Food Chemistry 156 (2014) 273–278

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of chemical elicitors on peppermint (*Mentha piperita*) plants and their impact on the metabolite profile and antioxidant capacity of resulting infusions

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ARTICLE INFO

Article history:
Received 29 September 2013
Received in revised form 17 December 2013
Accepted 27 January 2014
Available online 7 February 2014

Keywords:
Peppermint
Elicitor
Salicylic acid
Hydrogen peroxide

ABSTRACT

Infusions are widely consumed all over the world and are a source of dietary antioxidants, which can be improved in plants using elicitors. The aim of this study was to evaluate the foliar application of salicylic acid (SA) (0.5, 1 and 2 mM) or hydrogen peroxide (H_2O_2) (0.05, 0.1 and 0.5 mM) on peppermint (*Mentha piperita*) plants and its effect on the metabolite profile and antioxidant capacity of resulting infusions. Whereas 2 mM SA treatment improved plant growth parameters and metabolite profile (carbohydrates and amino acids), 0.5 and 1 mM SA treatments increased phenolic compound concentration. Sinapic acid, rutin and naringin were detected only in SA treatments; antioxidant capacity was also improved. Regarding H_2O_2 treatments, no differences in plant growth parameters, metabolite profile or antioxidant capacity were found. Therefore, the application of SA to peppermint is recommended in order to improve bioactive compounds and the antioxidant capacity of infusions.

1. Introduction

Herbal infusions are aromatic beverages prepared by pouring hot or boiling water over dry parts of plants. These infusions are some of the most widely consumed beverages in the world, providing a major source of dietary phenolic compounds, which are considered the most abundant natural antioxidants. In Mexico, approximately 80% of the population consumes infusions on a regular basis, and one of the most popular is prepared from peppermint (*Mentha piperita*) (Rivera et al., 2008).

Among the major components found in peppermint leaves are fatty acids such as linoleic, linolenic and palmitic acid. A variety of volatile compounds, mainly menthol, menthone and isomenthone have also been identified along with β-carotene, chlorophyll, α- and γ-tocopherols and ascorbic acid. Other important compounds found in peppermint are phenolic compounds. The proportion of phenolic compounds found in peppermint leaves is approximately 19–23% dry weight, of which 12% belongs to the flavonoids group, including eriocitrin, rosmarinic acid, hesperidin and luteolin 7-O-rutinoside, among others. 75 percent of these compounds can be extracted in an infusion (McKay & Blumberg, 2006). It has been reported that flavonoids exert many beneficial effects on health which is linked to their known biological functions as antioxidants, due to their free radical scavenging and metal chelating properties (Pawlak et al., 2010).

Phenolic compounds are involved in various plant processes such as growth and reproduction and are also synthesized as a defence mechanism against biotic or abiotic stress (Cohen & Kennedy, 2010); therefore, their production can be enhanced by treatment with certain compounds, termed elicitors, which are defined as a substance that, when introduced in small concentrations to a living system, initiates or improves the biosynthesis of specific compounds (Edreva et al., 2008; Ferrari, 2010). Salicylic acid (SA) is a phenolic compound that shows great potential as an elicitor in plants. It occurs naturally in plants in small amounts and participates in the regulation of physiological processes such as stomatal closure, nutrient uptake, chlorophyll and protein synthesis, transpiration and photosynthesis (Raskin, 1992). Low concentrations of exogenously applied SA interact with stress-signalling mechanisms and induce phenolic compound synthesis (Gharib, 2007; Ghasemzadeh & Jaafar, 2012; Khandaker, Akond, & Oba, 2011). Catalase and ascorbate peroxidase are the main enzymes involved in the removal of H_2O_2 in plants and their activities can be

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http://dx.doi.org/10.1016/j.foodchem.2014.01.101
0308-8146/

inhibited by SA; this inhibition leads to a local increase in H_2O_2 levels, which enhances adaptive responses in plants, such as an increase of the activity of phenylalanine ammonia-lyase (PAL), a key enzyme in the phenolic metabolic pathway (Ferrari, 2010; Klessig & Malamy, 1994). Based on the important role of H_2O_2 in the stress-signalling mechanisms and because this molecule is relatively stable and diffusible, it can be considered an elicitor.

At low concentrations, H_2O_2 can also induce the synthesis of plant defence metabolites, such as phenolic compounds (Kumar, Sirhindi, Bhardwaj, Kumar, & Jain, 2010; Moskova, Todorova, Vera, Sergei, & Sergiev, 2009). However, an increase in cellular levels of H_2O_2 can promote generation of reactive oxygen species (ROS), such as superoxide (O_2-) and hydroxyl radicals (HO), which at high concentrations are highly detrimental to cells. Similarly, oxidative stress occurs during the natural processes of senescence; it is a widespread phenomenon that can lead to growth inhibition as well as decreased yield and crop quality. Therefore, the use of plant elicitors and their concentration should be carefully considered in order to avoid detrimental effects (Cheeseman, 2007; Hung, Yu, & Lin, 2005). The purpose of this study was to apply different concentrations of salicylic acid or hydrogen peroxide on peppermint (*M. piperita*) leaves and to evaluate their effect on the growth of the plant, as well as the metabolite profile and antioxidant capacity of its infusions.

