SHORT COMMUNICATION

The effects of several abiotic elicitors on the expression of genes of key enzymes involved in the parthenolide biosynthetic pathway and its content in feverfew plant (Tanacetum parthenium L.)

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ABSTRACT

Feverfew is an herb used to treat different diseases such as migraine headaches. Due to the economic aspect of its metabolites in the pharmaceutical industry, establishing new approaches to produce the compounds on a large scale is essential. To investigate the effects of stimulators on parthenolide synthesis, feverfew plants were treated with different elicitors, including methyl jasmonate, salicylic acid, NaCl, aluminum oxide, and magnesium aluminate spinel nanoparticles. The expression of genes, E-beta-caryophyllene synthase, Germacrene A synthase, and Costunolide Synthase in the metabolite biosynthesis pathway was examined using qRT-PCR. In addition, parthenolide content, total flavonoids, and polyphenols antioxidant activity were evaluated by HPLC and spectrophotometry. Our results indicated that methyl jasmonate and salicylic acid were more effective on the final concentration of parthenolide, but magnesium aluminate spinel affected the genes’ expression, positively. The results show that the elicitors can be used to increase the metabolite in the plant, commercially.

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1. Introduction

Feverfew (Tanacetum parthenium L.) is a plant from the Asteraceae (pearl) family, which usually grows in forests and plains (Khajavi et al. 2019). Feverfew produces various compounds, including polar flavonoids, lipophilic parthenolide (in flowers and leaves), triterpene and sterol (in roots), and sesquiterpene lactones (Pourianezhad et al. 2016). The leaves and flowers of this plant contain compounds that have antifever, anti-inflammatory, and antipyretic activities, and they are used in traditional medicine to treat osteoarthritis, common colds, fever, and migraine headaches (Sur et al. 2009). Feverfew is also used as a laxative and vermifuge, as well as a spasmolytic in the treatment of colitis (Maggi 2019). In the present work, to find the effect of different abiotic elicitors and stresses on parthenolide content in feverfew, it was used two nanoparticles of aluminum oxide (Al₂O₃) (AO) and magnesium aluminate spinel (MgAl₂O₄) (MAS), NaCl, methyl jasmonate (MJ), and salicylic acid (SA). The effect of the nano-elicitors and others was also investigated on the expression of genes involved in the parthenolide biosynthesis pathway.

2. Results and discussion

2.1. The impacts of elicitors on total phenolic content

The total concentration of phenolic compounds in treated plants was examined by the spectroscopy method. As shown in figure S2, the content of these compounds was significantly increased in the samples treated with MJ (10 mM/48h) and AO elicitors (50 mg/L6 h) compared to control (without any treatment). Also, the least amount of total phenolic compounds belonged to the sample treated with SA (5 mM/6h).

Various studies have reported that MJ positively affects plant polyphenolic compounds and causes an increase in total phenols and anthocyanins in tomatoes (Tzortzakis and Economakis 2007), pomegranates (Sayyari et al. 2011), and strawberries (de la Peña Moreno et al. 2010). Anjum et al. studied the impact of metal nanomaterials (silver, copper, gold, cobalt, and zinc), metal oxide nanomaterials (ZnO, TiO₂, CuO, Cs₂O, Al₂O₃, and CdO), and carbon-related nanomaterials (SWCN, MWCNs, and C60), on producing specific metabolites (such as alkaloids, terpenoids, phenolics, and flavonoids) in medicinal plants. Their results have indicated that the use of different elicitors causes a significant increase in the content of total phenolic compounds (Anjum et al. 2019).

2.2. The effects of elicitors on antioxidant activity

To investigate the antioxidant activity of treated plants’ extract, the percentage of antioxidants at the different extract concentrations (100, 50, 20, 10, and 5 μg/mL) was measured. The sample treated with MJ (5 mM/6h) in the extract concentration of 100 μg/mL showed the highest percentage of scavenging activity. Also, in concentrations 50, 20, and 10 μg/mL of extract, the highest and least ratio of DPPH free radical scavenging activity was related to the SA-treated (10 mM/48h) and salt-treated (100 mg/L/48h) samples, respectively. Finally, at the concentration of 5 μg/mL of
extract, the samples treated with SA (5 mM/6h) and salt elicitors (50 mg/L/6h) had the highest percentage, and the feverfew plant treated with salt elicitor (100 mg/L/48h) had the least rate of scavenging activity among the samples. Dastyar et al. reported that SA (1 mM) and MJ (0.5 mM) induced the catalase and peroxidase activity, significantly (Dastyar et al. 2019). In another study, Jeong et al. investigated the production of antioxidant compounds by culturing Ginseng hairy roots with induction by various elicitors (salicylic acid, and acetylsalicylic). Their data showed that the use of SA (0.1–0.5 mM) and acyl salicylic acid (0.10 mm) increases the content of Ginseng saponin, significantly (Jeong et al. 2005).

