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THE CHANGES IN FATTY ACID PROFILE DURING SENESCENCE AND METHYL JASMONATE-INDUCED SENESCENCE OF *Ginkgo biloba* LEAVES

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ABSTRACT

The present study describes changes in fatty acid (FA) composition in *Ginkgo biloba* leaves subjected to senescence and to senescence induced by methyl jasmonate (MeJA). Green leaves were treated with MeJA in lanoline on the abaxial or adaxial side of the leaf blades. After three weeks of treatment, leaf blades and petioles were collected separately for FA analyses. Additionally, petioles and leaf blades were sampled for analyses before the experiment and after 6 weeks when leaf senescence was occurring. Linolenic (C18:3) and palmitic (C16:0) acids appeared to be the quantitatively most abundant FA in leaf blades and petioles of *G. biloba*. Both leaf senescence and that induced by MeJA caused a decrease in unsaturated FA content, especially linolenic (C18:3). However, the decrease in C18:3 acid in both leaf blades and petioles was greater when MeJA was applied to the abaxial side than when it was applied to the adaxial side of leaves or during senescence. At the same time, saturated FA content increased, resulting in a significant decrease in the ratio of total unsaturated to saturated FA. Since leaf fatty acids occur mainly as components of cell membranes, changes in their composition may have a crucial effect on membrane function and stability, as pointed out in the discussion of the results.

Key words: *Ginkgo biloba*, fatty acids, composition, methyl jasmonate, induced senescence, natural senescence

INTRODUCTION

Ginkgo biloba (L.) is currently the only naturally occurring species in the Ginkgoaceae family. It is also one of the most commercialized medicinal plants, as preparations produced from its leaves are currently the best-selling herbal products. *G. biloba* leaves contain unique terpene trilactones, bilobalides and ginkgolic acids as well as flavonoids, phenolic acids and other health-promoting compounds and the biological effect is due to the synergistic action of these compounds [van Beek and Montoro 2009]. Leaf extracts from *G. biloba* are popular herbal remedies for the treatment of Alzheimer's dementia, tinnitus, peripheral arterial disease and increase blood circulation [van Beek and Montoro 2009, Horbowicz et al. 2021].

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Leaf senescence is considered the last stage of leaf development that ultimately leads to its death [Lim et al. 2007]. During this process, nutrients released from aging leaves are accumulated in other organs to ensure successful growth the following season in perennial plants [Thomas 2013]. Among phytohormones cytokinins, auxin, and gibberellins delay leaf senescence, whereas ethylene, jasmonic acid abscisic acid accelerate this process [Jibran et al. 2013, Guo et al. 2021]. The process of leaf senescence is a coordinated action of plants regulated by hundreds of genes whose transcripts increase as the process progresses [Liu et al. 2008, Li et al. 2020, Saniewski et al. 2020]. Leaf senescence can be induced by many external biotic and abiotic factors as well as internal factors [Zhang and Zhou 2013, Jibran et al. 2013]. Detailed overview on the mechanisms and consequences of senescence have been presented in several papers [Wojciechowska et al. 2018].

An important phenomenon that occurs during leaf senescence is the degradation of membrane lipids. This disrupts the structural and functional integrity of cell membranes and is a major factor contributing to the senescence process [MacDonald et al. 2019]. Particularly important are the changes occurring in the membranes of chloroplasts because they affect the basis of photosynthesis. During *G. biloba* leaf senescence, the thylakoid membranes disrupted and chloroplasts were completely disintegrated [Shi et al. 2012].

Jasmonic acid (JA) and its derivatives, collectively known as jasmonates, are cyclopentanone derivatives whose biosynthesis begins with the oxidation of α-linolenic acid or 16:3 fatty acid. This reaction leads to the formation of the intermediate metabolite 12-oxophytodienoic acid (OPDA) [Wasternack and Strnad 2018]. The active form of JA is its conjugate with isoleucine. Other active JA metabolites include volatile signaling molecules, methyl JA (Me-JA) and *cis*-jasmone, which act as signaling molecules between plants by activating protein-coding defense genes and biosynthesis of various secondary compounds [Griffiths 2020].

Ueda and Kato [1981] were the first to show that jasmonic acid methyl ester (MeJA) strongly stimulated chlorophyll degradation and senescence in oat leaves. Methyl jasmonate (Me-JA) is formed by donating a methyl group from *S*-adenosyl-L-methionine to the JA molecule, using an appropriate carboxyl methyltransferase. Me-JA is volatile and is secreted from plant leaves during herbivore attack. It is a signaling compound detected by plants in the vicinity alerting them to the threat [Wasternack and Strnad 2018, Griffiths 2020]. Many original and review papers have been published on the biosynthesis, metabolism and role of jasmonates in plants [Wasternack and Hause 2018, Oliw and Hamberg 2019, Ghorbel et al. 2021].

JAs are well known as a stress hormones playing a key role in many other plant physiological and biochemical processes [Ali and Baek 2020]. It is likely that increased MeJA biosynthesis may be a response to abscisic acid (ABA) which leads to the release of C18:3 from membrane phospholipids and galactolipids [Wang et al. 2018]. In the MeJA treated plants, a genes involved in the crosstalk between the JA and ABA signaling pathways to regulate metabolism and growth were found to be up-regulated [Lackman et al. 2011]. Also several studies provide evidence for positive interactions between the jasmonates and ethylene signaling pathways, especially regarding the regulation of defense-related genes [Wasternack and Strnad 2018, Griffiths 2020].

