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Magnetic field effects on the magnetic properties, germination, chlorophyll fluorescence, and nutrient content of barley (*Hordeum vulgare* L.)

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ABSTRACT

The magnetic field (MF) interacts with biological systems and has the potential to increase germination, plant growth and productivity. Although it is known as a low cost and promising approach, the mechanism that increases growth is not fully understood yet. In this study, the effect of different MF strengths (20, 42, 125, and 250 mT) was investigated on barley (*Hordeum vulgare* L.). In addition to phenological parameters, possible cell damage, electron transport rate, chlorophyll fluorescence, magnetic character and elemental status of tissues were determined. Results showed that lower strengths (≤125 mT) of MF treatment improve germination. Confocal microscopy analyzes revealed MF-induced cell membrane damage in roots that could alter the elemental content of tissues. Elemental analyzes found that the content of macroelements (Ca, Mg, P, and K) are gradually reduced with increasing MF forces; in opposite the microelement contents (Fe, B, Cu, Mn, Zn, and Mo) are increased in roots. Diamagnetism is the dominant magnetic character in all root and leaf samples. However, the roots became surprisingly superparamagnetic in 250 mT application. It seems that MF treatment at higher strength (250 mT in this study) could influence the orientation of magnetic moments. These findings suggest that MF application: i) can alter the magnetic character of plants, ii) enhances the germination, photosynthetic machinery, and growth, and iii) affects the nutrient uptake and abundance in tissues, depending on the MF strength. This comprehensive study can help in understanding the interaction of magnetic field with plants.

1. Introduction

In nature, living beings are exposed to a constant magnetic field (MF) due to the magnetic field (geomagnetic field) of the earth which is about 25–65 μT (μT) (0.25–0.65 G) (Finlay et al., 2010). In addition, it has been shown that external MF can stimulate a variety of physiological responses of plants such as germination, flowering time, photosynthesis, biomass, activation of cryptochrome, and shoot growth (Maffei, 2014). Depending on its strength, frequency, and the plant type used, MF can alter transcriptome, proteome, and metabolome profiles of the plants (Herranz et al., 2013; Jin et al., 2019; Islam et al., 2020). Significant growth enhancement by MF has been achieved in several plants such as sunflower (*Helianthus annuus*) (Vashisth and Nagarajan 2010), rice (*Oryza sativa*) (Flórez et al., 2004), and chickpea (*Cicer arietinum*) (Vashisth and Nagarajan 2008). In agricultural practices, short-term pre-treatment of seeds (5–15 min) with external MF (5–15 mT) has increased the germination, growth, and fruit yield of wheat (Hussain et al., 2020). Not only in plants, but external MF application also improved the density and biomass (29.02% and 35.67%) of algae (Luo et al., 2020).

The enhancement in germination and growth parameters is attributed to the increased nutrient uptake (Hussain et al., 2020) because it was demonstrated that MF application affects seed membranes, leading to membrane impairment with reduced electron leakage during imbibition (Vashisth and Nagarajan 2008). Moreover, it was found that the lipid component of the cell membrane is significantly enhanced after

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external MF application (126 and 208 mT) in tomato, which could lead membrane deformation (Poinapen et al., 2013) and eventually membrane trafficking and nutrient uptake. Therefore, MF interaction with membrane system could be the key factor in controlling the plant physiological responses.

Instead of chemical stimulants, using magnetic treatment at the pre-sowing stage (magneto priming) protects plants from toxic residues, making it an environmentally friendly (effective, affordable, and safe) technique to increase plant growth and yield. However, information on how MF stimulates growth is limited. Most recently, the induction of plant hormones by MF has been shown. For instance, Jin et al. (2019) demonstrated that static MF treatment (600 mT) increases auxin concentrations at the root tip and promotes the root growth based on the applied MF strength and direction. Transcriptome and functional analyses suggested that auxin efflux (PIN3) and influx (AUX1) proteins may be involved in the regulation of plant growth enhancement. MF-induced Arabidopsis-flowering could be associated with gibberellin biosynthesis via cytochromes (CRYs), UV-A/blue-light photoreceptors (Xu et al., 2017). In addition, it has been suggested that CRYs could be positive regulators for the root growth under MF conditions (Dhiman and Galland, 2018; Jin et al., 2019). Also, MF stimulates cell multiplication, triggers molecular transformation which provide conditions for growth and further development achieved by the activation of protein synthesis (Zablotskii et al., 2016). Moreover, alleviation of various types of stresses by MF application has been shown in several studies such as drought (Karimi et al., 2012; Hasan et al., 2020), salt (Kataria et al., 2017), and heavy metals (Chen et al., 2017). Acquired resistance of mung bean seedlings against cadmium stress was observed upon 600 mT of MF application (Chen et al., 2011).

Although there are studies on the effect of the MF on several plants, more research is needed to understand how the magnetic field can contribute to plant growth, especially in crop species. Barley, which is the fourth most cultivated cereal in the world, is an important crop both economically and for food and feeding purposes. To improve its germination and growth performances, agricultural practices should focus on eco-friendly solutions. Although there are a limited number of studies showing the effect of MF on barley, there is no study elucidating the magnetic response, physiology, and nutrient uptake of the plant. Therefore, the purpose of this study is to find out i) how barley seed and seedlings physiologically react after being exposed to varying MF strengths, and ii) what is the underlying mechanism of MF-induced growth change. For these purposes, together with the magnetic and physiological responses, MF-induced changes on photosynthetic parameters and elemental content were examined. These findings could be important in understanding how MF contributes to plant growth. Also, it may contribute to further research, such as space exploration to understand the response of plants in high MF conditions.

2. Materials and methods

2.1. Growth conditions and magnetic field application

Barley seeds (*Hordeum vulgare* L. cv Tarm-92) were obtained from Field Crops Central Research Institute (Ankara, Turkey). The effect of different magnetic field (MF) strengths was assessed by growing the plants either on the germination (four days) or seedling (two weeks) stages. For the germination assay, the seeds (n = 20) were surface sterilized and soaked in distilled water for imbibing. Then, they were placed in Petri plates spread out by two layers of sterile tissue papers. Hoagland solution containing 6 mM KNO_3, 0.1 μM Fe-EDTA, 4 mM Ca $(NO_3)_2$, 1 mM $NH_4H_2PO_4$, 0.02 g MoO_3 (85%), 2 mM $MgSO_4$, 50 μM H_3BO_3, 9 μM $MnCl_2$, 0.8 μM $ZnSO_4$, and 0.3 μM $CuSO_4$ (Bostancioglu et al., 2018; Tombuloglu et al., 2019) was prepared. It was then poured onto the seeds in Petri plates. The plates were vertically placed at the center of variable gap magnets (EM-8618, Pasco, US) targeting the seeds. Each magnet's gap was fixed by measuring the magnetic field that hits the plates and yielded 14–20, 32–42, 110–130, and 240–250 mT values (Table 1). To provide a uniform magnetic field on the seeds, two flat pole pieces (8× 4.5 cm) were placed on each pole of the magnets (Supplementary Fig. 4). In this way, a constant and uniform MF covering all seeds was obtained. The MF measurements were carried out by using Teslameter 4060.50 from Frederiksen. The untreated sample was used as an MF-control. The plates were kept for 4 day at room temperature in a dark environment. Any seeds having a 0.5 cm root length were accepted as germinated. Afterward, the seedlings were transferred to a hydroponic system filled with Hoagland solution. An aquarium pump was run every 2 h to aerate the solutions. After being transferred, MF with different strengths (14–20, 32–42, 110–125, and 240–250 mT) was continuously applied to the seedlings. The growth conditions were adjusted as 20/25 °C, 60/70% humidity, and 16/8 light/dark regimes, with a 300 ± 20 μmol photon m^2/s of photosynthetically active radiation (PAR) which is accepted as normal-PAR for barley growth (Saini and Fricke, 2020). The seedlings (n = 8) were grown for two weeks and the whole leaf and/or root tissues were harvested, dried in an oven for three days, and then crushed for magnetic and elemental analyses.

Table 1
Magnetic field treatments.

