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Antioxidant capacity of parsley cells (*Petroselinum crispum* L.) in relation to iron-induced ferritin levels and static magnetic field

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This study was aimed to evaluate antioxidant response of parsley cells to 21 ppm iron and static magnetic field (SMF; 30 mT). The activity of catalase (CAT) and ascorbate peroxidase (APX) and the contents of malonyldialdehyde, iron and ferritin were measured at 6 and 12 h after treatments. Exposure to SMF increased the activity of CAT in treated cells, while combination of iron and SMF treatments as well as iron supply alone decreased CAT activity, compared to that of control cells. Combination of SMF with iron treatment reduced iron content of the cells and ameliorated mal effect of iron on CAT activity. All treatments reduced APX activity; however, the content of total ascorbate increased in response to iron and SMF + iron. The results showed that among the components of antioxidant system of parsley cells, enhanced activity of CAT in SMF-treated cells and increase of ascorbate in SMF + Fe-treated ones were responsible for the maintenance of membranes integrity. Ferritin contents of SMF- and SMF + Fe-treated cells also decreased significantly 12 h after treatments, compared to those of the control cells. These results cast doubt on the proposed functions of ferritin as a putative reactive oxygen species detoxifying molecule.

Keywords: antioxidant system, ferritin, iron, lipid peroxidation, parsley, *Petroselinum crispum*, static magnetic field

Introduction

Life on the earth has evolved in a sea of natural magnetic fields (MFs). Over the last century, the spectrum of man-made MFs has grown. Several experimental studies have suggested accumulation of reactive oxygen species (ROS) and an MF-induced oxidative damage in living organisms which has basically an adverse effect on physiological function, membrane integrity and cell structure (Mandal, Yadav, Yadav, & Nema, 2009; Sahebjamei, Abdolmaleki, & Ghanati, 2007). Effects of MF could be related to uncoupling of free-radical processes in membranes and enhanced ROS generation (Yao, Li, Yang, & Li, 2005). Protection of the organism against oxidative stress is based on antioxidant systems. Antioxidant constituents of

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plant material act as radical scavengers, including enzymatic and non-enzymatic systems. Enzymatic system includes superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase and phenol oxidase. Non-enzymatic system includes phenolic compounds, some essential oils, ascorbic acid (vitamin C) and carotenoids (Mandal et al., 2009). MF may also interact with other factors and atoms in the cell, particularly with iron (Dhawi, Al-Khayri, & Hassan, 2009). Iron is an essential micronutrient and an abundant ferromagnetic element in plant cells. Like other ferromagnetic elements, iron in an oscillatory MF oscillates in its magnetic moment along the field (Vaezzadeh, Noruzifar, Ghanati, Salehkotahi, & Mehdian, 2006). Moreover, it has important dual roles in free-radical chemistry in all organisms. Free iron can participate in fenton reactions and catalyze the generation of hydroxyl radical and other toxic oxygen species. However, iron is a constituent of major antioxidant enzymes, e.g. CAT, APX, POD and SOD (Becana, Moran, & Iturbe-Ormaetxe, 1998). The plants avoid iron overload toxicity and strictly control the homeostasis of iron by different mechanisms. Ferritins are a class of ubiquitous iron storage proteins, found in all living kingdoms (Briat et al., 2010; Chasteen & Harrison, 1999). They maintain iron in soluble form and protect the cells from the toxic effect of iron overload. Ferritin prevents direct deposition of ferric hydroxide colloids (especially at pH >6) by oxidizing Fe^{2+} and storing iron within the mineral core (Laulhère & Briat, 1993). When considering the effects of MFs at the molecular scale, the iron cage protein ferritin is an obvious candidate because it has the highest net magnetic moment of all proteins (Céspedes & Ueno, 2009). A multilayered system of genetic controls has evolved to regulate ferritin synthesis under the control of both iron and oxygen (Lescure et al., 1991), which emphasizes the key role played by ferritin in controlling iron and oxygen interactions.

The goal of this study was to determine the impact of short exposure to static magnetic field (SMF) on antioxidant system of suspension-cultured parsley cells supplemented with iron. This can improve our understanding about the mechanism behind the response of plant cells to SMF.

