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The Effect of Magnetic Iron Oxide Nanoparticles and Ferric Chloride on the Expression of Some Rosmarinic Acid Biosynthetic Genes in *Melissa Officinalis* L.

Simin Golkari

MSc Student, Department of Biotechnology, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran. E-mail address: golkari@agr.uk.ac.ir

Shahram Pourseyedi

*Corresponding author. Associate Professor, Department of Biotechnology, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran. E-mail address: spseyedi@uk.ac.ir

Ali Kazemipour

Assistant Professor, Department of Plant Genetics and Production Engineering, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran. E-mail address: ali.kazemi@uk.ac.ir

Mehdi Mansouri

Assistant Professor, Department of Biotechnology, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran. E-mail address: m.mansouri@uk.ac.ir

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Abstract

Objective

Rosmarinic acid (RA), an anticancer, antiallergic, and antimicrobial agent, is a secondary metabolite in many plants of the family Lamiaceae including *M. officinalis* L. The application of nanoparticles (NPs) as a novel elicitor for the biosynthesis of bioactive compounds shown that the NPs could affect the secondary metabolites in plants by eliciting the expression of biosynthetic pathway genes. The present paper aimed to assess the effect of Magnetic Iron Oxide Nanoparticles (MIONPs) in comparison with their dissolved counterpart.

Materials and methods

Foliar application of different concentrations: including 5, 10, 25, and 50 mg/L Fe of ferric chloride and MIONPs on the plant leaves was performed. The relative mRNA levels of *TAT*, *RAS*,

and *HPPR* were evaluated with quantitative real-time PCR (qRT-PCR) and compared between treated and untreated samples.

Results

This study showed the positive effects of ferric chloride and MIONPs on the expression of genes involved in the biosynthesis pathway of RA. The highest expression level of *TAT*, *HPPR*, and *RAS* genes was observed in plants treated with MIONPs at the concentration of 25 mg/L and the expression of genes decreased as the concentration increased to 50 mg/L. However, the genes expression was still higher compared to the control plant.

Conclusions

The results showed that the exposure of plants to ferric chloride and magnetic iron oxide nanoparticles led to an increase in the expression of the genes under study compared to control samples. However, the application of nano-scale iron particles had more effect than ferric chloride on the expression levels. An increase in the expression of genes involved in the biosynthesis pathway of RA through treatment with MIONPs provides an opportunity for induction of synthesis and accumulation of RA. Therefore, we hope to be able to enhance the production of RA in *M. officinalis* L. by using these NPs.

Keywords: Gene expression, lemon balm, medicinal plant, secondary metabolite, qRT-PCR.

Paper Type: Research Paper.

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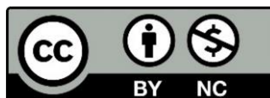

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Introduction

Lemon balm (*Melissa officinalis* L.) is a perennial herb, a member of the family Lamiaceae (Weitzel & Petersen 2010). This plant has three subspecies: *M. officinalis* subsp. Officinalis, *M.*

officinalis subsp. Altissima, and *M. officinalis* subsp. Inodora (Sari & Ceylan 2002). Lemon balm has been used as a medicinal plant to treat nervous and gastrointestinal disorders for over 2000 years (Weitzel & Petersen 2010). The leaves are used in herbal medicine due to their sedative, antispasmodic, and digestive properties (Miraj et al. 2017). Other medicinal effects of this herb such as antioxidant (Dastmalchi et al. 2008), anti-tumor (Saraydin et al. 2012), anti-inflammatory (Bounihi et al. 2013), and antiviral (Allahverdiyev et al. 2004) effects have also been recognized which are almost due to phenolic acids and the content of essential oil. Rosmarinic acid is one of the active components in various medicinal plants such as *Melissa Officinalis*, *Salvia officinalis*, *Mentha piperita*, *Symphytum officinale*, and *Thymus vulgaris* (Petersen et al. 2009). It is an ester of Caffeic acid and 3,4-dihydroxyphenyl lactic acids with the molecular formula $C_{18}H_{16}O_8$ (Petersen et al. 1993). This substance was first isolated from the rosemary plant by two Italian chemists, Scarpati and Oriente, and was named according to the aforementioned plant (Al-Dhabi et al. 2014). RA is found in the subfamily Nepetoideae of the family Lamiaceae and species of the Boraginaceae (Petersen 2013) and stored in the vacuole of plant cells (Pezeshki & Petersen 2018). As illustrated in Figure 1, RA biosynthesis initiates with the amino acids l-phenylalanine and l-tyrosine. Many of genes encoding enzymes involved in the RA biosynthesis pathway, such as hydroxyphenylpyruvate reductase (*HPPR*), rosmarinic acid synthase (*RAS*), phenylalanine ammonia-lyase (*PAL*) and 4-coumarate: CoA-ligase (*4CL*), have been identified in *M. officinalis* (Mansouri & Mohammadi 2021, Weitzel & Petersen 2010, Weitzel & Petersen 2011). The genes expression in eukaryotes is under multidimensionally and temporarily control (Shahsavari et al. 2021) and depends on the developmental stage. In each tissue type, only a relatively small set of genomes is expressed. (Mohammadabadi et al. 2021). Actually, the expression of genes in eukaryotes is specific to every tissue (Mohammadabadi et al. 2018), and the quantity of gene products that are made in the same tissue and other tissues manufacturer that product, regulates the expression of that gene (Mohammadabadi 2019). The accumulation of secondary metabolites often happens in plants subjected to stresses induced by different elicitors (Smetanska 2008). It can be achieved by affecting their metabolic synthesis pathways, i.e. the elicitors enhance the production of secondary metabolites by affecting the genes involved in their biosynthesis. Bacteria and fungi are biotic elicitors and mineral compounds and metal ions are abiotic ones (Zhao et al. 2005). The production of RA by biotic and abiotic elicitors in different herbal species was investigated concerning the pharmaceutical significance and high economic value of RA. An investigation carried out by Mizukami et al. (1992) showed that yeast extract affected and increased the content of RA in cultured cells of *Lithospermum erythrorhizon*. Also, a study reported that Ag^+ and yeast extract can be used as effective elicitors to increase the production of RA in hairy root of *Salvia miltiorrhiza* (Yan et al. 2006). Another study also reported the