	Magnetic field strength (mT)	Acronym
1	0 (Control)	0M
2	14–20	20M
3	32–42	42M
4	110–125	125M
5	240–250	250M

2.2. Magnetic behavior of root and leaf tissues

The Vibrating Sample Magnetometer (VSM) analyses of the root and leaf parts of the plants were carried out by using a Physical Property Measurement System (PPMS)-VSM instrument (Quantum Design, Model P525). The measurements were performed at room temperature. The accuracy of the instrument is $< 1 \times 10^{-5}$ emu/tesla with the Root Mean Square (RMS) sensitivity of $<10^{-5}$ emu or 0.5%. In order to find the net magnetization (M_{NET}) contribution created by different MFs in roots, the magnetization value of the control sample (M_{CTRL}) was subtracted from one of MF-treated samples (M_{Sub}).

$$M_{NET} = M_{Sub} - M_{CTRL}$$

2.3. Determination of photosynthetic parameters and the chlorophyll, carotenoids, and soluble protein content

The status of photosynthetic parameters was determined by measuring the chlorophyll fluorescence of the leaves treated by different MF strengths for two weeks. The 1st leaf of the seedlings was used for the measurements. For this purpose, a PAM fluorometer (Walz® GmbH, Effeltrich, Germany) was occupied. Before the measurement, the seedlings were incubated in dark for 30 min. A clip holder was placed on the leaf and an actinic pulse light with a maximum emission of 450 nm was applied (n = 3) (Fig. 5a). The photosynthetic photon flux density (PPFD) of blue measuring light was 1500 μmol photon $m^{-2}s^{-1}$ at a 1 mm distance from the tip of the light guide. Photosynthetic parameters such as Fo, Fm, Y(II), and ETR (minimum fluorescence, maximum fluorescence, effective photochemical quantum yield of photosystem II, and electron transport rate) were determined by using WinControl-3.29 software (Walz® GmbH, Effeltrich, Germany). The fluorescence signal was detected by using a PIN-photodiode. The variable fluorescence (Fv) and maximum quantum efficiency of PSII (Fv/Fm) were also calculated according to the formula stated by Kitajima and Butler (1975).

To determine the pigment content (chlorophyll *a, b,* and carotenoid),

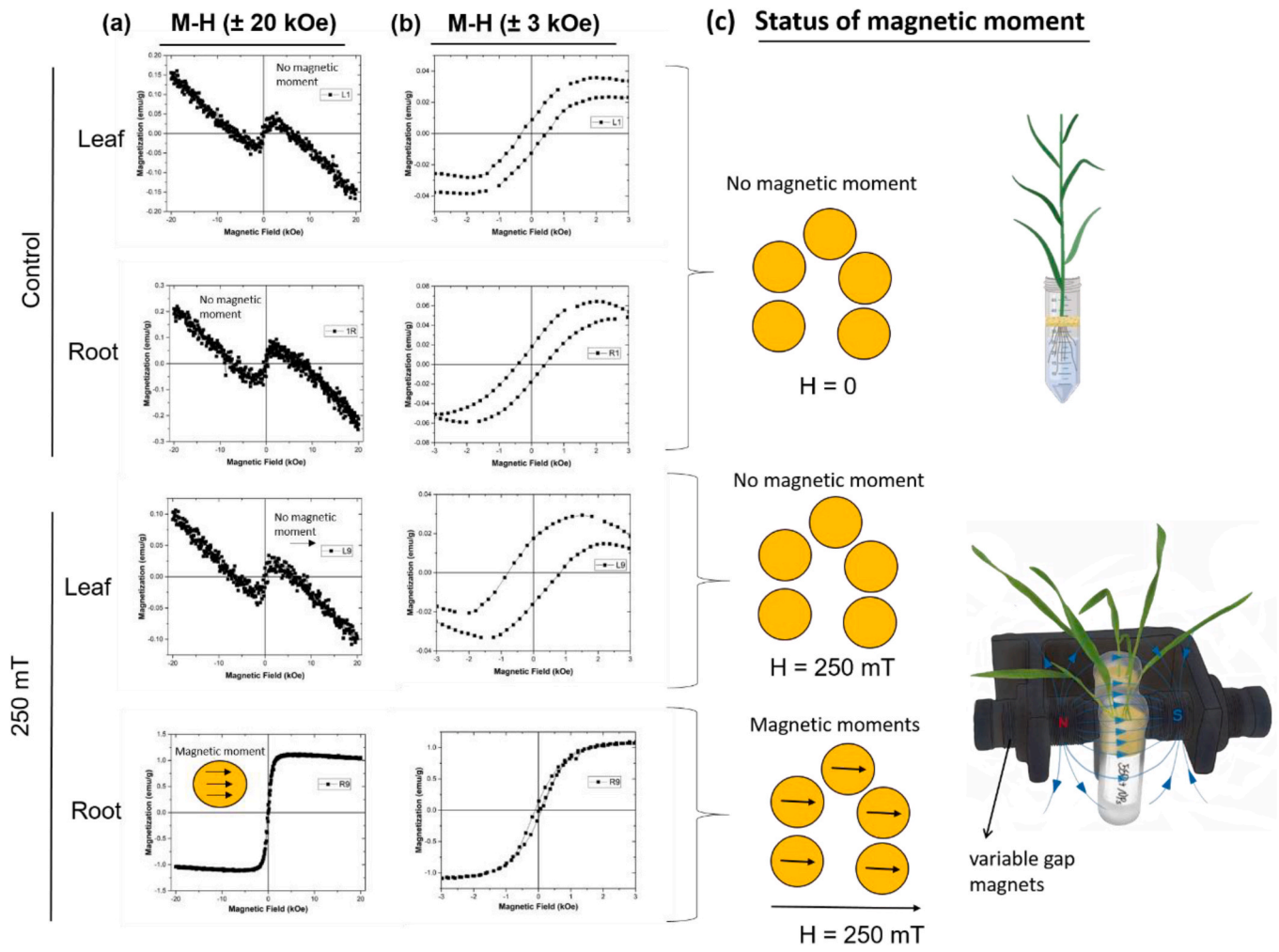


Fig. 1. Magnetic responses of root and leaf tissues versus the applied external magnetic field (MF, 250 mT). Control group (H = 0) is exempt from the MF treatment. **(a)** The internal magnetic response of the tissues (M–H) was observed between +20 and −20 kOe. **(b)** In addition, the M(H) curves around H = 0 kOe (±3 kOe) was depicted on the right-hand-side of each graph. **(c)** The magnetic moment of each sample was depicted where the empty circles represents no magnetic moment; while the circles with arrow indicates the existence of magnetic moments upon MF treatment. An expanded figure for the other MF-strengths are given in Supplementary Fig. 1.

the method described by Lichtenthaler and Wellburn (1983) was followed. The tip of the first leaf (50 mg) was freshly taken from the plant and crushed with the aid of 4 mL of 80% (v/v) acetone. After the centrifugation step at 4000×*g* for 15 min, the supernatant was separated, and it was measured spectrophotometrically using (Biotek, Synergy Neo2). As a result, the absorbance values at 663, 646, and 470 nm wavelengths were read and the following formulations were used to quantify chlorophyll *a*, chlorophyll *b,* and carotenoid content.

$$Chla = 12.21 \times A663 - 2.81 \times A646$$

$$Chlb = 20.13 \times A646 - 5.03 \times A663$$

$$Car = (1000 \times A470 - 3.27 \times Chla - 104 \times Chlb) \div 229$$

Soluble protein contents in tissues (root and leaf) were determined according to the Bradford method (Bradford, 1976). Accordingly, 50 mg of fresh root and leaf samples were crushed with 2 mL pre-cooled buffer (0.15 M phosphate buffer (pH 7.8) + 1 mM EDTA). The mixture was separated using a cooled centrifuge at 15,000×*g* for 20 min at 4 °C. The absorbance value of the obtained supernatant at 595 nm wavelength was determined using (Biotek, Synergy Neo2). The amount of protein corresponding to the reading value was calculated by using a calibration plot prepared for Bovine Serum Albumin (BSA) standards.

2.4. Confocal microscopy analysis of root tip cells

Propidium iodide (PI) dye was used to detect possible membrane damage to plant root tip cells. While PI, a cationic dye, cannot pass through intact cell membranes, it penetrates the cytoplasm from cells with damaged membranes, causing staining of the nucleus. In this way, it is possible to distinguish damaged cells under the microscope. The root samples (n = 5) for each experimental group were collected at the same time. For this, a 0.7–0.1 cm long tissue was excised from the plant and washed 3 times with PBS (pH 7.2). It was then left to stain for 5 min in freshly diluted PI solution (1%, P-4170, Sigma). The tissues were then rinsed with distilled water and observed under a confocal microscope (LSM 900, Zeiss, Germany). The parameters were chosen as follows: red-colored "PI dye"; excitation wavelength 536 nm; and maximum emission 617 nm.