Materials and methods

Cell growth conditions, treatment with iron and MF

A fast-growing cell line was established from shoot explants of parsley (*Petroselinum crispum* L.) on a modified MS medium without glycine (Sahebjamei et al., 2007). The medium was supplemented with 0.15 mg/l kinetin, 1.5 mg/l thiamin, 0.75 mg/l pyridoxine and 0.75 mg/l nicotinic acid, and its pH was adjusted to 5.8. Suspension cultures were established from this line, grown under darkness at a temperature of 27 ± 2 °C on rotary shaker at 123 rpm. The cells were subcultured weekly.

The cells at the end of their logarithmic growth phase (day 10 of subculture) were treated with or without iron in a final concentration of 21 mg/L in the form of Fe–EDTA. This concentration of iron was almost four times higher than its concentration in MS medium. Iron was added from a stock solution, after filter sterilization under laminar airflow. Exposure to 30-mT SMF was conducted for 4 h via a locally designed apparatus (Figure 1). SMF of 30 mT is the minimum level of MF used in magnetic therapy equipment (Sakhnini & Dairi, 2004). Exposure to SMF was performed by a locally designed MF generator. The electrical power was provided using a 220 V AC power supply equipped with variable transformer as well as a single-phase, full-wave rectifier. The maximum power and passing current were 1 kW and 50 A DC, respectively. MF generator system was consisted of two coils (each 3000 turns of 3 mm copper wire) equipped with a U-shaped laminated iron core (to prevent eddy

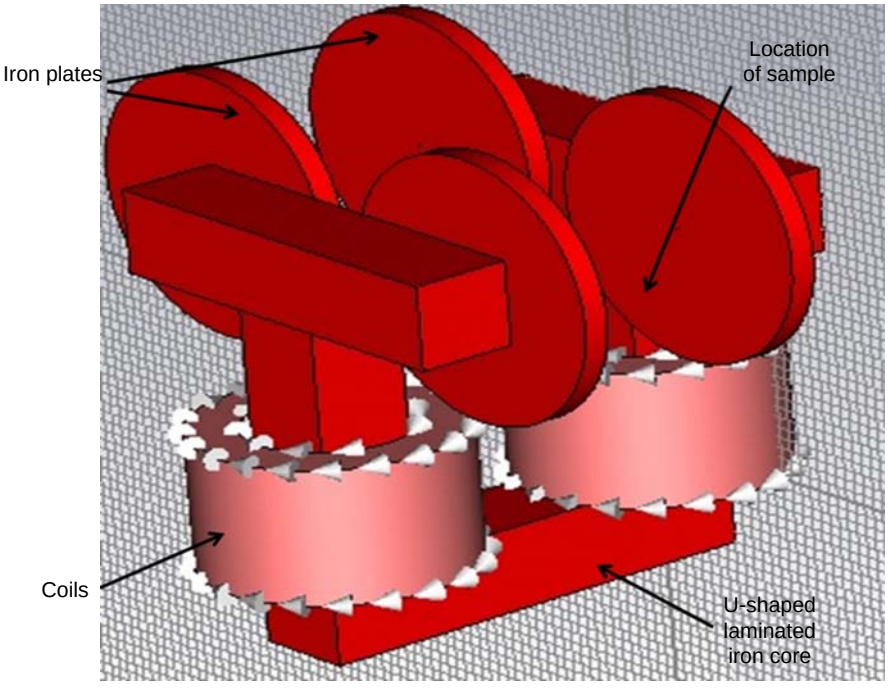

Figure 1. Schematic illustration of static magnetic producing apparatus. Coils are wrapped with copper pneumatic tube (not shown). Circulation of water in the tube prevents heating of the samples.

current losses). Using two vertical connectors, the arms of the U-shaped iron core were terminated to four circular iron plates covered with thin layer of nickel (each 23 mm thickness, 26 cm in diameter). A water circulation system via copper pneumatic tubes around the coils was employed to avoid the increase of the temperature. The temperature between the circular irons plates (where the samples were located) was measured by a thermometer and was almost the same as other parts of the room (e.g. the location site of the control plants) $\pm$ 1°C. Preliminary studies have shown that exposure to turned-off apparatus had no effects on physiological and morphological parameters of the plant cells. Since, no other electric appliance was working, the control samples were only exposed to the extremely low MF of the earth (GeoMF of 47 $\pm$ 5 μT, according to the Geophysics Institute of Tehran University) as the treatment group. Moreover, the control samples were kept far enough from the SMF-producing apparatus, to avoid any potential exposure to the MF. Presence of any pulsation in the current from rectifier into the SMF-generating apparatus was tested by an oscilloscope (8040, Leader Electronics Co., Yokohama, Japan), and a pulsation frequency of 50 Hz with a range of voltage variation about $\pm$ 1 V was shown. The presence of this pulsation frequency may be related to the shortcoming of the used single-phase, full-wave rectifier which provides a ripple voltage around 5%. This small ripple voltage confirmed that the generated MF is highly homogeneous. Calibration of the system as well as tests for the accuracy and uniformity of the SMF was performed by a Teslameter (13610.93, PHYWE, Gottingen, Germany).

