Potato Research 48 (2005) 107–115

Hydroxylated jasmonate levels during stolon to tuber transition in *Solanum tuberosum* L.

ANA CENZANO[1], ANA VIGLIOCCO[1], OTTO MIERSCH[2] and GUILLERMINA ABDALA[1,*]

[1] Universidad Nacional de Río Cuarto, 5800-Río Cuarto, Argentina
[2] Institut für Pflanzenbiochemie, Weinberg 3, 06120-Halle, Germany
*Corresponding author, Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, 5800-Río Cuarto, Argentina, e-mail: gabdala@exa.unrc.edu.ar

Accepted for publication: 13 January 2005

Additional keywords: jasmonic acid, hydroxylated forms, potato

Summary

Various octadecanoids and derived compounds have been identified in potato leaves. However, information regarding jasmonate hydroxylated forms in stolons or tubers is scarce. We investigated endogenous jasmonates in stolon material of *Solanum tuberosum* cv. Spunta. Stolons and incipient tubers were collected from 8 weeks old plants. The material was cut into apical regions and stolons. We identified jasmonic acid (JA), methyl jasmonate, 11-OH-JA, 12-OH-JA, 12-oxo-phytodienoic acid (OPDA) and a conjugate. The content of JA and 12-OH-JA decreased in the apical region but remained high in stolons during tuberization. Thus the apical region might be a site of JAs-utilization or metabolization and stolons might supply JAs to that region. The content of 12-OH-JA was higher than that of 11-OH-JA in all stages analyzed, both in apical regions and stolons. However, these compounds showed a different time-course in the apical region: while 11-OH-JA increased, 12-OH-JA decreased. Thus, JA from leaves or roots could be transported as 12-OH-JA to the apical region, stimulating tuber formation.

Introduction

Several developmental processes are influenced by jasmonates (JAs), including seed germination, root growth, flower and fruit development, senescence and tuber formation (Sembdner & Parthier, 1993; Creelman & Mullet, 1997; Abdala et al., 2002; Wasternack & Hause, 2002).

For several decades the development of a specific tuber-inducing stimulus in leaves exposed to short days (SD) was hypothesized based on grafting experiments. Koda et al. (1988) isolated a tuber-inducing compound from the leaves of *Solanum tuberosum* L. cv. Irish Cobbler. The structure of the compound was determined to be 3-oxo-2-(5'-ß-glucopyranosyloxy-2'-Z-pentenyl)-cyclopentane-3-acetic acid (Yoshihara et al., 1989). The aglycon of this glucoside was 12-OH-jasmonic acid (12-OH-JA), named tuberonic acid. These authors did not detect a glucoside of the 11-OH-jasmonic acid (11-OH-JA) in leaves of *Solanum tuberosum* L. The identification of a tuber-inducing compound from the leaflets of a potato plant however does not indicate that this compound is related to tuber formation.

Later, both forms, 11-OH-JA and 12-OH-JA, were found in *Solanum demissum*

Lindl leaflets grown under SD, the 11-OH-JA concentration being higher than the concentration of 12-OH-JA. None of these compounds could be detected in leaflets grown under long day conditions (Helder et al., 1993). Based on their results these authors suggested that 11-OH-JA might play a more important role than 12-OH-JA in tuberization.

Recently, the synthesis of a potato tuber-forming substance, methyl 12-OH-JA, and its epimer, was reported by Kiyota et al. (1999). However, the isolation of tuberonic acid glucoside methyl ester in potato leaves of *Solanum tuberosum* had been reported previously by Simko et al. (1996). These authors found high levels of this compound in the cv. Norchip grown under long days (LD). This cultivar tuberizes even under LD condition. The other cultivar was LT-1, which requires SD for tuberization. SD conditions appeared to reduce the level of the tuberonic acid glucoside methyl ester in both cultivars. The difference between LD and SD in the concentration of the glucoside methyl ester was the opposite of what was expected based upon the evidence that this compound promotes in vitro tuberization (Yoshihara et al., 1992).

