



# Magnetic field effects on the physiologic and molecular pathway of wheat (*Triticum turgidum* L.) germination and seedling growth

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## Abstract

The aim of this work was to investigate the influence of the magnetic field on seed germination percentage and wheat seedlings, such as root and stem length, water content, photosynthetic pigments, antioxidants, phenols, flavonoids, and gene tests. *Triticum turgidum* L. ssp. *durum* Desf. seeds were exposed to a magnetic field with an induction of 12.5 and 25 mT for 6 days with 15 and 30 min exposure times per day. The magnetic field effect increased the water, chlorophyll, and carotenoid contents while reducing the germination percentage and root length. Significant associations were found between gene expression and related parameters, especially under 25 mT magnetic induction for 30 min. Further research could contribute to explaining the relationship between the influence of the applied magnetic field and the qRT-PCR genes (*EF1*, *PhAL*, *Rubisco*, *CBP4*) and changes occurring in wheat seedling growth. The variability in wheat genotypes could be caused by the applied magnetic field with different magnetic induction and exposure times. In magnetic field induction of 25 mT for a 30 min exposure time, changes were observed in all genes compared to the control group. The findings have different and unexpected implications that germination can be regulated by gene expression and related enzymes, associated with induction, exposure method, and duration of the magnetic field.

**Keywords** Wheat · Magnetic field · Helmholtz apparatus · Gene expression

## Introduction

Magnetic field (MF) is a natural component and an unavoidable environmental factor for all organisms on the Earth, and thus all plants have to experience this force. Interactions between MF and plants are becoming increasingly popular as new evidence has emerged for the ability of plants to perceive and respond quickly to varying MF by regulating their metabolism and altering gene expression and phenotype (Maffei 2014). The studies on plant development and magnetic field interactions argue that the magnetic fields affect the membrane structure of plant cells, allowing the plants to absorb more water and nutrients. Naturally, this situation triggers other physiological and biochemical processes.

Furthermore, the great majority of biological molecules, such as hemoglobin, cytochrome, and ferritin, include metal ions that can be paramagnetic (Hozayn and Qados 2010). Also, it was proposed that magnetic fields influence the development characteristics and function of the mRNA regulation process, protein biosynthesis, and enzyme activities, resulting in changes in various organ and tissue activities (Atak et al. 2003). Although a number of theories had been proposed to explain this phenomenon, there is no complete conclusion on how these fields achieve such a change in the plants.

Previous research on the effects of MFs on organisms contains many conflicting reports. Because of the lack of understanding of the biological effects and mechanism of MFs, most of the studies are not systematic, lack testable theoretical predictions, and the results are not convincing (Harris et al. 2009). The effect of MF was described in a number of studies with different and conflicting results. In a previous study, wheat seeds were embedded in water overnight before being exposed to a 30mT static magnetic field (SMF) and 10 kHz EMF, 4 day and 5 h per day. Exposure to both MFs was found to increase the germination rate and results suggested that EMFs promote the membrane integrity and development

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properties of wheat seedlings (Payez et al. 2013). However, when compared to control groups, the mean germination time of rice (*Oryza sativa*) seeds exposed to one of two MF inductions (125 or 250 mT) for varied periods (1 min, 10 min, 20 min, 1 h, 24 h) was dramatically reduced. The results show that this form of magnetic treatment clearly influences rice germination and seedling growth (Florez et al. 2004).

Increased nutrient uptake is responsible for the improvement in germination and growth metrics after imbibition (Hussain et al. 2020). Indeed, application of MF has been shown to affect seed membranes, causing membrane damage due to reduced electron leakage upon absorption of water. However, this does not always mean that germination will be positively affected. The result of the effect differs according to the applied magnetic field and the plant species. The most important thing is which metabolic or molecular structures will be affected by the application of the magnetic field. Current study will try to understand the effectiveness of the two different magnetic field intensities with different exposure times on wheat (*Triticum turgidum* L.) germination and seedling growth, at physiologic, biochemical, and molecular levels. Finally, the aim of this study is to: I) determine how wheat seeds and seedlings react after being exposed to varied magnetic fields and II) comprehend the underlying process of MF-induced growth modification.

