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# Iron biofortification and activation of antioxidant system of wheat by static magnetic field

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**Abstract**

Effect of static magnetic field (SMF) on plants is a matter of debate. In the present study plausible useful effects of SMF on wheat plant (*Triticum aestivum* L.) via improvement of antioxidant system and iron/ferritin fortification were evaluated. The plants were cultivated in pots and in their reproductive stages were treated with or without a 30 mT SMF for 4 days, each 5h. The activity of antioxidant system, iron and ferritin contents was evaluated. In comparison with the control groups, SMF treatment significantly increased the contents of iron, ferritin, and Fe-bound proteins in edible parts of the plants. Treatment with SMF also significantly increased catalase activity and radical scavenging capacity of wheat seeds which led in turn to decrease of membrane lipid peroxidation. The results provide us with a new approach for application of physical stimulators such as SMF in order for iron biofortification and increase of antioxidant capacity of critical crops.

***Keywords:*** Bifortification; ferritin; iron; magnetic field; SMF; *Triticum aestivum*

## 1. Introduction

Modern agricultural efforts are now searching for an efficient eco-friendly production technology based on physical treatment of plants to improve yield quality and quantity. Exposure of plants to magnetic fields (MFs) is one of the safe and affordable potential physical treatments to enhance plant development and crop stand (Vashisth and Nagarajan, 2010). In spite of abundant reports on the adverse effects of MF on animal cells, there is scarce literature available on the beneficial effects of static and oscillating magnetic fields of very low to high field intensity on plant cells (Vashisth and Nagarajan, 2010; De Souza et al., 2005; Hajnorouzi et al., 2011). Magnetic fields interact with certain atoms, in particular iron, in the cell (Dhawi et al., 2009). Iron is an essential micronutrient for all plants and an abundant ferromagnetic element in the plant cells. Many of the vital functions of the plants, like enzyme and chlorophyll production, nitrogen fixation, development and metabolism are all dependent on iron. Although Fe is one of the most abundant metals in the earth's crust, its availability to plant roots is very low. Fe availability is dictated by the soil redox potential and pH. In soils that are aerobic or of higher pH, Fe is readily oxidized, and is predominately in the form of insoluble ferric oxides (Morrissey and Guerinot, 2009). The low solubility of Fe-bearing minerals restricts the available iron pools in most soils. The free iron concentration in soil solutions is usually less than 10-15 M (Romheld and Marschner, 1986). The ability of plants to respond to iron availability ultimately affects human nutrition, both in terms of crop yield and the Fe concentration of edible tissues. In human society iron deficiency is the most prevalent micronutrient deficiency, affecting mainly children under 5 years and women of childbearing age living in the poorer communities of the developing world (McLean et al., 2008). Deficiency of iron has a major negative impact on health and in pregnancy contributes to the risk of severe anemia, which is associated with higher maternal morbidity and mortality (Aikawa et al., 2006; Prasad and Nirupa, 2007). In this connection, biofortification of staple food crops is a sustainable approach to alleviate iron deficiency (Foyer et al., 2006; Petry et al., 2010). For the Fe biofortification, several challenges should be overcome. First, the plants have to increase Fe uptake. Second, Fe should accumulate in the edible parts of the plant such as leaves, seeds and fruits. Third, the nutrients should be preferentially stored in a form that renders them bioavailable for the human digestive system (Sperotto et al., 2012). Iron biofortification can be done either through

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conventional selective breeding, or through genetic engineering.

Ferritin, a ubiquitous class of iron storage nuclear encoded protein plays a major role in eukaryotic iron homeostasis (Harrison and Arosio, 1996). Plant ferritins are more likely than animal ferritins to be the source of ferritin in natural foods, and their mineral has a higher ratio of phosphate to iron (usually 4:1) than does that of animal ferritins (usually 1:8; Davila-Hicks et al., 2004). Plant ferritin genes have been obtained from many different plants such as *Lens esculenta* (Crichton et al., 1978), *Zea mays* (Lobreaux et al., 1992), and *Phaseolus vulgaris* (Spence et al., 1991). Constitutive expression of ferritin has been done in various crops like wheat, rice and lettuce and maize (Drakakaki et al., 2000; Goto et al., 2000). Ferritin genes were used in biofortification approaches, however, over-expression in vegetative tissues did not increase either ferritin or iron content (Drakakaki et al., 2000), and in some cases even caused iron deficiency symptoms (Van Wuytswinkel et al., 1999). Overall, ferritin over-expression genes involved need to be studied in more detail and it may be necessary to increase Fe uptake at the same time to have the full effect of Fe increases (Qu et al., 2005).

