Methyl Jasmonate-Induced Changes in Non- and Antioxidant-Enzymatic Defense in Peppermint (Mentha piperita)

Soheila Afkar¹, Ghasem Karimzadeh�, Mokhtar Jalali Javaran¹, Mozafar Sharifi² and Mehrdad Behmanesh³

Received: January 26, 2013     Accepted: May 11, 2013

¹Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
²Plant Biology Department, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
³Genetics Department, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Abstract
Peppermint (Mentha piperita L.), a herbaceous and perennial species which is produced mainly for the medicine and food. The peppermint plants were initiated from 10 cm-long rhizome cuttings followed by transferring into pots. The 48 h-treated plants with methyl jasmonate (MJ) concentrations (0, 0.1, 0.5 mM) were assessed for their total soluble proteins, malondialdehyde (MDA), chlorophylls a, b and total, anthocyanin, total carbohydrates, carotenoid, activity of antioxidant guaiacol peroxidase (POD) and superoxide dismutase (SOD) enzymes. The data were analyzed using completely randomized design (CRD) with three replications. Mean comparisons were carried out, using Duncan's multiple range test. MJ treatment caused significant changes in soluble proteins, chlorophylls (a, b and total), MDA, carbohydrates and antioxidant enzymes (SOD and POD) but had no effect on anthocyanin and carotenoid. These results indicate that MJ can effectively improve the defense system and antioxidant capacity of peppermint.

Keywords: Anthocyanin; Antioxidant enzymes; Chlorophyll; Malondialdehyde; Methyl jasmonate; Peppermint

Abbreviations
CAR–Carotenoid; CAT–Catalase; Chl–Chlorophyll; CHO–Carbohydrate; H₂O₂–Hydrogen peroxide; JA–Jasmonic acid; JAs–Jasmonates; MDA–Malondialdehyde; MJ–Methyl jasmonate; POD–Guaiacol peroxidase; ROS–Reactive oxygen species; SOD–Superoxide dismutase

Introduction
Peppermint (Mentha piperita L.) belongs to mint (Lamiaceae) family and is considered as a medicinal and aromatic plant species. Peppermint essential oil includes menthol, menthone, methylacetat, menthofuran and pulegone (Mahmoud and Croteau 2003; Tabatabaie and Nazar 2007). Its cultivation has economic importance, due to its ability to produce and store essential oil, whose main constituent is menthol, used in oral hygiene products, pharmaceuticals, cosmetics and foods. Menthol also has high antifungal and antibacterial potentials, thus becoming one of the most demanded substances by the scents and essences industry (Scavroni 2005). Because of this and other reasons, peppermint essential oil ranks high in terms of total sales volume (Orozco-Ca´rdenas et al. 2001). This herb synthesize and concentrate oils in its leaves in highly specialized epidermal secretory structures known as glandular trichomes (McCaskill et al. 1992). Jasmonates (JAs) including jasmonic acid (JA) and MJ are a family of cyclopentanone compounds synthesized from linolenic acid via the octadecanoic pathway. They inhibit plant growth generally but also promote diverse processes as a class of plant growth regulator consisting of fruit ripening, senescence,
tuber formation, tendril coiling, pollen formation and defense-related responses against mechanical and insect wounding and pathogen infection (Ueda and Kato 1980; Creelman and Mullet 1997). The jasmonates applied exogenously to plants exert various effects either inhibiting or promoting the morphological and physiological changes. It has been shown that MJ causes the generation of \( \text{H}_2\text{O}_2 \) (Orozco-Cárdenas and Ryan 1999; Orozco-Cárdenas et al. 2001; Hung and Kao 2004) and lipid peroxidation expressed as MDA production in plant cells (Hung and Kao 1998, 2004). Thus, MJ leads to oxidative stress in plant cells. Plants have an internal protective enzyme catalyzed clean up system to scavenge reactive oxygen species (ROS), thus ensuring normal cellular function. Superoxide dismutase (SOD) constitutes the first line of defense via detoxification of superoxide radicals (Sairam and Saxena 2000), thereby maintaining membranes of plant tissue. SOD detoxifies superoxide anion free radicals by forming \( \text{H}_2\text{O}_2 \); it can be further eliminated by concerted action of catalase (CAT) and POD. In addition, MJ helps in maintaining the pools of antioxidant enzymes and alleviating the oxidative stress (Li et al. 1998; Jung 2004). Both SOD and POD are important enzymes associated with anti-oxidative stress in plants. ROS scavenging group depends on the detoxification mechanism provided by an integrated system of non-enzymatic reduced molecules and enzymatic antioxidants (Jaleel et al. 2006). Exogenously applied JA and MJ lead to decreased expression of photosynthesis-related genes encoding for example the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), reduced translation and increased degradation of Rubisco and rapid loss of chlorophyll (Chl) in barley leaves (Weidhase et al. 1987; Parthier 1990). The MJ does not only regulate a variety of plant-developmental responses, but is also induced by pathogen attack or wounding, which often leads to the generation of ROS, including \( \text{H}_2\text{O}_2 \), superoxide anions (\( \text{O}_2^- \)) and hydroxyl free radicals (OH) (Faurie et al. 2009; Parra-Lobato et al. 2009). ROS have the potential to interact with many cellular components, triggering stresses in plant cell culture, leading to membrane damage and, as a result, there is an immediate cellular response to trigger plant defense signals. Plants possess antioxidant defense systems, consisting of enzymatic and non-enzymatic components, which normally maintain ROS balance within the cell. Plants contain substantial amounts of carotenoids that serve as non-enzymatic scavengers of ROS (Young and Britton 1990). Anti-oxidative enzymes include SOD, which catalyzes the disproportion of superoxide radicals to hydrogen peroxide and POD, which removes \( \text{H}_2\text{O}_2 \) (Kumari et al. 2006). The POD is associated with biochemical and physiological processes such as growth, cell formation, fruit development, ethylene biosynthesis, as well as the response to various stresses (Matamoros et al. 2003).