The iron-treated cells and their control group were harvested at 6 and 12 h after treatment. SMF-treated cells and their corresponding control groups were harvested at 6 and 12 h after finishing the exposure period to SMF. Harvesting of the cells was conducted on nylon mesh inside Buckner funnel under reduced pressure, and the cells were washed thoroughly with deionized water, frozen in liquid N_2 and kept at

−80 °C until used for biochemical analysis. The control plants were placed in the same conditions in terms of humidity, temperature and light but far enough from the apparatus being shielded from SMF.

Radical scavenging assay

The radical scavenging capacity of the cells was assessed using 1,1-diphenyl-2-picrylhydrazyl as a stable free radical (Khalaf, Shakya, AL-Othman, El-Agbar, & Farah, 2008). In brief, 0.002% DPPH was dissolved in methanol, and 1.5 mL of this solution was added to 0.5 mL of cell extract. The solution mixture was kept in dark for 30 min, and its optical density was measured at 517 nm by spectrophotometer (Cintra 6, GBC, Australia). The blank contained 1.5 mL of 0.002% DPPH solution. The capacity of cell extract to scavenge free radicals was calculated as follows:

$$\%\text{DPPH scavenging} = 100 \times (\text{absorbance of control} - \text{absorbance of sample})/\text{absorbance of control},$$

where absorbance of control is the absorbance in absence of standards or extracts and absorbance of sample is the absorbance in presence of standards or extracts. The positive control was prepared using ascorbic acid at a concentration of 10 mg/mL.

Scavenging of hydrogen peroxide

A modified method based on that of Oktay, Gülçin, and Küfrevioğlu I'rfan (2003) was used to determine the ability of the extracts to scavenge hydrogen peroxide. Hydrogen peroxide (43 mM) was prepared in phosphate buffer (pH 7.4). Standards (ascorbic acids) and extract solutions were prepared at concentrations of 0.1–1.0 mM. Aliquots of standard or extract solutions (3.4 mL) were added to 0.6 mL of hydrogen peroxide solution. The reaction mixture was incubated at room temperature for 10 min, and the absorbance was determined at 230 nm. The percentage of scavenging was calculated as follows:

$$\%H_2O_2 \text{ scavenging} = 100 \times (\text{absorbance of control} - \text{absorbance of sample})/\text{absorbance of control},$$

where absorbance of control is the absorbance in absence of standards or extracts; absorbance of sample is the absorbance in presence of standards or extracts.

Antioxidant enzymes assay

For measurement, the activity of CAT fresh samples (200 mg) was homogenized in 3 mL of 25 mM phosphate buffer (pH 6.8). The homogenate was centrifuged at 12 000 *g* for 20 min at 4 °C, and the supernatant was used for enzyme assay. Assay mixture in a total volume of 1 mL contained 450 μL of 25 mM phosphate buffer (pH 6.8), 500 μL of 10 mM H_2O_2 and 50 μL of enzyme (Ghanati, Abdolmaleki, Vaezzadeh, Rajabbeigi, & Yazdani, 2007).

For superoxide anion radical assay, the superoxide anion radicals were generated by a pyrogallol autoxidation system. A test tube containing 1 mL of Tris–HCl buffer solution (50 mM, pH 8.2) was incubated in a water bath at 25 °C for 20 min. Pyrogallol solution (45 mM of pyrogallol in 10 mM of HCl), which was also preincubated at 25 °C, was added to test tube and mixed up. The mixture was incubated at 25 °C for 3 min and then ascorbic acid was added dropwise into the mixture instantly to terminate the reaction. The absorbance was measured at 420 nm after 5 min

and denoted A_0 where A_0 is the speed of pyrogallol autoxidation (Marklund & Marklund, 1974).