The presence of malondialdehyde (MDA) is the result of the peroxidation of unsaturated fatty acids. Reactive oxygen species (ROS) can attack membrane lipids and generate MDA [Mittler 2002]. MDA is considered a marker of membrane lipid peroxidation because the decrease in membrane stability reflects the degree of lipid peroxidation caused by ROS. The increase in the level of lipid peroxidation preceded senescence and MeJA and ABA, were found to promote senescence and increase this process [Hung and Kao 1997].

On the other hand, MeJA positively influences the ability of plants to cope with stresses by activating defense enzymes, maintaining the integrity of cell membranes, activating the antioxidant system and inducing the accumulation of defense compounds [Yu et al. 2018]. Therefore, a reduction in membrane lipid peroxidation and MDA content was observed in some plants treated with MeJA during drought stress [Anjum et al. 2011].

Mature leaves of *Arabidopsis thaliana* degrade faster than young leaves treated with MeJA which induces onset of senescence [Jibran et al. 2013]. A high level of lipid remodeling in plant membranes in re-

sponse to environmental conditions such as temperature stress, salinity or drought has been demonstrated, and lipid composition analysis can be used to assess the physiological status of the plant [Reszczyńska and Hanaka 2020].

According to our previous study JA-Me clearly induced senescence of *G. biloba* leaves [Saniewski et al. 2020]. The experiment showed that MeJA applied to the abaxial side of *G. biloba* leaves increased the content of endogenous MeJA in the leaf blade about 30-fold compared with the control. Also, ABA content markedly increased during senescence and MeJA-induced leaf senescence.

The aim of the present study was to investigate how senescence of *Ginkgo biloba* leaves induced by MeJA affects the fatty acid profile, as well as rate of their oxidation by malondialdehyde content. The results were compared with those obtained for leaf senescence (yellowing). Analyses of the effect of MeJA were carried out separately for leaf blades and petioles, following the application of MeJA in lanolin paste placed on the abaxial or adaxial side of leaf blades.

MATERIALS AND METHODS

Plant material. Twelve-year-old *G. biloba* tree grown in Skierniewice, Poland (51°58'29.5"N, 20°09'05.1"E) was used in this study. Green leaves (ca. 30) were treated with methyl jasmonate (MeJA) at a concentration of 0.5% (w/w) in lanolin paste containing 30% water (w/w). The dimensions of the fully developed leaves used in analyses were: width 73 ±8 mm and height 45 ±5 mm, while the length of the petioles was 48 ±8 mm. MeJA was applied as a 2–3-mm-wide strip in the middle of the leaf across the vein on the adaxial and/or abaxial side of the leaf. Treatments were performed on September 9, 2017 on leaves grown on different branches of one tree. Lanolin was applied in the same way as a control on another set of leaves on the same branches of the tree. Three weeks after treatment, leaves treated lanolin with or without MeJA were harvested and subjected to analyses. Leaves collected were randomized and divided into three sub-samples. Only leaf fragments that were not coated with lanolin or lanolin containing MeJA were used for analysis. Leaf fragments coated with lanolin or lanolin containing MeJA were discarded. Additionally, petioles were analyzed after cutting them from the same leaves from which leaf blades. For comparison with MeJA-induced senescence, green leaf blades and petioles at the beginning of the experiment (September 9) as well as after senescence (October 20) were also analyzed. Neither lanolin nor MeJA in lanolin was used in these leaves. More details of the experiment, including weather conditions at that time, were described in our earlier papers [Saniewski et al. 2020, Horbowicz et al. 2021].

Analysis of fatty acids composition. Fatty acids were extracted with potassium hydroxide-methanol solution, which simultaneously combined extraction and saponification steps [Horbowicz and Obendorf 1992]. Briefly, samples (100 mg) of freeze-dried and pulverized plant tissues were homogenized in a mortar with 0.1 M potassium hydroxide in methanol (2 mL). After grinding the mixture was heated for 2 h at 80°C (heating block, Lab-Line Instruments, Tripunithura, India) to facilitate extraction and saponification. After the mixture cooled, 2 mL saturated solution of sodium chloride was added, and the mixture was extracted twice with 2 mL of hexane. The hexane and methanol-water layers were separated by centrifugation at 1000 g for 5 min, and the hexane fraction containing unsaponified compounds was discarded. The methanol-water layer contained fatty acid potassium salts was acidified with concentrated hydrochloric acid (1 mL), and extracted twice, each time with 1 mL hexane. Hexane fractions were pooled, and hexane was evaporated in a stream of cold nitrogen. Then the fatty acids were then methylated with a mixture (2 mL) of methanol, toluene, and sulfuric acid (80 : 20 : 2, v/v/v). The toluene layer containing fatty acids methyl esters (FAME), was used for analysis on a gas chromatograph. Potassium hydroxide, toluene, sulphuric acid, hydrochloric acid and sodium chloride were purchased from POCH (Gliwice, Poland), while methanol and hexane from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA), respectively.