To the best of our knowledge a possible strategy via the application of physical stimulators such as magnetic field in order to fortify iron storage proteins, e.g., ferritin has not been examined yet. The role of ferritin is to concentrate iron (as many as 4500 ions in each molecule) to an effective level that matches the cellular need (Goto, et al., 2000). In case of very high concentrations of iron, ferritin has a protective function by sequestering the iron inside the protein thus performing a detoxification function (Rama Kumar and Prasad, 2000; Fourcroy et al., 2004). A potential link between MF and its effects on living organisms is the fact that MF causes an oxidative stress, that is, increase in the activity, concentration, and lifetime of free radicals (Sahebjamei et al., 2007). It has been shown that static magnetic fields can cause an inconsistency in the function of antioxidant enzymes in plant cells and thereby lead to oxidative stress. Alteration of the activity of certain antioxidant enzymes e.g. catalase (CAT), superoxide dismutase (SOD), and peroxidase (PO) by external MF not only shows the relationship between MF and reactive oxygen species (ROS), but also suggest that antioxidant enzymes have the potential to function as magnetoreceptors (Sahebjamei et al., 2007; Payez et al., 2013; Haghighat etal. 2014). The cascade of antioxidant enzymes activity is initiated by the activity of SOD and conversion of superoxide anions to the most toxic species, $H_2O_2$. This species has a longer lifetime and also initiates the Fenton and Haber-Weiss reactions catalysed by iron, resulting in the generation of more reactive hydroxyl radicals (Hajnorouzi et al., 2011). Scavenging and detoxification of $H_2O_2$ is mainly achieved by CAT and it is more likely that among ROS scavenger enzymes, CAT is the key enzyme that effectively eliminates $H_2O_2$ (Sahebjamei et al., 2007). In the present study the effects of a static magnetic field (SMF) of 30 mT on iron status and antioxidant properties of wheat (*Triticum aestivum* L.) in seed production stage was evaluated.

## 2. Materials and Methods

### *2.1. Plant materials and exposure systems*

Wheat seeds (*Triticum aestivum* L. cv. Kavir) of uniform size and shape (at least 100 seeds) were planted in 5 kg of a clam-loam soil in pots. The soil analysis showed that it was composed of 28% clay, 29% silt, and 43% sand containing 4.02 meq/L calcium, 3.2 meq/L sodium, and 7mg/Kg iron, pH 7.0, EC 1.47 ds/m. The levels of other nutrients were also in the ranges adequate for normal growth of the plants. The plants were allowed to grow for ca.3 months and were watered every three days with tap water (pH 7.1) and every one week with a ½ Hoagland nutrient solution. The magnetic field was generated by a locally designed apparatus (Fig. 1a).

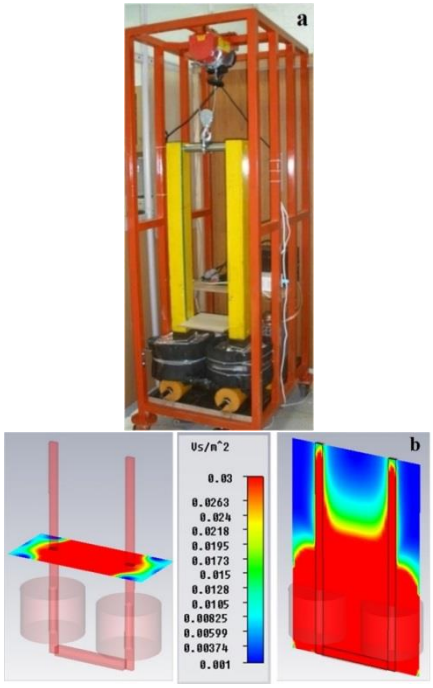


**Fig. 1.** Face view of SMF-generating device (a) and simulation of the homogeneity of a 30 mT magnetic field by CST (b). Homogeneity of the field in sample locations is indicated by red band between iron conductors