## Materials and methods

### Plant materials and growth conditions

The research material was the seeds of durum wheat (*Triticum turgidum* L.) cultivar Kızıltan-91 (Field Crops Central Research Institute, Ankara). Uniform and undamaged seeds

from the previous harvest season were chosen and sterilized for 5 min in 2.5% sodium hypochlorite. The experiment was performed on glass jars in climatic chambers. Two layers of sterile filter paper were placed on the bottom of each sterile glass jar (8×5×5.5 cm) and three replications with 12 seeds per replication were sown in each jar. Seeds were soaked in 6 ml of Hoagland's solution (Hoagland and Arnon, 1950) and placed in a temperature-controlled chamber at 24 °C with a 16/8 day/night photoperiod, 2500 lx light intensity, and 70% relative humidity. Wheat seeds were exposed to different durations and inductions of magnetic field.

### Magnetic field application

The experimental factors were composed of two levels of magnetic field induction and exposure time. Two different Helmholtz coils (30 cm diameter/1200 turns and 40 cm diameter/1500 turns) and three sources of direct current (DC) power supply were used for the generation of the magnetic field of 12.5 and 25 mT, respectively, according to preliminary experiment experience. By adjusting the current in the Helmholtz coils, the requisite magnetic field induction was obtained (Fig. 1).

The generated magnetic field was measured regularly by a tesla meter (HT 20 Digital Gaussmeter, SMA, China) during the exposure period. Seeds placed in glass jars were exposed adjusted magnetic field for the same period in a day during the germination period. The experimental group had two different magnetic field induction and durations (Table 1).

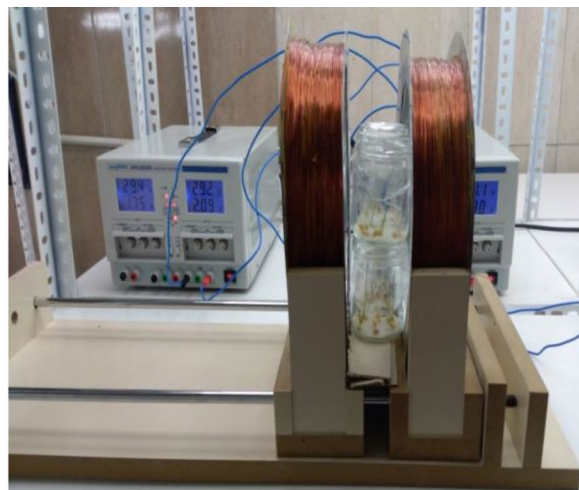


Fig. 1 Magnetic field apparatus and application process

## Seed germination and growth parameters

At the end of the magnetic field application (6 days after imbibition), the germination ratios were calculated and expressed as a percentage in all groups. Root and stem lengths (cm) were manually measured. Also, fresh and dry weights were determined using a precision scale  $\pm 0.01$  mg. Then relative water content of the seedlings was calculated according to the formula based on three measured values (dry, fresh weights, and turgor) following Hu et al. (2010).

Relative water content (%)

$$= \left[ \frac{\text{fresh weight} - \text{dry weight}}{\text{turgorous weight} - \text{dry weight}} \right] \times 100.$$

## Pigment contents

Chlorophyll a, b and carotenoids were determined by using a spectrophotometer (FLUOstar Omega Microplate Reader with UV/Vis Spectrometer, BMG LABTECH, Germany). 0.1 g healthy leaf samples were homogenized in 80% acetone to determine chlorophyll concentration. The chlorophyll and carotenoid contents were determined by measuring absorbance at 480, 645, and 663 nm. Arnon (1949). Lichtenthaler and Wellburn (1983) method was used to calculate the chlorophyll and carotenoid contents.