2.3. The effects of different elicitors on parthenolide concentration

The results of HPLC analysis (figure S3) show an increase in parthenolide content in SA (5 mM/6h) and MJ (10 mM/6h) treated plants. It can be suggested that these elicitors can affect the expression of the TpPTS gene at the end of the parthenolide biosynthesis pathway and cause increased parthenolide production. Ahmadi et al. investigated the impact of spherical nanocarbon (as foliar usage at five levels 0, 0.125, 0.25, 0.5, and 1 g/L), and SA (at two concentrations of 0 and 0.2 mmol) on biochemical traits and parthenolide content of two species of feverfew. Their data showed that spherical nanocarbon (at concentration of 500 mg/L) and SA (0.2 mmol), increased the parthenolide synthesis in both feverfew cultivars (Ahmadi et al. 2018). Majdi et al. evaluated parthenolide content using HPLC and the expression of genes involved in parthenolide biosynthesis pathway during external use of MJ and SA in feverfew. They reported both induced and increased parthenolide biosynthesis by 3.1 and 1.96 times within 24 h after treatment, respectively (Majdi et al. 2015). The results are similar to the present work. Pourianezhad et al. examined the effect of MJ (100 μM), silver ion (100 μM), and yeast extract (2.5 mg L⁻¹) on parthenolide concentration and the related genes in the cultivated feverfew roots. They showed that a high concentration of parthenolide (0.05 mg g⁻¹DW) was obtained with yeast extract (2.5 mg L⁻¹) and MJ (100 μM) elicitors after 48 h (Pourianezhad et al. 2016).

2.4. Effects of elicitors on genes expression

The gene expression analysis was performed using the real-time RT-PCR to evaluate the effects of the elicitors on genes in the parthenolide biosynthesis pathway. The expression of the E-beta-caryophyllene synthase (CarS), Germacrene A synthase (GAS), and Costunolide Synthase (COST) genes in feverfew plants treated with all elicitors (all concentrations and times) was analyzed. The relative expression of the COST, CarS, and GAS genes in the elicitor treated plants compared to control is depicted in figure S4. As shown in this figure, the most upregulation and downregulation of the genes (with different folds) occur in MAS nanoparticles (100 mg/L/6h) treatment. Also, the AO nanoparticles (100 mg/L/48h) and NaCl (100 mg/L/6h) among all treatments have positively been affected the genes. Moreover, the GAS gene was also influenced by AO nanoparticles (50 mg/L/48h), and SA (10 mM/6h), and its expression was increased (Fig. S4). The results show that nanoparticles had a positive effect on the expression
of GAS, COST, and CarS genes. It is a positive correlation between the expression of the genes and parthenolide concentration. Majdi et al. analyzed the genes in the pathway of terpenes (TpHMGR, TpGAS, and TpHDR) in chamomile. They reported a significant positive correlation between the behavior of the GAS gene and parthenolide content among the studied genes (Majdi et al. 2015).

3. Conclusion
Nowadays, with the progression in biotechnology techniques, researchers are trying to replace chemical drugs with natural materials from medicinal plants (José Bagur et al. 2017). Medicinal plants, due to their availability, to have natural compounds with low side effects, and high effectiveness, are among the most attractive organisms for bio-researches (Lachenmayer et al. 2010). In the current study, we investigated the effect of MJ, SA, salt, and two nanoparticles (AO and MAS), on phenolic and flavonoids content and the expression of genes (TpCars, TpGAS, and COST) involved in parthenolide biosynthesis pathway in feverfew. The highest parthenolide concentration by the HPLC test was observed in the samples treated with MJ and SA (all concentrations). Although Brown et al. previously studied the effect of chemical mutagens on the plant and its parthenolide content (Brown et al. 1995), the present work is the first report on the impact of new nanomaterials (AO and MAS nanoparticles) on the expression of genes involved in the parthenolide biosynthesis pathway in feverfew. These results open a new window for more research on the application of novel elicitors for more production of active medicinal compounds, commercially.

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