2.5. Determination of nutrient elements in the tissues

The mixtures of root or leaf samples taken from 10 plants were dried in a 60 °C oven for three days and then crushed. Prior to this procedure, the wet tissues were first sonicated for 5 s (Powersonic 410, Hwashin Technology, Korea) then washed thoroughly with DI water. The digestion of the samples was performed according to the recommended

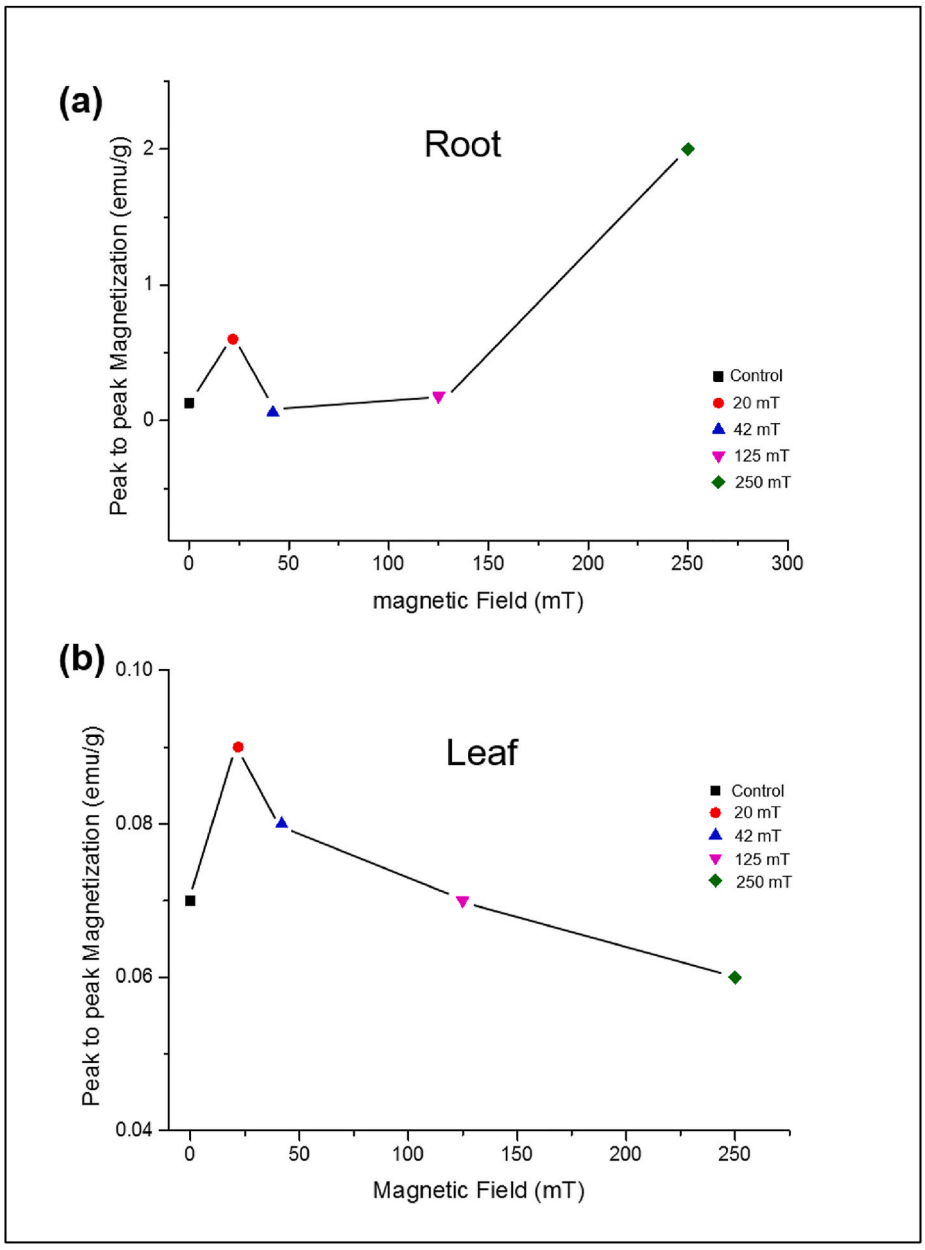


Fig. 2. Peak to peak magnetization signal values around H = 0 kOe of **(a)** the root and **(b)** leaf samples upon two weeks of MF (20, 42, 125, and 250 mT) application.

protocol of the U.S. Environmental Protection Agency (USEPA) method 3051 (USEPA, 1995). The mixture of concentrated nitric acid (HNO_3, 65%) (SCP Science, Thermo Fisher Sci) and 5 mL of DI water (ELGA, 21–22 MΩ) was poured into the vessel. The sample digestion was carried out in a microwave oven (Multiwave 3000, Anton Paar, equipped with a pressure/temperature sensor to control the microwave power). An inductively coupled plasma optical emission spectroscopy instrument (ICP-OES, PerkinElmer AvioR 145 500 ICP-OES Scott/Cross-Flow, USA) was used to quantify elements found in the tissues.

2.6. Statistical analyses

All experiments were carried out using sets consisting of at least three randomly selected biological samples unless indicated otherwise. The measurements were made with at least three technical replicates. The data obtained were analyzed by *t*-test analysis of variance. *P*-value was taken as a basis to detect significant variation between data. Significant changes according to the *P*-value (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$) were indicated. SPSS program version 15.0 (SPSS Inc., Chicago, USA) was used for the statistical analyses.

3. Results

3.1. Magnetic character of root and leaf tissues upon external magnetic field application

To probe the magnetic responses of roots and leaves versus the applied external magnetic field (MF), Vibrating Sample Magnetometer (VSM) analysis was conducted. The internal magnetic response of the tissues (root and leaf) (M-H) was observed between +20 and −20 kOe. According to the results, the roots have diamagnetic behavior under control and 20, 42, and 125 mT MF conditions (Fig. 1a; Supplementary Fig. 1). However, the sample treated with 250 mT of external MF exhibited super paramagnetic behavior. Additionally, weak ferromagnetism of around 0 kOe was observed in all root specimens. As seen from Fig. 1b (right side), observed weak ferromagnetic behaviors are

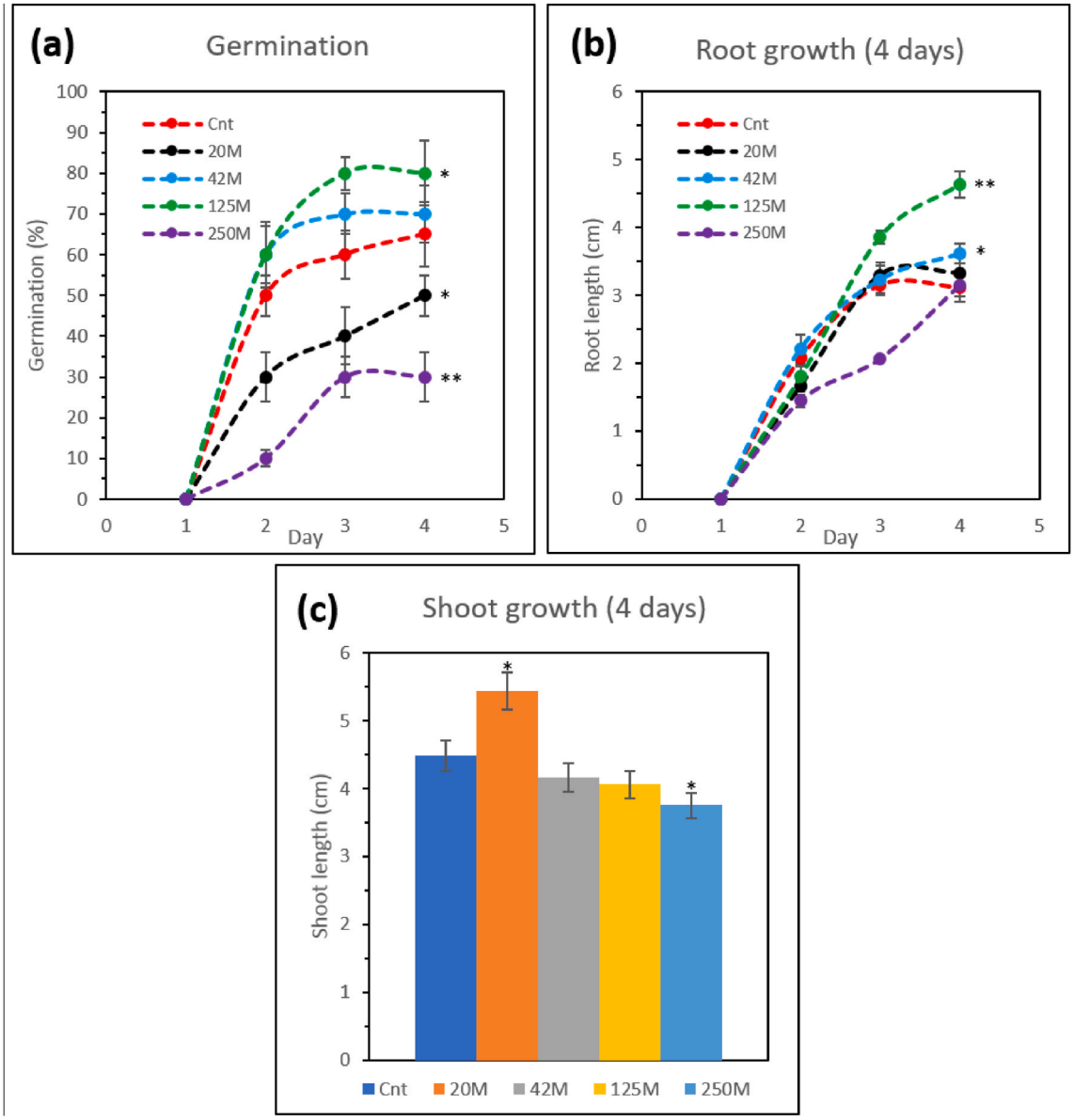


Fig. 3. Impact of magnetic field (MF) on **(a)** germination, **(b)** root growth and **(c)** shoot growth of barley after four days of seed sowing. The MF strengths were 20, 42, 120, and 250 mT. The data were statistically analyzed by *t*-test (*p < 0.05, **p < 0.01) (n = 15).

investigated in the images magnified around H = 0 kOe (M-H ±3 kOe). When the peak-to-peak magnetization values of these images were investigated as a function of the applied magnetic field (Fig. 2a), it is obvious that external MF application increases magnetic signals in barley roots with increasing MF intensity.

