ACTA AGROBOTANICA
Vol. 47, z. 1 – 1994
89-95

Fatty acid and sterol contents during tulip leaf senescence induced by methyl jasmonate

MARIAN SANIEWSKI, JANUSZ CZAPSKI*, MARCIN HORBOWICZ*

Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland and
*Research Institute of Vegetable Crops, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

(Received: October 10, 1994)

Abstract

It has been shown previously that methyl jasmonate (JA-Me) applied in lanolin paste on the bottom surface of intact tulip leaves causes a rapid and intense its senescence. The aim of this work was to study the effect of JA-Me on free and bound fatty acid and sterol contents during tulip leaf senescence. The main free and bound fatty acids of tulip leaf, in decreasing order of their abundance, were linolenic, linoleic, palmitic, oleic, stearic and myristic acids. Only the content of free linolenic acid decreased after treatment with JA-Me during visible stage of senescence. ß-Sitosterol (highest concentration), campesterol, stigmasterol and cholesterol were identified in tulip leaf. Methyl jasmonate evidently increased the level of ß-sitosterol, campesterol and stigmasterol during induced senescence. It is suggested that the increase in sterol concentrations under the influence of methyl jasmonate induced changes in membrane fluidity and permeability, which may be responsible for senescence.

INTRODUCTION

Jasmonic acid (JA) and methyl jasmonate (JA-Me) are widely distributed in the plant kingdom, showing various biological activities in the regulation of plant growth and development (P a r t h i e r, 1990, 1991; K o d a, 1992; S e m b d n e r and P a r t h i e r, 1993). These compounds are the major representative of a group of native plant bioregulators called jasmonates (S e m b d n e r and P a r t h i e r, 1993). Jasmonic acid is biosynthesized from linolenic acid and the first step of the reaction is catalyzed by lipoxygenase (S e m b d n e r and P a r t h i e r, 1993).

U e d a and K a t o (1980) isolated methyl jasmonate from *Artemisia absinthium* and found that the compound has strong stimulatory effect on oat leaf senescence (chlorophyll degradation) and from that time these compounds (jasmonates) attracted the attention of plant physiologists.

Methyl jasmonate stimulated respiration and proteolytic activity (S a t l e r and T h i m a n n, 1981) and peroxidase activity (W e i d h a s e et al., 1987 a) during leaf senescence. W e i d h a s e et al. (1987) showed that JA-Me caused rapid decrease activity of ribulose-1,5-biphosphate carboxylase and immunoreactive protein content of the enzyme. Further studies of W e i d h a s e et al. (1987) showed that methyl jasmonate-promoted leaf senescence is characterized also by the de novo synthesis of new abundant cytoplasmic polypeptides. JA-Me induced (increased) chlorophyllase activity in cotyledons of cucumber (A b e l e s et al., 1989). C h o u and K a o (1992) suggested that methyl jasmonate-promoted senescence of detached rice leaves may be mediated through blocking the entrance of calcium ions into the cytosol. The results of H e r r m a n n et al. (1989) clearly indicated that JA and JA-Me are capable of affecting gene expression in senescing leaves mainly at the transcriptional level. The senescence symptoms caused by jasmonate can be restored by cytokinins.

It is well known that lipid peroxidation is an inherent feature of senescence and there is increasing evidence that mobilization of fatty acids from membrane phospholipids serves as a source of substrate for lipid peroxidation. In particular, there is a dramatic decline in membrane phospholipid during the early stages of senescence, which becomes manifested as an increased ratio of membrane sterol: fatty acids (T h o m p s o n et al., 1987).

Possibly lipoxygenase plays a role in the regulation of plant senescence, either by a direct mediation of membrane lipid peroxidation, or via the production of jasmonic acid, a senescence promoting factor.

It was shown previously that methyl jasmonate at a concentration of 1.0 % in lanolin paste caused the rapid and strong senescence of the leaves of intact tulip (P u c h a l s k i et al., 1985). The morphological changes indicated by visible yellowing moving upwards from the insertion place were followed by the degradation of chlorophyll a and b (P u c h a l s k i et al., 1985).

The mechanism of action of methyl jasmonate on leaf senescence is still unknown. In this study we examined the effect of methyl jasmonate on fatty acid and sterol contents during induced senescence of tulip leaf.