For analysis of FAME, a Shimadzu GC 2010 Pro Gas Chromatograph (Kyoto, Japan) with a flame ionization detector (FID) was used. FAME were analyzed on a Fused Silica Carbowax Column, 30 m × 0.25 mm × 0.25 μm (Quadrex, Woodbridge, CT, USA). Separation and detection were performed under the following conditions: column oven temperature was raised

from 80 to 230°C at a heating rate of 4°C min^{-1} and then held isothermally at 230°C; injector temperature was 220°C, detector 250°C and injection volume 1 µL (split mode). The carrier gas was helium (He) at a total flow rate of 12 mL min^{-1}. The detector gases were hydrogen at 40 mL min^{-1}, air at 400 mL min^{-1}, and nitrogen (auxiliary gas) at 30 mL min^{-1}. Identification of the FAME was carried out using a retention times of standard mixture of methyl esters (Supelco 37 Component FAME Mix, Sigma-Aldrich, St. Louis, MO, USA). The content of each of fatty acid was expressed as % of total fatty acids content. The limit of quantification of FA was established at 0.2%.

For identification of the FAME a gas chromatograph coupled to a quadrupole mass spectrometry analyzer (GC/MS-QP2010 Plus, Shimadzu, Kyoto, Japan) equipped with a ZEBRON ZB5-MSi Guardian column (length of 30 m, ø 0.25 mm, and 0.25 µm film thickness Phenomenex, Torrance, CA, USA) was used. The samples were loaded onto the column using the split method (10 : 1); the injector temperature was 230°C. Helium at a flow rate of 1.19 mL min^{-1} was used as a carrier gas. The column oven was operated at a programmed temperature: initial temperature of 70°C adjusted to 130°C at 20°C min^{-1}, then adjusted to 210°C at 8°C min^{-1}, adjusted to 220°C at 3°C min^{-1}, adjusted to 300°C at 10°C min^{-1}, and (held for 3 min) and finally adjusted to 335°C, and held for 13 min. The temperature of the interface was 280°C, the ion source temperature was 250°C, and 0.8 kV electron ionization was used. The spectra were scanned in a range from 50 to 600 *m/z*, at a rate of 0.5/sec. FAME as well as other compounds were identified by comparison mass spectra of original standards as well as mass spectra from the NIST 05 library (National Institute of Standards and Technology, Gaithersburg, MD, USA).

Analysis of lipid peroxidation (MDA). The analysis was performed according to the method developed by Hodges et al. [1999]. Samples (25 mg) were homogenized with 4 mL of 80% ethanol and centrifuged for 10 min (3000 g). To 1 mL of the supernatant, 3 mL of 0.01% butylated hydroxytoluene (BHT) in 0.1% trichloroacetic acid (TCA) was added. Then, 1.5 mL of extract was then taken and 1.5 mL of 0.5% TBA in 20% TCA or 1.5 mL of 20% TCA was added for negative samples. BHT, TCA, and ethanol were purchased from POCH (Gliwice, Poland). Samples were incubated for 25 min at 95°C in a heating block and then rapidly cooled on ice. After cooling, samples were centrifuged 20 min (3000 g) and extinction was measured at wavelengths: 440 nm, 532 nm, 600 nm. Malondialdehyde (MDA) content was calculated using the appropriate formula.

Statistics. Analysis of variance and Tukey's post hoc test were used to check the significance of differences. Calculations were performed using Statistica 12PL software (StatSoft, Tulsa, OK, USA). Results presented as means marked with the same letter were not significantly different at $P < 0.05$ (Tukey's post hoc test). Comparisons were made within each fatty acid and analysed tissues separately.

RESULTS

In leaf blade and petiole tissues of *G. biloba*, 14 fatty acids (FA) were identified and assayed. Of these, there were 7 unsaturated, 2 monounsaturated and 5 polyunsaturated FA. The unsaturated FA were butyric acid (4:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0) behenic (C22:0), and lignoceric (C24:0). Monounsaturated FA were heptadecenoic (C17:1) and oleic (C18:1), while polyunsaturated were linoleic (C18:2), linolenic (C18:3), eicosadienoic acid (C20:2), arachidonic (C20:4) and docosadienoic acid (C22:2) (Tab. 1 and 2). Due to the presence on the chromatograms of several compounds whose retention times were not fully compatible with the standards, their identification was performed using a gas chromatograph coupled to a quadrupole mass spectrometry analyzer. This analysis revealed the presence of (Z)-3-(heptadec-10-en-1-yl)phenol (cardanol C17:1; 96%) and (Z)-3-(pentadec-8-en-1-yl)phenol (cardanol C15:1, 97%). The presence of 22-hydroxy-docosanoic acid methyl ester (89%) and hexadecanedioic acid dimethyl ester (94%) was also demonstrated. The probabilities of the presence of these compounds were confirmed using the NIST 05 database. Besides, GC/MS analysis showed the absence of eicosapentaenoic acid (C20:5) methyl

Table 1. Changes in the percentage composition of fatty acids in *Ginkgo biloba* leaf blades during senescence and methyl jasmonate-induced senescence. Mean results ±SD in rows followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test. Limit of quantification = 0.2%