The purpose of this study was to determine the changes in the physiological characteristics and in the activities of antioxidant enzymes capacity in *Mentha piperita* treated with different concentrations of MJ. It was hypothesized that MJ could improve non- and antioxidant enzymatic-defense in peppermint.
Materials and Methods
This experiment was carried out in the greenhouse of Tarbiat Modares University, Tehran, Iran. The peppermint plants were supplied from Iranian Institute of Medicinal Plants, Karaj, Iran. They initiated from 10 cm-long rhizome cuttings followed by transferring into pots. The 48 h-treated plants with MJ on three different concentrations (0, 0.1, 0.5 mM) were assessed for their total soluble proteins, chlorophylls (a, b, and total), MDA, total carbohydrates, carotenoid, anthocyanin and antioxidant enzymes (SOD and POD).

Determination of protein, lipid peroxides, carbohydrates and chlorophyll content in leaf extract
Soluble protein extraction was carried out according to Ausubel et al. (1995) and determined with Folin-Ciocalteu reagent according to Lowry et al. (1951) and Bradford (1976). The level of lipid peroxidation was measured in terms of MDA content, a product of lipid peroxidation, following the method of De Vos (1991). MDA is a major cytotoxic product of lipid peroxidation and acts as an indicator of free radical production. Total carbohydrates were estimated spectrophotometrically according to the method of Dubois et al. (1956). Chlorophyll was extracted in 80% (v/v) acetone from the leaf samples according to the method of Arnon (1949).

Measurement of carotenoid, anthocyanin content, POD and SOD activity
Carotenoid and anthocyanin were estimated spectrophotometrically according to the methods of Helrich (1990) and Krizek et al. (1993), respectively. POD activity was determined as an increase in optical density due to the formation of guaiacol dehydrogenation product according to Kar and Mishra (1976). SOD activity was assayed by using the photochemical NBT following the method of Giannopolitis and Ries (1977).