## Preparation of seedling extract

100 mg of seedling material (ground in liquid nitrogen) was mixed thoroughly with 10 ml of ethanol (80%) and shaken on a magnetic stirrer for 5 min at room temperature. The solutions were then sonicated at 37 °C for 40 min and incubated at 4 °C for 24 h and then centrifuged at 10,000 RPM. Supernatants were taken and stored at -20 °C until further analysis.

**Table 1** The induction and duration of the applied magnetic fields to *Triticum turgidum* seeds

Group	Magnetic field induction (mT)	Exposure time (min)
A	12.5	15
B	25	15
C	12.5	30
D	25	30
Control	0	0

## Antioxidant capacities

The antioxidant activities were carried out by using the extract samples obtained from the seedlings. The DPPH radical scavenging activity was measured according to Blois (2002) at 520 nm. The measurements of DPPH radical scavenging activities of samples that were exposed to the magnetic field application and control groups were calculated by using the following formula: Inhibition % =  $(AC - AS)/AC \times 100$  (AC: absorbance of control, AS: absorbance of the sample) (Moraes-de-Souza et al. 2008).

Total reducing capacity (TPTZ technique) was measured with a spectrophotometer at 595 nm for the FRAP test. Based on an iron sulfate standard (FeSO<sub>4</sub>) curve against a blank control, the extracts' reducing ability was represented as mol of iron (Fe<sup>2+</sup>) per gram of dry weight (mol Fe<sup>2+</sup>/gDW) (Sudha et al. 2012).

## Phytochemical content analysis

The total phenolic content was measured at 600 nm using the Folin–Ciocalteu method. According to gallic acid standard curve and a blank control, the results were calculated as mg of total phenolic content (gallic acid equivalent) per gram dry weight (mg GAE/gDW). At 510 nm wavelength, the total flavonoid content was determined. The extracts were tested in triplicate and the results were represented as milligrams of total flavonoid content (rutin equivalent, RE) per gram of dry weight of the ground powder (mg RE/gDW), based on the rutin standard curve. The analyses were carried out in triplicate (Dalar and Konczak 2013).

## cDNA synthesis and qRT-PCR

Seven-day-old seedlings were harvested to perform q-PCR analysis and ground in liquid nitrogen immediately. Powdered tissue was poured into 2 ml tubes and used for total RNA extraction. For the RNA extraction process, a commercial Rneasy kit (QIAGEN Rneasy Mini Kit 250) was used according to the manufacturers' instructions, and DNase I (Fermentas) kit was used to remove DNA residues in RNA. PolyA-RNA cDNA synthesis was carried out utilizing reverse transcriptase and oligodT18 primers to generate the cDNA. Table 2 shows the gene-specific primers used in the qRT-PCR tests using complementary DNAs as templates.

The spectrophotometric quantification of RNA was additionally validated using a NanoDrop (Thermo Fisher Scientific, Inc). Following the manufacturer's instructions, real-time PCR was done with the cDNA using the q-PCR SYBR

**Table 2** Genes and primer pair sequences are used for qRT-PCR

Genes and primers sequences			
Genes	Sequence	Size	Tm
1. Chlorophyll a, b binding protein	F- 5'- AAGGAGATCAAGAACGGGCG- 3'	140	60.11
	R- 5'-GATGTCGCCGATGTGTTGTG- 3'		
2. Phenylalanine ammonia lyase	F- 5'-ATCTCATCCAGGAAGACGCC- 3'	220	59.24
	R- 5'-CGGTCAGCAAATCCTTCTCG- 3'		
3. Ribulose 1,5 bisphosphate	F- 5'-TGGTTTCAAAGCCCTACGTG- 3'	215	58.4
	R- 5'- GTCCACCACGTAGACTCA- 3'		
4. Elongation factor1	F- 5'-CAGATTGGCAACGGCTACG- 3'	164	60.6
	R- 5'-CGGACAGCAAAAACGACCAAG- 3'		
5. Actin	F- 5'-AGCGTTGTTGTGAGGGAGT- 3'	180	66.4
	R- 5'-TGCTATCCTTCGTTTGGACCTT- 3'		

Green master mix. The Ct-method was used to compile and evaluate data from three independent experiments. Fold changes were calculated by the  $2^{-\Delta\Delta C_t}$  method referenced to actine (Livak and Schmittgen 2001).