Fatty acid	Treatment day (September 9)	Control (September 30)	Adaxial treatment (September 30)	Abaxial treatment (September 30)	Senesced (October 20)
Saturated fatty acids					
C4:0	1.4 ±0.2 ^{ab}	1.0 ±0.2 ^{ab}	0.7 ±0.1 ^{b}	1.6 ±0.1 ^{a}	2.3 ±0.2 ^{a}
C14:0	1.3 ±0.1 ^{c}	1.9 ±0.1 ^{b}	3.3 ±0.2 ^{a}	3.3 ±0.2 ^{a}	3.2 ±0.1 ^{a}
C16:0	21.3 ±2.0 ^{a}	20.5 ±0.7 ^{a}	17.8 ±0.5 ^{a}	18.7 ±0.3 ^{a}	20.6 ±0.5 ^{a}
C18:0	1.1 ±0.2 ^{c}	1.1 ±0.1 ^{c}	1.8 ±0.1 ^{b}	3.7 ±0.2 ^{a}	1.4 ±0.1 ^{c}
C20:0	<0.2	0.7 ±0.1 ^{d}	6.8 ±0.2 ^{b}	17.1 ±0.8 ^{a}	1.3 ±0.1 ^{c}
C22:0	<0.2	0.5 ±0.1 ^{b}	0.6 ±0.1 ^{b}	0.8 ±0.2 ^{b}	2.1 ±0.1 ^{a}
C24:0	4.2 ±0.2 ^{c}	3.5 ±0.4 ^{c}	5.9 ±0.1 ^{b}	7.1 ±0.3 ^{a}	5.9 ±0.1 ^{b}
Unsaturated fatty acids					
C17:1	4.8 ±0.1 ^{d}	5.3 ±0.2 ^{c}	4.4 ±0.1 ^{d}	6.6 ±0.3 ^{ab}	5.7 ±0.2 ^{bc}
C18:1	5.9 ±0.3 ^{a}	6.0 ±0.5 ^{a}	5.8 ±0.1 ^{a}	4.1 ±0.1 ^{b}	5.7 ±0.2 ^{a}
C18:2	5.8 ±0.3 ^{a}	5.3 ±0.2 ^{a}	5.6 ±0.1 ^{a}	5.5 ±0.2 ^{a}	3.3 ±0.1 ^{b}
C18:3	44.4 ±1.7 ^{ab}	45.1 ±1.4 ^{a}	38.6 ±0.6 ^{b}	25.5 ±0.1 ^{d}	33.8 ±0.9 ^{c}
C20:2	2.1 ±0.3 ^{a}	1.8 ±0.1 ^{a}	1.6 ±0.1 ^{a}	2.1 ±0.4 ^{a}	1.7 ±0.1 ^{a}
C20:4	2.2 ±0.2 ^{ab}	2.0 ±0.2 ^{a}	2.1 ±0.1 ^{a}	1.8 ±0.2 ^{ab}	1.7 ±0.1 ^{b}
C22:2	5.5 ±0.2 ^{b}	5.0 ±0.7 ^{b}	5.0 ±0.1 ^{b}	2.1 ±0.4 ^{c}	11.3 ±1.9 ^{a}

Table 2. Changes in the percentage composition of fatty acids in *Ginkgo biloba* petioles during senescence and methyl jasmonate-induced senescence. Mean results ±SD in rows followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test. Limit of quantification = 0.2%

Fatty acid	Treatment day (September 9)	Control (September 30)	Adaxial treatment (September 30)	Abaxial treatment (September 30)	Senesced (October 20)
Saturated fatty acids					
C4:0	<0.2	1.5 ±0.1 ^{b}	1.5 ±0.1 ^{b}	6.9 ±1.9 ^{a}	3.3 ±0.6 ^{a}
C14:0	<0.2	1.4 ±0.1 ^{b}	1.3 ±0.1 ^{b}	2.7 ±0.2 ^{a}	2.9 ±0.2 ^{a}
C16:0	24.6 ±1.9 ^{a}	22.6 ±0.6 ^{a}	22.7 ±0.5 ^{a}	18.3 ±1.8 ^{a}	18.9 ±0.9 ^{a}
C18:0	1.5 ±0.1 ^{b}	2.1 ±0.3 ^{ab}	2.8 ±0.2 ^{a}	2.9 ±0.1 ^{a}	3.2 ±0.1 ^{a}
C20:0	<0.2	<0.2	1.3 ±0.3 ^{b}	8.2 ±0.6 ^{a}	8.3 ±0.1 ^{a}
C22:0	<0.2	<0.2	<0.2	<0.2	<0.2
C24:0	7.2 ±0.6 ^{a}	4.7 ±0.3 ^{b}	4.7 ±0.2 ^{b}	7.1 ±0.9 ^{a}	7.6 ±0.5 ^{a}
Unsaturated fatty acids					
C17:1	2.1 ±0.3 ^{ab}	2.0 ±0.1 ^{b}	2.0 ±0.1 ^{b}	2.9 ±0.2 ^{ab}	3.2 ±0.1 ^{a}
C18:1	9.9 ±0.1 ^{b}	13.1 ±0.6 ^{a}	13.0 ±0.5 ^{a}	5.3 ±0.5 ^{c}	8.2 ±0.1 ^{b}
C18:2	10.3 ±0.6 ^{a}	10.4 ±0.1 ^{a}	10.3 ±0.2 ^{a}	11.7 ±1.3 ^{a}	8.3 ±0.1 ^{b}
C18:3	30.1 ±1.8 ^{a}	29.7 ±1.2 ^{a}	29.5 ±1.4 ^{a}	20.3 ±1.3 ^{b}	20.7 ±0.4 ^{b}
C20:2	3.1 ±0.3 ^{ab}	3.6 ±0.1 ^{b}	3.6 ±0.1 ^{b}	4.3 ±0.7 ^{ab}	4.1 ±0.1 ^{a}
C20:4	2.3 ±0.3 ^{a}	2.5 ±0.1 ^{a}	2.5 ±0.1 ^{a}	2.3 ±0.2 ^{a}	2.7 ±0.2 ^{a}
C22:2	10.3 ±2.2 ^{ab}	4.7 ±0.3 ^{b}	4.7 ±0.5 ^{b}	6.1 ±0.8 ^{b}	8.6 ±0.5 ^{ab}