significant role of methyl jasmonate, salicylic acid, and yeast extract in enhancing the amounts of RA in culturing cell suspension of *Solenostemon scutellarioides* (Sahu et al. 2013).

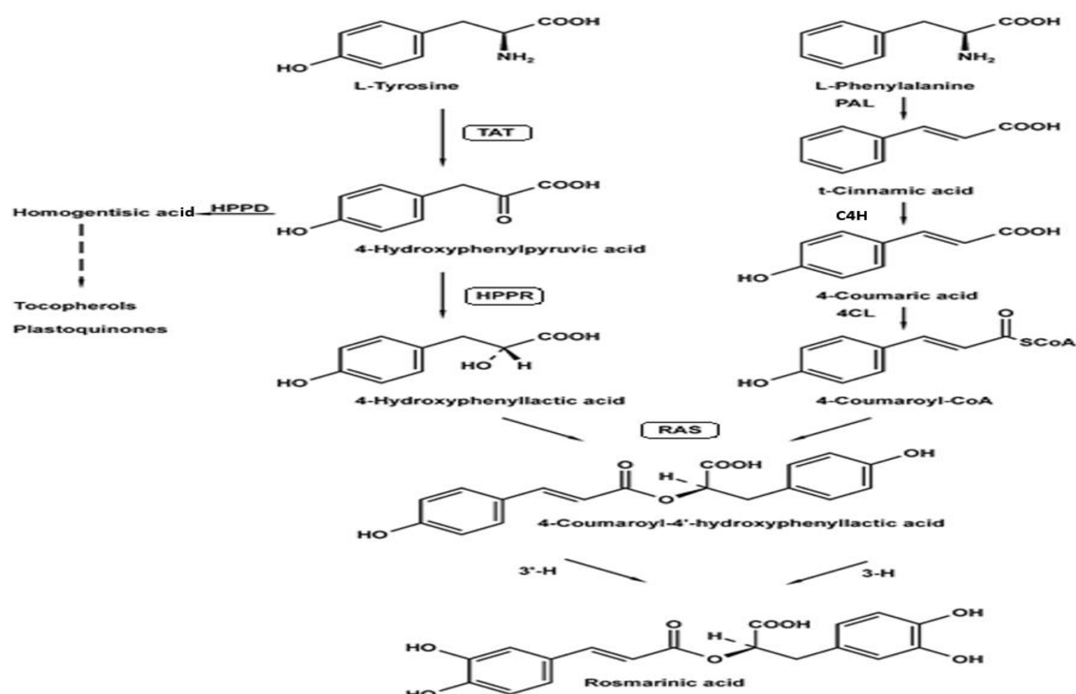

Figure 1. Biosynthetic pathway for rosmarinic acid. TAT=tyrosine aminotransferase, HPPR=hydroxyphenylpyruvate reductase, PAL=phenylalanine ammonia-lyase, C4H=cinnamic acid 4-hydroxylase, 4CL= 4-coumarate: CoA-ligase, RAS=rosmarinic acid synthase, 3-H, 3´-H=3- and 3´-hydroxylases, HPPD=hydroxyphenylpyruvate dioxygenase (Weitzel & Petersen 2010)

In recent years, nanoscience and nanotechnology have attracted the attention of many researchers. A key advance in nanoscience is the production and application of nanoparticles. NPs possess unique physical and chemical properties with 20-15000 atoms and a size of fewer than 100 nanometers (Liu 2006). The small size of NPs enhances the rate of adsorption, transfer, and specific surface area of these compounds compared to normal particles (Oloumi et al. 2015). Functional nanomaterials in the form of nano-additives, nano-fertilizers, nano-sensors, nano-pesticides and herbicides, etc., which are biocompatible, costeffective, and biodegradable, have received a lot of attention in the field of agriculture (Usman et al. 2020). These materials are used in agriculture with the aim of minimize the cost of production to maximize efficiency (De Oliveira et al. 2014), and reduce nutrient losses to increase yield (Usman et al. 2020). Since magnetic metal oxide nanoparticles, such as Fe_3O_4 have high stability and low toxicity, received much attention in various research fields (Zhu et al. 2018). Different studies have focused on replacing

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conventional Fe fertilizers with Fe_3O_4 and Fe_2O_3 nanoparticles as fertilizers. Nanoparticles of Fe have been used in field conditions (Kumari & Khan 2018, Rui et al. 2016) and hydroponic applications (Alkhatib et al. 2019, Konate et al. 2018, Li et al. 2021). Magnetite nanoparticles (Fe_3O_4) is considered as the most ideal magnetic nanoparticles for a wide range of fundamental investigations due to their nanoscale size, low toxicity, large surface area, low sedimentation rates, high thermal stability and low sedimentation rates (Cardoso et al. 2018). In this paper, we investigated the changes in expression patterns of *HPPR*, *TAT*, and *RAS* genes in the presence of different treatments with magnetic iron oxide nanoparticles and ferric chloride with respect to the economic significance and pharmaceutical value of RA found in *M. officinalis* L. and regarding the fact that three above-mentioned enzymes are involved in biosynthetic pathway of RA.

