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Long-term water deficit modulates antioxidant capacity of peppermint (*Mentha piperita* L.)

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ABSTRACT

Study of metabolite profile changes in peppermint is crucial due to its food and medicinal properties. In the current study, the effect of water deficit (normal irrigation as control, 0.75 field capacity (FC), 0.5 and 0.25 FC) investigated on the growth parameters, physiological response, phenolic compounds and content of L-ascorbic acid in peppermint plant. The morphological properties of peppermint reduced due to water deficit. The maximum essential oil percentage observed in 0.25 FC treatment (1.1%). Water deficit leads to a significant increment in the concentration of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). In the other word, the highest concentration of H_2O_2 and MDA measured in plants which exposed to the 0.25 FC treatment. Moreover, polyphenol oxidase (PPO) and superoxide dismutase (SOD) show the highest activity in the 0.5 FC and 0.25 FC, respectively. However, drought stress had a negative impact on the total phenol content (TPC) and flavonoid content which cause a reduction from 72.5 to 68.1 in control and 0.25 FC treatments. The elevated content of flavone observed in the 0.5 FC treatment. The radical scavenging activity of peppermint extracts against stable DPPH· (2,2-diphenyl-2-picrylhydrazyl hydrate) decreased under long-term water stress. While the content of L-ascorbic acid in water deficit treated plants was more than control plants. These results suggest that moderate water deficit (0.5–0.75 FC) in peppermint will be appropriate to enhance its biological properties.

1. Introduction

Environmental stresses limit crop performance and make many changes in their molecular processes like variation in metabolite profile (Akula and Ravishankar, 2011). Drought as the most important stress after perception in plants leads to an unusual increment in reactive oxygen species (ROS) production (Gill and Tuteja, 2010). There is a wide variety of free radical ROS such as superoxide radicals, hydroxyl radical, perhydroxyl radical and alkoxy radicals, and non-radical forms include hydrogen peroxide and singlet oxygen (Gill and Tuteja, 2010). A part of produced ROS participate in drought tolerance signal transduction and impact on a biological process like cell division and programmed cell death (PCD), which is caused by a change in expression of drought tolerance responsible genes (Suzuki et al., 2012). ROS can damage cells membrane and increase production of malondialdehyde (MDA) content (Choudhury et al., 2017). However, Plants employ both enzymatic and non-enzymatic system to cope with produced ROS (Gill and Tuteja, 2010). The enzymatic antioxidant processes involved the activity of enzymes like catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and polyphenol oxidase (PPO) (Gill and Tuteja, 2010; Talbi et al., 2015; Dietz et al., 2016).

Plants produce a wide variety of free radical scavenging molecules, such as nitrogen compound, phenolic compounds, vitamins, terpenoids against environmental stresses (Zheng and Wang, 2001; Cai et al., 2004; Huang et al., 2009; Iqbal et al., 2015). Phenolic compounds are the important constituents which participating in the cell defense system against free radicals in abiotic and biotic stress, as well as involved in various plant processes such as growth and reproduction (Szeto et al., 2002; Balasundram et al., 2006; Dias et al., 2016). It has shown that antioxidant compounds like phenolic products could be isolated and used as antioxidants for the prevention and treatment of free radical-related disorders (Krishnaiah et al., 2007). Researchers have been interesting in realization natural antioxidants for application in foods and medicinal materials instead of synthetic antioxidants with their carcinogenicity effects (Velioglu et al., 1998; Kumaran and Karunakaran, 2007 Krishnaiah et al., 2011).

Many medicinal plants with preservative effect suggest the presence of antioxidative and antimicrobial constituents in their tissues (Islam et al., 2017). Chemical constituents with antioxidant activity synthesized in plants play an important role in the prohibition of various

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putrefactive diseases (Hu and Willett, 2002). Previous studies demonstrated that plant materials with the potential of the antioxidant constituents for the preservation of health and protection from cancer and coronary heart disease are raising interest among scientists and food manufacturers (Pandey and Rizvi, 2009).

Peppermint (*Mentha piperita* L.) belongs to *Lamiaceae* family known as an important medicinal plant around the world. The essential oil of peppermint made from the beginning of the growth in vegetative organs and stored in glandular trichomes of leaves, which affected by various environmental factors (Croteau and Gershenzon, 1994). Peppermint oil is extracted from the stem, leaves, and flowers that containing a large number of important terpenoids such as menthol, menthone, limonene, isomenthone, menthofuran, pulegone, neomenthol, β-caryophyllene, 1, 8-cineole and sabinene (Rohloff, 1999; İşcan et al., 2002). As well as, other compounds that produced in *Metha piperita* including flavonoids, polyphenols, carotene, alpha-tocopherol, betaine and choline (Farnad et al., 2014). These compounds used as antipyretic, antispasmodic, analgesic, carminative, diaphoretic, analgesic, anti-diarrhea, anti-microbial as well as the treatment of irritable bowel syndrome (IBS), inflammatory bowel disease, inflammation and dysfunction of the gallbladder and liver diseases (McKay and Blumberg, 2006).