### Statistical analysis

All experiments were performed as three replications. The results were presented as the mean value  $\pm$  standard deviation. The obtained results were analyzed using Minitab software (v. 19.1.0, State College, PA: Minitab, Inc.), by applying one-way analysis of variance (ANOVA) at a significance level of  $p \leq 0.05$ .

### Results

The effects of the magnetic field on the germination of the seeds were examined, and it was interestingly determined that the germination percentage of all seeds decreased compared to the control group. It was noticed that there was a significant interaction between germination and magnetic field induction and duration, especially at the time point of 30 min. So it was found that the magnetic field applications significantly reduced the germination percentage at both 12.5 and 25 mT induction (Fig. 2a).

According to the data obtained in the study, an inverse symmetrical situation was observed between germination and relative water content. It was found that the relative water content in the seedlings with a low germination percentage was found to be significantly higher than that in the others (Fig. 2b). There was no significant difference between the root and stem lengths of the seedlings exposed to the magnetic field; however, it was determined that 25 mT and 30 min of exposure significantly reduced both root and stem lengths (Fig. 2c).

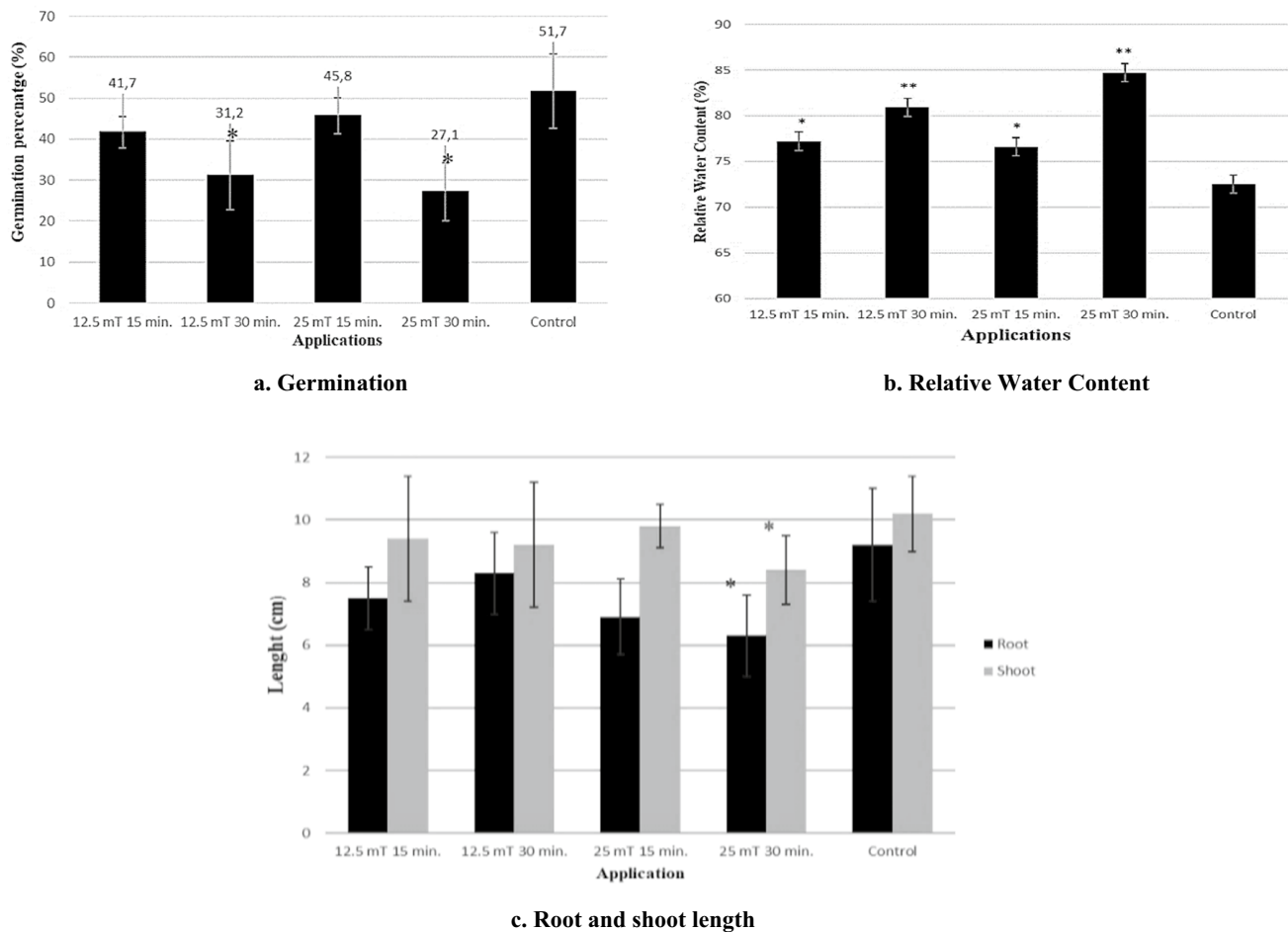
Chlorophyll pigment contents were evaluated, magnetic field applications caused contrasting results. According