Material and methods

Plant cultivation: In this research, the seeds of *M. officinalis* L. were prepared by Pakan Bazr Company in Esfahan, Iran. For sterilization, the seeds were immersed in 70% alcohol for 30 seconds, then washed in distilled water. They were disinfected with sodium hypochlorite 2.5% for 10 minutes and then three times washed in sterilized distilled water. The seed germination happened on wet filter papers in sterile Petri dishes in darkness. Two weeks after germination, the seedlings were transferred to 15 mL falcon tubes containing 1/2 Hoagland solution. We used a modified Hoagland's nutrient solution (Munns 2013). Each seedling was placed inside a falcon and placed on a shaker at 110 rpm for two months under phytotron conditions (16-hour of light and 8-hour of dark cycle, temperature of 25±1°C). We renewed the nutrient solution with a fresh solution every day.

Treatments and sampling: The MIONPs under study were synthesized using the green chemistry method from *M. officinalis* L. leaf extract in the biotechnology laboratory of the Shahid Bahonar University of Kerman, Iran. These nanoparticles were purified and free of any additional compounds such as secondary metabolites (Taheri et al. 2018). We determine the magnetic iron oxide nanoparticles' diameters by performing analysis with a dynamic light scattering (DLS) instrument (VASCO, France). $FeCl_3 \cdot 6H_2O$ under study was purchased from Merck Company of Germany. Foliar application of different concentrations (5, 10, 25, and 50 mg/L Fe) of ferric chloride and magnetic iron oxide nanoparticles on the plant leaves was carried out two times at intervals of 72 hours. We used double distilled water to control plants. Three replicates were considered for each concentration. In this study, foliar application of was performed on two-month old lemon balm plants (Figure 2). 48 hours after the last foliar application, the plant leaves were harvested, frozen in liquid nitrogen, and stored at -80ºC until RNA extraction.

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Figure 2. Two-month old lemon balm plant in falcon tubes containing Hoagland solution before treatment. Plants were grown under the condition of 16-hour of light and 8-hour of dark at temperature of 25±1°C

RNA extraction and cDNA synthesis: Total RNA was extracted from the fully expanded leaves of both treated and control plants using RNA Isolation Kit (Dena Zist, Asia), according to the manufacturer's instructions. Then, the extracted RNAs were treated with DNaseI (SinaClon BioScience, Iran) enzyme to remove possible contaminations of DNA. RNA concentration was measured using NanoDrop 1000. RNA quality was evaluated by 1% agarose gel electrophoresis. Ethidium bromide was used to stain the gel. The photograph of the gel was captured by using a gel documentation system. Next, 2 µg of total RNA was transformed to cDNA using a cDNA synthesis kit (Pars Tous, Iran) and following the manufacturer's instructions.

Gene expression analysis: Gene-specific primers (Table 1) were designed using the Primerquest tool (http://eu.idtdna.com/primerquest/home/index) and confirmed for specificity by Primer-BLAST searches in National Center for Biotechnology Information (NCBI). Since there was no information about the sequence of elongation factor 1-alpha (*EF1α*), using RNA-seq data previously generated from *M. officinalis*, we assembled and identified the gene sequence of *EF1α*. To this end, high-quality (Phred Score ≥ 30) reads were subjected to de novo assembly using Trinity software (Grabherr et al. 2011). All generated transcript sequences were aligned to the NCBI non-redundant (nr) protein database using the BLASTX program. *EF1α* was used as the internal control gene. Also, the *TAT*, *HPPR* and *RAS* genes were retrieved from the NCBI website. Quantitative Real-Time PCR reaction was carried out in a volume of 20 µL with Rotor-Gene Q system (Qiagen, Germany) by using 4 µL of 5x HOT FIREPol EvaGreen qPCR Mix Plus (Solis BioDyne, Estonia); 2 µL of sample cDNA; 1 µL of each primer with a concentration of 10 pmol/µL, and 12 µL of distilled water. The thermal profile used for reactions is as follows: 94ºC for 12 minutes, 40 cycles including: 94ºC for 30 seconds, 58-62ºC (Table 1) for 20 seconds, and 72ºC for 20 seconds. The specificity of products was assessed using agarose gel electrophoresis. We analysed the melting curve at temperatures ranging from 65 to 95ºC with an increase of temperature by 1 ºC. The experiment was repeated twice. The expression ratios of target genes

(*TAT*, *HPPR*, and *RAS*) were normalized with the expression of the *EF1α* gene and calculated using REST software (Pfaffl et al. 2002). Analysis of variance was performed using IBM SPSS Statistics 22, and Duncan test ($p \leq 0.05$) was used to compare means.