The electrical power was provided using a power supply working in range of 0–50 V and 0–20 A with a maximum power of 1 kW. The power supply equipped with a variable transformer as well as a single-phase, full-wave rectifier. Magnetic field generator system consisted of two coils (10000 loops of 2.5mm coated copper wire) equipped with a U-shaped laminated iron core (to prevent eddy current losses). Magnetic field fitting for exposure of plants with different height up to 1 m was provided by two vertical connectors (10×10×100 cm) joined to the iron core. Cooling of the system was performed through refrigeration system (using R12 cooling gas).The temperature of the samples location was continuously checked before, during, and after exposure of samples to the field. Uniformity of SMF in the location of samples was tested by a teslameter (13610.93, PHYWE, Gottingen, Germany) with a probe type Hall Sound effect. The accuracy of the teslameter was 0.1% for MF. Presence of any pulsation in the efferent current was tested by an oscilloscope (8040, Leader Electronics Co., Yokohama, Japan). The homogeneity of the generated magnetic field was theoretically estimated with Complete Technology for 3D EM Simulation software (http://www.CST.com) (Fig. 1b).

The local geomagnetic field was 47 ± 5 μT, according to the Geophysics Institute of Tehran University. During exposure time other electric appliances were off. The plants were exposed to SMF (30 mT) for 4 days, each 5 h during reproductive stage and ripening of the seeds. The period of exposure and the intensity of MF was decided based on previous studies (Payez et al., 2013).The control plants were placed in another room with the same condition but far enough from the MF producing apparatus. At the end of treatment period, the plants were harvested, washed thoroughly, wiped, frozen with liquid $N_2$, and stored at -80°C until used for biochemical analysis.

### *2.2. Quantification of ferritin and Fe-bound proteins*

Extraction and quantification of ferritin by ELISA sandwich method was conducted according to the method described by Lukac et al. (2009) using a kit (Pishtaz teb Zaman Diagnostics, Tehran, Iran). Ratio of Fe-bound to total protein contents of the aforesaid extract was determined by the method of Bejjani et al. (2007). Total protein and Fe-bound protein were estimated respectively at 280 and 420 nm, by a double beam UV-visible spectrophotometer (Cintra 6, GBC, Victoria, Australia).

### *2.3. Determination of antioxidant activity and iron content*

Activities of CAT and PO, radical scavenging capacity (RSC), membrane lipid peroxidation (LPO) of seeds, and measurement of iron contents of wheat roots, shoots and seeds were performed according to the following methods. In brief, frozen samples were homogenized in 50 mM Tris–maleate buffer (pH 6.0) and centrifuged at 12 000× g for 20 min at 2 ºC. The supernatant was used for enzyme assay. Activity of CAT was monitored by decomposition of $H_2O_2$ at 240 nm. Activity of PO was monitored by peroxidation of guaiacol at 470 nm (Cakmak and Horst, 1991). Activity of the enzymes was expressed against protein content of the extract. Protein content was determined by the method of Bradford (1976), using bovine serum albumin as a standard. The level of damage to the membranes was determined by measuring malonyldialdehyde (MDA) as the end product of the peroxidation of membrane lipids. To avoid the possible interference conferred by carbohydrates and pigments, improved and modified methods have been developed. An aliquot (0.5 g) of frozen powder was added to 5.0 mL of 0.1% (w/v) trichloroacetic acid and centrifuged at 10000× g for 5 min. Aliquot of the supernatant (1 mL) was added to 1mL of 0.5% (w/v) thiobarbituric acid in 20% (w/v) trichloroacetic acid. The mixture was heated at 100 ºC for 30 min and quickly cooled in an ice bath. After centrifugation at 10000 ×g for 10 min, the A532, A600 and A440 values of the supernatant were recorded. The value for the nonspecific absorption at 600nm was subtracted, and a standard curve of sucrose (2.5–10 mM) was used to correct the results from the interference of any soluble sugars in the samples by reading at A532 and A440. The MDA content was calculated using its absorption coefficient of $157 mM^{-1} cm^{-1}$ (Hodges et al., 1999). Evaluation of the total radical scavenging capacity of the cell extracts was conducted by using the stable 2.2'diphenylpicrylhydrazyl radicals (DPPH). In this method, the hydrogen atom level (or electron-donating ability) is measured from the bleaching of the purple-colored ethanol solution of DPPH. One-half mL of various ethanol extracts were diluted 1/10 and were added to 2.5 mL of a 1 mM ethanol solution of DPPH. After 40 min of incubation at room temperature, the absorbance was read against a blank at 517 nm. The total capacity of scavenging of DPPH in percent was calculated as follows (Stef et al., 2009): Total radical scavenging capacity (%) = [(A blank – A sample)/A blank]× 100.The iron content of the samples was measured in acid-digested ashes by an atomic absorption spectrometer (Shimadzu AA-670, Japan).

