Plant Physiology and Biochemistry 112 (2017) 207–217

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Magnesium and manganese affect photosynthesis, essential oil composition and phenolic compounds of *Tanacetum parthenium*

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ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form
1 January 2017
Accepted 1 January 2017
Available online 3 January 2017

Keywords:
Essential oil
Feverfew
Magnesium
Manganese
Phenolic compounds

ABSTRACT

The accumulation of plant defense metabolites is closely associated with the concentration of nutrient elements, yet data related to the interactive effects of two nutrients on the deployment of phenolics and terpenoids are scare. In the present study, the interaction between magnesium (Mg) and manganese (Mn) on nutrient uptake, photosynthesis, oxidative status and the accumulation of phenolics and terpenoids in the leaves of feverfew plants grown at different concentrations of Mg and Mn was investigated. Nutrient uptake and photosynthesis were associated with the amount of applied Mg but could be modified by the concentration of Mn. Phenolic biosynthetic enzymes and individual phenolics were not only induced by Mg, but their levels were also dependent on the Mn supply. Additionally, the proportion of monoterpenes was enhanced by a deficiency of Mg rather than an excess of Mn. Deprivation of Mg also decreased the proportion of sesquiterpenes in the essential oil. Therefore, it appears that a high Mg and a low Mn supply lead to a marked shift from monoterpene to sesquiterpene production. Phenolic compounds also differentially accumulated under varying Mg and Mn concentrations. These results suggest a profound effect of the combined supply of Mg and Mn on the biosynthesis of terpenes and phenolics.

1. Introduction

Mineral element interactions are widely recognized as one of the major causes of deficiency and toxicity symptoms in higher plants (Marschner, 1995; Fageria, 2001; El-Jaoual Eaton et al., 2012). The literature strongly suggests that the interaction between mineral elements occurs when the supply of one nutrient influences the uptake, transport, utilization, and function of another nutrient within a wide range of plant tissues (Fageria, 2001; Hermans et al., 2011). Magnesium (Mg) and manganese (Mn) are essential plant nutrients. Mg is involved in a wide range of biological activities, including energy metabolism, nucleotide metabolism, photosynthetic carbon fixation, and nucleic acid folding (Karley and White, 2009; Hermans et al., 2011). However, Mn primarily serves as an activator of several enzymes, including those that catalyze oxidation-reduction reactions, decarboxylation, hydrolysis and group transfers, and its role may be filled by other nutrients, especially magnesium (Chatterjee et al., 1994; El-Jaoual Eaton et al., 2012; Millaleo et al., 2013). High concentrations of Mn in media have also been reported to cause Mg deficiency in plants (Chatterjee et al., 1994; El-Jaoual Eaton et al., 2012). Alternatively, increasing the amount of Mg suppressed Mn uptake in the shoots and roots of some plant species (Harrison and Bergman, 1981; El-Jaoual Eaton et al., 2012). It has also been found that Mg and Mn antagonistically affect the Hill Reaction and synergistically promote the activities of aldolase and ATPase enzymes (Chatterjee et al., 1994; Agarwala et al., 1988).

Feverfew (*Tanacetum parthenium* L. Schulz Bip.) is an aromatic plant widely distributed across Asia, Europe, and North America (Palevitch et al., 1997). According to recent studies, the essential oil terpenes of feverfew plants exhibit anti-inflammatory, antibacterial, antifungal and insecticidal effects (Hough-Golstein and Hahn, 1992; Neszmelyi et al., 1992; Brown et al., 1997). In addition to its terpenoid components, the biological activity of feverfew is also due to its content of phenolic compounds including phenolic acids and flavonoids (Williams et al., 1999; Wu et al., 2006).