In the leaf, the control and MF-treated samples (20, 42, 125, and 250 mT) exhibited diamagnetic behavior (Fig. 1a; Supplementary Fig. 1). Similar to root samples of the same plant, diamagnetic behavior was the dominant character which can be attributed to the properties of organic materials. In addition, a weak ferromagnetic behavior was observed when focused around H = 0 kOe (Fig. 1b). These behaviors are also similar to the results obtained from the root samples in Fig. 1a. The peak-to-peak magnetization values as a function of the applied magnetic field were shown in Fig. 2b (right side). The signal value in the control group was 0.07 emu/g that increased to 0.09 emu/g at 20 mT, then a gradual decrease was observed at higher MF doses. It seems that other than the roots, MF application does not alter the orientation of the magnetic moments in leaves (Fig. 1c). This could be attributed to the distance of the aerial tissues to the MF applied.

As shown in Fig. S2, the M_{NET} of the root treated with 250 mT possesses super paramagnetic behavior, while lower MF strengths (20, 42, and 125 mT) reflected diamagnetic behavior. In addition, a weak ferromagnetic behavior has been observed at 20 and 42 MF treatments.

3.2. *Effects of the magnetic field on the germination and growth*

Fig. 3a depicts the germination percentage of barley seeds either non-treated (control) or MF-exposed conditions. Four days after the seed soaking, the germination of the control seeds was 65%, which increased to 70% and 80% upon 42 and 125 mT MF treatments, respectively. However, 250 mT application dramatically decreased the germination to 30%. This shows that MF strength is a critical factor affecting the seed germination on barley. While lower doses (*i.e.*, 42 and 125 mT) enhanced the germination, 250 mT caused a negative impact. In parallel to this finding, the root length was found to be improved in MF-treated seedlings than in the control (Fig. 3b). Among the applied MF doses, 125 mT led to the highest root growth. Similarly, the shoot length was significantly increased upon 20 mT treatment ($P < 0.05$). The increase in

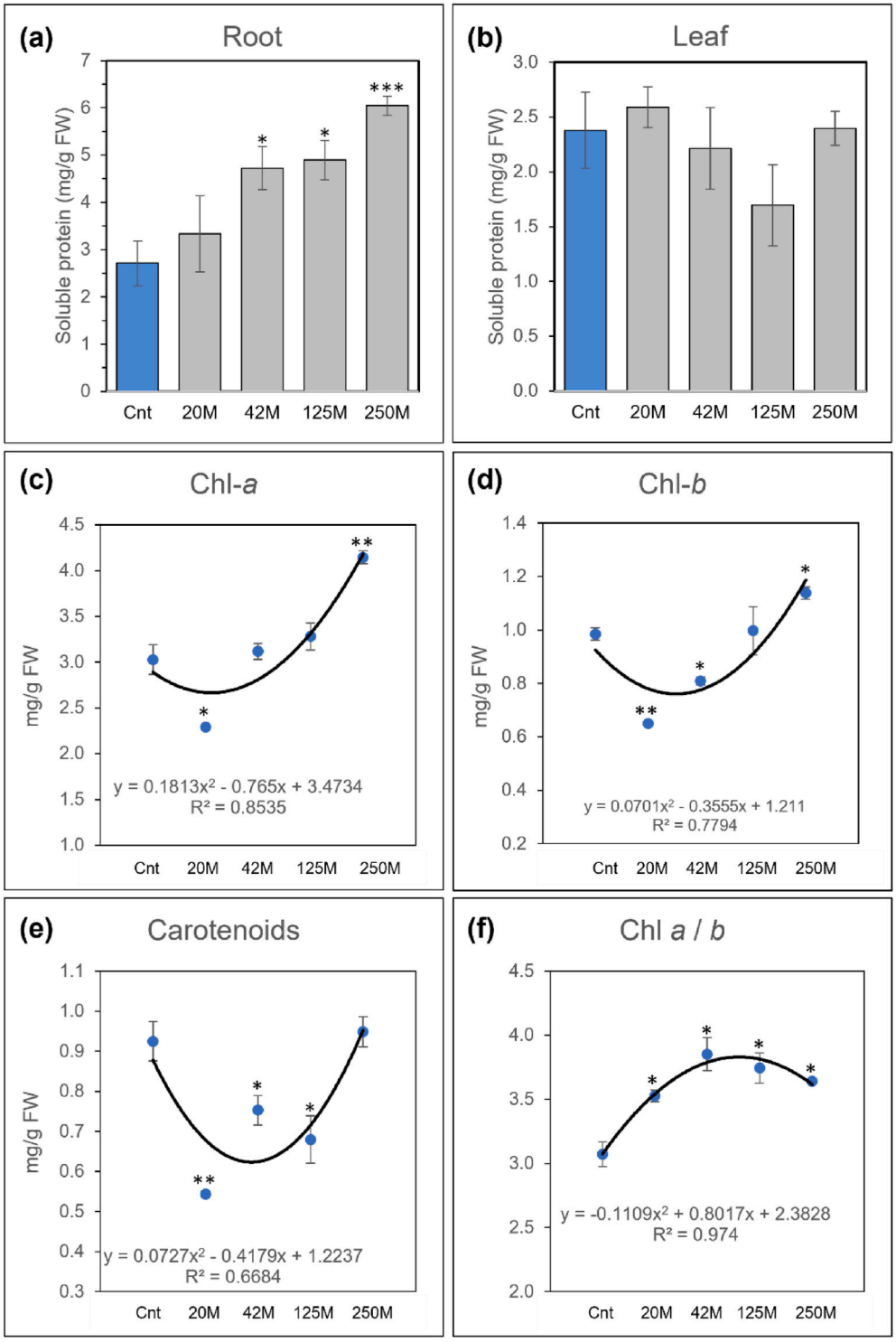


Fig. 4. Total soluble protein content of **(a)** the root and **(b)** the leaf tissues in control (Cnt) and MF-treated seedlings (20, 42, 120, and 250 mT) (FW = fresh weight). The content of **(c)** chlorophyll-*a*, **(d)** chlorophyll-*b*, and **(e)** carotenoids, and **(f)** Chl-*a* to -*b* ratio in the leaves. The data were statistically analyzed by *t*-test (*p < 0.05, **p < 0.01). Data are mean ± SE of three replicates.

the MF strength led to a gradual decrease in the shoot length (Fig. 3c). Similarly, after two weeks of the treatment, it was observed that the root length increased in parallel with increasing MF strengths, except 250 mT (Fig. S3). The highest growth increase (about 30%) compared to control was observed in the seedlings treated with 125 mT. Together with, it was found that 125 mT significantly increase the shoot length ($P < 0.05$) and lead to a slight increase in the leaf one. Fresh weight (FW) of the stem and leaf tissues did not change significantly ($P > 0.05$). However, 250 mT treatment led to a significant increase of the root FW (Fig. S3; $P < 0.001$).