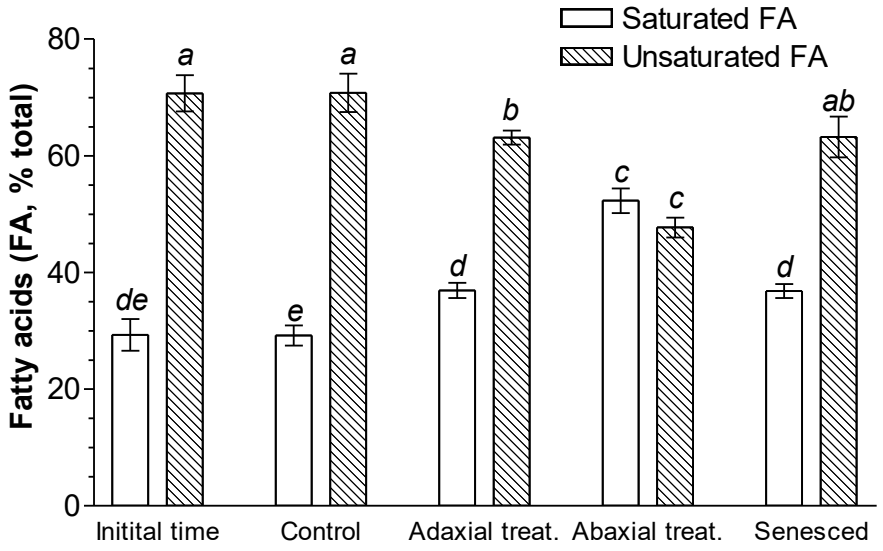

Fig. 1. Percentage of total saturated and unsaturated fatty acids in *Ginkgo biloba* leaf blades during senescence and methyl jasmonate-induced senescence. Mean results ±SD followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test. Abbreviations: Adaxial treat. and Abaxial treat. means MeJA applied adaxially and abaxially, respectively

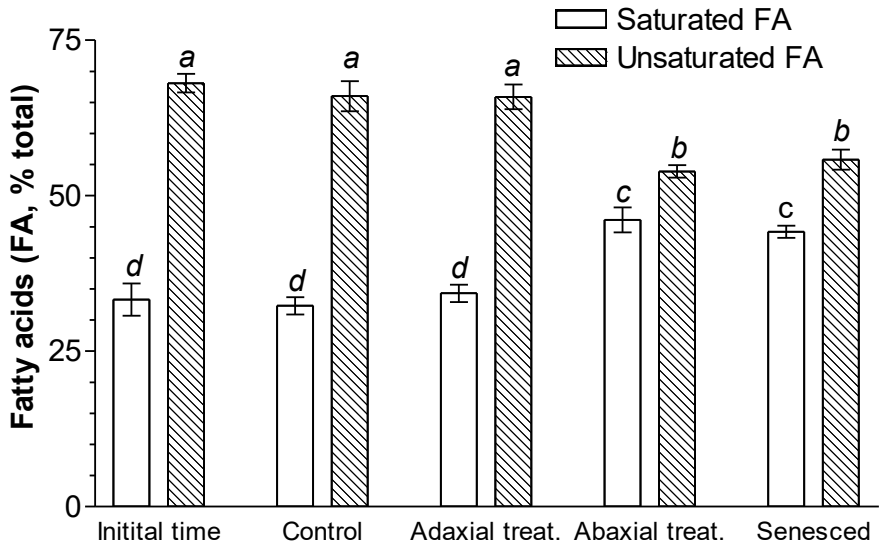

Fig. 2. Percentage of total saturated and unsaturated fatty acids in *Ginkgo biloba* petioles during senescence and methyl jasmonate-induced senescence. Mean results ±SD followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test. Abbreviations: Adaxial treat. and Abaxial treat means MeJA applied adaxially and abaxially, respectively

