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Changes in jasmonates and 12-oxophytodienoic acid contents of *Medicago sativa* L. during somatic embryogenesis

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Abstract

Jasmonic acid (JA), its methyl ester (MeJA) and the biosynthetic precursor 12-oxophytodienoic acid (OPDA) were detected quantitatively during somatic embryogenesis of *Medicago sativa* L. Using GC-MS analysis, these compounds were found in initial explants, in calli and in somatic embryos in the nanogram range per gram of fresh weight. In distinct stages of somatic embryogenesis, JA and 12-OPDA accumulated preferentially in cotyledonary embryos. Initial explants exhibited about five-fold higher JA content than OPDA content, whereas in other stages OPDA accumulated predominantly. These data suggest that also in embryogenic tissues OPDA and JA may have individual signalling properties.

List of abbreviations: 2,4-D – 2,4-dichlorophenoxyacetic acid, JA – jasmonic acid, MeJA – methyl jasmonate, OPDA – 12-oxophytodienoic acid, RP – regeneration protocol

Introduction

Jasmonic acid (JA) and its methyl ester (MeJA) are ubiquitously occurring plant growth regulators which were found in different plant tissues and organs such as flowers, fruits, seeds, buds, shoots, roots and leaves (Wasternack and Hause 2002). Beside the native isomer (+)-7-*iso*-jasmonic acid, metabolites such as hydroxylated, hydrated or dehydrated jasmonates and their methyl ester were identified. Also jasmonates conjugated with amino acids, amids, sugars or sulphate could be found.

Distinct developmental stages are clearly dependent on jasmonates. Since the initial observations on senescence promoting effects (Ueda and Kato 1980; reviewed in Parthier 1990), tuber formation, tendril coiling, root growth inhibition and flower development were shown to be influenced by JA and/or related compounds (reviewed in Wasternack and Hause 2002). Jasmonates were also shown to affect seed germination of some plant species (Corbineau *et al.* 1988, Kępczyński and Białecka 1994, Ranjan and Lewak 1992). Most convincing data on the role of jasmonates in different developmental processes are given by analysis of JA deficient mutants which are affected in JA biosynthesis. In *Arabidopsis*, all of these mutants were found to be male-sterile due to affected pollen development (reviewed in Wasternack and Hause 2002, Park *et al*. 2002, Von Malek *et al*. 2002).

Jasmonates are lipid-derived compounds. The initial substrate, α-linolenic acid, is metabolised by three plastid-located enzymes, the lipoxygenase (LOX), the allene oxide synthase (AOS) and the allene oxide cyclase (AOC), to the JA precursor OPDA (reviewed in Wasternack and Hause 2002). Subsequent peroxisomal-located steps lead to (+)-7-*iso*-JA then can equilibrate to the more stable (−)-JA.

Jasmonic acid and its precursor 12-oxophytodienoic acid (OPDA) are important signals in plant responses to biotic and abiotic stresses (Creelman and Mullet 1995, Stintzi *et al.* 2001).

In most plant stress responses, the role of JA is indicated by its endogenous rise following the onset of stress or pathogenic attack. Also distinct developmental stages, organs and tissues exhibit remarkable different and specific contents of JA, OPDA and related compounds. The tomato flower organs differ characteristically in the ratio of several JA- and OPDA related compounds (Hause *et al.* 2000, Miersch *et al*. 2004). There is increasing evidence for a spatial and temporal pattern of generation of jasmonates in plant development attributing to the expression of specific sets of genes.

To analyse a putative role of jasmonates in somatic embryogenesis we inspected tissues of *Medicago sativa* L. Somatic embryo development is well-known to be triggered by distinct balance of plant hormones. Beside the key regulators, auxins and cytokinins (Komamine *et al*. 1992), abscisic acid and ethylene (Kępczyński *et al.* 1992) were also shown to influence somatic embryogenesis. For JA, only data on treatment with JA during induction and differentiation of somatic embryos are available (Ruduś *et al*. 2001, Tokuji *et al*. 1995). However, such data are ambiguous due to artificial imbalance of the hormone levels.