Statistical analysis
The data were analyzed using completely randomized design with three replications by Minitab 16. Means and standard errors (SE) were used to compare MJ treatments, using Duncan's multiple range test. Moreover, correlation coefficients were calculated among all physiological characteristics.

Results
The result of ANOVA showed that MJ had significant effect on most measured physiological characteristics in the leaves of peppermint (Table 1). Antioxidant reactions in MJ-treated Mentha piperita caused a significant decrement in photosynthetic activities and pigment levels. The contents of Chls a, b and total decreased significantly in 0.1 mM MJ-treated leaves (Figures 1A, B, C, respectively) compared with the control, but no significant changes in those characters were detectable in 0.5 mM MJ-treated leaves. The ratio of Chls a + b/CAR also decreased remarkably in the MJ treated leaves (Figure 1D), indicating that the changes of total Chls a + b takes place faster than that of total carotenoids (CARs). In the present study, the MDA concentration increased significantly when plants were subjected to 0.1 mM MJ treatment.
compared with the control. CAR content was not changed by inducing MJ treatment, while protein content decreased in 0.5 mM MJ-treated leaves (Figure 1F). Total carbohydrates (CHO; glucose) content in 0.5 mM MJ-induced leaves was detected to be lower than in those of controls (Figure 1G). Our data showed that treatment of Mentha piperita plants with 0.5 mM MJ leads to a significant increase in POD activity. Total POD and SOD activities showed prominently about 2.36- and 1.81-fold increase, respectively at 48 h of MJ induction (Figures 1H, I). Antioxidant enzymes exhibited the highest activities at 0.5 mM MJ exposure compared to those in the control plants.

### Table 1. Effect of MJ on physiological characters in Mentha piperita

<table>
<thead>
<tr>
<th>MS</th>
<th>SOV</th>
<th>df</th>
<th>Protein</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Total Chl</th>
<th>CAR</th>
<th>MDA</th>
<th>POD</th>
<th>SOD</th>
<th>Total CHO</th>
<th>Chl a+b/CAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MJ</td>
<td>2</td>
<td>0.18</td>
<td>0.00008</td>
<td>2.728</td>
<td>3.564</td>
<td>2.275</td>
<td>0.0169</td>
<td>0.156</td>
<td>2.995</td>
<td>2.995</td>
<td>102.94</td>
<td>2.728</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td></td>
<td>0.00004</td>
<td>0.258</td>
<td>0.182</td>
<td>0.409</td>
<td>0.0053</td>
<td>0.013</td>
<td>0.169</td>
<td>0.169</td>
<td>9.43</td>
<td>0.258</td>
</tr>
</tbody>
</table>

**ns**, *", **", and ***Non significant and significants at 5%, 1% and 0.1% probability levels, respectively

Correlation coefficients among physiological characters (Table 2) showed positive and highly significant relationship between POD and SOD ($r = 0.985^{***}$) and negative and highly significant correlations between soluble protein and either SOD ($r = -0.911^{**}$) or POD ($r = -0.918^{***}$).

Similarly, total CHO negatively and significantly correlated with either Chl a ($r = -0.781^*$), Chl b ($r = -0.745^{**}$) or total Chl ($r = -0.704^*$). There were positive and significant correlations between total CHO and either protein ($r = 0.727^*$) or MDA ($r = 0.847^{**}$).