Another substance related to the hydroxylated forms of JA is potassium ß-D-glucopyranosyl 11-hydroxyjasmonate, which was isolated as a very active leaf-closing substance in the leguminosa *Albizzia julibrissin* (Ueda & Yamamura, 1999).

Although the levels of hydroxyjasmonates have been quantified in potato leaves, we do not know yet whether the concentration of hydroxyjasmonates changes during the transition from stolon to tuber. Also, the role of octadecanoids in the tuberization process is still not completely understood.

The present study investigates the variations in the endogenous content of octadecanoids in the apical region and in stolons of *Solanum tuberosum* cv. Spunta, during the transition of stolons to tubers.

Materials and methods

Potato tubers of *Solanum tuberosum* L., cv. Spunta, were planted in soil, in a greenhouse under controlled environmental conditions (15 h photoperiod, 30 °C/12 °C day/night temperature at planting time and 35 °C/18 °C day/night at tuber set). Plants were harvested between 8 and 10 weeks after planting and individual organs were collected according to Fig. 1 in the stages of: stolon with hooked apex (1st stage), stolon with initial swelling (2nd stage), stolon with advanced swelling (3rd stage) and tuber of 1.2 cm of length (4th stage). Three independent experiments were performed using 25 plants each. For octadecanoid analysis the material was cut into two regions, the apical region and the stolon part. The analysis of these compounds was done in triplicate (each determination on a pool of material selected from 10 plants).

Extraction, purification and assessment of octadecanoids

Fresh plant material (1 g) was homogenized with 10 ml methanol and 100 ng ($^2H_6$)JA, 100 ng ($^2H_5$)OPDA, 100 ng 11-($^2H_3$)OAc-JA, 100 ng 12-($^2H_3$)OAc-JA and

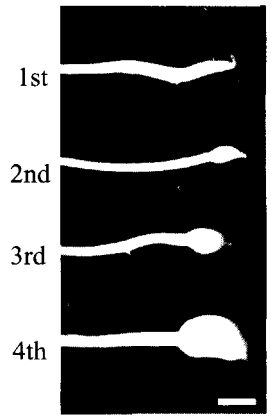

Fig. 1. Stages of the transition of stolons to tubers: 1st stage, stolon with hooked apex; 2nd stage, initial stolon swelling; 3rd stage, stolon with advanced swelling; 4th stage, tuber of 1.2 cm length. Bar = 1 cm.

200 ng JA-($^2H_3$)leucine conjugate as internal standards. The homogenate was filtered under vacuum on a column with cellulose filtre. Then, elute was evaporated and acetylated with pyridine/acetic acid anhydride (2:1) at 20 °C overnight. The extract was dried, dissolved with 10 ml methanol, and placed on columns filled with 3 ml DEAE-Sephadex A25 (Amersham Pharmacia Biotech AB, Sweden) (Ac^--form, methanol); the column was washed with 3 ml methanol. After washing with 3 ml 0.1 M acetic acid in methanol (all elutes combined gave fraction A), eluents with 3 ml of 1 M acetic acid in methanol and 3 ml of 1.5 M acetic acid in methanol were collected, evaporated and separated on preparative HPLC, and analyzed by GC-MS.

Fraction A (neutral jasmonates) was saponified with 5 ml 1 N NaOH and carboxylic acids were recovered with $CHCl_3$ after acidification with 1 N HCl to pH 4. The $CHCl_3$ solution was evaporated, separated on HPLC and analyzed by GC-MS.

HPLC: Eurospher 100-C18, (5 µm, 250 × 4 mm) (Knauer, Germany), Solvent A-MeOH, Solvent B-0.2% acetic acid in H_2O. Gradient 40% A to 100% A in 25 min. Fractions at R_t 10 to 11.30 min were collected in 1 vial (sample 1), 12 to 13.30 min and 20.30 to 22 min were collected in 1 vial (sample 2) and fraction 16.10 to 18.15 min was collected separately (sample 3).