The cells (0.2 g) were homogenized in 3 mL of Tris–HCl buffer solution (50 mM, pH 8.2). The homogenate was centrifuged at 12 000 *g* for 20 min at 4 °C, and 1 mL of the supernatant was transferred into a test tube. Incubation at 25 °C, addition of pyrogallol solution and termination with adding ascorbic acid were repeated as mentioned above. The absorbance was measured at 420 nm after 5 min and denoted as A_1. Simultaneously, a blank control of reagent was obtained as A_2. The scavenging percentage was calculated according to the following formula:

$$\text{Scavenging percentage} = [A_0 - (A_1 - A_2)] \times 100/A.$$

For APX assay, the cells (0.2 g) were homogenized in 1 mL of 50 mM Na-phosphate buffer (pH 7.8) containing 5 mM ascorbate, 5 mM dithiothreitol (DTT), 5 mM EDTA, 100 mM NaCl and 2% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 15 000 *g* for 15 min at 4 °C. The reaction was initiated by adding H_2O_2 to a final concentration of 44 μM. The reaction rate was monitored by the decrease in absorbance at 290 nm. The rate constant was calculated using the extinction coefficient of 2.8/mM/cm and corrected for the rate obtained prior to the addition of H_2O_2. Protein contents were determined by the method of Bradford (1976) using BSA as standard (Sahebjamei et al., 2007).

Total content of AsA was assayed based on the reduction of Fe^{3+} to Fe^{2+} by AsA in acidic solution. Fresh samples were extracted with trichloroacetic acid (TCA, 5%, w/v) followed by centrifugation (18 000 *g*, 15 min). Reaction mixture consisting of phosphate buffer (150 mM, pH 7.4), EDTA (5 mM), deionized water, TCA (10%), phosphoricacid (44%), α,α-dipyridyl (4% in 70% ethanol), $FeCl_3$ (3%) and 100 μL of cell extract was incubated at 40 °C for 40 min, and the absorbance was read at 532 nm, using spectrophotometer (Cintra 6, GBC, Australia) (Singh, Ma, Srivastava, & Rathinasabapathi, 2006).

Membrane lipid peroxidation rate

Rate of membrane lipid peroxidation was assayed by thiobarbituric acid (TBA) method by measuring malondialdehyde (MDA) as a final product of lipid peroxidation. Samples were homogenized in 3 mL of aquatic solution of TCA (10%, w/v). The homogenate was centrifuged at 15 000 *g* for 10 min, and 1 mL of the supernatant was added to 1 mL of TBA (0.25%). The mixture was incubated at 100 °C in a water bath for 30 min, after which the reaction tubes were placed in an ice-water bath to stop the reaction. Then, the absorbance of MDA was read at 532 nm followed by correction for the non-specific absorbance at 600 nm. The amount of MDA–TBA complex was calculated from the extinction coefficient of 155/mM/cm (De Vos, Schat, De Waal, Wooijs, & Ernst, 1991).

Iron content

The samples were ashed in a muffle furnace at 250 °C for 2 h followed by 2 h at 550 °C. One milliliter of a mixture of highly concentrated HCl and water (1:1) was added in order to digest ash and then dried on sand bath (110 °C). Then 5 mL of 1 N HCl was added, and total iron content in digested ash was measured by atomic absorption spectrometer (Shimadzu AA-670, Japan) (Katyal & Sharma, 1980).

Extraction of ferritin and quantification of it by ELISA

An indirect ELISA protocol was adapted from the method described by Flowers et al. (1986). Frozen samples (1 g) were homogenized on ice in 2 mL of extraction buffer

(10 mM sodium phosphate buffer, 100 mM sodium chloride, 2% polyvinylpyrolidone and 1 mM phenylmethanesulfonyl fluoride, pH 7.2). The slurry was centrifuged at 15 000 *g* for 10 min at 4 °C, and the supernatant was used (Lukac, Aluru, & Reddy, 2009). Fifty microliters of extract or standard were added to plates whose wells were precoated with anti-ferritin antibody, following manufacturer's instruction (Pishtazteb.co, Tehran, Iran). Ferritin content was determined according to the result of complexes formed during the antigen–antibody reaction, using an ELISA kit (Pishtazteb Zaman Diagnostics, Tehran, Iran), and the absorbance was detected at 450 nm using an ELISA reader.

Statistical analysis

All of the experiments were carried out with at least three independent repetitions, each with three samples. It should be noted that each treatment was conducted with its own control group. Data were expressed as the mean values ± SD. ANOVA procedure of the statistical program SPSS 16.0 was used. The significance of differences between treatments was evaluated using least significant difference (LSD) test at level of $p \leq 0.05$.