MATERIAL AND METHODS

The experiments were performed with tulip bulbs cv. Gudoshnik from commercial stocks. After lifting, the bulbs with a circumference 10-12 cm were stored at temperature 18-24°C, until flower differentiation (stage G) was completed. From October 20 the bulbs were transferred into temperature 5°C and stored there for a minimum of 12 weeks. Later, these cooled bulbs were used for planting and they were cultivated in greenhouse at a temperature of about 17°C. Three days before flowering the leaves of intact tulips were treated with methyl jasmonate at a concentration of 1.0 % in lanolin paste (prepared by mixing the lanolin with distilled water

1 : 3). The paste was applied on the bottom surface of the first leaf. The second half of the leaf, treated only with lanolin was used as control tissue. The experiments with methyl jasmonate application were conducted 3 times between January and April. Six days after treatment with JA-Me first symptoms of tulip leaf senescence were well visible.

Three and 6 days after treatment with JA-Me the leaves above the place of application were cut off and frozen at – 20°C. The same procedure was used for control leaf pices. A mixed sample of leaf tissue from 7 plants was prepared from the control and JA-Me treated leaves. Frozen samples were used for analysis of fatty acid and sterol contents.

Samples were weighed and blended with 40 ml 0.05 N NaOH and a few drops of silicon antifoam solution in an Ultra-Turrax tissue grinder at 13 500 rpm. The slurry was filtered with 1 g Celite-545 as a filter aid through Whatman No. 1 paper under reduced pressure. The filtrate was acidified with 1 ml 6 N HCl. After adding 50 µg internal standard (margaric acid), free fatty acids were extracted twice with 50 ml hexane. Combined hexane extracts were dried by passing through a 5 g anhydrous sulphate layer, and then evaporated to dryness on a rotary evaporator. Methyl esters of fatty acids were prepared with boron trifluoride-methanol reagent (M e t c a l f e et al., 1966). Quantitative and qualitative determinations of fatty acid methyl esters were performed by GLC using a Pye Unicam 204 gas chromatograph equipped with a 200 x 0.2 cm column (packed with 10 % Silar 10 C on Chromosorb W, 80/100 mesh) and a flame ionization detector. The column temperature was programmed to increase from 121°C to 210°C at 6°C min^{-1}. The amounts of individual fatty acids were calculated from standard curves of appropriate acid esters.

The total amounts of individual fatty acids were determined after lipid hydrolysis (3 h at 80°C) using 2 ml 5 % KOH in methanol. After cooling and adding 2 ml 10 % NaCl solution, the mixture was extracted twice with 2 ml isooctane. The water fraction contained fatty acid potassium salts while the isooctane layer contained sterols. The total amounts of indivitual fatty acids were then analyzed (after acidification of the water fraction with 6 N HCl to pH = 1) according the procedure for free fatty acids, as described above. Bound fatty acid content was calculated from the total and free fatty acid content by subtraction.

The isooctane fraction containing sterols was dried using 100 mg anhydrous sodium sulphate; after filtration, it was evaporated to dryness at room temperature using a stream of nitrogen. Sterols were silylated (H o r b o w i c z and O b e n d o r f, 1992) and analyzed by GLC, using Hewlett-Packard gas chromatograph model 5890 equipped with HP-1 capillary column (12.5 m x 0.2 mm) and mass selective detector model 5917.

Identification of sterols was achived by camparison retention time and examining in SCAN mode mass spectrum of particular peak with trimethylsilyl (TMS) derivatives of authentic sterol standards (purchased from Larodan Fine Chemicals and Sigma). The mass spectra similarity was expressed as match quality using the probability – based matching algorithm. The mass spectral database of TMS sterol standards was saved in computer in our own created library. Amount of individual

sterols was calculated from standard curve of appropriate standard-TMS derivative measured in the SIM mode using 9 characteristic mass ions (129.10, 255.25, 340.30, 382.35, 396.40, 458.40, 472.45, 484.45, 486.50).

All the results were subjected to an analysis of variance and evaluated using t-test at 5 % level of significance.

RESULTS AND DISCUSSION

The main free and bound fatty acids of tulip leaf, in decreasing order of their abundance, were linolenic, linoleic, palmitic, oleic, stearic and myristic (Tab. 1 and 2). Only the content of free linolenic acid decreased after treatment with JA-Me during visible stage of senescence (Tab. 1). ß-Sitosterol (highest concentration), campesterol, stigmasterol and cholesterol were identified in tulip leaf (Tab. 3). Methyl jasmonate evidently increased the level of ß-sitosterol, campesterol and stigmasterol during induced senescence. It is suggested that the increase in sterol concentrations under the influence of methyl jasmonate induced changes in membrane fluidity and permeability, which may be also responsible for senescence.