to results, 25 mT and 30 min magnetic field applications caused an increase in chlorophyll pigments, while other applications caused negative (decrease) or nonsignificant changes in chlorophyll pigments. The effects of the magnetic field on chlorophyll a and b varied according to time and induction ratios. Short exposure of 25 mT for 15 min was effective to decrease photosynthetic pigments; however, prolonged exposure time for 30 min at the same induction increased the level of the pigments significantly (Table 3).

The effect of MF treatments on total phenolic content was observed only at 25 mT/30 min. Moreover, the flavonoid content decreased in all magnetic field groups compared to the control. The antioxidant activities of DPPH and FRAP decreased compared to the control. The exception was the insignificant increase at 12.5 mT induction for both exposure times (DPPH). The 25 mT magnetic field treatment with a duration of 15 and 30 min significantly reduced the FRAP content by 25 and 28%, respectively, compared to the control group (Table 4).

Figure 3 indicates the graphs comparing the gene expression patterns of seedlings exposed to 12.5 and 25 mT magnetic fields for 15 and 30 min. Within each treatment, values are shown as fold induction or repression relative to the control.

The expression levels of all studied genes were strongly affected by magnetic field applications. In general, a proportional increase in the expression of all genes was determined, depending on the increase in magnetic field induction and duration. The qRT-PCR results indicate that representative genes show more than a twofold difference in expression between the exposure time and intensities especially in *Rubisco* and *CBP4* genes (Fig. 3) Fig. 2c.



**Fig. 2** Effects of magnetic fields on growth parameters. **a** Germination rates (%), **b** relative water content (%) and **c** root and shoot length (cm) (\*/\*\* indicates statistically significant difference)

**Table 3** Effects of magnetic fields on chlorophyll pigment concentrations (mg/g)

Applications	Chl_a	Chl_b	Tot. Chl	Car
12,5 mT, 15 min	102.7 ± 9.9*	77.3 ± 7.8	180.0 ± 3.1	9.9 ± 1.3
12,5 mT, 30 min	117.9 ± 6.1	81.7 ± 8.8	199.6 ± 5.9	9.6 ± 2.4
25 mT, 15 min	88.1 ± 8.8**	65.9 ± 7.5**	154.0 ± 6.1**	5.9 ± 1.1**
25 mT, 30 min	146.6 ± 4.9	84.2 ± 9.6*	230.8 ± 9.9*	10.4 ± 2.1*
Control	144.4 ± 3.8	70.3 ± 7.8	214.7 ± 5.9	9.7 ± 1.4

\*/\*\* indicates a statistically significant difference compared to control at  $p < 0.05$  and  $0.01$ , respectively

