Antioxidant Enzymes Activity and Anthocyanin Content in Fe$^{2+}$-treated Lemon Balm Seedlings

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ABSTRACT: Lemon balm (Melissa officinalis) is a perennia lherb belonging to Lamiaceae family. This plant has many beneficial effects including anti-bacterial, anti-oxidative, anti-viral, anti-cancer, anti-allergic and anti-inflammatory effects. In order to investigate the plant response to tentative oxidative stress, effects of different concentrations of Fe$^{2+}$ (0, 10, 20, 30 and 40 µM) on antioxidant enzymes activities as well as anthocyanin content were analyzed in M. officinalis seedlings after treatment for 24 h. Results show that the activities of peroxidase (POD) and superoxide dismutase (SOD) were higher than control while in the case of catalase (CAT) activity, all investigated subjects showed activities lower than control. Furthermore, treatment of M. officinalis 45-day-old seedlings with Fe$^{2+}$ ions leads to a significant increase in anthocyanin content in comparison with control treatment. Therefore, it can be suggested that this plant seeks to attenuate the induced oxidative stress by augmenting enzymatic (by increasing CAT and SOD activities) and non-enzymatic (by increasing anthocyanin content) antioxidant system.

Keywords: Anthocyanin, Antioxidant, Iron, Lemon balm

INTRODUCTION

Lemon balm (Melissa officinalis L.) is a well-known medicinal plant belonging to Lamiaceae family. Its leaves are used in traditional medicine for digestive aid, anti-inflammatory, anti-spasmodic, antinociceptive, tonic and diuretic purposes (Sadraei, 2003). Besides, this plant has various biological activities such as anti-viral, anti-bacterial, anti-oxidative which are due to presence of essence content and phenolic acid esters (Weitzel and Petersen, 2011). In order to investigate the plant response to tentative oxidative stress, we exposed 45-day-old M. officinalis seedlings to Fe$^{2+}$ ions for 24 h. Iron as an essential microelement for most plants, plays important biological roles in processes such as photosynthesis, chloroplast evolution and chlorophyll biosynthesis. On the other hand, this element is a main part of cellular oxidation reduction systems such as heme proteins including cytochromes, catalase (CAT), peroxidase (POD), leghemoglobin, iron-sulphur proteins eg. ferredoxin, aconitase and superoxide dismutase (SOD) (Marschner, 1995). As well as, the role of this element in nitrogen fixation and activities of some enzymes such as CAT, POD and cytochrome oxidase (CO) is well recognized (Balakrishnan, 2000; Ruiz, 2000). However, an increase of its concentration in plants leads to iron toxicity and production of various oxygen species such as superoxide anion (O$_2^-$), hydroxyl radical (OH) and hydrogen peroxide (H$_2$O$_2$) which induces oxidative stress in plants (Asada, 1999; Bhattacharjee, 2005; Dat, 2000). Plants prevent damages caused by radicals through enzymatic system (catalase, superoxide dismutase, peroxidase, ascorbate peroxidase and glutathione reductase) and non-enzymatic antioxidants(ascorbic acid, glutathione, tocopherols, flavonoids) (Ignat,
2011). In present study, effects of different concentrations of Fe$^{2+}$ on some antioxidant enzymes activities as well as anthocyanin content were analyzed in *M. officinalis* seedlings after treatment for 24 h.

**MATERIALS AND METHODS**

**Plant Culture and Treatment**

For surface sterilization, the seeds were exposed to 2% sodium hypochlorite solution for 5 min. After rinsed several times using sterilized distilled water, the seeds were planted in MS medium (Murashige and Skoog, 1962) solidified with 0.8% (w/v) agar. They were transferred to a dark condition with a relative humidity of 55±5% and a temperature of 28±2 °C. After 2 weeks, they were transferred to 16h: 8h (Light: Dark) condition at 30±2 °C. For applying treatments to the 45-day-old seedlings, they were collected from their medium and rinsed thoroughly in sterile water. Then after, they were transferred to liquid MS medium containing different concentrations of Fe$^{2+}$. The media were prepared in 4 different concentrations (0 (control), 10, 20, 30 and 40 µM) separately by using FeSO$_4$.7H$_2$O salt and treatments were applied for 24 h. After collecting treated seedlings, they were rinsed in distilled water for several times and then they were frozen in liquid nitrogen, and kept at -80 °C until used.

**Measurement of Antioxidant Enzymes Activity**

Firstly, 0.5 g of wet tissue was ground into powder in 50 mM potassium phosphate buffer (pH 7.5) containing 1% polyvinylpyrrolidone (PVP) and 1 mM EDTA. The suspension was centrifuged at 11000 rpm at 4 °C for 20 min. The supernatant was used for measuring protein content and investigating enzyme activities (Zhang, 2008).

Superoxide dismutase (SOD) activity was measured according to the method of Giannopolitis and Reis (1977), based on the enzyme's capability for preventing photochemical reduction of nitroblue tetrazolium (NBT) and absorption amount was measured at wavelength of 650 nm (Giannopolitis and Ries, 1977).

Catalase (CAD) activity was measured according to the method of Dhindsa, (1981), based on the enzyme's capability for degrading H$_2$O$_2$ in 1 min using an extinction coefficient of 33000 mol$^{-1}$cm$^{-1}$ (Dhindsa, 1981).