Derivatization: Evaporated samples were dissolved in 200 µl $CHCl_3$/*N*,*N*-diisopropylethylamine (1:1) and derivatized with 10 µl pentafluorobenzylbromide at 20 °C overnight. The evaporated samples 1 and 2 from HPLC were dissolved in 5 ml n-hexane and passed through a SiOH-column (500 mg; Machery-Nagel, Germany). The pentafluorobenzyl esters were eluted with 7 ml n-hexane/diethylether (2:1). The evaporated sample 3 were dissolved in n-hexane/ether (2:1) and eluted with n-hexane/ether (1:1). Elutes were evaporated, dissolved in 100 µl MeCN and analyzed by GC-MS using methods A and B.

GC-MS: (GCQ Finnigan), 70 eV, negative chemical ionization, ionization gas

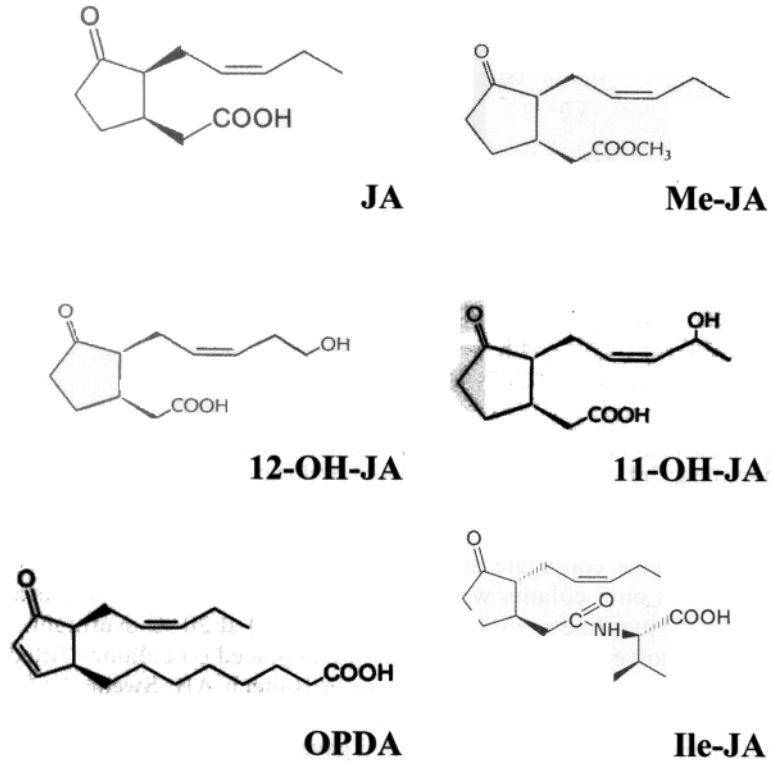

Fig. 2. Chemical structures of jasmonates found in potato stolons and tubers. JA: jasmonic acid; Me-JA: methyl-jasmonate; 12-OH-JA: 12-hydroxylated jasmonic acid; 11-OH-JA: 11-hydroxylated jasmonic acid; OPDA: 12-oxo-phytodienoic acid; JA-Ile: jasmonic acid conjugated with isoleucine.

NH_3, source temperature 140 °C, column Rtx-5w/Integra Guard (Restek, Germany) (5m inert precolumn connected with column 15 m × 0.25 mm, 0.25 µm film thickness, crossbond 5% diphenyl – 95% dimethyl polysiloxane, injection temperature 250 °C, interface temperature 275 °C; helium 20 cm s^{-1}; splitless injection.