Table 1. The primers used in this study for qRT-PCR

Gene name	Primer sequence (5′→3′)	Amplicon size (bp)	Annealing Temperature (°C)	Accession number
TAT	Forward: CAAGACCGTGTTTCCCAATC Reverse: TCAACTTCCCATCCCTTCTC	97	58	JN863949.1
HPPR	Forward: GTGAGTGCGATAAGTATGTGAG Reverse: CAACTGCTAACCCGATTCTG	120	60	MW118284.1
RAS	Forward: CCAACTACCACACACTGAG Reverse: GTAAGGGTAGAAGTCAACGAG	116	62	FR670523.1
EF1α	Forward: CGGACATCTCATCTACAAGC Reverse: GTCAAGAACCCAGGCATAC	112	62	ERR2040574 SRR5150719

Results and Discussion

Hydrodynamic diameter of magnetic iron oxide nanoparticles: Concerning the results obtained by performing analysis with a DLS instrument, the diameter of MIONPs was measured to be 68 nanometers showing that synthesized particles have favorable dimensions on nanometer scale (Figure 3).

Quality control of extracted RNA and specificity of primers: A nanodrop system was used to quantity and quality the extracted RNA, and a value of 1.87 was obtained for OD260/OD280 ratio. The quality of extracted RNA was determined using 1% agarose gel electrophoresis. Two clear bands on the gel, including 25S rRNA and 18S rRNA, showed that the extracted RNA was complete and perfect. In all qRT-PCR reactions, only one amplified fragment was observed over the 1% agarose gel for every one of the *TAT*, *HPPR*, *RAS*, and *EF1α* genes. The presence of four single bands with the lengths of 112 bp, 97 bp, 120 bp, and 116 bp, respectively, for *EF1α*, *TAT*, *HPPR*, and *RAS* genes over the gel suggest the specific amplification of each gene (Figure 4). Moreover, the amplification of only one fragment for each one of the genes was confirmed by the obtained melting curve (Figure 5).

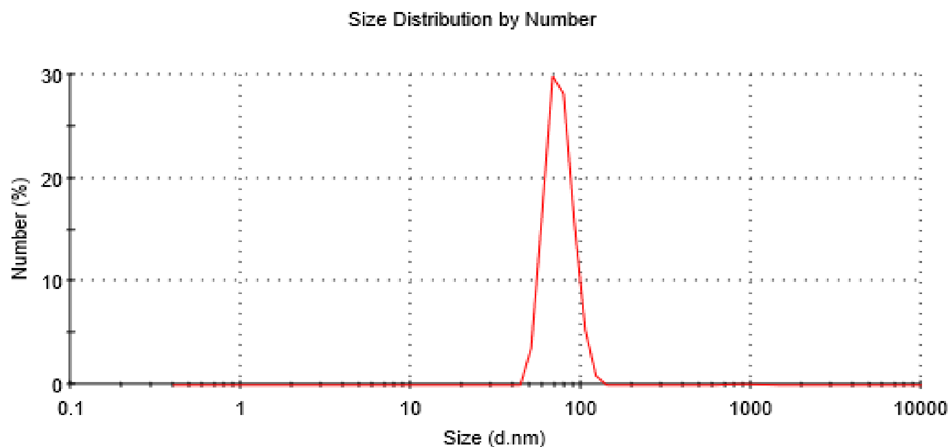

Figure 3. Size distribution of magnetic iron oxide nanoparticles using dynamic light scattering (DLS)

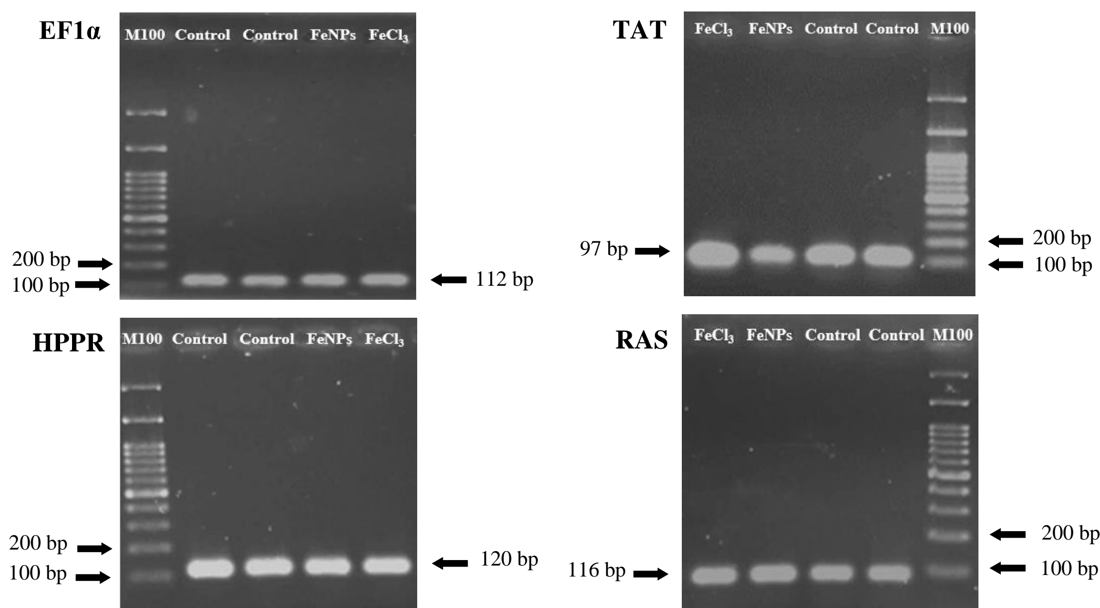

Figure 4. Agarose gel (1%) showing specific amplification of each gene in quantitative real-time PCR reaction. The single bands with the lengths of 112 bp, 97 bp, 120 bp, and 116 bp, belong to the *EF1α*, *TAT*, *HPPR* and *RAS* genes in Lemon balm, respectively