Table 1

The effect of methyl jasmonate on the content of some free fatty acids (μg g^{-1} fresh weight) in tulip leaves

Fatty acids	3 days after tratment		6 days after treatment		$LSD_{0.05}$
	Control	JA-Me	Control	JA-Me	
Myristic (C 14 : 0)	8	6	5	4	NS
Palmitic (C 16 : 0)	86a	85a	50b	33b	20
Stearic (C 18 : 0)	8a	4bc	7ab	3c	4
Oleic (C 18 : 1)	14	14	12	15	NS
Linoleic (C 18 : 2)	117a	133a	50b	30b	41
Linolenic (C 18 : 3)	273a	290a	154b	70c	72

Table 2

The effect of methyl jasmonate on the content of some bound fatty acids (μg g^{-1} fresh weight) in tulip leaves

Fatty acids	3 days after tratment		6 days after treatment		$LSD_{0.05}$
	Control	JA-Me	Control	JA-Me	
Myristic (C 14 : 0)	46	40	36	38	NS
Palmitic (C 16 : 0)	934	897	722	760	NS
Stearic (C 18 : 0)	69a	38b	25b	27b	20
Oleic (C 18 : 1)	67a	63a	32b	23b	12
Linoleic (C 18 : 2)	1017ab	1107a	791c	893bc	154
Linolenic (C 18 : 3)	2067	2361	2003	2256	NS

Table 3

The effect of methyl jasmonate on the content of sterols ($\mu g\ g^{-1}$ fresh weight) in tulip leaves

Sterols	3 days after tratment		6 days after treatment	
	Control	JA-Me	Control	JA-Me
ß-Sitosterol	97b	117ab	79b	128a
Stigmasterol	18b	24b	25b	46a
Campesterol	26a	31a	25a	43b
Cholesterol	11a	10a	16a	17a

It is well known that ethylene plays a major role in the senescence processes in most plant organs, mainly in fruits and flowers, and several studies have tried to relate ethylene production to degradation of membrane phospholipids. Generally speaking, the ethylene production of leaves is not a factor in their senescence (T h i m a n n, 1980). Tulip leaf senescence induced by methyl jasmonate did not affect the ethylene production, ACC (1-aminocyclopropane-1-carboxylic acid) content and ACC oxidase activity (P u c h a l s k i et al., 1989). Recently, P o r a t et al. (1993) showed that methyl jasmonate, applied to *Dendrobium* and *Petunia* flowers as a aqueous solution through the cut stem or stigma, or as a vapour, accelerated the senescence and increased ethylene production and ACC content in proportion to the dose of the compound. Authors concluded that JA-Me enhanced *Petunia* and *Dendrobium* flower senescence via the promotion of ACC and ethylene production.

It was found that JA-Me stimulated the activity of two isoforms of superoxide dismutase in tulip leaf (P u c h a l s k i et al., 1989). The senescence process induced in tulip leaf by JA-Me is not connected with the activity of superoxide dismutase since the activity of superoxide dismutase declines as leaf tissue naturally senesces (T h o m p s o n et al., 1987). The enzyme, superoxide dismutase, claims the superoxide radical ($0_2^{\cdot-}$) by catalyzing its dismutation to hydrogen peroxide and oxygen. It is well known that initiation of lipid peroxidation can be induced by free radicals ($0_2^{\cdot-}$, $\cdot OH$) or singlet oxygen (T h o m p s o n et al., 1987).

N i i n o m i i et al. (1987) suggest that lipid peroxidation by the [peroxidase/H_20_2/phenolic] system might play also important role in leaf senescence, however the role of methyl jasmonate in the process was not investigated yet.

The mechanism of induction of tulip senescence by methyl jasmonate will be further studied.

Acknowledgements

We wish to thank Dr. E. D e m o l e, Firmenich SA, Switzerland, for the gift of authentic (±)-methyl jasmonate. This work was support by a Grant No. 5 5212 92 03 from State Committee for Scientific Research (Poland).

REFERENCES

Abeles F. B., Hershberger W. L., Dunn L. J., 1989. Hormonal regulation, and intracellular localization of a 33-kD cationic peroxidase in excised cucumber cotyledons. Plant Physiol. 89: 664-668.

Chou C. M., Kao C. H., 1992. Methyl jasmonate, calcium, and leaf senescence in rice. Plant Physiol. 99: 1693-1694.

Herrmann G., Lehmann J., Peterson A., Sembdner G., Weidhase R., Parthier B., 1989. Species and tissue specificity of jasmonate-induced abundant proteins. J. Plant Physiol. 134: 703-709.