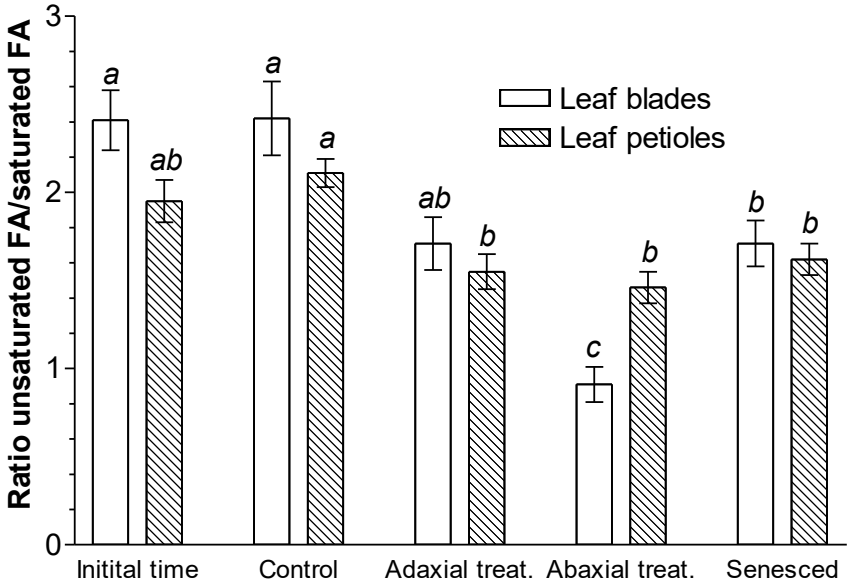

Fig. 3. Ratio of total unsaturated to saturated fatty acids in *Ginkgo biloba* leaf blades and petioles during senescence and methyl jasmonate-induced senescence. Mean results ±SD followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test. Abbreviations: Adaxial treat. and Abaxial treat means MeJA applied adaxially and abaxially, respectively

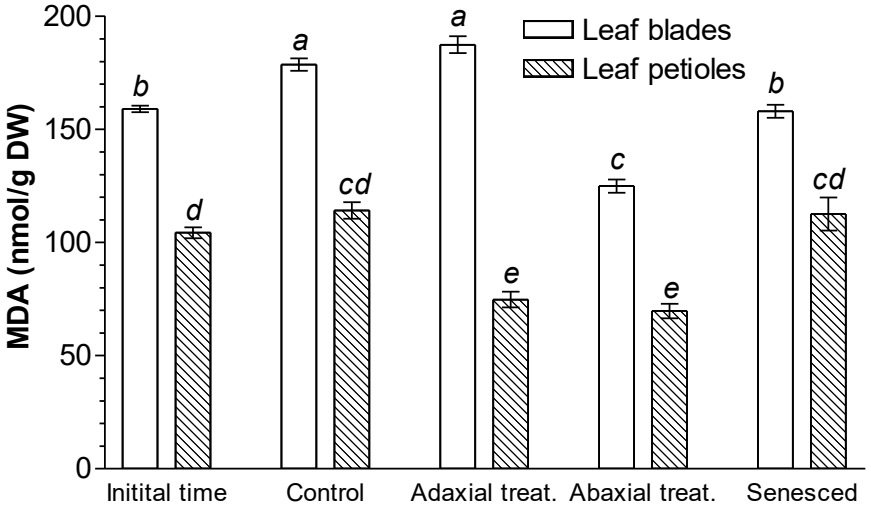

Fig. 4. Contents of malondialdehyde (MDA) in *Ginkgo biloba* leaf blades and petioles during senescence and methyl jasmonate-induced senescence. Mean results ±SD followed by the same letter were not significantly different ($p < 0.05$) according to Tukey's test. Abbreviations: Adaxial treat. and Abaxial treat means MeJA applied adaxially and abaxially

ester and docosahexaenoic acid (C22:6) methyl ester. Linolenic acid (C18:3) was quantitatively major acid, accounting for 25.5– 45.1% of total FA in leaf blades and 20.3–30.1% in petioles, followed by palmitic acid, which accounted for 17.8–21.3% in leaf blades and 18.3–24.6% in petioles of total FA, respectively (Tab. 1 and 2).

Both senescence and MeJA-induced senescence significantly altered the FA composition in *G. biloba* leaf blades and petioles. The leaf blade tissue showed an increase in the percentage of saturated acids, especially C20:0 and C24:0 and to a lesser extent C4:0, C20:0 and C22:0 (Tab. 1). The highest percentages of saturated FA in the total acid pool occurred in leaf blades treated with MeJA on the abaxial side. Under the abaxial application of MeJA, a particularly large increase was observed for C20:0 acid. Among the unsaturated FA, the percentages of C17:1, C18:1, C20:2, and C20:4 acids varied slightly. On the other hand, there was a clear decrease in the percentage of the quantitatively major acid, which was C18:3 (Tab. 1). A particularly large decline in the C18:3 acid content occurred in leaf blades treated with Me-JA on the abaxial side. The losses of this acid were 25% higher than in senescing (yellow) leaf blades and 34% higher than in adaxially treated leaf blades and nearly 54% higher than in initial control (green) leaf blades (Tab. 1). In petioles, the tendency was quite similar (Tab. 2).

Assessing the total contents of saturated and unsaturated FA, it can be noted that the proportion of unsaturated FA exceeded 70% in the leaf blades of the initial leaves (harvested on September 9) and control leaves (harvested on September 30) (Fig. 1). This proportion decreases very slightly in leaf blades treated with MeJA on the adaxial side of leaves. However, MeJA applied abaxially resulted in equalization of the share of both FA types. On the other hand, in untreated senesced leaf blades collected on October 20, saturated FA accounted for more than 60% of their total amount (Fig. 1). Similar changes in the percentages of FA occurred in the petioles of *G. biloba* (Tab. 2, Fig. 2).

There was a marked increase in C20:0 and C24:0 saturated as a result of both during leaf senescence and MeJA-induced leaf senescence. Among the unsaturated FA of petioles, similar to that of leaf blades, there was a marked decrease in the percentage of C18:3 acid (Tab. 2). In the case of C18:1 and C18:2 acids, the percentage decrease was small. On the other hand, in the case of C22:2 acid, an increase in its content in the total amount of FA was observed under abaxial use of JA-Me and during senescence. The total unsaturated FA content in petioles of initial leaves (collected September 9), control leaves (collected September 30), and in those treated abaxially also exceeded 70% (Fig. 2). However, MeJA applied to the abaxial side of leaves as well as after senescence process resulted in a predominance of saturated FA over unsaturated FA in petioles.