Column temperature programme: 1 min 60 °C, 25 °C min^{-1} to 180 °C, 5 °C min^{-1} to 270 °C, 10 °C min^{-1} to 300 °C, 10 min 300 °C; R_t of pentafluorobenzyl esters: ($^2H_6$)JA 10.30 min, JA 10.36 min, 11-($^2H_3$)OAc-JA 13.60 min, 11-OAc-JA 13.63 min, 12-($^2H_3$)OAc-JA 15.39 min, 12-OAc-JA 15.42 min, JA-($^2H_3$)Leu 19.63 min, JA-($^2H_3$)Ile 19.93 min ($^2H_5$)OPDA 20.10 min, OPDA 20.16 min, Fragments *m/z* 209, 215 (standard), *m/z* 267, 270 (standard), *m/z* 267, 270 (standard), *m/z* 322, 325 (standard) and *m/z* 291, 296 (standard) were used for the quantification of JA, 11-OH-JA, 12-OH-JA, JA-Ile and OPDA, respectively.

Statistical analysis

Statistical analysis of JAs measurements was performed by an ANOVA and a posteriori test LSD. The software used was Statistica (StatSoft Inc., 1999).

Results

The JA-derivatives, 11-OH-JA, 12-OH-JA, Me-JA, JA-Ile, and the JA-precursor OPDA, were identified in stolon parts and in the apical region of potato plants (Fig. 2). The levels of endogenous JA, Me-JA, 11-OH-JA and 12-OH-JA were measured during the transition of stolons to tubers. Changes in JA and hydroxylated forms were found along this process.

Despite the identification of JA-Ile and OPDA in stolons and tubers, we were unable to quantify these compounds. This was due to the very low level of JA-Ile and to specific problems of the biological material of OPDA.

In the apical region we could observe a decrease in JA concentration from the 1st stage to the 4th stage, whereas stolons maintained the same level along the stages of the transition of stolon to tuber. In the 4th stage, JA content in the stolon was higher than in the apical region (Fig. 3A).

The 12-OH-JA form decreased in the 3rd and 4th stages compared with the 1st and 2nd stages in apical region. On the contrary, no significant changes were found in stolon for the four stages analyzed (Fig. 3C).

In the apical region and stolon at the 4th stage an increase was observed in relation to the derivative 11-OH-JA (Fig. 3D).

The content of 12-OH-JA was higher than that of 11-OH-JA in the four stages of tuber formation, either in the apical region or in the stolon. However, both compounds showed a different time-course in the apical region: while 11-OH-JA increased from the 1st stage to the 4th stage, the content of 12-OH-JA decreased (Fig. 3C and D).

As shown in Fig. 3B, Me-JA did not show significant modifications between the apical region and the stolon and its basal level was much lower than for the other JAs. The low level of Me-JA may be due to the volatilization of this compound to the atmosphere (Boland et al., 1995).

Discussion

There have been many studies on the effects of growth regulators on tuberization in potato (Koda, 1997; Castro et al., 1999; 2000; Abdala et al., 2002), but no unequivocal tuberizing factor has yet been identified (Jackson, 1999). However, hormonal signals are common in morphogenetic events and they need transduction pathways and receptive tissues to exert their action. For the morphogenetic process of tuberization the target tissue is placed in the meristem region of the stolons. For this reason it is important to study the changes in JAs along the transformation of stolon to the tuber.