Along with the well-known roles of Mg and Mn, these elements are two major cofactors of terpene synthase enzymes (TPS). For instance, sesquiterpene synthases prefer Mg, but monoterpene

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http://dx.doi.org/10.1016/j.plaphy.2017.01.002
0981-9428/

synthases are less selective in their divalent cation requirements (Rohdich et al., 2006; Köllner et al., 2008). The catalytic site of TPS requires Mg and Mn for substrate binding. In other words, the reaction chemistry of TPS is dependent on the availability of these two divalent elements to produce a diverse array of terpenes (Green et al., 2009). In addition, the biosynthetic cost of various terpene components differs due to their varying levels of chemical reduction. Therefore, changes in the balance between Mg and Mn could activate different groups of terpene synthases that in turn could alter the composition of terpenes (Picaud et al., 2005; Köllner et al., 2008). Moreover, high Mn concentrations act indirectly to increase the accumulation of phenolic compounds (Rouphael et al., 2012; Ribera et al., 2013; Gerendás and Führs, 2013; Kováčik et al., 2014).

Hence, we hypothesized that exposure to varying concentrations of Mg and Mn may depress the concentration and uptake of each other, and this may affect the photosynthesis, phenolic metabolism, and terpene profile of feverfew plants.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of feverfew (*Tanacetum parthenium* L. Schulz Bip., diploid 'Zardband' Asteraceae) were kindly provided by Esfahan Agricultural and Natural Resources Research Center, Esfahan, Iran. Seeds were sterilized with 0.4% sodium hypochlorite for 10 min and incubated at 15/25 °C for 2 days in the light to ensure germination. Seeds were sown in a nutrient medium (Hoagland and Arnon, 1950) in a growth chamber with a light/dark regime of 14/10 h, relative humidity of 40–45%, temperature of 25/17 °C (day/night), and photosynthetic photon flux density (PPFD) of 300 μmol m^{-2} s^{-1} and germinated after 10 days. Fifty uniform seedlings were then transferred to each of 6 L plastic containers containing 2.5 mM $Ca(NO_3)_2$, 2.5 mM KNO_3, 1.0 mM $MgSO_4$, 0.5 mM KH_2PO_4, 32 μM Fe-EDTA, 45 μM H_3BO_3, 9.1 μM $MnCl_2$, 0.76 μM $ZnCl_2$, 0.31 μM $CuCl_2$ and 0.21 μM Na_2MoO_4. The medium was renewed every 4 days. Plants were grown hydroponically for 40 days and subjected to four Mg levels (1 mM (control), Mg-free, 2 and 4 mM Mg) and four Mn levels (9.1 μM (control), Mn-free, 50 and 150 μM Mn) in a factorial combination. The Mg was removed from the medium by substituting the $MgSO_4$ with equivalent moles of K_2SO_4 to keep the sulfur supply and medium osmoticum constant. The $MnCl_2$ was also removed from the solution to cause a deficiency of Mn. Concentrations of Mg and Mn were selected based on prior studies in which different plant terpene synthases produced alternate types of enzymes at varying concentrations of Mg and Mn (Rohdich et al., 2006; Köllner et al., 2008). Fourteen days after the induction of Mg and Mn, feverfew plants were harvested after the determination of photosynthetic parameters. Feverfew leaves were collected and kept at −80 °C for biochemical analyses. Selected feverfew leaves were dried in an oven at 70 °C for 24 h and weighed to analyze the individual phenolics.

2.2. Quantification of mineral nutrients

Plant roots and shoots (50 mg) were separated, weighed, dried by a freeze-dryer and ground using a mixer mill. Then, the samples were digested with nitric acid (v/v 65%), and concentrations of the elements were measured with inductively coupled plasma atomic emission spectrometry (ICP-AES) (Kováčik et al., 2009a, b).