Therefore, the aim of this study was to quantify endogenous JA, its methyl ester and OPDA in dependence of somatic embryogenesis in tissues of *Medicago sativa* L. The data are discussed in terms of putative role of these compounds in embryogenesis.

Material and methods

Regeneration protocols

Plants of *Medicago sativa* L. were grown in a growth chamber at 24±2 °C under a 16 h photoperiod (350 $\mu E \cdot m^{-2} \cdot s^{-1}$). Petioles were used as initial explants in two different regeneration protocols (RP I and RP II).

RP I: This protocol consists of two steps which allow a separation of induction and differentiation phases (Meijer and Brown 1987). Petiole-derived explants of about 15 mm in length (5 per Petri dish) were incubated for ten days on SH medium (Schenk and Hildebrandt 1972) containing 22.6 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 4.7 μM kinetin (induction medium). They were then transferred onto SH medium lacking 2,4-D and kinetin (differentiation medium). The cultures were incubated at 25±1 °C under a 16 h photoperiod with 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity.

RP II: This is a protocol in which two main phases of somatic embryogenesis, induction and differentiation are separated by an additional step of embryogenic tissue proliferation that leads to high numbers of synchronised somatic embryos (McKersie *et al*. 1989). During induction, explants (10 mm length) were incubated for 14 days on SH medium containing 4.5 μM 2,4-D and 0.9 μM kinetin. Callus was sub-cultured into 40 ml liquid B_5g which is a standard B_5 medium (Gamborg *et al*. 1968) modified to contain 4.5 μM 2,4-D and 0.5 μM NAA for 7 days. A sequential sieving of cell suspensions through 800 mm and 200 mm nylon sieves separated the embryogenic fraction. For embryo differentiation, the embryogenic fraction was spread on a 200 μm nylon screen on a hormone-free B0i2Y medium (Bingham *et al*. 1975) containing 0.15 M sucrose. Two weeks after sieving, most of somatic embryos achieved cotyledonary stage of development. They were transferred with nylon screen onto B0i2Y medium containing 20 μM abscisic acid (ABA) and incubated for another week to become mature and acquired in desiccation tolerance.

Preparation of samples

For determination of endogenous jasmonates sampling was performed at specific stages of somatic embryogenesis. In RP I samples were collected at the beginning (4^{th} day) and at the end (10^{th} day) of the induction phase as well as prior to (7^{th} day) and just after (14^{th} day) the onset of somatic embryos which occur about 10^{th} day of differentiation phase. In case of RP II, the analyses comprised 14 day old embryogenically induced callus, embryogenic fraction of suspension, immature and mature cotyledonary embryos. The levels of endogenous jasmonates were also evaluated in petioles of *M. sativa*. In each case samples of 0.5 – 1.0 g fresh weight were frozen in liquid nitrogen and stored at -80 °C until analysis. Three to five replicate analyses were carried out for each sample combination.