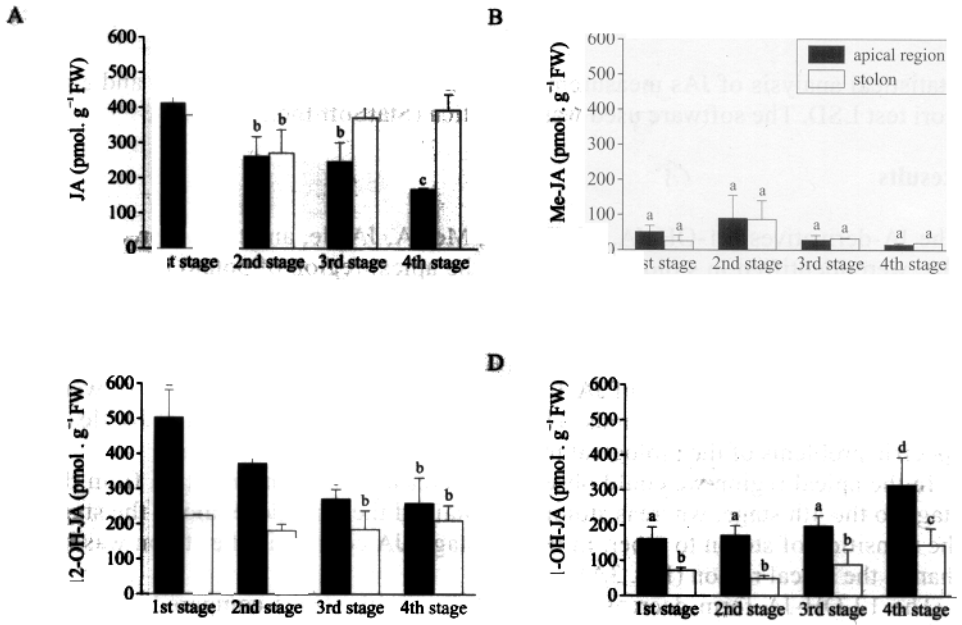

Fig. 3. A: Jasmonic acid (JA); B: methyl-jasmonate (Me-JA); C: 12-hydroxylated jasmonic acid (12-OH-JA); D: 11-hydroxylated jasmonic acid (11-OH-JA) content in apical region and stolon during the transition of potato stolons into tubers (n = 3, ± SE; *P<0.05). Data with the same letter are not significantly different.

In addition to JA and Me-JA, we identified and quantified for the first time the hydroxylated derivatives 11-OH-JA and 12-OH-JA in all the stages of the stolon to tuber transition. One of the JA-hydroxylated forms, 11-OH-JA, had been detected as a endogenous substance in higher plants by Helder et al. (1993). Previously, both hydroxylated JA-derivatives had been isolated from the culture filtrate of the fungus *Botryodiplodia theobromae* (Miersch et al., 1991), and the presence of 12-OH-JA and its methyl esther in potato leaves had been reported by Simko et al. (1996).

The similar changes in 12-OH-JA and JA content along the transition of stolon to tuber, and the maintenance of a high and constant level of both compounds in stolons concomitant with a decrease in the apical region, suggest that the apical region might be the site where the hydroxylation or utilization/metabolism occurs; stolons supply JAs to the apical region. Our previous data suggest a role for JA not only in cell expansion of non-swelling stolons exogenously treated with JA, but also in the early appearance of xylem elements in the regions proximal to the tunic. Protoxylem elements such as tracheal elements with thin primary cell walls were present in JA-treated stolons. The early vascular tissue differentiation in the apical region may be related to water and nutrient movement (Cenzano et al., 2003). We can conclude that endogenous as well as exogenous JA cause cell expansion, enlargement of the meris-

tems accompained with leaf primordium modifications, and early vascular tissue differentiation, resulting in the formation of tubers in detriment of shoots.

In relation to JAs transport in plants, a highly polar compound, the glucoside of tuberonic acid (TAG), was identified after feeding potato leaf with [2-^{14}C] (±) JA (Yoshihara et al., 1996). The radioactivity was found in the stolons and tubers, meaning that TAG metabolized from JA moved to belowground parts of the potato plants. TAG is more favoured than JA for translocation due to its polar nature. When TAG concentration reaches a high enough level, the production of tubers starts; however, it is still not certain whether a high accumulation of TAG in tubers is a cause or a consequence of tuberization. Based on our results we can assume that JA from leaves or roots could be transportated as 12-OH-JA and/or TAG to the apical region stimulating tuber formation by means of cell expansion and vascular tissue differentiation.