No changes in MDA content was observed in *G. biloba* petioles after senescence, whereas a slight decrease was observed in the leaf blades (Fig. 4). MeJA applied to the adaxial side of leaves caused a decrease in MDA content in petioles but did not affect its content in leaf blades. On the other hand, MeJA applied on the abaxial side of leaves caused a pronounced decrease in MDA content compared with control leaf blades and petioles. MDA content in leaf blades and petioles treated abaxially with MeJA was lower than in senesced leaves.

DISCUSSION

There are limited data in the available literature on the fatty acid composition of *G. biloba* leaves. A Korean study carried out in the 1970s showed that the main FA in the leaves of these species were C18:3 (45.2%) and C16:0 (25.1%) [Chung and Shin 1978]. Later, the taxonomic study of gymnosperm species showed in *G. biloba* leaves the occurrence of 6 saturated FA (C12:0, C14:0, C16:0, C18:0, C20:0 and C22:0), and among unsaturated FA, C16:1, C16:2, C16:3, C18:1, C18:2, C18:3, C20:2, C20:3, and C20:4 were present [Mongrand et al. 2001]. Similar to Korean study, the quantitatively dominant FA were C18:3 (39.3–41.5%), and C16:0 (23.6–24.8%). The composition of FA of *G. biloba* leaves in our study mostly confirms those obtained by Chung and Shin [1978] as well as Mongrand et al. [2001]. In later paper, in methanolic extract from *G. biloba* leaves grown in Turkey the main FA were C16:0 with 37.70% and C18:1 with 26.03% [Maltas et al. 2011]. But in the acetone extract, C18:1 was the major FA with 37.64%. In addition, C14:0, C18:0 and C16:1 were present in lower amounts in these extracts. Also, according to study by Pereira et al. [2013] in *G. biloba* leaves major FA was C16:0, following by

C18:3, C18:1, C18:2 and C18:0. Such discrepancies from ours and previously reported results may be the effects climatic conditions on FA composition or the method of analysis used.

FA in leaves occur mainly as components of cell bio-membranes as phospholipids, galactolipids and long-chain sphingolipids [Gomez et al. 2018]. Degradation of membrane is an essential process during leaf senescence and several studies have reported changes in membrane lipids during this process [Thompson et al. 1998]. According to these studies, generally in plastid galactolipids and phospholipids of leaves C18:3 is the predominant FA [Mongrand et al. 2001]. The high percentage of C18:3 acid in FA of *G. biloba* leaves obtained during the present study confirms these data.

G. biloba leaves also contain FA in epicuticular waxes [Gülz et al. 1992]. The wax content was approximately 0.84% dry weight, or 36.4 μg wax per cm^2 of surface area. The major components of the waxes were FA (38.6%), primary alcohols (25.9%), and nonacosan-10-ol (15.0%). Among the FA, the main ones were those with the number of carbon atoms from 20 to 34 which accounted for about 90% of all acids, but detailed data was not included.

One of the earliest symptoms of senescence is the loss of selective membrane permeability. The appearance of membrane leakage is caused by changes in the structure and organization of lipid bilayers due to hydrolysis of phospholipids and galactolipids [Ananieva et al. 2007]. Senescence induced either by placing plants in the darkness or MeJA-induced resulted in an approximately 2-fold increase in saturated FA (C14:0; 16:0, 18:0, 20:0, 22:0, and 24:0) and monoenoic FA content (16:1 and 18:1) in zucchini (*Cucurbita pepo*) leaves [Ivanova et al. 2012]. The highest increase (about 10-fold) was observed in the content of C20:0, C22:0 and C24:0 acids. At the same time, senescence caused a 2-fold decrease in the content of polyunsaturated FA (C18:2 and C18:3). The reduction in the proportion of unsaturated FA, especially C18:3, shown in our study confirms these results, as well as earlier report by Yang and Ohlrogge [2009]. They observed that there is a preferential reduction in unsaturated FA content during senescence of *Arabidopsis* leaves. Also, in senescent rose leaves, the percentage of C18:3 in chloroplast lipids was lower than in green tissues [Mishra and Sangwan 2008].

The relatively high percentage reduction in C18:3 acid shown in senescing leaves and in MeJA-treated leaves on the abaxial side reveals a particularly intense induction of leaf senescence by MeJA. This was probably due to the synergistic effect of exogenous MeJA with this internal and other phytohormones [Saniewski et al. 2020]. Whereas all plant hormones are involved in all stages of the plant life cycle, abscisic acid, ethylene, and jasmonates are primarily active in leaf senescence [Lim et al. 2007, Zhang and Zhou 2013, Jibran et al. 2013]. During senescence and MeJA-induced leaf senescence, the level of endogenous abscisic acid increased significantly, whereas the level of 1-aminocyclopropanecarboxylic acid, an ethylene precursor, did not change [Saniewski et al. 2020]. In addition, the contents of cytokinins, gibberellins, and auxins changed to a small extent. The authors suggest that jasmonates interact with abscisic acid in regulation on the senescence process in *G. biloba* leaves [Saniewski et al. 2020].