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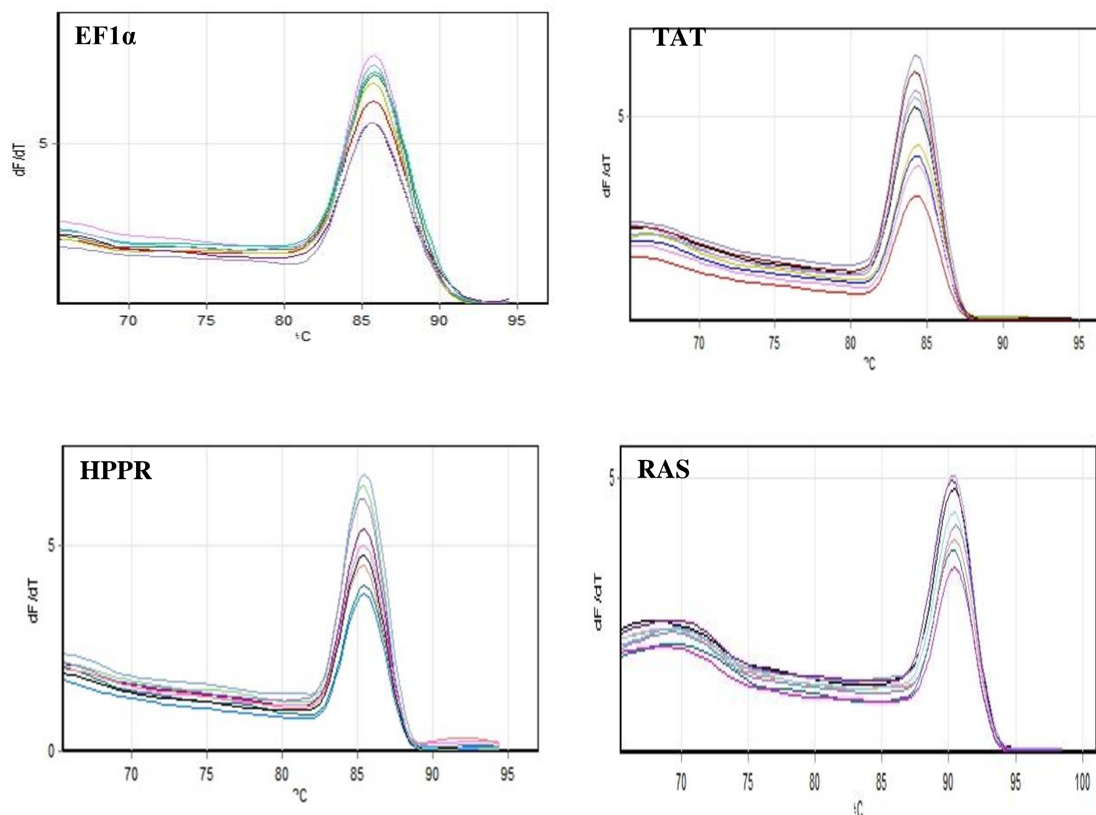

Figure 5. Specific amplification in real-time PCR. The melting curve of *EF1α*, *TAT*, *HPPR* and *RAS* genes with single peak

Study of gene expression in plants treated with magnetic iron oxide nanoparticles: Our results showed that different concentrations of MIONPs caused a meaningful increase in the expression of *TAT* gene compared to the control samples. The maximum level of the *TAT* expression was observed in plants treated with a MIONPs concentration of 25 mg/L. At a concentration of 25 mg/L, the expression of the gene was 12.7 times higher compared to the control samples. There were no meaningful differences between treatments with concentrations of 5 and 10 mg/L (Figure 6a). In addition, treatments with all concentrations of MIONPs significantly increased the expression level of the *HPPR* gene compared to the control. According to the results, the expression level of the *HPPR* gene was the highest (20 times) at a concentration of 25 mg/L. The lowest expression (7.7 times) was observed in plants were treated with 50 mg/L MIONPs (Figure 6b). We also found that the expression level of *RAS* gene in the plants treated with MIONPs concentrations of 10 and 25 mg/L meaningfully increased by 5.1 and 12.6 times, respectively, compared with the control samples. In addition, an increase was observed in the expression level of that gene at MIONPs concentrations of 5 and 50 mg/L, but it was not meaningful (Figure 6c). Generally, the results showed that the expression of the *TAT*, *HPPR*, and

RAS genes decreased as concentration increased to 50 mg/L. But, the level of expression was higher compared to the control plant.

