BIOLOGIA PLANTARUM 34 (1-2): 71-76, 1992

The effect of methyl jasmonate on free fatty acids content in ripening tomato fruits

J. CZAPSKI*, M. HORBOWICZ* and M. SANIEWSKI**

*Research Institute of Vegetable Crops, 22 Lipca 3/5, 96-100 Skierniewice, Poland**
*Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland***

Abstract

The effect of methyl jasmonate (JA-Me), applied to mature green tomato fruits cv. Modena, on the content of some fatty acids in ripe fruits was studied. Methyl jasmonate greatly increased content of linolenic acid and in the lesser degree decreased the amount of linoleic acid. The ratio of linolenic acid to linoleic acid content increased 4.5 - 7.7 times in methyl jasmonate treated samples in comparison to untreated-controls. JA-Me did not affect the contents of lauric, myristic, palmitic, stearic, palmitoleic and oleic acids.

Introduction

Jasmonic acid (JA) and methyl jasmonate (JA-Me) are known to be widespread native plant compounds (Meyer *et al.* 1984, Sembdner and Gross 1986), the biosynthesis of which comes from linolenic acid (Vick and Zimmerman 1984). The complete reaction sequence of jasmonic acid biosynthesis was presented by Vick and Zimmerman (1984) and the first step of the reaction is catalyzed by lipoxygenase.

It has been found that methyl jasmonate strongly influences some physiological processes in tomato fruit: the inhibition of lycopene and stimulation of β-carotene accumulation (Saniewski and Czapski 1983, Czapski and Saniewski 1985), the stimulation of chlorophyll degradation (Saniewski *et al.* 1987a), the stimulation of ethylene production, mostly through enhancement of the activity of ethylene-forming enzyme (Saniewski and Czapski 1985, Saniewski *et al.* 1987b,c), the inhibition of polygalacturonase activity (Saniewski *et al.* 1987a), the stimulation of polyphenol oxidase and inhibition of peroxidase activity (Czapski and Saniewski 1988), and the decrease in tocopherols content (Czapski *et al.* 1991).

In the present work the effect of methyl jasmonate on some free fatty acids content in ripening tomato fruits is documented.

Received 10 September 1990, *accepted* 1 February 1991.
Acknowledgements: We wish to thank Dr. E.Demole, Firmenich SA, Geneva, Switzerland, for the gift of authentic (±)-methyl jasmonate.

Material and methods

Mature green tomato (*Lycopersicon esculentum* L. cv. Modena) were used. The experiments were made between July and September, 1989. Fruits were harvested and treated with (±)-methyl jasmonate at a concentration of 1 % m/m in lanolin paste. This was applied on one side of the fruit over an area of 5.0 cm^2. As a control, the other side was treated with lanolin paste without JA-Me. Fruits were kept at about 20 °C under natural light and then they were taken for analysis after 7, 11, 12 and 15 d in ripe stage. Skin with 2-3 mm pericarp tissue were cut off from an area of about 4.0 cm^2 from control - untreated tissue (CU), control - treated with lanolin only (CL), and JA-Me treated tissues (JA-Me), and frozen at -20 °C until analysis. Each experiment was performed in triplicate.

Frozen samples were weighed and mixed with 40 ml of 0.05 M NaOH and with few drops of silicon antifoam solution, in an *Ultra-Turax* tissue grinder at 13500 rpm. The slurry was filtered with 1 g *Celite-545* as a filter aid through *Whatman No 1* paper in a Büchner funnel under reduced pressure. Then filtrate was acidified with 1 cm^3 of 6 M HCl. After adding of 50 μg of internal standard (margarinic acid) free fatty acids were extracted twice with 50 cm^3 of hexane. After each shaking step the mixture was filtered through *ca.* 1 g of *Celite-545* for better separation of hexane and water layers. Combined hexane extracts were dried by passing through 5 g anhydrous sodium sulphate layer, and then evaporated to dryness on rotary evaporator. Methyl esters of fatty acids were prepared by boron-trifluoride methanol (Metcalfe *et al.* 1966). Quantitative and qualitative determinations of fatty acid methyl esters were performed by GLC using *Pye Unicam 204* gas chromatograph equipped with 200 × 0.2 cm column (packed with 10 % *Silar 10C* on *Chromosorb W* 80/100 mesh) and flame ionization detector. The column temperature was programmed from 121 °C up to 210 °C at temperature increasing 6 °C min^{-1}. Argon was used as a carrier gas. Identification was made by comparison of retention times with those of authentic fatty acid standards. Amount of individual fatty acids were calculated from standard curves of appropriate acid esters. The data were treated with analysis of variance and evaluated using *t*-Duncan test at a 5 % level of confidence.