Results and discussion

In this study, supplied iron was rapidly absorbed by parsley cells and led to a remarkable increase in total iron contents of the cells after 6 and 12 h of treatments (Table 1). With an adequate amount of iron in MS medium, treatment with SMF did not affect iron uptake by the cells; therefore, the iron content of SMF-treated cells was identical to that of the control (Table 1). However, under high iron supply, SMF affected iron uptake by parsley cells, so that iron content of SMF + Fe-treated cells was even lower than treatment with iron alone (Table 1).

No significant increase was observed in total scavenging capacity of Fe-treated cells (Table 1). Interestingly, the activity of CAT in parsley cells was reduced by treatment with iron, compared to that of the control (Table 1). However, applying SMF with similar magnitude on parsley cells induced the activity of CAT, mainly after 12 h (Table 1). Nevertheless, APX activity reduced in all treatments.

Total content of ascorbate of parsley cells increased after treatment with 21 ppm of iron, compared to that of the control cells (Figure 2). Treatment with SMF did not change ascorbate content of the cells, but combined treatment of iron and SMF increased ascorbate, 6 h of treatment. However after 12 h, ascorbate content of SMF + Fe-treated cells returned to the level of control cells (Figure 2).

Table 1. Iron content and antioxidant activity of parsley cells treated with iron, SMF or combination of both. CAT and APX activities were expressed as ΔAbs 240/mg protein and ΔAbs 290/mg of protein, respectively. Data show means ± SE, $n = 3$.

Treatment time (h)	Total iron (mg/g FW)	CAT activity	Scavenging of O_2 (%)	APX activity	Scavenging capacity (%)
Control 6	0.13 ± 0.00[a*]	1.95 ± 0.0[a]	78.58 ± 0.4[a]	0.30 ± 0.0[a]	55.22 ± 0.2[a]
12	0.14 ± 0.04[a]	1.99 ± 0.0[a]	75.97 ± 2.6[a]	0.31 ± 0.0[a]	54.04 ± 0.1[a]
Fe 6	0.36 ± 0.03[b]	0.61 ± 0.0[b]	79.80 ± 0.0[a]	0.11 ± 0.0[b]	54.91 ± 0.2[a]
12	0.28 ± 0.00[b]	0.30 ± 0.0[c]	80.56 ± 0.3[a]	0.16 ± 0.0[b]	55.45 ± 0.2[a]
SMF 6	0.14 ± 0.04[a]	2.70 ± 0.1[d]	79.80 ± 1.5[a]	0.21 ± 0.0[c]	58.08 ± 1.4[a]
12	0.13 ± 0.01[a]	31.45 ± 4[e]	77.50 ± 1[a]	0.09 ± 0.0[d]	57.52 ± 0.9[a]
SMF + Fe 6	0.08 ± 0.01[c]	1.28 ± 0.3[f]	81.64 ± 2.4[a]	0.17 ± 0.0[c]	51.39 ± 0.4[a]
12	0.10 ± 0.02[c]	1.49 ± 0.3[g]	79.17 ± 1.5[a]	0.22 ± 0.0[e]	53.15 ± 0.3[a]

*Data within the same column followed by different letters are significantly different ($P \leq 0.05$).

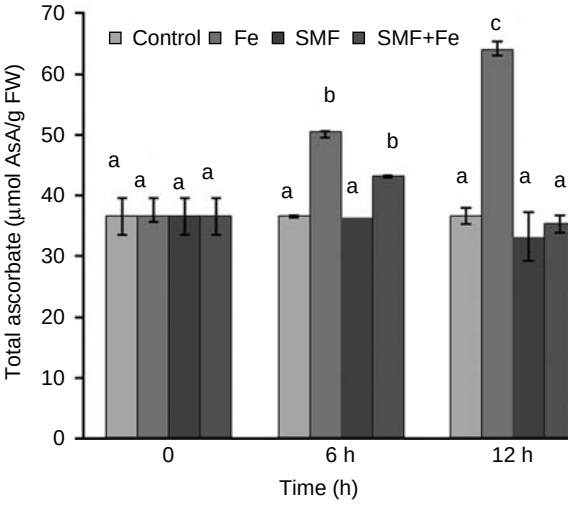

Figure 2. Effect of iron and SMF on ascorbate content of parsley cells 6 and 12 h after treatments. Data show means ± SE, $n = 3$. Different letters represent significant differences at $p \leq 0.05$. It should be noted that each treatment was conducted with its own control group.

