Response of peppermint to methyl jasmonate application

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Abstract

Jasmonate compounds are known as new plant hormones that play important roles in the regulation of plant growth and development. Mints have been used and valued as aromatic herbs for thousands of years. Peppermint (*Mentha piperita* L.) is used for medicinal and food purposes and its essential oil is considered industrially important. Peppermint plants were grown in a greenhouse in pots. At flowering phase, the plants were treated with different concentrations of MeJA (0, 0.1, 0.5 mM) and after 24 h were evaluated for their carotenoid, anthocyanin, phenol, flavonol, flavonoid, H_2O_2, and proline. Analysis of variance indicated that different concentrations of MeJA caused significant variation in all measured traits except proline. As the latest studies indicated that components such as polyphenols, carotenoids, and flavonoids are natural antioxidants, the results of this experiment showed a significant increase in the antioxidant potential of *Mentha piperita* treated with 0.1 mM MeJA.

Keywords: peppermint; phenol; carotenoid; anthocyanin; H_2O_2

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Introduction

Jasmonates including Jasmonic acid (JA) and methyl jasmonate (MeJA) are widely distributed in the plant kingdom. They play important roles in the regulation of plant growth and development, so they are known as new plant hormones. In the abiotic and biotic stress conditions endogenous levels of jasmonates increase rapidly and transiently in plants (Saniewski et al., 2004). Medicinal and aromatic plants are suitable and economical for primary health care and are used by more than 80% of world's population. Mints have been used and valued as aromatic herbs for thousands of years (Chakraborty and Chattopadhyay, 2008). *Mentha piperita* is one of the most popular medicinal plants known to modern human (Abbaszadeh et al., 2009) which is a tetraploid (2n = 72) and sterile natural hybrid of *M. aquatica* L. (2n = 96) and *M. spicata* (2n=48) (Aflatuni, 2005). Peppermint (*Mentha piperita* L.) is used for medicinal and food purposes (Scavroni et al., 2005) and its essential oil is considered industrially important (Aflatuni, 2005). The essential oils of peppermint which is mainly composed of monoterpenes are produced in glandular trichomes, and this is the main reason of the aroma of peppermint (Krasnyanski et al., 1999). Peppermint mainly is used as spice in preparing variety of foods (Areias et al., 2001) and its essential oil is used worldwide mostly in the confectionary and pharmaceutical industries

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(Krasnyanski et al., 1999). Indians have used peppermint as digestive system diseases treatment for two centuries. Studies showed that peppermint is directly and/or indirectly used in traditional and modern medicine (*Abbaszadeh et al.,* 2009). Various environmental stresses lead to an excessive production of ROS and high levels of ROS cause damage to the photosynthetic pigments, membrane lipids, proteins, and nucleic acids (Torabi and Farzami Sepehr, 2015). Phenolic compounds, polyphenol, carotenoid, flavonoid, and anthocyanin are natural antioxidants that have a remarkably high scavenging activity against free radicals (Chanjirakul et al., 2006; Cao et al., 2009). Also, antioxidants have an important role in preventing a variety of life style-related diseases and aging because these are closely related to the active oxygen and lipid peroxidation. These phenolic compounds and flavonoids are potent antioxidants, free radical scavengers, and metal chelators (Jayasinghe et al., 2003). Previous studies showed that MeJA stimulates anthocyanin biosynthesis in soybean seedlings, *Arabidopsis thaliana*, and in cell cultures of *Vaccinium pahalae* (Saniewski et al., 2003). Also it was indicated that MeJA and SA induce the accumulation of phenolic compounds in ginseng root by altering the phenolic synthesis enzymes (Ali et al., 2007). The purpose of this study was the use of methyl jasmonate as a chemical elicitor compound to stimulate secondary metabolites compounds production and antioxidant capacity of *Mentha piperita.*

Materials and Methods

Peppermint plants were grown in a greenhouse in pots with a temperature of 25/20 °C (day/night). At flowering phase, the plants were treated with different concentrations of MeJA (0, 0.1, 0.5 mM) and they were evaluated for their carotenoid, anthocyanin, phenol, flavonol, flavonoid, H_2O_2 and proline contents after 24 h.