## Discussion

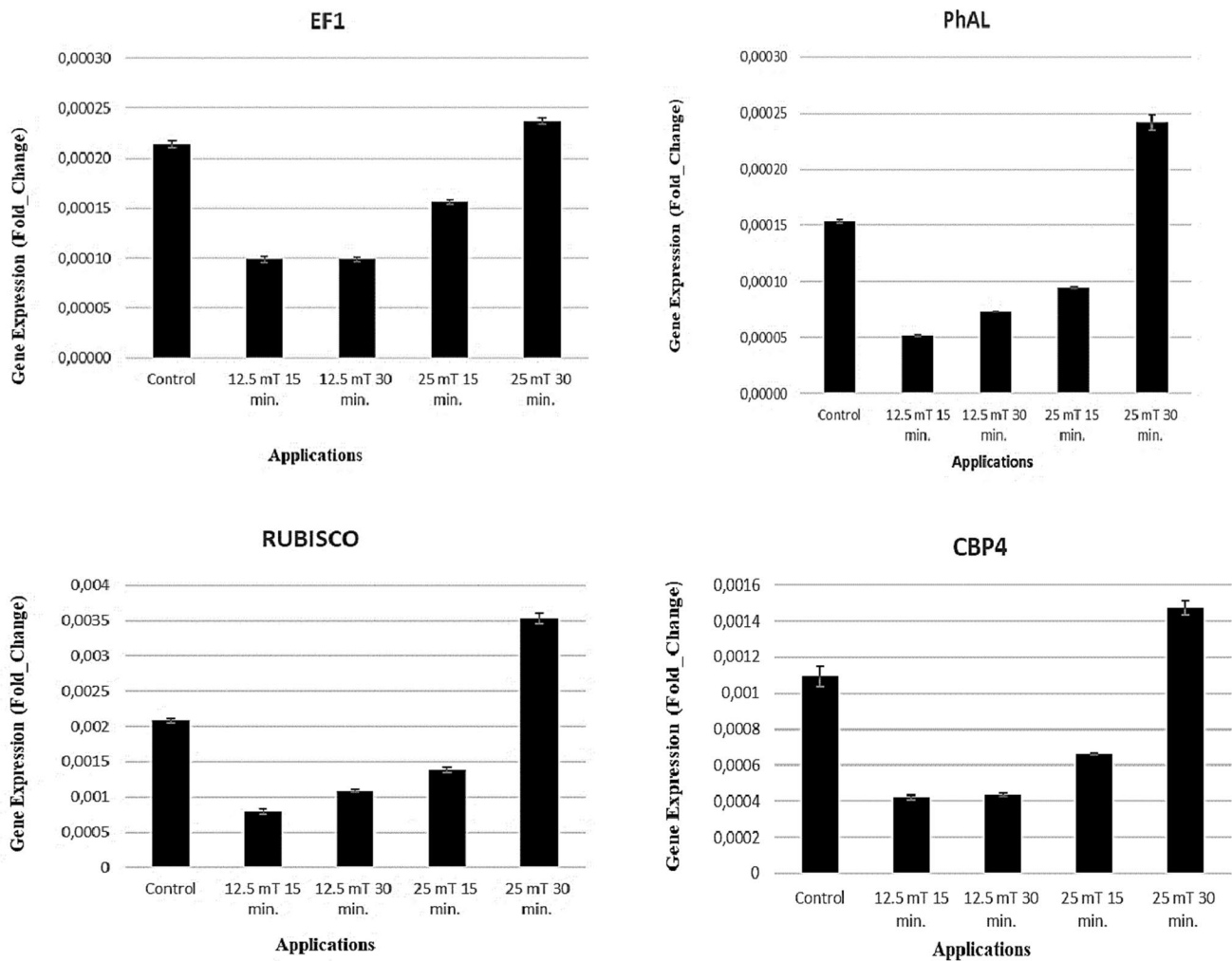
In the current study, a significant effect was observed for both magnetic field and exposure times, which had a negative effect on the wheat seeds' germination percentage compared to the control. However, the duration of exposure time is thought to be more dominant on the negative effect, because of the prominent effect of 30 min than 15 min. The effect of magnetic field application, is dependent not only on its intensity, but also on plant genotype and duration.