Determination of jasmonates and 12-oxophytodienoic acid

Frozen plant tissues (1.0 g fresh weight) were homogenised in a mortar in liquid nitrogen and extracted with 10 ml of methanol, and 100 ng of [$^2H_6$]-JA (Miersch 1991) and 100 ng of [$^2H_5$]-OPDA prepared from [17-$^2H_2$,18-$^2H_3$]-linolenic acid according to Zimmerman and Feng (1978) were added as internal standards. The filtrate was loaded onto 3 ml DEAE-Sephadex A25 columns (acetate form, methanol), and the columns were washed with 3 ml of methanol. The eluents were collected as a neutral fraction for methyl jasmonate determination. After subsequent washing with 3 ml 0.1 M acetic acid in methanol, eluents obtained with 3 ml of 1 M acetic acid in methanol and with 3 ml of 1.5 M acetic acid in methanol were collected as the acidic fraction for JA and OPDA detection. The neutral fraction was hydrolysed with 2 ml 1 M NaOH overnight, then after partial evaporation acidified with a few drops of 4 M HCl and extracted with chloroform. Evaporated samples were next extracted with methanol and fractionated on 3 ml DEAE-Sephadex A25 columns (acetate form, methanol). The column washed sequentially with 3 ml of methanol, 0.1 M, 1.0 M and 1.5 M acetic acid in methanol. The acidic fraction of the neutral fraction was collected and analysed. Each collected fraction was then evaporated and separated by HPLC on an Eurospher – C18 column (5 µm, 250 mm x 4 mm), (Knauer, Germany) with a flow rate of 1 ml·min^{-1}. Separation was performed with solvent A (methanol) and solvent B (0.2 % acetic acid in water) with a gradient of 40 % solvent A to 100 % within 25 min. Fractions eluting between 12.0 and 13.3 min and between 20.3 and 22.0 min were collected in a vial, and then evaporated. For subsequent derivatization, samples were dissolved in 200 µl $CHCl_3$ and N,N-diisopropylethylamine (1:1, v/v) and derivatized with 10 µl pentafluorobenzylbromide at 20 °C overnight. The evaporated samples were dissolved in 5 ml of n-hexane and passed through SiOH-columns (500 mg; Machery-Nagel, Germany). The pentafluorobenzyl esters were eluted with a mixture of 7 ml of n-hexane and diethyl ether (2:1, v/v). After evaporation, residues were dissolved in 100 µl acetonitrile and subjected to GC-MS analysis with a Finnigan Mwt GCQ equipped with 5 m inert precolumn and a 30 m x 0.25 mm, 0.25 µm film thickness Rtx-5w/Integra Guard column (Restek, Germany). The analyses were conducted under the following conditions: 70 eV, NCI, ionisation gas - NH_3, source temperature 140 °C. The He carrier gas linear velocity was 40 cm·s^{-1}. The splitless injection port temperature was 250 °C and the interface temperature was 275 °C. The GC temperature program for JA and OPDA determination was: 100 °C for 1 min, 100 to 200 °C at 25 °C·min^{-1}, then to 300 °C at 5 °C·min^{-1} and held for 20 min. Retention times were as follow: for [$^2H_6$]JA-pentafluorbenzyl ester 11.92 min, for JA-pentafluorobenzyl ester 11.98 min, for [$^2H_5$]OPDA-pentafluorobenzyl ester 21.31 min, OPDA-pentafluorobenzyl ester 21.39 min. Fragments *m/z* 209 (JA), 215 (JA-standard) and 291 (OPDA), 296 (OPDA-standard) were used for quantification.

Data analysis

Endogenous hormonal concentrations were determined in three to five biological replications and analysed using Statistica for Windows version 6.1 software (StatSoft Inc., Tulusa, Oklahoma, USA). The Post-Hoc Fisher's Least-Significant-Difference-Test (LSD) was used to determine significant differences in hormone concentrations means (<0.05).

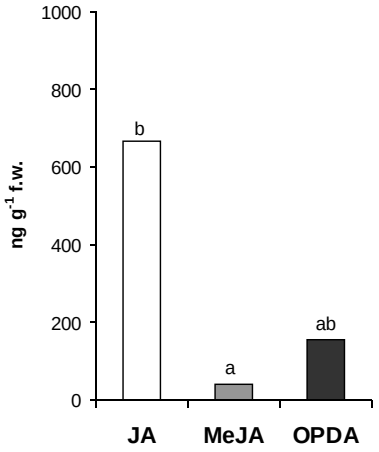

Fig. 1. The content of JA, MeJA and OPDA in initial explants (petioles) for embryogenic cultures of *Medicago sativa* L. Values are means of three replicates. Significant differences ($p< 0.05$) according to LSD test are marked with distinct letters.