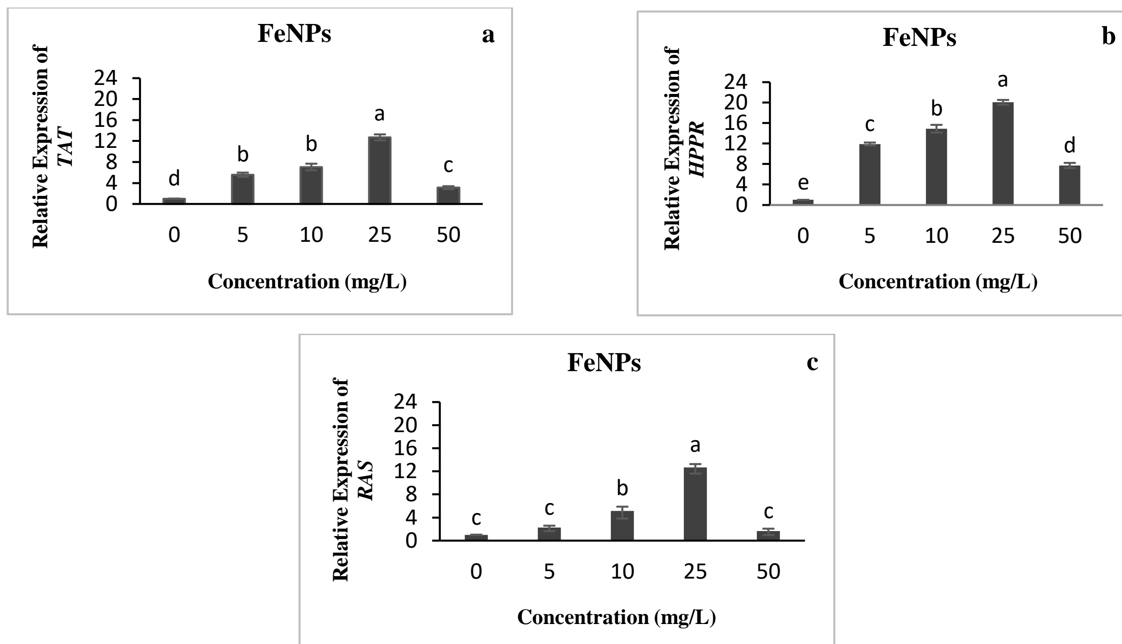

Figure 6. Changes in the expression affected by different concentrations of magnetic iron oxide nanoparticles, which are indicated by a, b, and c and belong to the tyrosine aminotransferase (*TAT*), hydroxyphenylpyruvate reductase (*HPPR)* and, rosmarinic acid synthase (*RAS*) genes, respectively. Different letters indicate a significant difference at the 5% probability level. Error bars show standard error values

Study of gene expression in plants treated with ferric chloride: In another test, ferric chloride affected the expression of the *TAT* gene at all concentrations. The *TAT* expression level increased meaningfully with treatments of ferric chloride. The highest expression was obtained at a concentration of 25 mg/L (6.7 times higher). However, there were no meaningful differences between treatments with concentrations of 10 and 25 mg/L (Figure 7a). As illustrated in Figure 7b, the plants treated with different concentrations of ferric chloride also exhibited changes in the expression level of the *HPPR* gene. The expression of the *HPPR* increased meaningfully at concentrations of 5, 10, and 25 mg/L (3.9, 4.1, and 4.6 times, respectively) compared to the control. However, there was no meaningful increase in the gene expression in the plants treated with a concentration of 50 mg/L. In treatments with ferric chloride, the concentration of 25 mg/L caused an increase in the mRNA level of the *RAS* gene as well, compared to the control samples. The expression level of the *RAS* gene increased meaningfully (2.6 times) at this concentration,

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but in plants treated with other concentrations, the increase in the expression level was not meaningful (Figure 7c).