In iron treatment, H_2O_2 was more than those of the control cells (6 h of the treatment) but increase in ascorbate compensated it, resulting in consistency of MDA content of iron-treated cells (Figure 3). Our results showed that SMF stimulated CAT activity, however, the cells were not able to prevent peroxidation of their membrane lipids (Figure 3). Consistent amount of ascorbate accompanied by decreased activity of CAT in SMF + Fe treatment resulted in accumulation of H_2O_2 and increase of MDA (Figure 3).

Under controlled conditions, suspension-cultured parsley cells contained 230 ng ferritin/g FW (Table 2). However, ferritin content of 21 ppm-treated cells significantly reduced, 12 h after treatment (Table 2). Ferritin contents of SMF- and SMF + Fe-treated cells also significantly decreased after 12 h of the treatments, compared to those of the control cells.

During the past decades, great experimental and theoretical insights have been provided with respect to the effects of MFs on living organisms, nonetheless their exact mechanism(s) is still unclear. Currently, two biophysical mechanisms have emerged as the most promising magneto-detection candidates, namely iron–mineral-based magnetoreception and chemical (photoreceptor based) magnetoreception (Johnsen & Lohmann, 2005). Iron is a ferromagnetic element, therefore could be affected by MF (Penuelas, Llusia, Martinez, & Fpntcuberta, 2004). A few evidences have been emerging recently to show a plausible interaction between MF and iron content of plants (Ghanati et al., 2007), although the mechanisms involved have received little attention. External MFs influence both the activation of ions (in particular iron cations) and the polarization of dipoles in plant cells (Dhawi et al., 2009). Little information is available on the actual plants iron requirement for optimal growth. Iron requirement of various plant species can differ appreciably. These differences are rather attributed to the range of iron uptake efficiency (Christ, 1974). Under high and sufficient concentrations of iron, it is absorbed by a large family of metal transporters, Natural Resistance Associated Macrophage Protein (NRAMP), which have a capacity to transport Ni, Zn, Cu, Co, Mn and Cd as well (Curie & Briat, 2003).

Previous research has reported less iron uptake by plants under treatment with low-intensity MF (Hajnorouzi, Vaezzadeh, Ghanati, Jamnezhad, & Nahidian, 2011), and occurrence of some modifications on the function of the ion channels has been

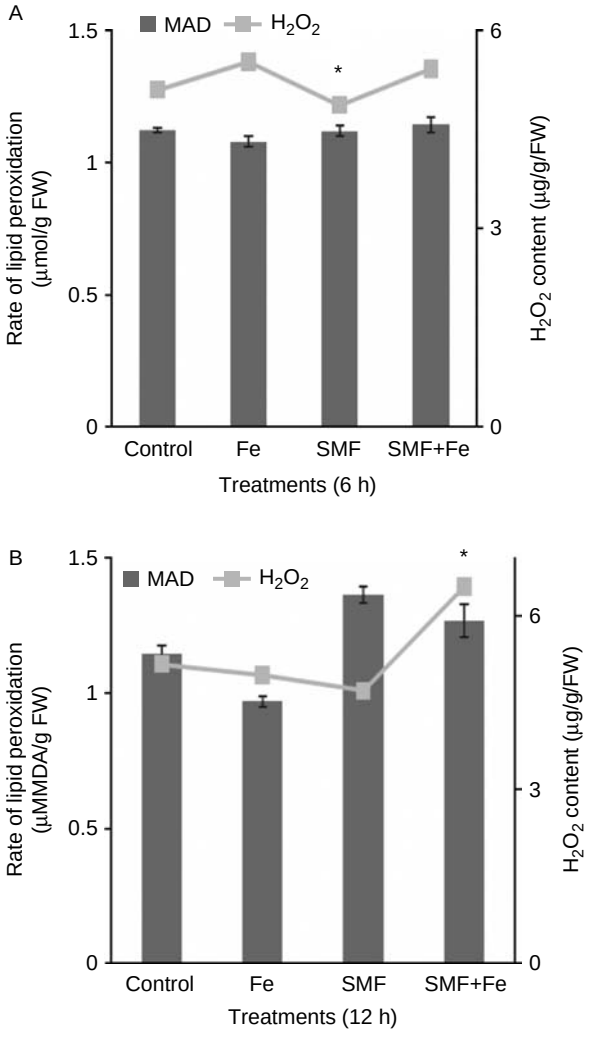

Figure 3. Effects of iron and SMF on membrane lipid peroxidation and hydrogen peroxide content of parsley cells, 6 (A) and 12 h (B) after treatments. Data show means ± SE, $n = 3$. Asterisk indicates significant differences with control group at $P \leq 0.05$. It should be noted that each treatment was conducted with its own control group.