Results and discussion

The experiments confirmed our previous observations that green tomatoes treated with methyl jasmonate developed a yellow colour, while the control untreated area was red. It is due to the inhibition of lycopene and stimulation of β-carotene accumulation (Saniewski and Czapski 1983, Czapski and Saniewski 1985). Methyl jasmonate greatly increased content of linolenic acid (Fig. 1). Amount of linolenic acid during ripening period in methyl jasmonate treated tomatoes increased more than 2.8 times compared with controls. The content of linolenic acid in untreated mature green tomato fruit was on the level of corresponding red fruit (Fig. 1).

The effect of methyl jasmonate on linoleic acid content was less evident than that on linolenic acid (Fig. 2). JA-Me treated tomato fruit had about 65 - 36 % less linoleic acid than that in control fruit treated lanolin paste only. Amount of linoleic acid in untreated mature green tomato fruit was about 2-fold higher than that in red fruits.

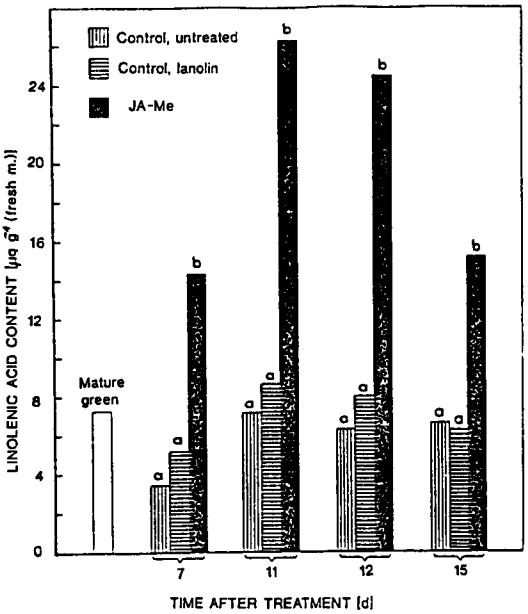

Fig. 1. The effect of methyl jasmonate (JA-Me), applied to mature green tomatoes, on the content of linolenic acid ($C_{18:3}$), analyzed in ripe stage after different time from treatment. Means followed by the same letter do not differ at the 5 % level of significance using *t*-Duncan test.

Table 1. The effect of methyl jasmonate (JA-Me), applied to mature green tomatoes, on the content of some fatty acids [μg kg^{-1}(fresh mass)], analyzed in ripe stage after different time from treatment. Control fruit were treated with lanolin paste without JA-Me (CL) or untreated (CU).

Fatty acids	Mature green	Time after treatment [d] 7 CU	CL	JA-Me	11 CU	CL	JA-Me	12 CU	CL	JA-Me	15 CU	CL	JA-Me
Lauric	127	123	137	127	213	223	257	120	120	187	177	203	180
Myristic	810	790	887	713	1140	1070	1570	567	740	963	767	887	867
Palmitic	9610	6580	7830	6680	11000	10660	14350	6820	8510	11380	8740	8910	9250
Stearic	2790	2400	2940	2340	3240	3380	4270	2070	2860	3400	2750	3130	3290
Palmitol	2680	2580	2600	1860	3750	3130	5690	1650	2370	3400	1500	1390	1620
Oleic	10090	7400	8170	6700	11280	10630	15370	6740	8610	11320	11130	10150	8770

Means of both controls and JA-Me treatment do not differ significantly at 5 % level in *t*-Duncan test.