### Table 2. Correlation coefficients among physiological characters in Mentha piperita

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Antocyanin</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Total Chl</th>
<th>CAR</th>
<th>MDA</th>
<th>POD</th>
<th>Total CHO</th>
<th>Chl a+b/CAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antocyanin</td>
<td>-0.189**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chl a</td>
<td>-0.511**</td>
<td>-0.336</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chl b</td>
<td>-0.398**</td>
<td>-0.398**</td>
<td>0.964**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Chl</td>
<td>-0.391**</td>
<td>-0.413**</td>
<td>0.979**</td>
<td>0.985**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAR</td>
<td>-0.792**</td>
<td>0.124**</td>
<td>0.521**</td>
<td>0.456**</td>
<td>0.461**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>0.713*</td>
<td>0.150**</td>
<td>-0.786</td>
<td>-0.777</td>
<td>-0.778*</td>
<td>-0.666*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POD</td>
<td>-0.918**</td>
<td>0.280**</td>
<td>0.445**</td>
<td>0.318**</td>
<td>0.295**</td>
<td>0.583**</td>
<td>0.576**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td>-0.911**</td>
<td>0.256**</td>
<td>0.461**</td>
<td>0.353**</td>
<td>0.336**</td>
<td>0.585**</td>
<td>-0.664**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CHO</td>
<td>0.727*</td>
<td>0.174**</td>
<td>-0.781</td>
<td>-0.745</td>
<td>-0.704*</td>
<td>-0.638**</td>
<td>0.847**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chl a+b/CAR</td>
<td>0.074**</td>
<td>-0.494**</td>
<td>0.867**</td>
<td>0.904**</td>
<td>0.918***</td>
<td>0.075**</td>
<td>-0.568**</td>
<td>-0.497**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ns**, *", **", and ***Non significant and significants at 5%, 1% and 0.1% probability levels, respectively
**Discussion**

Hormones inclusive jasmonates may mediate the response of plants to environmental stresses and may interact with other cellular metabolites and environmental factors in the regulation of stress responses (Parthier 1990). In previous reports, it was found that MJ stimulates the production of \( \text{H}_2\text{O}_2 \) (Orozco-Ca´rdenas and Ryan 1999, Orozco-Ca´rdenas et al. 2001; Hung and Kao 2004), leading to oxidative stress in plant cells. Reduced oxygen species such as hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) and superoxide radicals (\( \text{O}_2^- \)) are formed due to oxidative stress and they can produce free radicals, inducing lipid peroxidation and protein denaturation. The MDA is an oxidized product of membrane lipids and its level can show the extent of oxidative stress. When plants were treated with 0.1 mM MJ, the MDA concentration significantly increased compared to the control. Smaller amount of MDA by 0.5 mM MJ application in our study (Figure 1E) indicated that it had better efficiency to endure the damage of cellular membranes than 0.1 mM MJ concentration. MDA, produced by lipid peroxidation of cell membrane, is often used as an indicator of salt and oxidative damages (Mandhania 2006).

Decrease in MDA level by 0.5 mM MJ application may be the result of increased activities of antioxidant enzymes that can help to clean up ROS and alter ratio of membranes fatty acids as a major source of ROS production (Wang 1999). However, lipid peroxidation operated under exogenous application of MJ in peanut (Kumari et al. 2006). Previous studies showed soluble protein content as a main index of physiological condition of plants. We can express the disturbance in protein metabolism as a reason for decreasing the total soluble protein amount of the treated plants (Figure 1F). Many results suggested a connection between photosynthesis and jasmonates in plants. The contents of Chls a, b and total decreased significantly in 0.1 mM MJ-treated leaves (Figures 1A, B, C, respectively) compared with the control. It was reported that Chl a is more intensely degraded than Chl b (Wolf 1956). Exogenously applied MJ reduced translation and increased degradation of Rubisco and resulted in rapid loss of Chl in barley leaves (Weidhase et al. 1987; Parthier 1990). Results of the present study indicated that significant increase in total CHO is somehow associated with reduction in Chl. Sugar, mainly glucose, accumulation in the cell is responsible for the regulation of photosynthetic process (Moore et al. 1999). The highly significant correlations among CHO, protein and MDA (Table 2) suggested that physiological traits have a close relationship with each other. The important components of thylakoid membranes are CARs which can effectively suppress the exited Chl a, Chl b and total Chl (Knox and Dodge 1985).