Results and Discussion

In order to test the levels of jasmonates and OPDA in the tissue used for regeneration and embryogenic culture, petioles of *M. sativa* (second, third and fourth from the top of a mother plant) were collected and analysed. The level of JA was four times higher than OPDA and even sixteen times higher than MeJA in initial petioles of *Medicago sativa* L. (Fig. 1). Relatively high levels of JA and less OPDA in petioles serving as primary explants for *in vitro* culture initiation might be caused by wound response accompanied with sampling. More probable is high biosynthetic capacity in petioles. For tomato, it was clearly shown that the JA biosynthetic enzyme allene oxide cyclase (AOC) occurs mainly in vascular bundles and preferentially in petioles (Hause *et al.* 2000). Further studies revealed the occurrence of AOC proteins in sieve elements (Hause *et al.* 2003). This tissue specific occurrence of JA was accompanied with a preferential formation of JA in vascular bundles (Stenzel *et al.* 2003a). It is interesting to note that the JA content in petioles substantially exceeded that of OPDA. In fully developed unwounded and wounded leaf tissue OPDA level was observed to be 5-10-fold higher than the JA level as shown for *A. thaliana* (Stenzel *et al*. 2003b) and tomato (Stenzel *et al*. 2003a)

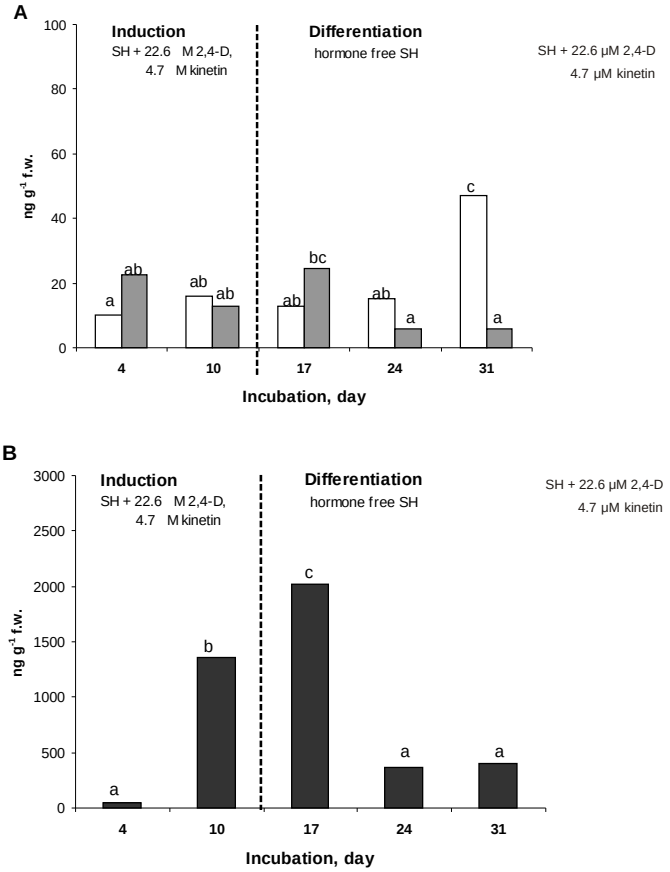

Fig. 2. Levels of JA (white), MeJA (grey) (A) and OPDA (black) (B) during induction and differentiation of *Medicago sativa* L. somatic embryogenesis in RP I.. Values are means of three replicates. Significant differences ($p< 0.05$) according to LSD test are marked with distinct letters.

Changes in plant growth regulators in embryogenic culture media that modify the concentrations of endogenous phytohormones seem to be undoubtedly the most important trigger for establishment of the desired developmental steps. Induction of regeneration in RP I in the presence of 2,4-D (22.6 µM) and kinetin (4.7 µM) revealed a dramatic alteration in the ratio of JA, MeJA and OPDA compared to the original tissue (Fig. 2). Whereas JA level decreased below 20 ng per g fresh weight in the first 24 days of induction and differentiation (Fig. 2A), OPDA increased dramatically up to above 2000 ng per g fresh weight at day 17 (Fig. 2B). These changes represent a 33-fold decrease in JA level and an 13-fold increase in OPDA level compared to the initial tissue taken from petioles (Fig. 1). Following the dynamics of changes of endogenous jasmonates in the course of somatic