3.3. Impact of constant magnetic field on protein content, pigmentation, and chlorophyll fluorescence

The increasing MF strengths led to a gradual increase of the total soluble protein content in roots (Fig. 4a). Interestingly, the protein

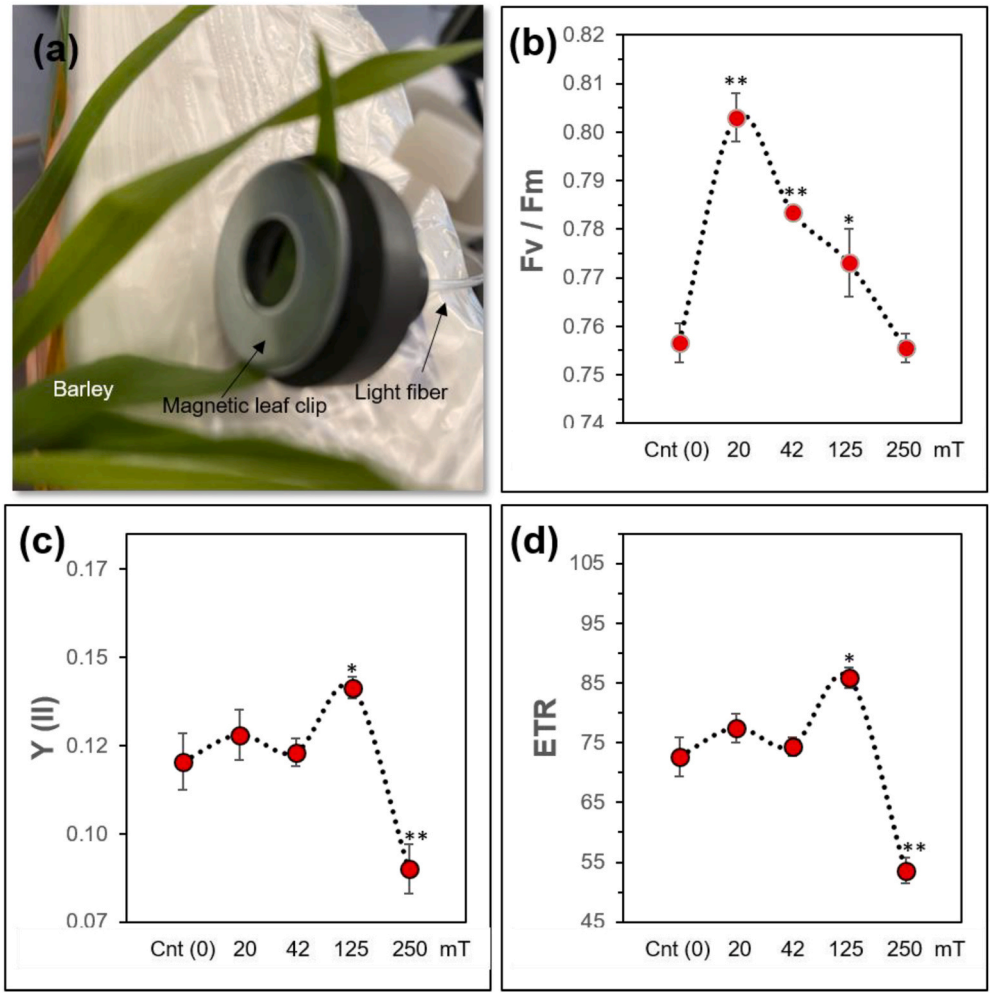


Fig. 5. The impact of different MF strengths (20, 42, 125, and 250 mT) on chlorophyll fluorescence parameters. **(a)** Measurement of barley leaves by PAM fluorometer. **(b)** Fv/Fm ratio represents the maximum quantum efficiency of PSII, **(c)** Y(II) represents the effective photochemical quantum yield of photosystem II, and **(d)** ETR shows the electron transport rate. The data were statistically analyzed by *t*-test (*p < 0.05, **p < 0.01). Data are mean ± SE of three replicates.

content was 6.1 mg/g FW at 250 mT, which is about 2 times the control. The total soluble content was found to be significantly changed in roots, but not in the leaves (Fig. 4b, $P > 0.05$). Fig. 4c–f shows the content of chlorophyll-*a*, chlorophyll-*b*, carotenoids, and Chl-*a* to -*b* ratio after two weeks of MF treatment. Compared to untreated leaf, MF-application at 20 mT decreased pigmentation. On the contrary, increasing MF-strengths caused a gradual increase. At 250 mT, Chl-*a* and -*b* was about 35% and 18% higher than those of control, respectively. Carotenoid content was reduced at lower doses (20, 42, and 125 mT), however, it was increased at the highest strength (250 mT) to the control level. As shown in Fig. 4f, the chlorophyll-*a*/-*b* ratio gradually increased by increasing MF-strengths. However, higher MF strengths led to a slight reduction compared to 42 mT. Overall, the results showed that MF application in barley increased Chl-*a* and Chl-*b* contents as well as Chl-*a*/*b* ratio but did not significantly affect carotenoids.

In addition to protein and pigment contents, the impact of different MF strengths on chlorophyll fluorescence parameters (the maximum quantum efficiency of PSII (Fv/Fm), effective photochemical quantum yield of photosystem II (Y(II)), and electron transport rate (ETR)) were determined (Fig. 5). According to the results, the Fv/Fm ratio dramatically increased at 20 mT, then it was gradually declined under 42, 125, and 250 mT applications (Fig. 5b). In parallel to this finding, MF-treatments enhanced the Y(II) and ETR up to 125 mT (Fig. 5c and d). Interestingly, these parameters dramatically reduced at 250 mT, which is in line with the results obtained for the chl-*a*/-*b* ratio (Fig. 4f), germination, and growth parameters (Fig. 3). While the MF applications up to 125 mT increase the efficiency of the photosystem, it has been observed that the efficiency decreases at a high magnetic field (250 mT).

3.4. Observation of plant roots with a confocal microscope

The cell membrane is among the first structure of a plant affected by stress. Microscopic observation at the root tips stained with PI showed that continuous application of MF caused deformation of the cell membrane. Apparently, the damaged membranes cannot exclude the cationic PI dye, eventually causing staining of the nuclei (Fig. 6). The membrane injury was observed in all tested MF-treated samples, even under a lower magnetic field (20 mT). In addition, in the control roots, a negligible amount of cell nuclei was found stained.

3.5. Impact of the magnetic field application on the nutrient contents of tissues

In order to find out the status of nutrients in plant tissues (leaf and root), ICP-OES analysis was conducted. As shown in Fig. 7, magnesium (Mg), potassium (K), phosphorus (P), and calcium (Ca) abundances in the control roots were respectively 0.58, 18.1, 8.9, and 7.4 g/kg in dry weight (DW). However, increasing the strength of MF from 20 to 250 mT led to a gradual and dramatic decline. They were recorded as 0.08, 1.9, 1.1, and 0.9 g/kg DW at 250 mT, which corresponds to approximately

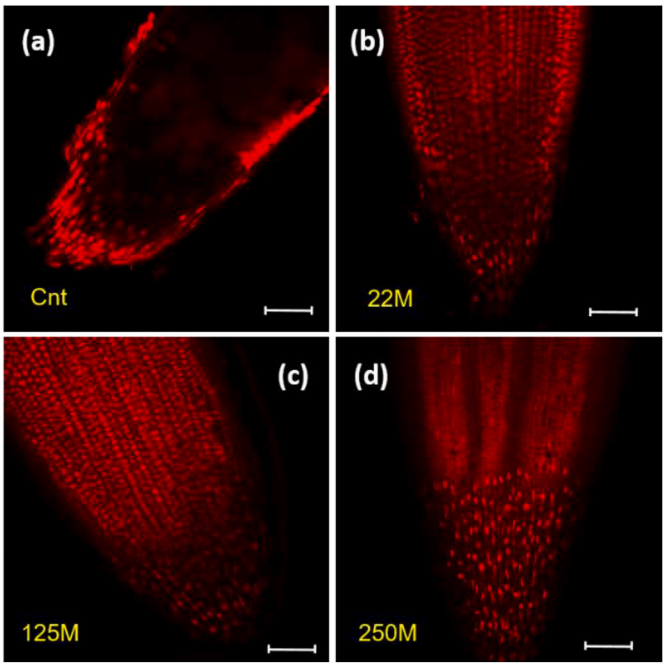


Fig. 6. Confocal microscopy analyses of root tip cells grown under different MF strengths (control (0), 20, 125, and 250 mT). The root tips (0.5 cm) were stained with propidium iodide (PI) dye. The scale bar = 100 μm.

7.0–9.5-fold reduction over the control roots. However, there was no such dramatic change in the leaves. Nevertheless, a significant increase was observed in the content of K at 250 mT ($P < 0.01$). These results indicated that MF application led to a considerable decline of the macronutrients in barley roots, but not in the leaves. The decline was proportional to the applied MF strength. However, the reduction of those elements in roots does not seem to affect the leaf content of the same elements much.

The contents of micro-elements including iron (Fe), boron (B), copper (Cu), manganese (Mn), zinc (Zn), and molybdenum (Mo) were determined both in root and leaf tissues of MF-treated and untreated barley (Fig. 8). Compared to the control, the bioavailability of these elements in the leaves is increased by the MF exposure. For Fe, B, Cu, Mn, Zn, and Mo, the increase was approximately 3, 3, 50, 3, 30, and 8 times that of the control, respectively. In addition to leaves, the abundances of these elements in the roots were found to be strikingly raised by the MF treatment. Interestingly, the root content of magnetic elements, Fe and Mn, were found to be dramatically increased upon MF treatments.