Determination of H_2O_2, proline and carotenoid content in leaf extract

Proline content was measured following the methods of Bates et al. (1973). Frozen leaf samples (0.2 g) were homogenized with 3 ml sulphosalicylic acid (3%), and then were centrifuged at 10,000 rpm. Supernatant (2 ml), acid ninhydrin (2 ml), and glacial acetic acid (2 ml) were mixed and boiled at 100 °C for 1 h. Reaction mixture was cooled at room temperature and absorbance was read at 520 nm after adding toluene (4 ml).

The H_2O_2 content was measured according to method of Velikova et al. (2000). The samples (0.2 g) were homogenized in 3 ml of trichloroacetic acid (0.1%) on ice and centrifuged at 12000 rpm. potassium phosphate buffer (0.5 ml) and KI (1 ml) were added to 0.5 ml aliquot of the supernatant. The absorbance of the reaction mixture was recorded at 390 nm.

Measurement of carotenoid and anthocyanin content

Carotenoid content was determined in 80% acetone extract. The samples were centrifuged (20000 g, 20 min) and absorbance was estimated spectrophotometrically at 510 nm according to the method of Amon (1949). Anthocyanin was measured with method of Giannopolitis et al. (1977). Plant tissue (0.1 g) was pulverized with 3 ml of hydrochloric acid and methanol with the ratio of 99 to 1 at absorbance of 550 nm. To calculate the concentration, the extinction coefficient of 33000 cm^{-2} M^{-6} was used.

Measurement of phenol, flavonol and fFlavonoid content

Extraction of phenol was carried out according to the method of Ranganna (1986) and it was estimated using the method reported by Boonyuen et al. (2009). Methanolic extract (1 ml), Folin-Ciocalteu reagent (5 ml), and sodium carbonate solution (4 ml) were mixed and absorbance was measured at 765 nm. For drawing the calibration curve, Gallic acid was used as a standard. Total flavonoid and flavonol contents were estimated by the aluminium chloride method, according to Akkol and Goger (2008). For total flavonoid, 250 µL of aluminium chloride solution, 250 µL of potassium acetate and 1 mL of methanolic extract were mixed and remained for 30 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm

Table 1
Mean squares (MS) of ANOVA for physiological characters in *Mentha piperita*

S.O.V.	Df	MS						
		Anthocyanin	Carotenoid	Flavonoid	Flavonol	Phenol	H_2O_2	Proline
MeJA	2	0.0001^{**}	0.199^{**}	1.56^{*}	2.01^{**}	2.24^{*}	185.48^{**}	1.13^{ns}
Error	6	0.000009	0.01	0.32	0.07	0.4	2.5	0.79

Table 2
Correlation coefficients between physiological characters in *Mentha piperita*

	Anthocyanin	Carotenoid	Flavonoid	Flavonol	Phenol	H_2O_2
Carotenoid	0.191^{ns}					
Flavonoid	-0.759^{*}	0.323^{ns}				
Flavonol	-0.644^{ns}	0.494^{ns}	0.791^{*}			
Phenol	-0.644^{ns}	0.513^{ns}	0.828^{**}	0.922^{**}		
H_2O_2	-0.357^{ns}	0.817^{**}	0.689^{*}	0.896^{**}	0.901^{**}	
Proline	-0.7^{*}	-0.177^{ns}	0.456^{ns}	0.439^{ns}	0.48^{ns}	0.191^{ns}

^{ns}, *, ** and *** , Non-significant and significant at 5%, 1% and 0.1% probability levels, respectively

with a spectrophotometer. Reaction mixture (1 mL of methanolic extract, 1 mL of aluminium chloride solution and 3 mL of sodium acetate) was read at 445 nm for total flavonol assay. Rutin was used as a standard for calibration curve.

Statistical Analysis

A completely randomized design with three replications was used for data collection. Duncan's multiple range tests were used to compare MeJA treatments. Moreover, correlation coefficients were calculated among all physiological characteristics.