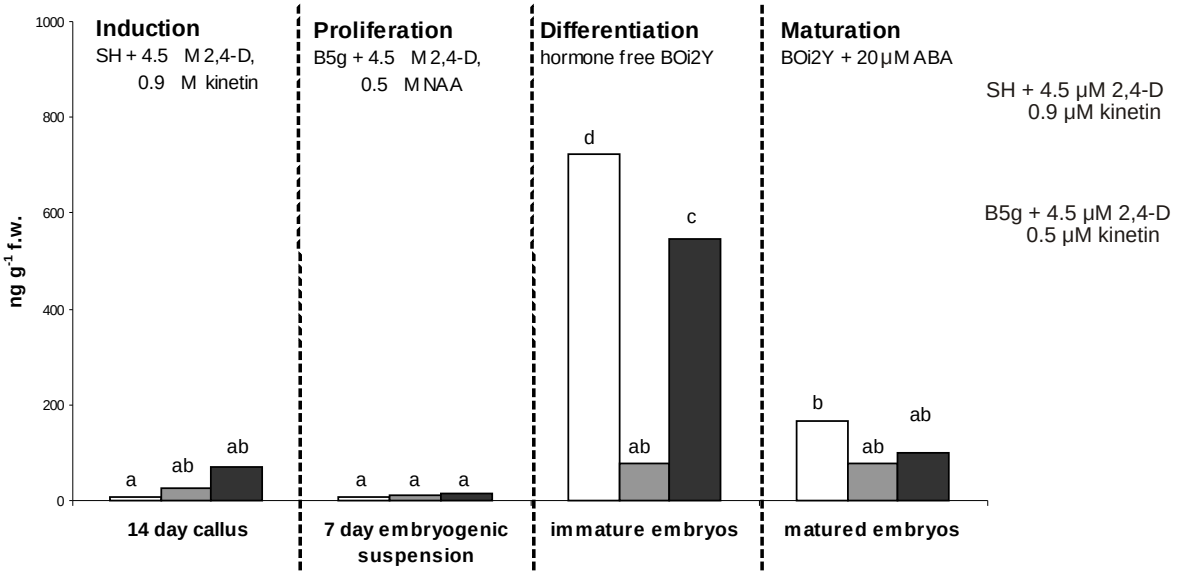

Fig. 3. Levels of JA (white), MeJA (grey) and OPDA (black) at distinct stages of *Medicago sativa* L. somatic embryogenesis in RP II. Values are means of three replicates or five replicates in case of experiments with somatic embryos. Significant differences ($p < 0.05$) according to LSD test are marked with distinct letters.

embryogenesis in *Medicago sativa* cultures it appeared that their contents remained on a stable and rather low level from the beginning of induction up to 24th day of differentiation phases in RP I (Fig. 2A). Only at the end of differentiation (31st day of incubation on SH hormone free medium), when the emergence of cotyledonary embryos occurred, an elevation in JA content was observed. This suggests that the endogenous level of jasmonates was independent from exogenous 2,4-D and kinetin used as embryogenic inducers, although it is well known that levels of other hormones can be altered, *e.g*. by 2,4-D (Dong *et al*. 1997, Ivanova *et al*. 1994, Michalczuk *et al*. 1992). Here it is worthwhile to mention that genetic and biochemical evidences exist on a link between JA-dependent and auxin-dependent signalling (Xu *et al.* 2002).

In contrast to JA and MeJA, the OPDA content increase remarkable elevating by one order of magnitude until the onset of differentiation (17th day of incubation) followed by a rapid decrease (Fig. 2B). This might be caused by the high 2,4-D level added in the induction phase. Due to the constant JA level in this phase and the already mentioned cross-talk between JA-dependent and auxin-dependent signalling (Xu *et al*. 2002), 2,4-D may affect reactions upstream of OPDA formation.