The ratio of linolenic to linoleic acid contents which are two of three major unsaturated fatty acids in tomato fruits (Jadhav *et al.* 1972) increased 4.5 - 7.7 fold after JA-Me treatment as compared to the proportions of untreated fruits being 1.0 in red ripe or 0.5 in mature green fruit, respectively (Fig. 3). Worthy of note is the 2-fold increase in the linolenic/linoleic acid ratio in red tomatoes as compared to mature green ones.

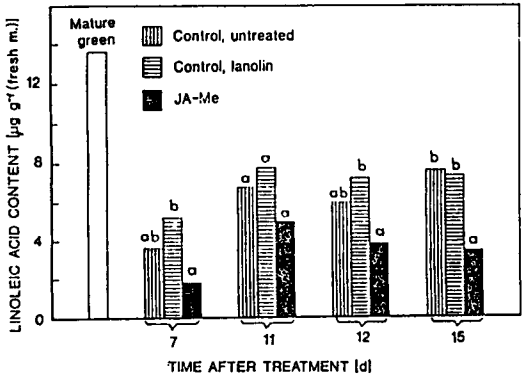

Fig. 2. The effect of methyl jasmonate (JA-Me), applied to mature green tomatoes, on the content of linoleic acid ($C_{18:2}$), analyzed in ripe stage after different time from treatment. Means followed by the same letter do not differ at the 5 % level of significance using *t*-Duncan test.

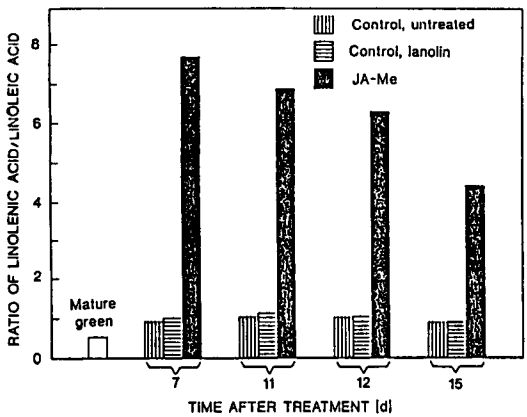

Fig. 3. The effect of methyl jasmonate (JA-Me), applied to mature green tomatoes, on the ratio of linolenic to linoleic acid content, analyzed in ripe stage.

The fatty acids composition in mature green and red ripe tomatoes is presented on Table 1. Oleic and palmitic acids as well as linolenic and linoleic acids (Figs. 1, 2) were shown to be principal fatty acids in tomatoes. Methyl jasmonate applied to mature green tomato fruit did not affect significantly the lauric, myristic, palmitic, stearic, palmitoleic and oleic acid contents determined in ripe fruit (Table 1).

The principal substrates for lipoxygenase activity in higher plants are linolenic and linoleic acids (Hildebrand 1989). It is known that lipoxygenase is involved in the first step of biosynthesis of jasmonic acid from linolenic acid (Vick and Zimmerman 1984, Hildebrand 1989). Lipoxygenase activity greatly increased during ripening of tomatoes and consequently its activity is higher in the ripe fruit than in mature green one (Jadhav *et al.* 1972, Sekiya *et al.* 1983). The great increase of the amount of linolenic acid in tomato tissues treated with JA-Me may be explained through inhibitory effect of JA-Me on lipoxygenase activity (feed back) and/or through stimulation of synthesis of linolenic acid from linoleic acid which amount decreases in methyl jasmonate treated tissues. The synthesis of linolenic from linoleic acid is catalyzed by linoleoyl desaturase, but information concerning the desaturation of linoleate to linolenate is currently very limited (Wang and Hildebrand 1988). It was also demonstrated that linoleic and linolenic acids are the precursors of carbonyls in which hexanal is one of the predominant aldehydes of tomato volatiles (Dalal *et al.* 1968, Jadhav *et al.* 1972). Kazeniac and Hall (1970) implicated lipoxygenase as the enzyme involved in the chain of reactions leading to hexanal formation.

