5 days after elicitation, yielding RA content of about 40 mg g^{-1} dry wt. This amount was 75% higher than that of the control (fig. 1d). The RA biosynthesis stimulating properties of MeJA have also been demonstrated in cultures of other plant species. Elicitation of *Lavandula vera* cell suspension culture with 50 μM MeJA enhanced the content of RA 2.5 fold [11]. MeJA increased also the accumulation RA in *Lithospermum erythrorhizon* cell culture [12]. On the other hand, Nitzsche et al. [17] did not observe changes in rosmarinic acid biosynthesis, when *Lavandula officinalis* cells were elicited with 10 μM jasmonic acid.

Our results are in agreement with the fact that changes in plant gene expression are induced by micro molar concentrations of MeJA [11-13] and that the time required to reach the maximum level of gene expression is different for each biosynthetic pathway and even each compound type. In sage shoot culture, MeJA rapidly stimulated secondary metabolite production. The level of diterpenoids (CA and Car) started to increase 24 h after MeJA treatment and the maximum effect was observed after 72 h. In case of RA, the levels of the compound were quite similar on day 3 and 5 after elicitation, but the amount of RA in sage shoot culture decreased 7 days after elicitation (fig. 1d). A rapid response to MeJA was also observed in respect to scopolamine accumulation in *Atropa baetica* hairy root culture [10]. The content of the alkaloid reached a maximum after 4 h of exposure to 100 μM MeJA and decreased after 72 h. However, a longer period of elicitor contact (10 days) was required to achieve maximal level of valepotriate production in transformed root culture of *Valerianella locusta* [14].

It is also noteworthy that MeJA did not have a negative effect on the growth of sage shoots when the elicitor was added on 14th day of cultivation period. The dry and fresh weight decreased to 8% in comparison to the control only when the cultures were

treated with 20 μM of MeJA after 5 days of MeJA exposure. In general, elicitation could have a negative effect on plant growth [13]. Therefore, in our study MeJA was added to 14-day-old cultures, after the end of intensive growth (data not shown).

CONCLUSION

The above results clearly show that, MeJA could be an important factor in enhancing the biosynthesis of compounds with antioxidant properties in sage shoot cultures at the concentrations which do not suppress biomass accumulation.

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WPŁYW JASMONIANU METYLU NA WYTWARZANIE ZWIĄZKÓW O WŁAŚCIWOŚCIACH PRZECIWUTLENIAJĄCYCH W KULTURACH PĘDÓW *SALVIA OFFICINALIS* L.

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S t r e s z c z e n i e

Aby zwiększyć wytwarzanie metabolitów wtórnych o właściwościach przeciwutleniających (kwasu karnozolowego, karnozolu i kwasu rozmarynowego) w kulturze pędów szałwii lekarskiej, w 14-tym dniu hodowli do podłoża płynnego dodano jasmonian metylu. Wpływ elicytora zależał od jego stężenia, czasu elicytacji i rodzaju badanego metabolitu. Działanie MeJA obserwowano już po 24 godzinach od rozpoczęcia elicytacji. Najwięcej diterpenów, w przeliczeniu na sumę karnozolu i kwasu karnozolowego (ok. 8mg/g s.m.), wytwarzały kultury pędów po 3 dniach elicytacji jasmonianem metylu w stężeniu 20 μM. W przypadku kwasu rozmarynowego najlepszy efekt osiągnięto po 5-dniowym okresie elicytacji, kiedy jasmonian użyto w stężeniu 50 lub 100 μM. W tych warunkach zawartość RA w pędach (ok. 41 mg/g s.m.) była prawie dwukrotnie wyższa w porównaniu z kontrolą, którą stanowiły kultury uzupełnione jedynie etanolem (rozpuszczalnik jasmonianu metylu).

Słowa kluczowe: *diterpenoidy, jasmonian metylu, kwas rozmarynowy, Salvia officinalis L.*