### 2.4. Statistical analysis

All of the experiments were carried out with at least three independent repetitions, each with three samples. Data were expressed as the mean values ± standard deviation (SD). Statistical analysis was performed using the Student's t-test, and the differences between the treatments were expressed as significant at a level of $p \leq 0.05$.

## 3. Results

Treatment of wheat plants with SMF remarkably increased ferritin content of shoots (1.3 × of the control). However, treatment with SMF did not cause significant change in ferritin content of seeds, compared to the seeds of control plants (Fig. 2).

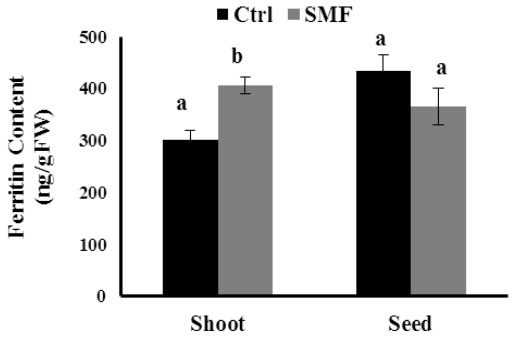


**Fig. 2.** Effects of SMF treatment on the ferritin content of wheat plants. Data are means obtained from three independent repetitions, each at least with three samples ± SD. Different letters indicate significant differences at $p \leq 0.05$ according to Student's t-test

The ratio of Fe-bound to total protein content in shoots and seeds increased by SMF to 1.4 and 1.3 fold of the control, respectively (Fig. 3a).The iron contents of roots of SMF-treated and control plants were identical, whereas, iron contents of shoots and seeds of SMF-exposed plants were significantly more than those of the control plants (Fig. 3b). Effect of exposure to SMF on antioxidant system of wheat plants is shown in Table 1. The activity of CAT, the main H2O2 scavenging enzyme, in seeds of SMF-treated plants was remarkably high (16 fold of the control seeds). The part of hydrogen peroxide which was not detoxified by CAT was detoxified by other enzymes, in particular PO. High activity of CAT in SMF-treated plants resulted in a lower activity of PO (86%) in comparison with the control seeds (Table 1). Treatment of wheat seeds with SMF increased their radical scavenging capacity (113%) compared to the control group. SMF treatment caused a significant decrease (43%) in the rate of lipid peroxidation of wheat seeds plasma membranes, compared to the control ones (Table 1).