It is well known that JA and derivatives are synthezised in leaves (Sembdner & Parthier, 1993; Creelman & Mullet, 1997; Mueller, 1997) and roots (Abdala et al., 2003), although there is no data about JA-synthesis in stems or stolons. However, we cannot discard this idea since Hause et al. (2000) reported the occurrence of allene oxide cyclase, an enzyme related to JA synthesis, in the vascular bundles of tomato stems and other structures. Moreover, LOX_1 transcripts accumulation, the first enzyme of JA-biosynthesis, was found in the tuber apical and subapical regions, specifically in the vascular tissue of the perimedular region, the major site of cell growth (Kolomiets et al., 2001). In addition, mutants with diminished expression of LOX_1 showed a decreased activity of this enzyme with the consequent reduction of tuber number and size. These results point out the participation of some metabolites derived from LOX in the tuberization induction and tuber enlargement.

Also, the parallel 12-OH-JA decrease and the 11-OH-JA increase in the apical region could indicate either a conversion pathway of 12-OH-JA into 11-OH-JA, or a preferential hydroxylation in C-11 with respect to C-12. Metabolic studies performed with [^{14}C]-(±)-JA and [^{14}C]-9,10-dihydro-JA incorporated into six-day-old barley plants showed that in both cases hydroxylation was larger in C-11 than in C-12 (Sembdner et al., 1990). So far it is not known if there is a causal relationship between the presence of JA-hydroxylated forms and tuber formation; however, it is reasonable to assume that these compounds are involved in the formation of the plant storage organs.

Little is known about the physiological action of the JA-hydroxylated derivatives. To date, 12-OH-JA was found in *Arabidopsis thaliana* (Gidda et al., 2003), and its glucoside was also detected in *Helianthus tuberosus* (Matsura et al., 1993) and *Astragalus complanatus* (Cui et al., 1993). The presence of 12-OH-JA in *Arabidopsis thaliana* indicates that the distribution of this compound is not only restricted to tuber-producing plants. Taken together 12-OH-JA might have different biological function, e.g. delayed flowering initiation by decreased levels of 12-OH-JA (Gidda et al., 2003).

Acknowledgements

We thank the contribution of language consultant Professor Iliana A. Martínez to this paper. This work was supported by a grant from SECYT-UNRC to G.A. and fellowships from CONICET to A.C. and A.V.

References

Abdala, G., G. Castro, O. Miersch & D. Pearce, 2002. Changes in jasmonates and gibberellins during development of potato plant (*Solanum tuberosum* L.). *Plant Growth Regulation* 36 (2): 121–126.

Abdala, G., O. Miersch, R. Kramell, A. Vigliocco, E. Agostini, G. Forchetti & S. Alemano, 2003. Jasmonate and octadecanoid occurrence in tomato hairy roots. Endogenous level changes in response to NaCl. *Plant Growth Regulation* 40(1): 21–27.

Boland, W., J. Hopke, J. Donath, J. Nüske & F. Bublitz, 1995. Jasmonic acid and coronatin induce odor production in plants. *Angewandte Chemie Internationale Edition England* 34: 1600–1602.

Castro, G., T. Kraus & G. Abdala, 1999. Endogenous jasmonic acid and radial cell expansion in buds of potato tubers. *Journal of Plant Physiology* 155: 706–710.

Castro, G., G. Abdala, C. Agüero & R. Tizio, 2000. Interaction between jasmonic and gibberellic acids on in vitro tuberization of potato plantlets. *Potato Research* 43: 83–88.

Cenzano, A., A. Vigliocco, T. Kraus & G. Abdala, 2003. Exogenously applied jasmonic acid induces changes on the apical meristem morphology of potato stolons. *Annals of Botany* 91(7): 917–921.

Creelman, R.A. & J.E. Mullet, 1997. Biosynthesis and actions of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 48: 355–381.

Cui, B., M. Nakamura, J. Kinjo & T. Nohara, 1993. Chemical constituents of astragali semen. *Chemical Pharmaceutical Bulletin* 41: 178–182.

Gidda, S.K., O. Miersch, A. Levitin, J. Schmidt, C. Wasternack & L. Varin, 2003. Biochemical and molecular characterization of a hydroxyjasmonate sulfotransferase from *Arabidopsis thaliana*. *Journal of Biological Chemistry* 278: 17895–17900.