Peroxidase (POD) activity was assayed according to the method of Plewa et al. (1991) based on the amount of tetraguaiacol absorbed after formation by oxidation of guaiacol catalyzed by this enzyme in 3 min at a wavelength of 470 nm using an extinction coefficient of tetraguaiacol, $\varepsilon = 26.6$ mM$^{-1}$ cm$^{-1}$ (Plewa, 1991).

**Measurement of Anthocyanin Content**

Anthocyanin content was measured according to the method of Krizek, (1993). Absorbance was measured at 550 nm and the extinction coefficient of 33000 mol$^{-1}$cm$^{-1}$ was used to calculate anthocyanin content (Krizek, 1993).

**Statistical Analysis**

All experiments were done with 3 independent replications with a completely randomized design. Data means were used for Duncan's multiple range test after that one way analysis of variance (ANOVA) with a significance level of 0.05 was used for analyses of data with SAS 9.1.3 (SAS Institute, Cary, NC).

**RESULTS AND DISCUSSION**

In present study, effects of different concentrations of Fe$^{2+}$ on anthocyanin content and antioxidant enzymes activities were analyzed in *M. officinalis* seedlings after treatment for 24 h. As mentioned above, increase in Fe$^{2+}$ concentration leads to toxicity and induction of oxidative stress. Worth mentioning that plants protect themselves against damages caused by these oxidative stresses by augmenting enzymatic and non-enzymatic antioxidant systems. In oxidative stress conditions, SOD, the enzyme which is responsible for converting superoxide radicals into H$_2$ and O$_2$ (Fukai and Ushio-Fukai, 2011), is the first enzyme that proceeds to detoxify ROS (Garnczarska and Ratajczak, 2000). In seedlings, which were treated with Fe$^{2+}$ for 24 h, SOD activity was increased significantly compared to control (Figure 1A). Increase in activity of this enzyme may be attributed to the effect of iron ion on producing oxidative stresses and hence, to its effects on preventing damages produced by oxidative stresses. SOD activity leads to production of hydrogen peroxide free radicals, which are scavenged in plants by enzymes such as CAT and POD. As shown in Figure 1C, in many subjects treated with Fe$^{2+}$, POD activity is increased. On the other hand, there is a significant decrease for CAT activity in treated subjects compared to control (Figure 1B). Previous studies have shown that POD tends to H$_2$O$_2$, more than CAT (Jimenez, 1997). Therefore, it seems that increase in POD leads to scavenging these radicals or the resulted stress leads to deactivation of CAT. Hence, it can be suggested that plant helps to remove free radicals against the applied oxidative stress by activating SOD and POD.
enzymatic systems. Many studies on antioxidant systems have shown that an integrated activity of SOD, CAT and POD is needed for protection against active toxic oxygen species (Garnczarska and Ratajczak, 2000). In tomato (Lycopersicon esculentum), increased arsenic concentrations leads to significant increase in POD enzyme activity which indicates confrontation with harmful effects of oxidative stress (Miteva, 2005). As well as, in red cabbage red cabbage (Brassica oleracea var. capitata), as plant full of enzymatic and non-enzymatic antioxidants, significant increase in activity of enzymes such as POD and SOD has been observed under heavy metal stress (Posmyk, 2009). Wang, (2004) showed that treatment of Brassica junica seedlings with copper leads to increase in activities of SOD, POD and ascorbate peroxidase (ASPX) and decrease in CAT activity (Wang, 2004) which is consistent with the results of present study. Decrease in CAT activity can be due to POD high affinity to combine with H₂O₂ (Zhang, 2013) or suppression of its activity in presence of high concentrations of heavy metals such as iron and copper (Choudhary, 2007; Posmyk, 2009). Worth mentioning that pea (Pisum sativum), cadmium leads to oxidation of CAT and hence decrease in its activity (Maksymiec, 2005).

Figure 1. Activities of SOD (A), CAT (B), POD (C) and anthocyanin content (D) in seedlings treated with different concentrations of Fe²⁺ for 24 h. different letters above the bars indicate significance at 0.05 percent level

On the other hand; in condition of heavy metal toxicity, lack of neutralization of free radicals and remaining of hydrogen peroxide leads to Fenton and Haber-Weiss reaction which in presence of multiple-charged ions like iron converts into very dangerous hydroxyl radical (Mittler, 2004) which may lead to a irreversible change in enzymes activities, change in gene expression, irreversible damages to cell wall and nucleic acid which are reported by many authors (Mishra, 2006). As can be seen decrease in activities of all of the studied enzymes in treatments may be attributed to production of hydroxyl radicals which is resulted by oxidative stress and subsequent degradation of these enzymes. Among non-enzymatic defense systems, phenolic compounds, flavonoids, and particularly anthocyanins and even carotenoids are active in plants exposed to high concentrations of heavy metals and preserve the plant against toxicity (Dai, 2006; Posmyk, 2005; Posmyk, 2009). As can be seen in Figure 1D, anthocyanin content in seedlings has increased significantly under Fe²⁺ treatment in harmony with increase of the ion in medium. Therefore, increase in anthocyanin content in M. officinalis seedlings under treatment with this ion may be due to plant confrontation against this type of stress.

CONCLUSION

In sum, our findings suggest that treatment of 45-day-old M. officinalis seedlings with iron ions leads to production of free radicals and oxidative stress. M. officinalis proceeds to conquer such a condition through augmenting activities of antioxidant enzymes such as SOD and POD as well as augmenting non-enzymatic compounds content such as anthocyanin.

REFERENCES


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