4. Discussion

4.1. Effect of magnetic field on the magnetic character of barley tissues

The magnetic analyses showed that the tissues have diamagnetic behavior under control and MF conditions. In addition to diamagnetism, interestingly, the roots treated by 250 mT MF exhibited super paramagnetic behavior (Fig. 1a). Additionally, weak ferromagnetism of around 0 kOe was observed in all root and leaf specimens, which can be attributed to the Fe or Co ions found in plants (Fig. 1b). In fact, since the vast majority of biological materials are diamagnetic (Till et al., 2021), it is expected that root and leaf tissues exhibit diamagnetic behavior. Together with, some proteins containing metal ions such as hemoglobin, cytochrome, ferritin, *etc,* exhibit paramagnetic character (Piruzyan et al., 1980; Peñuelas et al., 2004). In this study, diamagnetism dominated ferromagnetism with the increasing external MF. In all tested specimens except root 250 mT, there is no magnetic moments. However, for 250 mT in the root sample, magnetic moments at the saturation magnetization are parallel to the MF (Fig. 1c). The observed super paramagnetism is thought to be due to the alignment of the magnetic moments of Fe or Co ions in the same direction with the strong 250 mT MF application. Similarly, there is an increase in M_{NET} around 0 kOe with the contribution of the increased MF due to the existence of magnetic elements in barley naturally (Fig. S1). This is possibly due to the increase in the number of magnetic moments oriented in the same field direction with MF increase. The results suggested that the application of MF at 250 mT has the capacity to change the orientation of the magnetic moments, which can change the magnetic behavior and eventually the physiological responses of barley.

4.2. Impact of external magnetic field application on physiology, membrane integrity, and nutrient status

This study showed that the MF strengths up to 125 mT enhance the germination of barley. However, 250 mT causes a negative impact on germination (Fig. 3). Acceleration of seed germination by external MF has been shown on different plants, such as wheat (Gholami et al., 2010), tomato (Moon and Chung, 2000), and sunflower (Vashisth and Nagarajan, 2010; Kornarzyński et al., 2020). During germination, the seeds require high energy for respiration. The proteolytic enzyme such as α-amylase reaches the highest activity which is stimulated by external MF (Ramakrishna and Rao, 2005). Vashisth and Nagarajan (2010) found that protease activity of sunflower seeds is higher in 50 mT MF-treated seeds (2 h) than the untreated ones. It can be argued that MF treatment may enhance germination and the speed of germination by accelerating the activity of proteolytic enzymes. Contrarily, a serial MF treatment (from 5 to 120 mT) on sunflower seeds for 7 days caused mostly a negative effect on germination (Kornarzyński et al., 2020). The effective MF strength that stimulates growth and germination may vary according to the plant type used and the strength, frequency, and exposure time of MF. Among the tested MFs, this study found that 125 mT is the most effective MF which improved the germination by 23%, the root length about 30%, and the stem length about 13% upon two weeks of MF-treatment (Fig. S2). However, the same result was not observed in the leaves. In agricultural practice, it can be used to improve germination, speed up the growth, and enhance the yield of barley seeds.

The status of soluble protein content is a parameter to show the response of plants against a particular stimulant. Depending on the plant species and sensitivity, as well as the type and severity of the stress, the soluble protein abundance varies (Verslues and Sharma, 2010). For instance, Shine et al. (2011) indicated that 150 and 200 mT of MF increased the total soluble content in soybean leaves. Similarly, pulsed-MF (1.5 mT at 10.0 Hz for 5 h per day) enhanced the protein content, plant height, fresh and dry weight of soybean (Radhakrishnan and Kumari, 2012). Novitsky et al. (2001) showed a two-fold increase of protein level after ~403 A/m (5.06 Oe) of MF treatment to onion. In parallel, this study found that the plant roots treated with 250 mT MF had approximately 2 times the protein compared to the control (Fig. 4a), However, varied MF strengths did not cause a significant change in the leaves (Fig. 4b, $P > 0.05$). This result suggests that MF treatment increases the stress level of plants particularly in roots, which was found beneficial for plants, leading to growth enhancement up to 125 mT. However, 250 mT was not tolerable resulting in a decline in both germination and growth stages relative to the control (Fig. 3). This regression may be related to the change in the magnetic character of the roots (orientation of the magnetic moments) at 250 mT as shown in Fig. 1. Chlorophyll-*a*/-*b* ratio (Chl-*a*/*b*) is another parameter that displays oxidative damage, which is inversely proportional to each other; a lower Chl-*a*/*b* ratio indicates higher damage (Kasajima, 2017). As shown in Fig. 4f, the Chl-*a*/*b* ratio gradually increased by increasing MF-strengths. However, higher MF strengths (125 and 250 mT) led to a slight reduction compared to lower ones (20 and 42 mT). The average Chl-*a*/*b* ratio varies according to plant type, which is about 3.5 in rice

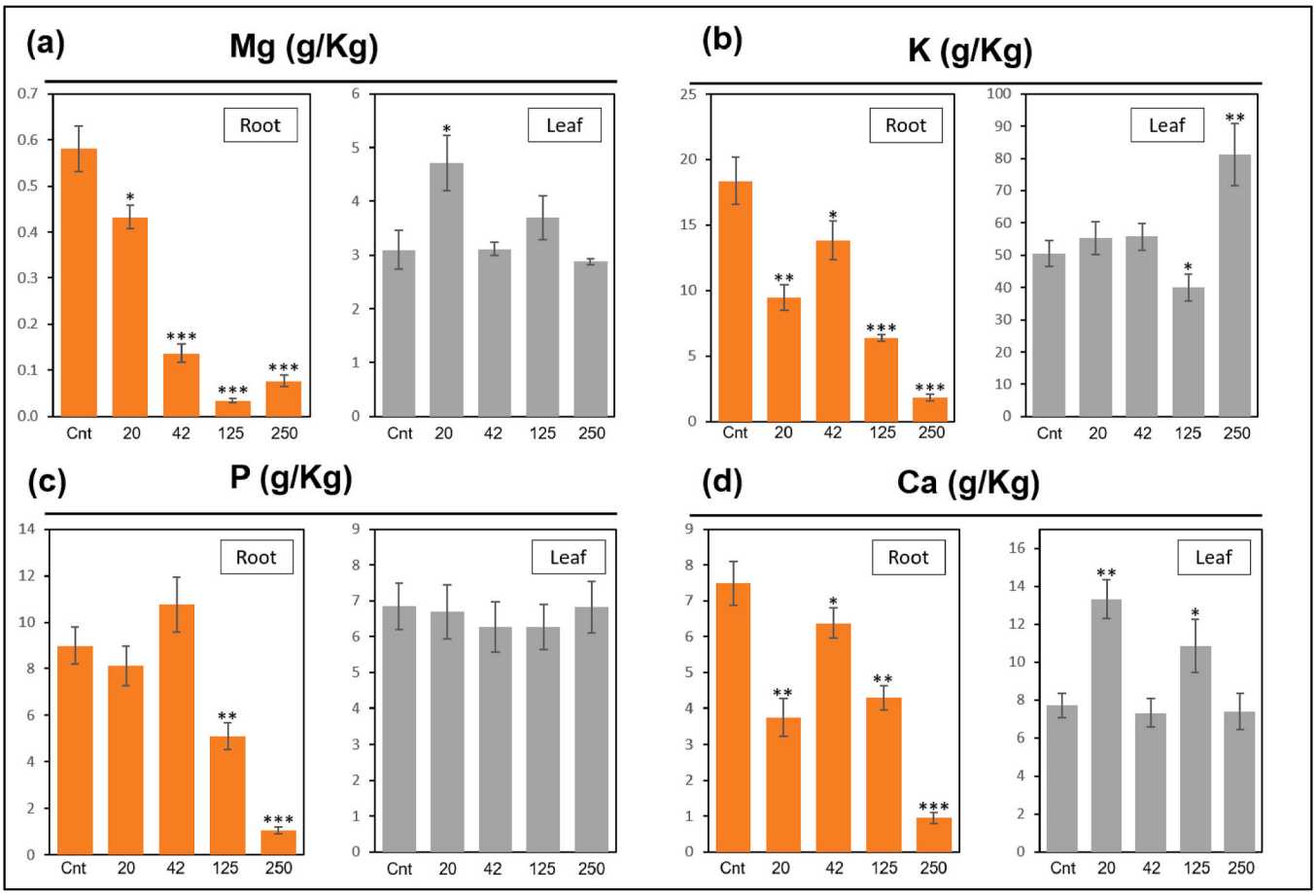


Fig. 7. The status of macro-elements in the root and leaf of barley seedlings after two weeks of MF treatments (0 (control), 20, 42, 125, and 250 mT). **(a)** Magnesium (Mg), **(b)** potassium (K), **(c)** phosphate (P), and **(d)** calcium (Ca). The quantity of elements was represented as g element in kg dried tissue (g/kg in DW). Error bars represent standard errors obtained from three technical repetitions of samples (n = 10). *t*-test was performed to calculate the significant changes over the control (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$).

(Kasajima, 2019), 3.9 in wheat (Al-Amri et al., 2020), and 3.1 in barley (Tombuloglu et al., 2021). In consistence with those studies, the Chl-*a*/*b* ratio in control leaves was about 3.1.