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

Helder, H., O. Miersch, D. Vreugdenhil & G. Sembdner, 1993. Ocurrence of hydroxylated jasmonic acids in leaflets of *Solanum demissum* plants grown under long- and short-days conditions. *Physiologia Plantarum* 88: 647–653.

Jackson, S.D., 1999. Multiple signaling pathways control tuber induction in potato. *Plant Physiology* 119: 1–8.

Kiyota, H., D. Nakashima & T. Oritani, 1999. Synthesis of ( )-methyl tuberonate, a potato tuber-forming substance, and its epimer. *Bioscience Biotechnology Biochemistry* 63: 2110–2117.

Koda, Y., E. Omer, T. Yoshihara, H. Shibata, S. Sakamura & Y. Okazawa, 1988. Isolation of a specific tuber-inducing substance from potato leaves. *Plant Cell Physiology* 29:

1947–1051.

Koda, Y., 1997. Possible involvement of jasmonates in various morphogenic events. *Physiologia Plantarum* 100: 639–646.

Kolomiets, M.V., D.J. Hannapel, H. Chen, M. Tymeson & R.J. Gladon, 2001. Lipoxygenase is involved in the control of potato tuber development. *Plant Cell* 13: 613-626.

Matsura. H., T. Yoshihara, A. Ichihara, Y. Kikuta & Y. Koda, 1993. Tuber-forming substances in Jerusalem artichoke (*Helianthus tuberosus* L.). *Bioscience Biotechnology Biochemistry* 57: 1253–1256.

Miersch, O., G. Schneider & G. Sembdner, 1991. Hydroxylated jasmonic acid and related compounds from *Botryodiplodia theobromae*. *Phytochemistry* 30: 4049–4051.

Mueller, M.J., 1997. Enzymes involved in jasmonic acid biosynthesis. *Physiologia Plantarum* 100: 653–663.

Sembdner, G., A. Meyer, O. Miersch & C. Brückner, 1990. Metabolism of jasmonic acid. In: R.P. Pharis & S.B. Rood (Eds), Plant Growth Subtances. Springer-Verlag, New York, pp. 374–379.

Sembdner, G. & B. Parthier, 1993. The biochemistry and the physiological and molecular actions of jasmonates. *Annual Review of Plant Physiology and Plant Molecular Biology* 44: 569–589.

Simko, I., E.A. Omer, E.E. Ewing, S. Mcmurry, J.L. Koch & P.J. Davies, 1996. Tuberonic (12-OH-JA) acid glucoside and its methyl ester in potato. *Phytochemistry* 43: 727–730.

Ueda, M. & S. Yamamura, 1999. Potassium ß-D-glucopyranosyl 11-hydroxyjasmonate, a leaf-closing substance of *Albizzia julibrissin* Durazz. *Tetrahedron Letters* 40: 7823–7826.

Wasternack, C. & B. Hause, 2002. Jasmonates and octadecanoids - signals in plant stress response and development. In: K. Moldave (Ed.), Progress in Nucleic Acid Research and Molecular Biology. Academic Press New York 72, pp. 165-221.

Yoshihara, T., E. Omer, H. Koshino, S. Sakamura, Y. Kikuta & Y. Koda, 1989. Structure of a tuber-inducing stimulus from potato leaves (*Solanum tuberosum* L.). *Agricultural and Biological Chemistry* 53: 2835–2837.

Yoshihara, T., H. Matsura, A. Ichihara, K. Kikuta & K. Koda, 1992. Tuber forming substances of Jerusalem artichoke (*Helianthus tuberosus* L.). In: C.M. Karssen, L.C. van Loon & D. Vreugdenhil (Eds), Progress in Plant Growth Regulation. Kluwer Academic Publishers, Dordrecht, pp. 286–290.

Yoshihara, T., M. Amanuma, T. Tsutsumi, Y. Okumura, H. Matsuura & A. Ichihara, 1996. Metabolism and transport of [2-14C] (±) jasmonic acid in the potato plant. *Plant Cell Physiology* 37: 586–590.