suggested as the mechanism involved. It has been postulated that if a ferrimagnetic nanocrystal is fixed to an ion channel, it has the potential to directly influence ion transport and would alter ion movement across a membrane (Kirschvink, Kobayashi-Kirschvink, & Woodford, 1992). It has been assumed that the ion in the channel is forced through the channel because of its spiral structure (Liboff, 1986). Using plant plasma membrane vesicles, Koch, Sommarin, Persson, Salford, & Eberhardt (2003) showed that suitable combinations of static and time varying MFs directly interact with the calcium channel protein in the cell membrane. MF may play an important role in cation uptake capacity and has a positive effect on immobile plant nutrient uptake. However, Radhakrishnan and Kumari (2012) argued that the diamagnetic anisotropic properties of membrane phospholipids in MF treatment reorient the phospholipids and cause deformation of membrane channels. Therefore, there is a possibility that iron was desorbed from parsley cells in SMF + Fe treatment.

Similar to other redox metals, iron can induce the production of ROS in plants. Iron is also responsible for the production of hydroxyl radical (OH·) through the

Table 2. Effect of iron and SMF on ferritin content of suspension-cultured parsley cells.

	Control		Fe		SMF		SMF + Fe	
	6 h	12 h	6 h	12 h	6 h	12 h	6 h	12 h
Ferritin (ng/g FW)	$230 \pm 1^{a,*}$	230 ± 3^{a}	216 ± 17^{a}	176 ± 10^{b}	188 ± 10^{b}	191 ± 2^{b}	218 ± 1^{a}	196 ± 3^{b}

Notes: Data show means ± SE, $n = 3$.
*Data followed by different letters are significantly different ($P \leq 0.05$).

Fenton and Haber–Weiss reaction (Dat et al., 2000). Hydrogen peroxide in turn is the main substrate for one major ROS scavenging enzyme, CAT. In the presence of high amounts of iron, plants may function to increase the production of metal-binding compounds or scavenge excessively produced ROS by antioxidant enzymes (Ghanati et al., 2007). Iron content of parsley plants has been reported as 0.0192% (Peterson & Elvehjem, 1928). Our results show that 21 ppm of iron is not an excess amount for parsley cells since it does not induce ROS production or activation of ROS scavenging enzymes. No significant increase was observed in total scavenging capacity of Fe-treated cells (Table 1).

The activity of CAT in parsley cells reduced by treatment with iron, compared to that of the control (Table 1). Decrease in CAT activity with high supply of iron has been previously reported by other researchers as well (Chatterjee, Gopal, & Dube, 2006; Mehraban, Abdolzadeh, & Sadeghipour, 2008). High iron supply might inhibit the activity of CAT, and the other ROS scavenging enzyme APX, by blocking the essential functional groups like —SH in enzymes (Ali & Alqurainy, 2006).

Adverse bio-effects of SMF are also attributed to production of free radicals or increase of their longevity in biological systems. Accumulation of these radicals leads to abnormalities in gene expression, damage of proteins and change in the activity of certain enzymes, damage of membranes, as well as reduction in the level of antioxidant hormones (Robison, Pendleton, Monson, Murray, & O'Neill, 2002; Shine, Guruprasad, & Anjali, 2011, 2012). Treatment of suspension-cultured tobacco cells with an SMF of 30 mT lowered the activity of ROS scavenging enzymes, namely CAT (Sahebjamei et al., 2007), resulting in damage of membrane lipids. However, applying SMF with similar magnitude on parsley cells in this study induced the activity of CAT, mainly after 12 h (Table 1). CAT is a metalloenzyme whose activity can be affected by MF (Regoli et al., 2005). It has been proposed that MF changes the molecular conformation of the enzyme and hence the substrate binds more easily to the enzyme (Batcioglu et al., 2002). CAT is the key enzyme that effectively eliminates H_2O_2, thereby regulates the activity of APX (Sahebjamei et al., 2007). Higher activity of CAT in SMF-treated parsley cells resulted in lower activity of APX, in comparison with that of the control cells (Table 1). Combination of SMF with iron treatment ameliorated mal effects of iron on CAT activity (Table 1). This effect may be, at least in part, resulted from lower absorption of iron in this treatment.