Horbowicz M., Obendorf R. L., 1992. Changes in sterols and fatty acids of buckwheat endosperm and embryo during seed development. J. Agric. Food Chem. 40: 745-750.

Koda Y., 1992. The role of jasmonic acid and related compounds in the regulation of plant development. Inter. Rev. Cytol. 135: 155-198.

Metcalfe L. D., Schmitz A. A., Pelka J. R., 1966. Rapid preparation of fatty acid esters from lipids for gas chromatography analysis. Anal. Chem. 38: 514-517.

Niinomi A., Morimoto M., Shimizu S., 1987. Lipid peroxidation by the [peroxidase/H_2O_2/phenolic] system. Plant Cell Physiol. 28: 731-734.

Parthier B., 1990. Jasmonates. hormonal regulators of stress factors in leaf senescence ? J. Plant Growth Regul. 9: 57-63.

Parthier B., 1991. Jasmonates, new regulators of plant growth and development: many facts and few hypotheses on their actions. Bot. Acta 104: 446-454.

Porat R., Borochov A., Halevy A. H., 1993. Enhancement of petunia and dendrobium flower senescence by jasmonic acid methyl ester is via the promotion of ethylene production. Plant Growth Regul. 13: 297-301.

Puchalski J., Klim P., Saniewski M., Nowacki J., 1989. Studies of some physiological processes during tulip leaf senescence induced by methyl jasmonate. Acta Hortic. 25: 107-114.

Puchalski J., Saniewski M., Klim P., 1985. The effect of methyl jasmonate on tulip leaf senescence and peroxidase patterns. Acta Hortic. 167: 247-257.

Satler S. O., Thimann K. V., 1981. Le jasmonate de methyle: nouveau et puissant promoteur de la senescence de feuilles. C.R. Acad. Sci. Paris. Ser. III 293: 735-740.

Sembdner D., Parthier B., 1993. The biochemistry and the physiological and molecular actions of jasmonates. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44: 569-589.

Thimann K. V., 1980. The senescence of leaves. [In:] Senescence in Plants, Ed. by K.V. Thimann, CRC Press, Inc., Boca Raton, Florida, pp. 85-115.

Thompson J. E., Legge R. L., Barber R. F., 1987. The role of free radicals in senescence and wounding. New Phytol. 105: 317-344.

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

Weidhase R. A., Kramell H. -M., Lehmann J., Liebisch H. -W., Lerbs W., Parthier B., 1987. Methyl jasmonate-induced changes in the polypeptide pattern of senescing barley leaf segments. Plant Sci. 51: 177-186.

Weidhase R. A., Lehmann J., Kramell H., Sembdner G., Parthier B., 1987 a. Degradation of ribulose-1,5-bisphosphate carboxylase and chlorophyll in senescing barley leaf segments triggered by jasmonic acid methyl ester, and counteraction by cytokinin. Physiol. Plant. 69: 161-166.

Zawartość kwasów tłuszczowych i steroli w czasie indukowanego starzenia się liści tulipana przez ester metylowy kwasu jasmonowego

Streszczenie

Wykazano poprzednio, że ester metylowy kwasu jasmonowego (JA-Me) podany w paście lanolinowej na dolną powierzchnię liści tulipana powodował szybkie i intensywne ich starzenie się. Celem tej pracy było zbadanie wpływu JA-Me na zawartość wolnych i związanych kwasów tłuszczowych i steroli w czasie

indukowanego starzenia się . Głównymi wolnymi związanymi kwasami tłuszczowymi w liściach tulipana, w kolejności zmniejszającej się ich zawartości, były kwasy: linolenowy, linolowy, palmitynowy, olejowy, stearynowy i mirystynowy. Tylko zawartość wolnego kwasu linolenowego uległa obniżeniu po traktowaniu liści JA-Me w czasie widocznego starzenia się. ß-Sitosterol (najwyższe stężenie), kampesterol, stigmasterol i cholesterol zostały zidentyfikowane w liściach tulipana. JA-Me zasadniczo wpływa na wzrost zawartości ß-sitosterolu, kampesterolu i stigmasterolu w czasie indukowanego starzenia.

Sugeruje się, że wzrost zawartości steroli pod wpływem estru metylowego kwasu jasmonowego w liściach tulipana może indukować zmiany w przepuszczalności i płynności membran, a zmiany te mogą być odpowiedzialne za indukcję starzenia się liści.