It is known that in chloroplast lipids of senescing leaves, the contribution of C18:3 is lower than in green leaves [Koiwai et al. 1981, Mishra and Sangwan 2008]. As a result, the ratio of unsaturated FA to saturated FA at the end of leaf senescence was much lower than in green leaves. A reduction in unsaturated FA content, especially C18:3, was also found in barley (*Hordeum vulgare* L.) leaves as a result of their senescence in the dark and cocklebur (*Xanthium strumarium* L.), and abscisic acid accelerated these changes [Newman et al. 1983]. In the present study we also observed a reduction in the ratio of unsaturated to saturated FA, both during senescence and senescence induced by MeJA (Fig. 3).

Jasmonates are known to be involved in plant growth and development processes, including senescence, and in responses to various biotic and abiotic stresses [Jibran et al. 2013], reviewed by Wasternack and Hause [2013]. The involvement of jasmonates in senescence regulation was first observed on detached oat leaves [Ueda and Kato 1981]. In a study carried out by He et al. [2002], four times more endogenous JA was produced in senescing leaves compared to non-senescing ones, and JA treatments initiated senescence in attached and detached leaves. The results of previous studies showed a marked stimulation of the increase in endogenous MeJA after MeJA treatment,

as well as jasmonic acid and 12-oxo-phytodienoic acid, an intermediate metabolite in JAs biosynthesis [Saniewski et al. 2020]. Moreover, recently Czigle et al. [2019] demonstrated in the volatile constituent in *G. biloba* leaves the presence of linolenic acid methyl ester, whose biosynthesis may be related to the loss of C18:3 observed in our study during senescence of *G. biloba* leaves. These reasons are possible contributors to the observed decline in linolenic acid, and should be taken into account in explaining the causes of this phenomenon.

In an earlier study of green and senescing leaves of *G. biloba*, saturated FA having 12 to 32 carbon atoms in the chain were found, the main ones being C16 and C24 acids [Tu et al. 2001]. The authors analyzed the contents and overall composition of external (epicuticular) and internal lipids during senescence of *G. biloba* leaves. They found that epicuticular lipids constituted 3% and 4% of all FA in green and in senescent leaves, respectively. They also showed that total lipid content during senescence of *G. biloba* leaves decreased from 17.6% dry weight (DW) to 13.4% DW, and this decline was primarily in leaf internal lipids [Tu et al. 2001]. It is possible that this decrease is a result of the reduction in C18:3 acid found in our study during senescence and MeJA-induced senescence of *G. biloba* leaves.

The extent of membrane lipid peroxidation is an indicator of tissue oxidative stress. This process disrupts the integrity and function of membranes, membrane proteins and is a source of harmful compounds such as MDA [Procházková and Wilhelmová 2007]. In our study, we found no changes in MDA content in leaf petioles and small decline in leaf blades of *G. biloba* after its senescence. This means that senescence process does not necessarily result in increased lipid peroxidation and MDA content. However, MeJA applied on the adaxial side of leaves caused an increase of MDA in leaf blades. Similarly, MDA content increased significantly after treatment for 21 days with MeJA, suggesting that it induces senescence in *G. biloba* leaves [Li et al. 2020].

On the other hand, MeJA applied on the abaxial side of leaves caused a pronounced decrease in MDA compared to control leaf blades and petioles. These contents were also lower than those found in blades and petioles of senesced leaves. This may indicate some inhibitory role of MeJA in the oxidation of unsaturated FA which leads to the lower formation of MDA. Earlier, a reduction in membrane lipid peroxidation and MDA content was observed in soybean plants treated with MeJA during drought stress [Anjum et al. 2011]. The authors suggested that this could be explained by increased levels of antioxidants, which help scavenge ROS, and altered fatty acid ratios in membranes, making them less susceptible to ROS [Wang 1999].

The mechanism for regulating leaf senescence in *G. biloba* remains unclear. Autumn leaf senescence is a complex process and a complex phenomenon. By comparing the leaves of *G. biloba* yellow and green mutants physiologically and transcriptomically, it was possible to identify 116 differentially expressed genes and 46 factors involved in chloroplast development, chlorophyll metabolism, pigment biosynthesis and photosynthesis [Li et al. 2018]. Besides analysis of senescence-related genes showed that the expression levels of most ABA and JA-related genes increased, while genes related to the cytoskeleton, photosynthesis and antioxidation decreased after the transition from the green leaf to the yellow leaf stage [Li et al. 2020].

CONCLUSIONS

The exogenously applied methyl jasmonate (MeJA) effectively induced changes in the percentage composition of fatty acids (FA) contained in leaf blades and petioles on *Ginkgo biloba* tree. The clear reduction in the proportion of linolenic acid was accompanied by an increase in the percentage of saturated FA in both leaf organs. This resulted in a decrease in the proportion of all unsaturated fatty acids in their total pool during senescence and MeJA-induced senescence of both leaf organs examined. However, the application of MeJA accelerated this process. This may cause a deterioration in the quality of preparations obtained from late harvested leaves. The senescence process, as well as that induced by abaxially applied MeJA, resulted in a decrease lipid peroxidation as determined by MDA content. In the future, these obtained results should be supplemented with analyses of composition of particular types of membrane lipids (phospholipids, galactolipids and long-chain sphingolipids), as well as advanced genetic studies in this area on the transcripts of genes related to the senescence of *G. biloba* leaves and the participation in this process of MeJA.

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