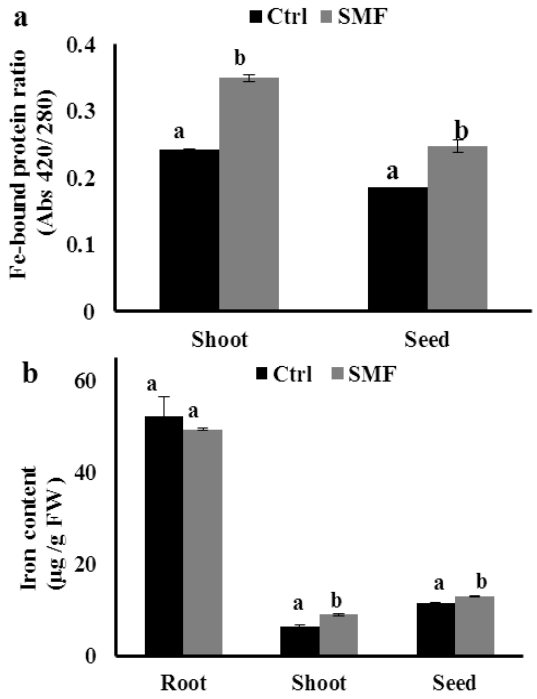


**Fig. 3.** Effects of SMF treatment on the ratio of Fe-bound to total protein contents (a) and total iron content of different plant parts (b). Data are means obtained from three independent repetitions, each at least with three samples ± SD. Different letters indicate significant differences at $p \leq 0.05$ according to Student's t-test

**Table 1.** Effects of SMF on the activity of wheat seeds antioxidant system. The activity of catalase and peroxidase were expressed as ΔAbs 240 /mg protein and ΔAbs 470 /mg protein, respectively. Radical scavenging capacity calculated as DPPH % and the rate of lipid peroxidation of membranes as mM MDA per gram of fresh weight

| | CAT | PO | RSC | LPO |
|---|---|---|---|---|
| Ctrl | $0.06\pm0.00^{a*}$ | $17.62\pm1.66^{b}$ | $44.86\pm2.4^{a}$ | $6.88\pm0.46^{b}$ |
| SMF | $0.96\pm0.12^{b}$ | $2.49\pm0.09^{a}$ | $51.01\pm1.86^{b}$ | $3.89\pm0.07^{a}$ |

*Data are means obtained from three independent repetitions, each with at least three samples ± SD. In each column, different letters indicate significant differences at $p \leq 0.05$ according to Student's t-test

## 4. Discussion

Biofortification of crops with iron contributes to the improvement of food quality and may help reduce the prevalence of Fe deficiency anemia world-wide (Sperotto et al., 2012). Green plants require a continuous supply of Fe as they grow, because Fe does not move from the older to the newer leaves (Brown, 1978). 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 and Briat, 2003).

The iron content of roots of SMF-treated and not treated plants were identical. Nonetheless, iron content of shoots and seeds of SMF-treated plants was significantly higher than those of the control ones. These results suggest that not only did SMF accelerate the delivery of absorbed iron from roots towards upper parts and filling seeds, but it also increased uptake of iron by roots so that total iron content of SMF-treated plants was higher than that of the control ones. While there is a substantial body of information on the effects of externally applied electrical field on ion channels in plants, there is no adequate literature on the exact mechanism of magnetic field effects on the ion uptake by them (Serdyukov and Novitskii, 2013). It has been postulated that if a ferromagnetic nanocrystal (such as ferritin) is fixed to an ion channel, it has the potential to directly influence ion transport and would alter ion movement across a membrane (Kirschvink et al., 1992).The plants avoid iron overload toxicity and strictly control the homeostasis of iron (Briat et al., 2010). Ferritin prevents direct deposition of ferric hydroxide colloids by oxidizing $Fe^{2+}$ and storing it within the mineral core (Laulhere and Briat, 1993). Increase of iron content in SMF-treated wheat plants was accompanied by increase of ferritin and Fe-bound protein contents. A little literatures is available regarding the effects of magnetic fields on the plant antioxidant system (Serdyukov and Novitskii, 2013; Sahebjamei et al., 2007). Exposure of wheat plants in their reproductive phase increased the activity of seed CAT and RSC but decreased PO activity. Significant increase of CAT activity in SMF-treated wheat plants sufficiently scavenged reactive oxygen species and resulted in decrease of lipid peroxidation and increase of membranes integrity. Peroxidase is also an antioxidant but due to its role in the stiffening of the walls, a lower activity of it will be more beneficial for the growth (Ghanati et al., 2005). Altogether, the results presented here provide us with a new approach for application of physical stimulators such as SMF in order for iron biofortification and to increase the antioxidant capacity of critical crops.

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