The jasmonate and OPDA levels of somatic embryogenesis in RP I, were compared with that of 14 day old callus obtained in RP II on SH medium which contained 10-fold lower levels of 2,4-D and kinetin than in RP I, as well as of 7 day old embryogenic cell suspension derived from that callus (Fig. 3). The callus and the embryogenetic cell suspension exhibited much lower levels of JA, MeJA and OPDA. The OPDA level were 10-fold lower in the callus and nearly 30-fold lower in the embryogenic cell suspension compared to induction conditions in RP I. Under these conditions, OPDA levels again exceeded the levels of JA and MeJA.

Finally, we analysed contents of jasmonates and OPDA in somatic embryos obtained in RP II which achieved cotyledonary stage of development and were then matured in the presence of exogenous ABA. The level of JA was very high in immature cotyledonary embryos reaching 723 ng per g fresh weight and decreased down to 166 ng per g fresh weight during maturation phase. Similar levels and changes were observed for OPDA.

Although the levels of jasmonates and OPDA presented here are overall levels neglecting putative cell type- and tissue-specific differences, the remarkable differences are indicative for distinct stages of differentiation and embryo development.

We can not role out that other jasmonate compounds than the free acid and its methyl ester accumulate in some of developmental stages analysed here. For tobacco, bright yellow-2 cell suspension cultures metabolism of JA to its glucose and gentiobiose esters as well as to their C-11 and C-12 hydroxylated form has been described recently (Świątek *et al.* 2004). To best of our knowledge, however, such metabolites if observed, are minor constituents, and JA and OPDA are the major constituents in most plant species analysed so far. Three points of interest can be drawn from the presented data:

(i) JA and OPDA levels changed independently from each other

(ii) In RPI somatic embryogenesis OPDA levels exceeded that of JA remarkably in all stages

(iii) Immature embryos contained high levels of JA and OPDA.

To (i): The independent changes in JA and OPDA levels shown here might be of significance for the signalling properties of both compounds and accord in data for *Arabidopsis* and tomato. Initially, in *A. thaliana* and tomato a distinct ratio of octadecanoids and jasmonates was observed designated as "oxylipin signature" (Weber *et al.* 1997). Later on, the individual signalling properties of JA and OPDA were clearly shown with the JA-deficient *opr3* mutant of *A. thaliana* (Stintzi *et al.* 2001). Even the various organs of tomato flower carry completely different pattern of oxylipins (Hause *et al.* 2000). Since the pattern of OPDA, JA and MeJA differed remarkably between the developmental stages of somatic embryogenesis in RPI and RPII, it will be interesting to see whether a corresponding alteration of gene expression occurs specifically by JA and/or OPDA.

To (ii): High OPDA levels is a common phenomenon in many tissues. In untreated leaves of *A. thaliana* up to 1-2 nmoles per g f.w. were detected (Stintzi *et al.* 2001, Stenzel *et al.* 2003b) and even large amount of esterified OPDA was found (Stelmach *et al.* 2001). It is, however, still unclear how this high OPDA levels are biologically active. OPDA is formed within the plastids, but its release into peroxisomes, where further conversion takes place, is unclear. OPDA of untreated tissue might be confined to the plastids. This is supported by the fact that in untreated *Arabidopsis* leaves despite high OPDA levels OPDA-inducible genes are not activated (Reymond *et al.* 2000).

To (iii): The high level of JA and OPDA in cotyledonary embryos might be related to mobilization of carbon and nitrogen as well as formation of embryogenesis-specific proteins as known for the jasmonate-inducible vegetative storage proteins (VSPs), of the soybean which accumulate preferentially in embryos (Creelman and Mullet 1997). Furthermore, some of the embryogenesis-related proteins of *Nicotiana plumbaginifolia* are clearly JA-inducible (Reinbothe *et al.* 1994).

Summarising, the distinct pattern of JA and OPDA observed here, for different stages of somatic embryogenesis suggest specific roles for these compounds during embryogenesis. It will be interesting to see, whether this pattern is reflected in expression of genes inducible by JA and/or OPDA.