The effect of methyl jasmonate on lipoxygenase activity during ripening of tomato fruits will be subject of further study.

References

Czapski, J., Horbowicz, M., Saniewski, M.: The effect of methyl jasmonate on tocopherols content in ripening tomato fruit. - Bull. Pol. Acad. Biol. **39**: 41-45, 1991.

Czapski, J., Saniewski, M.: Effect of methyl jasmonate on carotenoids in tomato fruits. Gartenbauwissenschaft **50**: 35-37, 1985.

Czapski, J., Saniewski, M.: The effect of methyl jasmonate on polyphenol oxidase and peroxidase activities in tomato fruit. - Bull. Pol. Acad. Biol. **36**: 127-132, 1988.

Dalal, K.B., Salunkhe, K.D., Olsen, L.E., Do, J.Y., Yu, M.H.: Volatile components of developing tomato fruit grown under field and greenhouse conditions. - Plant Cell Physiol. **9**: 389-400, 1968.

Hildebrand, D.F.: Lipoxygenases. - Physiol. Plant. **76**: 249-253, 1989.

Jadhav, S., Singh, B., Salunkhe, D.K.: Metabolism of unsaturated fatty acids in tomato fruit: linoleic and linolenic acid as precursors of hexanal. - Plant Cell Physiol. **13**: 449-459, 1972.

Kazeniac, S.J., Hall, R.M.: Flavor chemistry of tomato volatiles. - J. Food Sci. **35**: 519-530, 1970.

Meyer, A., Miersch, O., Büttner, C., Dathe, W., Sembdner, G.: Occurrence of the plant growth regulator jasmonic acid in plants. - J. Plant Growth Regul. **3**: 1-8, 1984.

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

Saniewski, M., Czapski, J.: The effect of methyl jasmonate on lycopene and β-carotene accumulation in ripening red tomatoes. - Experientia **39**: 1373-1374, 1983.

Saniewski, M., Czapski, J.: Stimulatory effect of methyl jasmonate on ethylene production in tomato fruits. - Experientia **41**: 256-257, 1985.

Saniewski, M., Czapski, J., Nowacki, J.: Relationship between stimulatory effect of methyl jasmonate on ethylene and l-aminocyclopropane-l-carboxylic acid content in tomatoes. - Biol. Plant. **29**: 17-21, 1987b.

Saniewski, M., Nowacki, J., Czapski, J.: The effect of methyl jasmonate on ethylene production and ethylene-forming enzyme activity in tomatoes. - J. Plant Physiol. **129**: 175-180, 1987c.

Saniewski, M., Urbanek, H., Czapski, J.: Effects of methyl jasmonate on ethylene production, chlorophyll degradation, and polygalacturonase activity in tomatoes. - J. Plant Physiol. **127**: 177-181, 1987a.

Sekiya, J., Kajiwara, T., Munechika, K., Hatanaka, A.: Distribution of lipoxygenase and hydroperoxide lyase in the leaves of various plant species. - Phytochemistry **22**: 1867-1869, 1983.

Sembdner, D., Gross, D.: Plant growth substances of plant and microbial origin. - In: Bopp, M. (ed.): Plant Growth Substances 1985. Pp. 139-147. Springer-Verlag, Berlin - Heidelberg - New York - Tokyo, 1986.

Vick, B.A., Zimmerman, D.C.: Biosynthesis of jasmonic acid by several plant species. - Plant Physiol. **75**: 458-461, 1984.

Wang, X.M., Hildebrand, D.F.: Biosynthesis and regulation of linolenic acid in higher plants. - Plant Physiol. Biochem. **26**: 777-792, 1988.