2.3. Photosynthetic gas exchange and chlorophyll fluorescence

Gas exchange parameters were measured on fully developed leaves with an open gas-exchange system Li-6400 XT (Li-Cor, Lincoln, NE, USA) equipped with an integrated fluorescence chamber head (Li-6400-40; Li-Cor). To decrease the effect of light variation, measurements were performed between 10.00 and 12.00 h. Air temperature, air relative humidity, and PPFD were maintained at 25 °C, 40–45%, and 300 μmol m^{-2} s^{-1}, respectively. Light intensity (PPFD) and CO_2 inside the chamber were 1000 μmol m^{-2} s^{-1} (with 10% blue light to maximize stomatal aperture) and 370 μmol mol^{-1}, respectively. Maximum efficiency of PSII (F_v/F_m) was also measured on the third fully expanded leaves after 30 min of dark adaptation using leaf-clips designed for this purpose. The minimum fluorescence yield in the dark-adapted state (F_0) was determined using a modulated pulse (<0.05 μmol m^{-2} s^{-1} for 1.8 μs) that was too small to impose physiological changes in the feverfew plant. Maximum fluorescence level in this state (F_m) was measured by firing the saturation pulse of 8000 μmol m^{-2} s^{-1} for 0.7 s. Values of the variable fluorescence yield ($F_v = F_m - F_0$) and maximum quantum efficiency of PSII (F_v/F_m) were determined from F_0 and F_m (Schreiber et al., 1994).

2.4. Determination of hydrogen peroxide and superoxide

The hydrogen peroxide concentration (H_2O_2) was measured as described by Jana and Choudhuri (1981). H_2O_2 was extracted by homogenizing 250 mg of feverfew leaves with 3 mL of phosphate buffer (50 mM, pH 6.5). The homogenates were centrifuged at 10,000*g* for 15 min. Then, the supernatant was added to a tube containing 0.1% titanium sulfate in 20% (v/v) H_2SO_4. After mixing, the solution was centrifuged at 8,000*g* for 10 min, the absorbance was read at 410 nm, and the level of H_2O_2 was determined against a standard curve of H_2O_2. Quantification of superoxide ($O_2^{\bullet-}$) was performed by the method of Elstner and Heupel (1976). Fresh leaves (100 mg) were crushed in 2 mL of 65 mM potassium phosphate buffer (pH 7.8) and centrifuged at 10,000*g* for 20 min. Subsequently the supernatant was added to a tube containing 65 mM potassium phosphate buffer (pH 7.8) and 10 mM hydroxylammonium chloride and incubated at 25 °C for 30 min. The mixture was then incubated with 17 mM sulfanilic acid and 7 mM α-naphthyl amine at 25 °C for 30 min. The solution obtained was mixed with an equal volume of ethyl ether, and the absorbance of the pink phase was determined at 530 nm. The concentration of superoxide was determined based on a standard graph.

2.5. Phenolic biosynthetic enzymes

Phenylalanine ammonia-lyase (PAL) activity was assayed following the method of McCallum and Walker (1990). Feverfew leaves (300 mg) were extracted with a solution containing 5 mL 0.06 M sodium borate buffer (pH 8.8), 5 mM β-mercaptoethanol, and 3% (w/v) PVPP. Then, the supernatant was centrifuged and filtered, and the reaction was initiated by the addition of 5 mL of 0.06 M L-phenylalanine. After incubation at 37 °C for 1 h, the reaction was stopped by the addition of 0.5 mL of 35% (w/v) trifluoroacetic acid (TFA), and then centrifuged for 10 min at 5000*g*. The PAL activity was determined by the production of cinnamate during 1 h at 30 °C, as measured by the absorbance change of the supernatant at 290 nm.

The polyphenol oxidase (PPO) activity was measured following the method of Ghanati et al. (2002). Fresh leaves (200 mg) were ground in liquid nitrogen and extracted in a 100 mM potassium phosphate buffer (pH 6.8). After centrifugation at 10,000*g* for 15 min, the supernatant was added to 100 mM potassium phosphate buffer (pH 6.5) and freshly prepared 4-methylcatechol at a final concentration of 20 mM. The activity of PPO was expressed