2. Materials and methods

2.1. Reagents and Biological Materials

Peppermint plants were purchased from the local plant nursery (Floraplant S.A. de C.V). Caffeic, coumaric, sinapic and rosmarinic acids, as well as gallocatechin-gallate, naringin, rutin, quercetin and hesperidin were purchased from Sigma–Aldrich USA.

2.2. Plant cultivation, growth measurement parameters and elicitor treatment

Plants were grown in a greenhouse at the Universidad Autonoma de Queretaro, at 25 °C and 80% RH in pots with a diameter of 40 cm, with irrigation every 3 days. fertilisation was carried out in three doses at 15, 30 and 45 days after planting. Each pot was fertilised with the same fertiliser containing calcium nitrate (1.12 g/pot), magnesium sulpate (0.50 g/pot), potassium nitrate (0.36 g/pot), monobasic potassium phosphate (0.30 g/pot), iron chelate (0.07 g/pot) and manganese sulpate (0.01 g/pot). After 45 days, the plants were randomly treated with one of the two elicitors tested, which were dissolved in distiled water (SA: 0, 0.5, 1.0 and 2.0 mM; H_2O_2: 0, 0.05, 0.10 and 0.50 mM). Each treatment included 6 replicates, producing a total of 48 experimental units (pots). These solutions were sprayed at dew point (approximately 100 ml per plant) in two applications at days 45 and 52 and the leaves were collected for analysis at day 60.

The growth response of the plants to elicitor treatment was determined by measuring the increase in shoot length (longitudinal growth) and leaf expansion, which was evaluated by measuring the length of the leaf from the base to the apex (longitudinal growth) and at the broadest part (transverse growth). All of these parameters were measured before and after treatment (at days 45 and 60). Shoots ($n = 12$) and leaves ($n = 24$) were randomly chosen for each treatment.

2.3. Harvest and infusion preparation

Sixty days after planting, midlife leaves were collected from each experimental unit and dried at 45 °C for 24 h using a convection oven (Fisher Scientific, 650D, USA) followed by milling in a herb grinder (Krups GX4100, México) to a particle size of 0.7–1.0 mm. Infusions were prepared by adding 1 g of ground material to 100 ml of freshly boiled distiled water and allowed to stand for 10 min, and then filtered using a 0.5 mm pore size filter. These conditions simulate the recommended preparation of commercial infusions.

2.4. Total phenolic and flavonoid content

The total phenolic content of the peppermint infusions was determined according to the Folin–Ciocalteu colourimetric method (Singleton & Rossi, 1965), and the results were expressed as milligrammes of gallic acid (GA) equivalents per g of dry matter (mg GAE/g). The flavonoid content was determined according to Liu et al. (2002), and the results were expressed as mg of (+)-catechin equivalents per g of dry matter (mg CAE/g).

2.5. Identification of phenolic compounds

Phenolic compounds in the peppermint infusions were identified and quantified using a Waters 600 HPLC-DAD system, equipped with an auto-sampler (Waters 717 plus) and a UV/Vis detector (Waters 2487) (Milford, MA, USA). The separation was performed on a Phenomenex ODS-C18 column (250 × 4.6 mm, 5 μm). Samples (10 μl) were injected onto the reversed-phase column (Phenomenex C18 250 × 4.6 mm), at a flow rate of 1 ml/min. The mobile phase consisted of two solvents: (A) 1% v/v formic acid in water and (B) acetonitrile, in a ratio (A:B) of 98:2 at 0 min, 68:32 at 30 min, 45:55 at 48 min, 5:95 at 53 min, 98:2 at 57 min, a total run time of 67 min. Absorbance measurements were from 260–320 nm. Quantification was carried out using standards of phenolic acids and flavonoids (caffeic, coumaric, sinapic and rosmarinic acids as well as gallocatechin-gallate, naringin, rutin, quercetin and hesperidin).

2.6. Gas chromatography–mass spectrometry (GC–MS) analysis

A sample solution (1 mg/ml) was prepared in methanol, the methanol evaporated with nitrogen gas and then 50 μl of derivatizing agent, BSTFA (*N,O*-bis[trimethylsilyl]trifluoroacetamide) + 1% TMCS (trimethylchlorosilane), was added and stirred for 2 min at room temperature. Finally, 1 μl was injected into a GC–MS system.

The system consisted of an Agilent GC Series 7890A (Wilmington, DE, USA) and an Agilent single quadrupole MS detector (model 5975C), with electron energy set at 70 eV and the mass range at 50–700 *m/z*. An HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm) and a split/splitless injector (2 mm i.d.) were used. The injector was set at 250 °C. GC was performed in the splitless mode with 2.5 min splitless time. The initial oven temperature was held at 100 °C for 1 min and raised to 220 °C at 6 °C/min, held for 1.23 min, then raised to 290 °C at 10 °C/min, and raised to 310 °C at 40 °C/min, and held for 7.5 min. The flow rate of carrier gas (helium) was maintained at 1 ml/min. The GC–MS control and data processing was performed using Chem-Station (Agilent Technologies) software.

2.7. DPPH and ABTS free radical scavenging assays

DPPH free radical scavenging was measured as described by Brand-Wiliams, Cuvelier, and Berset (1995) and the ABTS assay was conducted based on the method by Re et al. (1999). Both results were expressed as the half maximal inhibitory concentration (IC_{50}) and compared with a (+)-catechin standard.