Chlorophyll fluorescence is a common method to understand plant health (Peña-Olmos and Casierra-Posada, 2013). When the plant encounters an unfavorable situation, the number of open reaction centers decreases, resulting in a lower Fv/Fm ratio (Baker, 2008; Tombuloglu et al., 2020). It seems that MF application at 20 mT has a positive impact on the efficacy of PSII. However, the increase in MF strengths (42, 125, and 250 mT) suppressed this effect, which may be associated with the oxidative damage caused by MF. Besides the Fv/Fm ratio, Y(II) is a parameter representing plant efficiency and the amount of energy used in PSII (Baker, 2008). The results showed that in line with the results obtained for the chl-*a*/*b* ratio (Fig. 4f), as well as the germination and growth parameters (Fig. 3), MF-treatments enhanced the Y(II) and ETR up to 125 mT (Fig. 5c and d). Interestingly, these parameters dramatically reduced at 250 mT. Overall, these findings point out that MF applications up to 125 mT increase the efficiency of the photosynthetic parameters, which might contribute to the growth enhancement of barley seedlings.

Together with these parameters, the potential effect of MF on the membrane integrity of root cells was examined. According to the results, continuous application of MF caused deformation of the cell membrane where cationic PI dye penetrates and stains the nuclei of the root cells (Fig. 6). The injury was observed in all tested MF-treated samples. Reina et al. (2001) stated that MF can help increase membrane permeability and facilitate the water absorption process. In addition, MF has been shown to induce cell membrane damage in HL-60 cells *in vitro*; wherein application of 6 mT MF resulted in a loss of the cell membrane's ability to exclude cationic dyes such as PI or trypan blue (Teodori et al., 2002). Also, Zablotskii et al. (2016) suggested that high-gradient MF (1 T) can significantly alter membrane potential and functionality of cells. Moreover, it was shown that low-frequency electro-MF (5.6 mT and 100 Hz) caused DNA strand breaks (Mihai et al., 2014). At the molecular level, MF application can alter cell signaling mechanisms by changing the membrane potential that leads to the alteration of gene and protein expression (Escobar et al., 2020). Overall, the results suggest MF-induced cell membrane damage in root cells, which would alter the membrane potential, ion transfer, and nutrient uptake of barley seedlings grown under MF conditions.

MF treatment decreased the macro-element contents in roots, but not in the leaves (Fig. 7). However, the content of micro-elements was dramatically improved by MF application both in roots and leaves of barley. Improved nutrient uptake by MF has been shown in different plants. For instance, 300 mT led to an increase in the microelement content (Fe, Zn, and Cu) of the leaves and roots in *Populus*. In addition, carbon (C) and phosphorus (P) but a decreased nitrogen (N) content was observed in the leaves (Liu et al., 2019). Irrigation of tomato seedlings with magnetized water increased the abundance of N, P, and K (El-Yazied et al., 2012; Zhang et al., 2014). Also, macro- and micro-elements such as N, K, Ca, Fe, and Zn contents have been found to increase upon MF treatment on snow pea and chickpea (Grewal and Maheshwari, 2011). In another study, increasing MF strengths up to 384 mT raised N, K, Ca, Mg, Cu, Fe, Mn, Na, and Zn levels, but reduced P and S contents

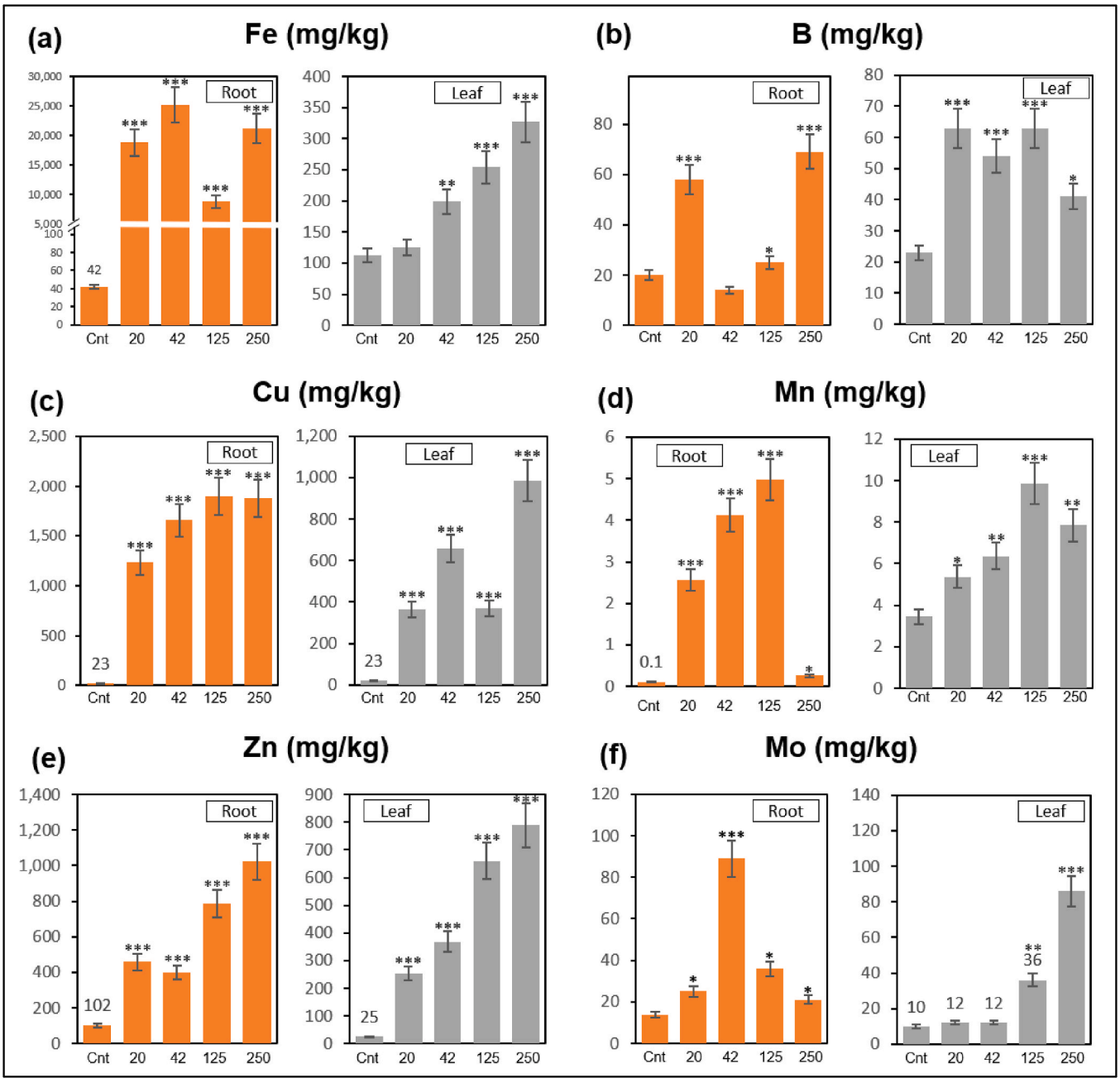


Fig. 8. The status of micro-elements in the root and leaf of barley seedlings after two weeks of MF treatments (0 (control), 20, 42, 125, and 250 mT). **(a)** Iron (Fe), **(b)** boron (B), **(c)** copper (Cu), **(d)** manganese (Mn), **(e)** zinc (Zn), and **(f)** molybdenum (Mo). Error bars represent standard errors obtained from three technical repetitions of samples (n = 10). *t*-test was performed to calculate the significant changes over the control (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$).

(Eşitken and Turan, 2004). In parallel to these studies, the current study found that MF-treatment affects the nutritional status of plants. The increase in growth performance upon MF applications could be due to the fluctuations in nutrient uptake, which could be associated with the cell membrane injury as shown in Fig. 6.