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References

Bingham E.T., Hurley L.V., Kaatz D.M., Saunders J.W. 1975. Breeding alfalfa which regenerate from callus tissue culture. Crop Sci. 15: 719-721.

Corbineau F., Rudnicki R.M., Come D. 1988. The effects of methyl jasmonate on sunflower (*Helianthus annuus* L.) seed germination and seedling development. Plant Growth Regul. 7: 157-169.

Creelman R.A., Mullet J.E. 1995. Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. Proc. Natl. Acad. Sci. USA 92: 4114-4119.

Creelman R.A., Mullet J.E. 1997. Oligosaccharins, brassinolides, and jasmonates: nontraditional regulators of plant growth, development, and gene expression. Plant Cell 9: 1211-1223.

Dong J.Z., Bock C.A., Dunstan D.I. 1997. Influences of altered phytohormone use on endogenous ABA and

mRNA populations during white spruce (*Picea glauca*) somatic embryo culture. Tree Physiol. 17: 53-57.

Gamborg O., Miller R., Ojima K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50: 151-158.

Hause B., Stenzel I., Miersch O., Maucher H., Kramell R., Ziegler J., Wasternack C. 2000. Tissue-specific oxylipin signature of tomato flowers: allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles. Plant J. 24: 113-126.

Hause B., Hause G., Kutter C., Miersch O., Wasternack C. 2003. Enzymes of jasmonate biosynthesis occur in tomato sieve elements. Plant Cell Physiol. 44: 643-648.

Ivanova A., Velcheva M., Denchev P., Atanassov A., Van Onckelen H.A. 1994. Endogenous hormone levels during direct embryogenesis of *Medicago falcata*. Physiol. Plant. 92: 85-89.

Kępczyński J., Białecka B. 1994. Stimulatory effect of ethephon, ACC, gibberellin A_3 and A_{4+7} on germination of methyl jasmonate inhibited *Amaranthus caudatus* L. seeds. Plant Growth Regul. 14: 211-216.

Kępczyński J., McKersie B.D., Brown D.C.W. 1992. Requirement of ethylene for growth of callus and somatic embryogenesis in *Medicago sativa* L. J. Exp. Bot. 43: 1199-1202.

Komamine A., Kawahara R., Matsumoto M., Sunabori S., Toya T., Fujiwara A., Tsukahara M., Smith J., Ito M., Fukuda H., Nomura A., Fujimura T. 1992. Mechanisms of somatic embryogenesis in cell cultures: physiology, biochemistry and molecular biology. In Vitro Cell. Dev. Biol. Plant 28: 11-14.

McKersie B.D., Senaratna T., Bowley S.R., Brown D.C.W., Krochko J.E., Bewley J.D. 1989. Applications of artificial seed technology in the production of hybrid alfalfa (*Medicago sativa* L.). In Vitro Cell. Dev. Biol. Plant 25: 1183-1188.

Meijer E.G.M., Brown D.C.W. 1987. A novel system for rapid high frequency somatic embryogenesis in *Medicago sativa*. Physiol. Plant. 69: 591-596.

Michalczuk L., Cooke T.J., Cohen J.D. 1992. Auxin levels at different stages of carrot somatic embryogenesis. Phytochemistry 31: 1097-1103.

Miersch O. 1991. Synthesis of (+/-)-(10-^{2}H,11-$^2H_2$,12-$^2H_3$) jasmonic acid. Z. Naturforsch. 46b: 1724-1729.

Miersch O., Weichert H., Stenzel I., Hause B., Maucher H., Feussner I., Wasternack C. 2004. Consititutive overexpression of allene oxide cyclase in tomato (*Lycopersicon esculentum* cv. Lukullus) elevates levels of some jasmonates and octadecanoids in flower organs but not in leaves. Phytochemistry 65: 847-857.

Park J.H., Halitschke R., Kim H.B., Baldwin I.T., Feldmann K.A., Feyereisen R. 2002. A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. Plant J. 31: 1-12.