Ascorbate content of parsley cells is shown in Figure 2. L-Ascorbic acid (vitamin C) is a multifunctional compound in both plants and animals. A plant-specific pathway of vitamin C biosynthesis has been described and appears to be controlled by both developmental triggers and environmental cues. In favorable conditions, it represents 10% of the total soluble carbohydrate pool (Smirnoff & Wheeler, 2000). Total content of ascorbate in parsley cells increased after treatment with 21 ppm of iron, compared to that of the control cells, (Figure 2) and may imply again that 21 ppm iron was beneficiary for parsley cells. Treatment with SMF did not change ascorbate content of the cells that can be expectable considering the stimulation of CAT and improvement of redox homeostasis of parsley cells. Combined treatment of iron and SMF increased ascorbate, 6 h of treatment. After 12 h, however, ascorbate

content of SMF + Fe-treated cells returned to the level of control cells (Figure 2). This may be related to lower uptake of iron by the cells under this treatment.

MDA is a cytotoxic product of lipid peroxidation and an indicator of free-radical production (Ohkawa, Ohishi, & Yagi, 1979). Although due to low activity of CAT in iron treatment, H_2O_2 was more than those of the control cells (6 h of treatment), but increase in ascorbate compensated it, resulting in consistency of MDA content of iron-treated cells (Figure 3). This implied again that 21 ppm of iron did not result in accumulation of ROS and membrane damage. Despite the stimulation of CAT activity by SMF, the cells were not able to prevent peroxidation of their membrane lipids (Figure 3). Consistent amount of ascorbate accompanied by decreased activity of CAT in SMF + Fe treatment resulted in accumulation of H_2O_2 and increase of MDA (Figure 3).

Ferritin is a protein able to store up to 4500 iron atoms in its central cavity (Harrison & Arosio, 1996; Theil, 2004). Although the use of serum ferritin as a sensitive indicator of iron deficiency in human diseases has been discussed controversially (WHO, 2007), phytoferritin has been considered as a ubiquitous iron storage protein that plays a crucial role in intracellular iron homeostasis and a good indicator for iron status in plants providing a non-toxic and bioavailable form of iron (Briat et al., 2010). Due to its ability to sequester iron, ferritin possesses the dual function of detoxification and storage (Ayala-Vela et al., 2008).

It has a highly conserved structure in plants and animals, but a distinct cytological location and a different level of control in response to iron excess. Iron-induced synthesis of ferritin was observed in suspension-cultured maize cells (Lobreaux, Hardy, & Briat, 1993), but their putative iron-storage function to furnish iron during various development processes is unlikely to be essential (Briat et al., 2010). In this study, suspension-cultured parsley cells, in control conditions, contained 230 ng ferritin/g FW (Table 2). Twelve hours after treatment of the cells with 21 ppm of iron, however, their ferritin content significantly reduced (Table 2). In an experimental analysis on iron content, ferritin gene activity and ferritin protein content of different cultivars of common beans, Ayala-Vela et al. (2008) found no relationship between ferritin transcriptional levels and ferritin protein contents with iron content. However, plant cells may not need to synthesize alternate ferritins for detoxification of iron because, in contrast to animals, plant vacuoles can be used, and plants are able to sequester excess iron in their vacuoles (Cvitanich et al., 2010; Kampfenkel, Montagu, & Inze, 1995; Lescure et al., 1991). Ferritin contents of SMF- and SMF + Fe-treated cells also significantly decreased after 12 h of treatments, compared to those of the control cells. Many studies have pointed out that regulation of ferritin biosynthesis is conducted at the level of transcription and post-transcriptional level (Ayala-Vela et al., 2008, Briat et al., 2010; Proudhon, Briat, & Lescure, 1989), but some additional control mechanisms remained to be elucidated to explain how ferritin is synthesized at the proper level in particular cells. Studies on plant ferritin functions and regulation of their synthesis revealed strong links between these proteins and protection against oxidative stress. Therefore, there is a possibility that increased activity of CAT in SMF-treated cells and increased ascorbate in SMF + Fe-treated cells have scavenged ROS and reduced its level so that being insufficient to induce ferritin biosynthesis.

Conclusion

Different components of antioxidant system of parsley cells were triggered by SMF, iron and their combination. In Fe and SMF + Fe treatments, ascorbate was

responsible for detoxification of ROS, while this task was achieved by increase of CAT in SMF treatment. Although there is a hypothesis that ferritin can ameliorate the risk of iron accumulation and detoxification, no direct relationship was observed between ferritin and iron content of parsley cells and their antioxidant capacity.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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