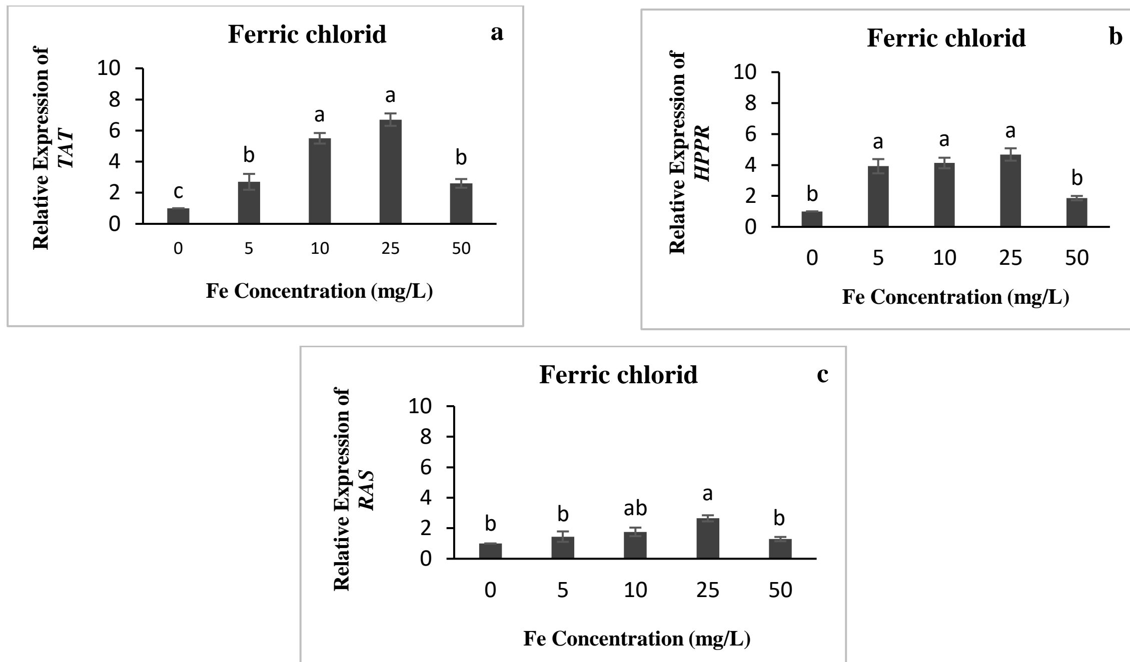

Figure 7. Changes in the expression affected by different concentrations of ferric chloride, which are indicated by a, b, and c and belong to the tyrosine aminotransferase (*TAT*), hydroxyphenylpyruvate reductase (*HPPR)* and, rosmarinic acid synthase (*RAS*) genes, respectively. Different letters indicate a significant difference at the 5% probability level. Error bars show standard error values

Overall, our results indicated that the application of MIONPs and ferric chloride-induced the expression of *TAT*, *HPPR*, and *RAS*, but the effect caused by MIONPs was further. Physicochemical properties of nanoparticles, including crystalline structure, size, and surface charge, impression their bioaccumulation and translocation in plants (Pacheco & Buzea 2018). One of the most important factors that affect the uptake of NPs in plants is the size of these particles (Wang et al. 2011, Zhu et al. 2008). The uptake of NPs is specific to the plant and can have a positive, negative or no effect on the plant (Pacheco & Buzea 2018). From the research done by MA et al. (2010), it can be realized that NPs with smaller size can be quickly absorbed by plants and cause more phytotoxicity at lower concentrations (Rico et al. 2011, Wang et al. 2013). Therefore, normally NPs that are smaller in size are more toxic than larger particles (Buzea et al. 2007). NPs less than 20 nm may easily pass via the plant cell wall, while large particles create pores in the cell wall and enter the plant cell (Rastogi et al. 2017). The effectiveness of NPs depends on their concentration and varies in different plants (Siddiqui et al. 2015).

Since research shows that expression of gene in plants is altered when exposed to NPs, it is important to comprehend the mechanism by which NPs may affect expression of gene (Rastogi et al. 2017). It has been reported that NPs lead to oxidative burst by causing metabolic imbalance (Hossain et al. 2015, Jiang et al. 2014). Electron leakage in chloroplast and mitochondria is known as the primary reason of ROS production, and peroxisomes are recognized to produce the superoxide anion radicale (Sharma & Dietz 2009). This hypothesis is confirmed by lipid peroxidation and detection of increased ROS levels as a result of plant exposure to NPs (Cvjetko et al. 2017; Jiang et al. 2014; Shaw et al. 2014; Song et al. 2016). Actually, ROS-mediated signaling pathway is hypothesized as the cause for gene regulation by NPs in various plants (Rastogi 2019).

Elicitors can increase the production of RA in plants by inducing the expression of genes involved in the biosynthesis pathway of the compound. A study showed that the Fe_3O_4 nanoparticles' application enhanced the *RAS* gene expression in *Dracocephalum kotschyi* hairy root cultures. RAS is an essential enzyme in the biosynthesis of RA. The authors reported a positive relationship between the expression of the *RAS* and RA (Nourozi et al. 2019). However, another ones showed the positive effect of yeast extract on the production of RA. The authors reported that the expression of the *TAT* gene in *M. officinalis* treated by yeast extract was accompanied with the production of RA (Nasiri-Bezenjani et al. 2014). However, Yan et al. (2006) reported a direct relationship between the TAT activity and RA accumulation. They stated that the increase in RA content in *Salvia miltiorrhiza* hairy roots was associated with an increase in the TAT activity. The TAT has been known as a chief enzyme in the biosynthesis pathway of RA (Huang et al. 2008). This enzyme catalyzes the reaction of Tyrosine to pHPP. Therefore, it is needed for the biosynthesis of Rosmarinic acid, Plastoquinones, and Tocopherols because it can prepare pHPP (Kim & Petersen 2002). In the present study, the increasing effect of MIONPs on the expression of genes involved in the biosynthesis pathway of RA was observed. The positive efficiencies of MIONPs was reported in diverse studies. An investigation carried out by Aminizadeh et al. (2016) showed that Fe_3O_4 nanoparticles affected and increased the content of Sulforaphane in *Lepidium draba* seedlings. In addition, it is a study reported that iron oxide nanoparticles can be used as effective elicitors to increase the production of scopolamine and hyoscyamine in the hairy root of *Hyoscyamus reticulatus* L. (Moharrami et al. 2017). Another study also reported the significant role of iron nanoparticles in enhancing the amounts of hypericin and hyperforin in culturing cell suspension of *Hypericum perforatum* L. (Sharafi et al. 2013).