4.3. Possible scenario on magnetic field-plant interaction

The cell membrane is the first structure that reacts when the cell is exposed to an external stimulus (Ozturk et al., 2010). Poinapen et al. (2013) demonstrated that external MF application on tomato seeds led to significant structural changes on the plasma membrane. The deformation in the lipid structure rather than proteins was observed which could influence membrane fluidity, signal transduction, and membrane trafficking (Poinapen et al., 2013). In line with these findings, the current study suggests MF-induced deformation of cell membranes in barley roots (Fig. 6), possibly due to the structural changes on the membrane lipids. Although the PI dye clearly penetrates injured cell membranes, the lowest MF power leading to membrane deformation is not clear. The damage could be associated with lipid peroxidation due to the accumulation of reactive oxygen species (ROS) such as singlet oxygen, superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot-}$), as shown elsewhere (Sahebjamei et al., 2007; Auten and Davis, 2009; Latef et al., 2020). In this study, the increase in the protein level can be attributed to the increase in stress-related proteins and ROS scavenging enzymes. When they are accumulated in the cell, antioxidant enzymes react to detoxify these substances. For instance, as a by-product of the catalase enzyme, oxygen radicals accumulate in the cytosol which softens the cell membrane due

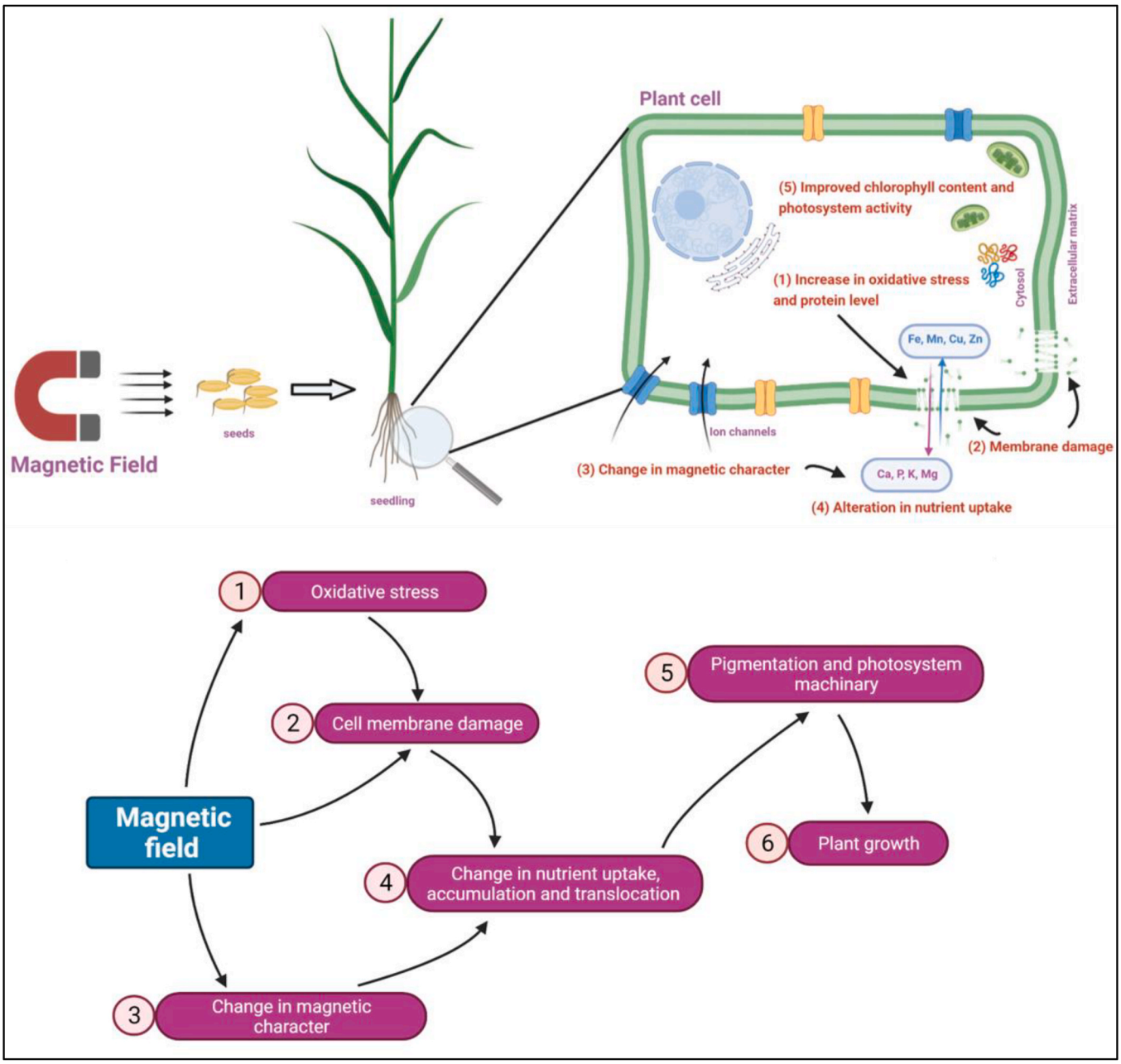


Fig. 9. Shema represents possible scenario underlining MF-induced improvement on seed germination and growth.

to the degradation of pectin polysaccharides of the cell wall. The loosening of the cell wall and deformation on the cell membrane leads to cell elongation and growth with aid of intrinsic turgor pressure (Kim et al., 2014). As a result of membrane damage, disruption in cellular trafficking and ion transport is inevitable and eventually could influence macro- and micro-element contents as shown in Figs. 7 and 8.

Together with the membrane damage, the MF application might influence ferromagnetic elements (Fe, Co content) by changing the orientation of their magnetic moments (Figs. 8 and 9). Especially the biological substances containing magnetic elements (Fe, Co, and Mn) could be more susceptible (Peñuelas et al., 2004). As indicated in Fig. 1, it is obvious that MF application altered the magnetic response of roots due to the expected changes in the magnetic orientations. This could maintain oriented movement of Fe-, Co-, or Mn-containing biological substances, such as ferritin, cytochrome, and the enzymes residing in one of these elements as a cofactor.

MF treatment enhanced chlorophyll level and photosynthetic activities, which can be associated with improved nutrient uptake. For example, a dramatic increase was observed in the amount of Fe and Mn, which act as a cofactor in photosynthetic reactions or are present in the chloroplast structure (Figs. 7 and 8). Another theory relates to the MF-induced orientation of biological materials. For instance, when external MF is applied, Mn^{2+} ions tend to be oriented in the same direction of the magnetic field rather than the direction of water movement (root-to-leaf or *vice versa*). Therefore Mn^{2+} ions do not migrate in the same direction as water and tend to accumulate (Dhawi and Al-Khayri, 2009; Da Silva and Dobránszki 2016). Similarly, iron (Fe) as a magnetic element may relatively trap in tissues depending on the MF applied. This could lead to unbalanced Fe and Mn levels in the leaves which can affect chloroplast maintenance and function, pigmentation, and photosynthetic reactions, and eventually the plant growth (Fig. 9). A dramatic increase in Fe and Mn levels in root and leaf tissues could lead to the improved photosynthetic performance of barley, preferably at lower MF strengths.

In the universe, the magnetic field is usually low, in the order of μGauss. On the Earth's surface, it is less than 100 μT. However, in compact objects, it assumes extraordinarily large values (Treumann et al., 2014). What we know today is the Earth's magnetic field protects plants from space radiation by shielding (Wolff et al., 2014). Besides, high magnetic field can be used easily for special purposes in which we can access features we didn't expect, such as drug delivery applications, pathogen control, bioremediation, growth enhancement of plants, etc. Understanding magnetic field and plant interaction are extremely important for understanding the evolution of life forms on our planet. However, studies on the effects of magnetic field on plants in space conditions such as the Moon and Mars, where there is no global magnetic field, are very limited (Maffei, 2014). Therefore, further studies are required to decipher the interaction between magnetic fields and living beings. This will help us to develop ideas for the design and survival of living forms for use in future space exploration.

5. Conclusion

This study describes the response of barley plants against different magnetic field (MF) strengths at physiological and cellular levels.

1) The external MF application significantly enhanced germination, photosynthetic activities, and plant growth. In general, the growth enhancement is limited at higher MF-strengths (*i.e.*, 250 mT), while 20–125 mT is appropriate for seed germination and plant growth. Although it is an easy and inexpensive method to increase plant germination and growth parameters, its large-scale use is limited yet. Therefore, new research is needed regarding their use in large areas.
2) Cell membrane damage in the root tip cells was observed at all MF strengths. The membrane damage might have disrupted the membrane trafficking and ion transport, which might lead to changes in the nutritional status of the plant.
3) Elemental analysis showed that the content of micro- and macro-elements is dramatically altered in MF-treated tissues. In general, calcium, magnesium, phosphorus, and potassium contents were decreased; while iron, boron, copper, manganese, zinc, and molybdenum levels were improved both in roots and leaves.
4) Together with the membrane damage, MF could alter the orientation of magnetic elements such as iron, cobalt, and manganese, which might contribute to their accumulation or re-localization in the plant body. Not only magnetic elements, but also the substances harboring these ions might be influenced. Analysis of the magnetic character of plant parts revealed that MF can change the magnetic response of tissues, where diamagnetic to super paramagnetic substitution was observed at 250 mT.

Overall, the results help to understand how the magnetic field contributes to plant growth and development. Also, it sheds light on the magnetoreception and response mechanism of crop plants especially at higher strengths compared to the earth's geomagnetic field. The magnetic field at lower strengths ($\leq$250 mT) has the potential to improve growth indices and can therefore be incorporated into plant breeding programs.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Author contribution

IE and HT conceptualized the study. SO and IE performed magnetic measurements. IE analyzed the iron content of the plant parts. NA, BA, MB, and RA performed physiological tests. HT, IE, and TK wrote the manuscript. All authors read and commented on the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2021.11.033.

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