The stimulatory effect of the application of different magnetic field levels and durations on the germination is reported in several species including rice (Florez et al. 2004), wheat and barley (Martinez et al. 2000), *Pisum* (Iqbal et al. 2012), sugar beet (Szajnsner et al. 2017), and chickpea (Mridha and Nagarajan 2014). The researchers associated their results with hydrolysis or the expression of hormones (indole, gibberellins, or zeatin) or amylolytic enzymes ( $\alpha$ - $\beta$  amylase). However, there is no complete and uniform theory explaining the function or effects of magnetic fields. Changes in hormone concentrations, enzyme activities or ion transport across the cell membrane and also changes

**Table 4** Effects of magnetic fields on total phenolic and flavonoid contents and antioxidant activities

Applications	Total phenolic content (mg/g)	Flanovoid (mg/g)	DPHH (%)	FRAP ( $\mu$ mol $\text{Fe}^{2+}$ /g)
12.5 mT 15 min	$4.3 \pm 0.3$	$1.7 \pm 0.2$	$19.5 \pm 3.6$	$182.3 \pm 11.4$
12.5 mT 30 min	$4.8 \pm 0.3$	$1.9 \pm 0.2$	$19.5 \pm 2.9$	$171.3 \pm 11.5$
25 mT 15 min	$4.5 \pm 0.5$	$1.7 \pm 0.3$	$18.3 \pm 0.6$	$149.9 \pm 3.2a^*$
25 mT 30 min	$6.2 \pm 0.1^*$	$1.7 \pm 0.7$	$17.9 \pm 0.2^*$	$142.6 \pm 18.5^*$
Control	$5.1 \pm 0.7$	$2.3 \pm 0.4$	$19.0 \pm 1.76$	$199.4 \pm 10.8$

\*/\*\* Indicates statistically significant difference compared to control



**Fig. 3** Effect of magnetic field on relative gene expression levels (Fold\_Changes); *Triticum* elongation factor-1 (EF1); phenylalanine ammonia lyase (PhAL); ribulose 1,5 bisphosphate carboxylase/oxygenase (Rubisco); chlorophyll a,b binding protein (CBP4)

in DNA synthesis or transmission can all be influenced by variable magnetic fields (Strasak et al. 2002). In our study, depending on the type of wheat seed and the duration/intensity, magnetic field treatment may act on imbibition of water, abscisic acid pathway, or hydrolytic enzymes so germination inhibition may have occurred depending on the type of magnetic field treatment at 25 mT and 30 min. The short

duration of the magnetic field is reported to cause a negative effect on most of the indicators characterizing sunflower seed germination and growth (Matwijczuk et al 2012). In a study, results showed that lower strengths ( $\leq 125$  mT) of MF treatment affects the germination of barley seeds. Confocal microscopy examinations demonstrated MF-induced cell membrane disruption in roots, which could affect tissue

elemental composition (Ercan et al. 2022). Similar effects were detected on root and shoot length. Germination delay caused a significant decrease in the lengths, especially in 25 mT—30 min application.

One of the most interesting results of our study was that the relative water content (RWC) was quite high in the groups that were more affected by the magnetic field and had germination inhibition. RWC percentage of all applications was significantly higher than that of control. The results indicated that there is a contrasting effect between germination inhibition and RWC. Magnetically treated water, acting on calcium ions, may improve the permeability of cell membranes in seeds. This may alter ion transport via cell membranes, causing an imbalance in ion concentration in the cell and variations in intracellular pH. It is possible to explain the response of magnetic field on variations in water characteristics and impurities (Matwijczuk et al. 2012). Another reason for the high RWC may be the effect of the magnetic field on aquaporin in the membranes.