Further increment in antioxidant enzyme activities is caused by exogenous application of MJ (Anjum et al. 2011). The modification of antioxidant enzymes (SOD, POD) can play important protective roles in avoiding the deleterious effects triggered by elevated levels of ROS observed at initial moments of MJ exposure. To minimize the damaging results of ROS, plants use a lot of evolved non- and enzymatic-antioxidant systems. Plants contain substantial amounts of CAR that serve as non-enzymatic
scavengers of ROS (Young and Britton 1990). The metabolism of ROS is dependent on several functionally interrelated antioxidant enzymes such as SOD and POD. Enzymatic antioxidant systems provide protection against the toxic effects of ROS (Scandalios 1993). PODs are involved in a large number of biochemical and physiological processes (Yip 1964) and may change quantitatively and qualitatively during growth and development (Shannon 1969). The SOD is believed to play a crucial role in antioxidant defense because it catalyzes the dismutation of \( \mathbf{O}_2^\cdot \) into \( \mathbf{H}_2\mathbf{O}_2 \), whereas CAT and POD destroy \( \mathbf{H}_2\mathbf{O}_2 \) (Scandalios 1993). A positive

---

**Figure 1.** Effects of methyl jasmonate (MJ) on A) Chl a (mg g\(^{-1}\) F.W), B) Chl b (mg g\(^{-1}\) F.W.), C) Total Chl (mg g\(^{-1}\) F.W.), D) Chl a+b/CAR, E) MDA (mM cm\(^{-1}\)), F) protein (mg g\(^{-1}\) F.W), G) total CHO (mg g\(^{-1}\)FW), H) POD (\( \Delta A_{470} \) mg\(^{-1}\) protein) and I) SOD (mg mg\(^{-1}\) protein) in *Mentha piperita*.

M0 = Control  M1 = 0.1 mM  M2 = 0.5
and high correlation between POD and SOD (Table 2) suggests that an increase of SOD activity was accompanied by an increase of POD activity as a result of high demand of quenching \( \text{H}_2\text{O}_2 \). Such findings in *Mentha piperita* are consensus with *Triticum aestivum* results reported by Ghobadi *et al.* (2011). In *Arabidopsis*, when JA was used at a concentration of 100 \( \mu \text{M} \), no significant alteration in the enzymes activities was detected until the third day of induction, but the activity was reduced remarkably after 6 d of treatment (Berger 2002). In barley, MJ mediated the stimulation of antioxidant enzymes including SOD, CAT and POD (Popova *et al.* 2003). JA ability to cause chlorosis led to the suggestion that this compound plays a role in plant senescence (Ueda *et al.* 1981), however, it was reputed by the fact that high JA levels were found in the zones of cell division, young leaves, and reproductive structures (Creelman and Mullet 1997). Application of MJ caused a senescence-like symptom as indicated by a great decline in photosynthesis and Chls and a strong increase in anthocyanins and antioxidant enzyme activities in *Arabidopsis thaliana* (Jung 2004). The most obvious character of leaf senescence is yellowing. Chl loss has been the principal criterion of senescence in the most reports. The protein degradation during leaf senescence has been realized in the earliest studies. In the present study, the senescence of *Mentha piperita* leaves was followed by measuring the decrease of Chl and protein contents. It is clear that MJ significantly promotes the senescence of peppermint leaves. These results are in agreement with those in the previous reports (Chao and Kao 1992; Tsai *et al.* 1996; Chen and Kao 1998). Several reports showed that the soluble sugar content often goes up, not down, in senescing leaves (Shiroya *et al.* 1961; Trippi 1965; Egli *et al.* 1980; Lazan *et al.* 1983; Crafts-Brandner *et al.* 1984). It has even been proposed that elevated sugar content actually causes senescence (Lazan *et al.* 1983). Our results showed that glucose (soluble sugar) content decreased in 0.5 mM MJ-treated peppermint leaves (Figure 1G), refuting the suggestion that sugar accumulation may cause leaf senescence. Moreover, the effect of exogenous MJ treatment on antioxidant enzymes (POD, SOD) and non-enzymatic defenses was evaluated, verifying that MJ can increase the activity of antioxidant enzymes in *Mentha piperita*.

**Acknowledgments**
Authors would like to thank Tarbiat Modares University for financially supporting this research work.

**References**