In this study, the highest expression level of the *TAT*, *HPPR*, and *RAS* genes was observed in plants treated with MIONPs at the concentration of 25 mg/L, and the expression of genes

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decreased as the concentration increased to 50 mg/L. Nevertheless, the expression was still higher in comparing to the control plant. High concentrations of iron can cause toxicity. When iron is excessively absorbed and accumulates in plant tissue, it can disturb the cellular redox balance towards peroxidation state and cause oxidative stress. Therefore, excess iron can cause excessive production of ROS (Lapaz et al. 2022). The results demonstrated that the highest expression belonged to the *HPPR* gene at the MIONPs concentration of 25 mg/L (20 times higher increase in expression). The *HPPR* gene contributes to the transformation of 4-Hydroxy Phenylpyruvate (pHPP) to 4-Hydroxy Phenyllactate (pHPL). It is a key gene in the tyrosine pathway of RA biosynthesis (Xing et al. 2014). Therefore, the application of MIONPs up to a concentration of 25 mg/L can have an increasing effect on the expression of genes and possibly an increase in the production of RA, which should be studied.

Conclusions: RA, as a secondary metabolite found in *M. officinalis* L., is an economic and pharmaceutical compound. Therefore, its rapid and massive synthesis is essential. Our results showed that the exposure of plants to ferric chloride and magnetic iron oxide nanoparticles led to an increase in the expression of *TAT*, *HPPR*, and *RAS* genes involved in the biosynthesis pathway of RA compared to the control samples. However, the application of NPs had more effect than that of ferric chloride on the expression levels. An increase in the expression of genes involved in the biosynthesis pathway of RA through treatment with iron oxide nanoparticles provides an opportunity for induction of synthesis and accumulation of RA in *M. officinalis* L. NPs have high reactivity and large specific surface area. Therefore, we hope to be able to enhance the production of RA in *M. officinalis* L. by using these NPs.

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دانشگاه شهید باهنر کرمان

اثر نانوذرات اکسید آهن مغناطیسی و فریک کلرید بر بیان برخی از ژن‌های بیوسنتز رزمارینیک اسید در بادرنجبویه (*Melissa Officinalis* L.)

سیمین گلکاری

دانش آموخته کارشناسی ارشد، گروه بیوتکنولوژی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان، ایران. رایانامه: golkari@agr.uk.ac.ir

شهرام پورسیدی

*نویسنده مسئول: دانشیار، گروه بیوتکنولوژی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان، ایران. رایانامه: spseyedi@uk.ac.ir

علی کاظمی‌پور

استادیار، گروه مهندسی تولید و ژنتیک گیاهی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان، ایران. رایانامه: ali.kazemi@uk.ac.ir

مهدی منصوری

استادیار، گروه بیوتکنولوژی، دانشکده کشاورزی، دانشگاه شهید باهنر کرمان، کرمان، ایران. رایانامه: m.mansouri@uk.ac.ir

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چکیده

هدف: رزمارینیک اسید یک ترکیب ضد سرطانی، ضد آلرژی و ضد میکروبی است که به عنوان متابولیت ثانویه در بسیاری از گیاهان خانواده نعناع از جمله بادرنجبوبه وجود دارد. کاربرد نانوذرات به عنوان یک الیسیتور جدید برای بیوسنتز متابولیت‌های ثانویه نشان می‌دهد که نانوذرات می‌توانند این ترکیبات را در گیاهان با تحریک بیان ژن‌های مسیر بیوسنتز تحت تأثیر قرار دهند. این پژوهش با هدف بررسی اثر نانوذرات اکسید آهن مغناطیسی در مقایسه با همتای محلول آن انجام شد.

مواد و روش‌ها: محلول‌پاشی غلظت‌های مختلف (۵، ۱۰، ۲۵ و ۵۰ میلی‌گرم بر لیتر آهن) فریک کلرید و نانوذرات اکسید آهن مغناطیسی روی برگ‌های گیاه انجام شد. سطوح بیان ژن‌های *TAT*، *HPPR* و *RAS* با استفاده از qRT-PCR بررسی شد، و نمونه‌های تیمار شده و تیمار نشده مورد مقایسه قرار گرفتند.

نتایج: این مطالعه اثرات مثبت فریک کلرید و نانوذرات اکسید آهن مغناطیسی را بر بیان ژن‌های دخیل در مسیر بیوسنتز رزمارینیک اسید نشان داد. بیشترین میزان بیان ژن‌های *TAT*، *HPPR* و *RAS* در گیاهان تیمار شده با نانوذرات اکسید آهن مغناطیسی در غلظت ۲۵ میلی‌گرم بر لیتر مشاهده شد، و بیان ژن‌ها با افزایش غلظت به ۵۰ میلی‌گرم بر لیتر کاهش یافت، با این حال، میزان بیان همچنان در مقایسه با گیاه شاهد بیشتر بود.

نتیجه‌گیری: نتایج نشان داد که قرار گرفتن گیاهان در معرض فریک کلرید و نانوذرات اکسید آهن مغناطیسی منجر به افزایش بیان ژن‌های مورد مطالعه در مقایسه با گیاهان شاهد می‌شود. اما، کاربرد ذرات آهن در مقیاس نانو تأثیر بیشتری نسبت به فریک کلرید بر سطوح بیان داشت. افزایش بیان ژن‌های دخیل در مسیر بیوسنتز رزمارینیک اسید از طریق تیمار با نانوذرات اکسید آهن مغناطیسی فرصتی برای سنتز و تجمع رزمارینیک اسید فراهم می‌کند. بنابراین، ما امیدواریم بتوانیم با استفاده از این نانوذرات تولید رزمارینیک اسید در بادرنجبویه را افزایش دهیم.

کلیدواژه‌ها: بیان ژن، بادرنجبویه، گیاه دارویی، متابولیت ثانویه، qRT-PCR.

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