Parthier B. 1990. Jasmonates: hormonal regulators or stress factors in leaf senescence? J. Plant Growth Regul. 9: 1-7.

Ranjan R., Lewak S. 1992. Jasmonic acid promotes germination and lipase activity in non-stratified apple embryos. Physiol. Plant. 86: 335-339.

Reinbothe C., Tewes A., Lehmann J., Parthier B., Reinbothe S. 1994. Induction by methyl jasmonate of embryogenesis-related proteins and mRNAs in *Nicotiana plumbaginifolia*. Plant Sci. 104: 59-70.

Reymond P., Weber H., Diamond M., Farmer E.E. 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. Plant Cell 12: 707-719.

Ruduś I., Kępczyński J., Kępczyńska E. 2001. The influence of the jasmonates and abscisic acid on callus growth and somatic embryogenesis in *Medicago sativa* L. tissue culture. Acta Physiol. Plant. 23: 103-107.

Schenk R.U., Hildebrandt A.C. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 50: 199-204.

Stelmach B.A., Müller A., Hennig P., Gebhardt S., Schubert-Zsilavecz M., Weiler E.W. 2001. A novel class of oxylipins, *sn*1-*O*-(12-oxophytodienoyl)-*sn*2-*O*--(hexadecatrienoyl)-monogalactosyl diglyceride, from *Arabidopsis thaliana*. J. Biol. Chem. 276: 1282-1283.

Stenzel I., Hause B., Maucher M., Pitzschke A., Miersch O., Ziegler J., Ryan C.A., Wasternack C. 2003a. Allene oxide cyclase dependence of the wound response and vascular bundle specific generation of jasmonate in tomato – amplification in wound-signalling. Plant J. 33: 577-589.

Stenzel I., Hause B., Miersch O., Kurz T., Maucher H., Weichert H., Ziegler J., Feussner I., Wasternack C. 2003b. Jasmonate biosynthesis and the allene oxide cyclase family of *Arabidopsis thaliana*. Plant Mol. Biol. 51: 895-911.

Stintzi A., Weber H., Reymond P., Browse J., Farmer E.E. 2001. Plant defence in the absence of jasmonic acid: the role of cyclopentanones. Proc. Natl. Acad. Sci. USA 98: 12837-12842.

Świątek A., Dongen W.V., Esmans E.L., Van Onckelen H.V. 2004. Metabolic fate of jasmonates in tobacco bright yellow-2 cells. Plant Physiol. 135: 161-172.

Tokuji Y., Mizue Y., Masuda H. 1995. Effects of methyl jasmonate and concanavalin A on embryogenesis and the induction of secondary somatic embryos of carrot. Biosci. Biotechnol. Biochem. 59: 1675-1678.

Ueda J., Kato J. 1980. Isolation and identification of a senescence-promoting substance from wormwood (*Artemisia absinthium* L.). Plant Physiol. 66: 246-249.

Von Malek B., Van Der Graaff E., Schneitz K., Keller B. 2002. The *Arabidopsis* male-sterile mutant dde2-2 is defective in the allene oxide synthase gene encoding one of the key enzymes of the jasmonic acid biosynthesis pathway. Planta 216: 187-192.

Wasternack C., Hause B. 2002. Jasmonates and octadecanoids: signals in plant stress responses and development. Progr. Nucleic Acid Res. Mol. Biol. 72: 165-221.

Weber H., Vick B.A., Farmer E.E. 1997. Dinor--oxo-phytodienoic acid: A new hexadecanoid signal. Proc. Natl. Acad. Sci. USA 94: 10473-10478.

Xu L., Liu F., Lechner E., Genschik P., Crosby W.L., Ma H., Peng W., Huang D., Xie D. 2002. The SCR^{COI1} ubiquitin-ligase complexes are required for jasmonate response in *Arabidopsis*. Plant Cell 14: 1919-1935.

Zimmerman D.C., Feng P. 1978. Characterization of a prostaglandin-like metabolite linolenic acid produced by a flaxseed extract. Lipids 13: 313-316.

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