Magnetic field has effects on leaf gas exchange performance as well as photosystem II (PSII) efficiency, photosynthetic pigments (chlorophyll *a* and *b*), and the performance index (Yano et al. 2004; Rochalska 2005; Baghel et al. 2018). In our study, especially chl\_b contents indicate significant differences caused by magnetic field application. In addition, different values were obtained in 25 mT/15 min compared to other applications, particularly in carotenoids. Magnetic field influences pigment content (carotenoids, chlorophyll *a*, *b*, and total pigments), with carotenoids and chlorophyll *a* being affected more than chlorophyll *b*. Shine et al. 2011. In our result, the chlorophyll and carotenoid contents were decreased during 25mT and 15 min application. MF exposure reduced the level of photosynthetic pigments in seedlings of *Zea mays* L. and *Robinia pseudoacacia* L. This effect could be related to the influence of MF on the reduction in plastids (Taia et al. 2007) or a decrease in carotenoids explained by a decrease in pigment content. In this context carotenoids are used in radical scavenging processes (Strzalka et al. 2003).

Unpaired electrons on metal ions may orient in the same direction as the applied magnetic field, gaining more energy in the enzymes. This energy may be transmitted to other molecules, causing additional radicals to develop and affect antioxidant capacity (Çelik et al. 2009). In many plant species, phenolic compounds and flavonoids act as antioxidants, and a significantly positive association between total phenols and antioxidant activity has been reported. (Rainha et al. 2011). According to the results obtained in our study, it was determined that there was a decrease in the antioxidant activities of DPPH and FRAP due to the increased phenolic content, especially in 25 mT/30 min application. Metabolic and biochemical activities improved because of magnetic treatments and changed the biochemical properties (Zareei et al. 2019).

Varying intensity of low-frequency MF affects several parameters, including CO<sub>2</sub> assimilation, pigment content, and activity of photosynthetic reactions (Sukhova et al. 2021), by the generation of electric potential (electromagnetic field). The results of the study demonstrated that low-frequency MF influence electric and signal reactions in wheat plants. This impact is achieved by the extremely low-frequency MF acting on the signaling systems that balance the intracellular concentration of Ca<sup>2+</sup> and the activity of H<sup>+</sup>-ATPase. Because a plant cell conducts various critical tasks, MF-induced variations in the electric potential can have a specific value for its functioning. Magnetic fields cause an increase in Ca<sup>2+</sup> concentration due to the activation of the H<sup>+</sup>/Ca<sup>2+</sup> antiporter, which was suggested as a key mechanism of electromagnetism (Grinberg et al. 2022).

The presence of a magnetic field may trigger signal transduction and gene regulation. Also in our study, q-PCR results indicate that magnetic fields have far-reaching effects on the genome of wheat seedlings. In particular, in all four different gene regions studied, the expression levels of 25 mT/30 min were higher than both control and other treatments. In general, researchers hypothesized that magnetic fields were powerful enough to disrupt the complex conformational dynamics involved in elements of gene regulation, resulting in the varied expression of a wide range of genes in plants (Paul et al. 2006).

A magnetic field can affect the local chemical structure of any of the multiple signaling pathways, which are linked to the differential expression of any number of genes involved in various aspects of plant growth (Weaver et al. 2000). However, further research is needed to fully comprehend the findings.

Finally, it should not be thought that the application of magnetic field will always have a positive effect on germination. The responses are quite variable and complex and depend on the pathway to be triggered on metabolism.

## Conclusion

The effects of the magnetic field on living organisms are evaluated differently because of its effects on various pathways. While some consider the magnetic field as an environmental stress condition for the growth of plants, others suggest the magnetic field as a promising technique for agricultural improvements. Now, the efficiency of magnetic field parameters (such as exposure time/intensity, combination with other stresses, or effects on nerves and treatments) on organisms appear to be species specific, but it remains a mystery and needs much more work.

**Author contribution statement** MEE contributed to the study conception and design. Material preparation, data collection and analysis were performed by MÖ. The first draft

of the manuscript was written by MEE and MÖ commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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