There is an interest to understanding agents influence the biosynthesis of peppermint essential oil in order to increase the commercially important component like (−)-menthol and (−)-menthone and reduction of the undesirable (+)-pulegone and (+)-menthofuran (Davis et al., 2005). However, previous studies have shown that peppermint oil constitutes affected by various factors such as UV-B (Dolzhenko et al., 2010), chemical elicitors (Figueroa-Pérez et al., 2014), and drought stresses (Rahimi et al., 2017). The focus of the present study was to evaluate the effect of long-term limited water on the morphological and physiological response, phenolic compounds and antioxidant capacity of peppermint.

2. Material and methods

2.1. Plant material

The peppermint plants were prepared from the Medicinal Plants Research Center, Institue of Medicinal Plants, ACECR, Karaj, Iran. The experiment carried out in a CRD design with four treatments (normal irrigation as control, 0.75 field capacity (FC), 0.5 and 0.25 FC) at three replications. Treatments were applied two weeks after planting and the establishment of the plant cuttings, and the FC of plots was checked twice a day and continued until the early flowering.

2.2. Measurement of H_2O_2 and MDA

Hydrogen peroxide (H_2O_2) extraction was measured according to Loreto and Velikova (2001), so that, 0.35 g of leaves were homogenized with 5 ml 0.1% (w/v) trichloroacetic acid (TCA) in an ice bath. The homogenate was centrifuged at 12,000× *g* for 15 min, then 0.5 ml of supernatant was transferred to a new tube and mixed with 0.5 ml 10 mM phosphate buffer (pH 7.0), and 1 ml 1 M KI. The assay mixture was kept in dark for 1 h and the absorbance was read at 390 nm. The concentration of H_2O_2 was determined from a standard curve and expressed as H_2O_2 μmol g^{-1} fresh weight. By measuring the concentration of MDA, Lipid peroxidation in leaves was detected according to Heath and Packer (1968). According to this method, 0.2 g of fresh leaves, powdered with 5 ml 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 8000× *g* for 5 min, 1 ml of supernatant transfer to new tube then added 4.5 ml 20% TCA. The homogenate was centrifuged at 4000× *g* for 10 min, the absorbance was read at 532 nm. The unspecific turbidity was corrected by A_{600} subtracting from A_{530}. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.3. Enzymatic antioxidant activity

Protein extraction was carried out according to Bradford (1976) using Bovine Serum Albumin V as a standard. For enzyme assays, frozen leaf samples were ground to a fine powder with liquid nitrogen and extracted with 50 nm phosphate buffer (pH 7.0). The extracts were centrifuged at 4 °C for 30 min at 20,000× *g* and after that supernatant was collected and used for protein content assay and enzyme activities. Superoxide dismutase activity was determined by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitroblue tetrazolium according to the method of Dhindsa et al. (1982). The reaction buffer containing 100 mM phosphate buffer (pH 8.7), 12 mM Methionine, 75 mM nitroblue tetrazolium, 100 μM EDTA, and 0–100 ml of enzyme extract was added 2 mM riboflavin. The reaction was allowed to run for 15 min, after which the light was switched off and the absorbance read at 560 nm. One unit of superoxide dismutase activity was defined as the amount of enzyme which caused 50% inhibition of photochemical reduction of nitroblue tetrazolium. Superoxide dismutase activity of the extract was expressed as ΔOD mg-1 protein min-1. The activity of polyphenol oxidase (PPO) was determined using Chance and Maehly (1955) method. In this method pyrogallol used as the substrate.

2.4. Assessment of antioxidant activity against DPPH·

The capacity of radical scavenging in extracts against stable DPPH· (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined by Brand-Williams et al. method (1995). The solutions were obtained using 0.02 g of dry extract in 10 ml of methanol and ethanol. Then, three different concentrations of DPPH· include 10, 50 and 100 μg were used in methanol and ethanol. Final mass of extracts obtained by mixed solution of both methanol and ethanol with 77 μl extract solution. The same concentrations were used as a standard, as well. After that, extracts were retained in the dark for 15 min and 25 °C. Then absorption of methanolic, ethanolic and standard were measured in three replications. Radical scavenging activity was estimated using following formula:

$$\% \text{ inhibition} = [(A_B \_ A_A)/A_B] \times 100$$

Where:

A_B: Absorption of blank sample (t = 0 min)

A_A: Absorption of tested extract solution (t = 15 min).

2.5. Determination of total phenolic compounds

The Folin and Ciocalteu methods (1927) in methanolic and ethanolic extracts were used to determine the content of total phenolic compounds. According to Temraz and El-Tantawy (2008) method, the calibration curve carried out using a different concentration of ethanolic gallic acid solutions include 0.024, 0.075, 0.105 and 0.3 mg/ml, in combination with 5 ml Folin–Ciocaleu reagent and 4 ml sodium carbonate (75 g/l). The calibration curve was drawn using obtained data in 765 nm after 35 min. Then leave extracts (10 g/l) were combined with described reagents and absorption was measured after 1 h to assess phenolic compounds in three technical replications. Finally, the total phenolic compound was estimated using following formula:

$$C = c.\ V/m$$

where:

C: total phenolic content

c: The concentration of gallic acid established from the calibration curve, mg/ml

V: The volume of extract, ml;

m: The weight of pure plant methanolic and ethanolic extract