Results

Analysis of variance results indicated that different concentrations of MeJA cause significant variation in all measured traits except proline (Table 1). Figure (I.E) shows anthocyanin accumulation in leaves of *Mentha piperita* during 24 h after MeJA treatment. Carotenoid content was enhanced at 0.1 MeJA, but there was no significant difference between 0.5 mM and control (Fig. I.F). The phenol, flavonol, and flavonoid content in comparison with the control decreased by 0.5 mM MeJA treatment and this decrease was significant according to Table 1. But no significant differences in phenol, flavonol, and flavonoid content were found between control and 0.1 mM MeJA treated plant after 24 h. In this study at the higher concentrations of MeJA, flavonol, flavonoid, and phenol content decreased more than lower concentrations (Figs. I.B, I.C, I.D). So MeJA at 0.5 mM has created the lowest total of secondary metabolite like phenol, flavonol, and flavonoid. Proline was decreased with MeJA gradually but this reduction was not statistically significant. In this study the production of H_2O_2 in *Mentha piperita* plants after MeJA treatment was assayed. The highest and lowest content was observed at 0.1 and 0.5 mM, respectively (Fig. I.A). These results also indicated that the H_2O_2 production was concentrated after 24 h at 0.1 mM but decreased at 0.5 mM treatment. H_2O_2 is an endogenous signalling molecule involved in plant responses to abiotic and biotic stresses. Due to the reduction of H_2O_2 at 0.5 mM of MeJA, decreased content of phenol, flavonoid, and flavonol as scavenging free radicals in this concentration is justified. A significant positive correlation was found between H_2O_2 and phenol, flavonol, flavonoid, and carotenoid ($r= 0.901^{**}$, $r= 0.896^{**}$, $r= 0.689^{*}$, $r= 0.817^{**}$, respectively) (Table 2).

Discussion

Anthocyanin synthesis was enhanced with MeJA treatment gradually. Anthocyanin is an important secondary metabolite that plays a role as an antioxidant whose biosynthesis is y controlled environmental and internal factors. Other possible functions of anthocyanin such as protecting against cold stress or providing drought resistance are likely to be associated with activities restricted to particular classes of plants (Horbowicz et al., 2008). On the other hand, anthocyanin belongs to a group of phenolic compounds which has positive effects on human health (Chanjirakul et al., 2006). Anthocyanin content of *mentha piperita* enhanced at 0.1 and 0.5 mM MeJA. This is in agreement with the study by Misczak et al. (2003) that also showed MeJA greatly stimulated anthocyanin accumulation in leaves of uncooled and cooled derooted tulip bulbs.

It is shown that carotenoids protect the photosynthetic machinery against the impact of ROS under stress conditions (Smolikova et al., 2011). It can be concluded that the increase in carotenoid is a refined method of free radicals and reduce adverse effects of the stress. It was found that proline can play a significant role in stress tolerance (Ghorbanli et al., 2015). In contrast to many results of various studied plants, MeJA prevented synthesis and accumulation of proline in peppermint. Previous study showed that salicylic acid (SA) and MeJA increased the H_2O_2 content in *Arabidopsis thaliana* and *Ricinus communis*, respectively (Soares et al., 2010). Flavonoids have important roles like the scavenging of oxygen-derived free radicals and their capacity to act as antioxidants (Nijveldt et al., 2001). Phenolic compounds in fruit and vegetables may produce beneficial effects by scavenging free radicals. Thus, phenolic compounds may help protect cells against the oxidative damage caused by free radicals (Chanjirakul et al., 2006). Plant flavonoids are generally identified as defence metabolites (Soriano et al., 2004) and on the other hand the ability of carotenoid, phenol, flavonol, and flavonoid to act as free radical scavengers confirms positive correlation between flavonoid, flavonol, phenol, carotenoid, and H2O2. It has shown that natural antioxidants such as polyphenols, carotenoids, and flavonoids have a remarkably high scavenging activity against chemically generated radicals (Soriano et al., 2004). MeJA increases anthocyanin content and phenolic compounds in raspberries and this was followed by increase in antioxidant enzymes activity and antioxidant capacity (Chanjirakul et al., 2006). Since anthocyanin and carotenoid have antioxidant effect, high levels of anthocyanin and carotenoid content at 0.1 mM of MeJA in our study promoted the defence mechanism in *Mentha piperita*.

Conclusion

Our results indicate that the regulation of anthocyanin biosynthesis by methyl jasmonate in *Mentha piperita* probably differs from its mode in other plants. In conclusion, this study shows that 0.1 mM MeJA treatment consistently produced higher antioxidant capacity in *Mentha piperita.*

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