Seed priming with electromagnetic field improved growth, nutrition and metabolism of *Salvia nemorosa* L.

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Abstract

In this study, the efficiencies of seed priming with electromagnetic field (0, 2, 4, and 6 mT; 30 minutes daily for 3 days) were investigated in *Salvia nemorosa*. The exposure to electromagnetic filed led to significant increases in biomass accumulation (mean=53.6 %). Electromagnetic filed treatments significantly increased K, Ca, Mg, and Fe contents in leaves by mean 39.5 %. The electromagnetic filed treatments at all applied intensities increased both chlorophyll and carotenoid contents. Moreover, the electromagnetic filed-treated seedlings had significantly higher protein levels (mean=47 %) than the control. The applications of electromagnetic filed treatments induced peroxidase activities (mean=34.5 %) in leaves. However, these treatments reduced the activities of superoxide dismutase and catalase enzymes. The PAL activities in the electromagnetic filed-treated seedlings were higher by 61 % over the control. With a similar trend, the electromagnetic filed treatments promoted flavonoid accumulations. These results support this hypothesis that the application of the electromagnetic field may improve plant growth and secondary metabolism. Further studies, especially at molecular levels may help to elucidate the complicated involved mechanisms.

Keywords: *Antioxidant enzymes; Electromagnetic field; Salvia nemorosa; Secondary metabolism; Seed priming*

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Introduction

Plants are constantly affected by various physicochemical environmental factors, among which the magnetic field is not well-considered and therefore involved mechanisms are elusive (Katsenios et al. 2016). Plants like other kinds of living organisms are exposing to the earth's magnetic field as a natural environmental factor (5×10^{-6} Tesla (T) having geographical-varied intensities ranging 25-65 μT (Da Silva and Dobránszki 2016). However, relatively limited studies have represented how biological systems may respond to the magnetic field as an external stimulus. The electromagnetic field is the combination of an electric field (resulted from

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the stationary charges) and a magnetic field (generated by moving charges). A multitude of factors, including magnet type, magnetic field intensity, orientation, polarity, and exposure time affect plant responses to stimuli caused by the magnetic field (Da Silva and Dobránszki 2016).

Bagheri et al. (2014) reported that seed priming with electromagnetic fields at 3.8 mT pretreated improved seed germination as well as biomass accumulation in Lepidium sativum L. Moreover, electromagnetic field pretreatment of soybean seeds also enhanced the fresh and dry weight, the plant height, and protein concentration (Radhakrishnan and Kumari 2012). Furthermore, this priming technique was also improved plant nutrition, indicted with considerable increases in Zn, Cu, Fe, Mn, Mg, K (Radhakrishnan and Kumari 2012). In Helianthus annus, electromagnetic fields also promoted seed germination and seedling growth (Mildažienė et al. 2019).

Likewise, seed priming with the electromagnetic field at 60, 120, and 180 mT led to the linear increase in root biomass (Iqbal et al. 2012). Also, the increment of chlorophyll a and b content was noted for 60 mT and 120 mT (Iqbal et al. 2012). In cotton, the potential benefits of electromagnetic filed treatment on the root growth have become evident (Bilalis et al. 2013).

The electromagnetic field approach is a physical treatment, exhibiting beneficial potentials toward the biological system. The potential benefits of magnetic field applications have been underlined by several lines of evidence in various plant species (Vashisth and Nagarajan 2010; Leelapriya et al. 2003; Mildaziene et al. 2018; Mildažienė et al. 2019). However, data on the effects of electromagnetic fields on plant nutrition, antioxidant machinery, and secondary metabolism is rare and contributing mechanisms are elusive.

Plant species belonged to the Salvia genus are a rich source of significant terpenoid derivatives. These natural bioactive agents have been found to exhibit promising anti-proliferative and anticancer properties (Bahadori et al. 2018). S. nemorosa is considered as a pharmaceutical valuable plant. Hence, attempts have been made to improve the secondary metabolism and production of pharmaceutically valuable secondary metabolites of this plant (Kuzma andWysokinska, 2003). Various steroids, flavonoids, and terpenoids, including ursolic, aamyrin, oleanolic acids, luteolin, eupatilin, apigenin, salvipisone, acetylhorminone, horminone, nemorone, nemorosin, salvinemorol, pachystazone, methylenecycloartanol, sitosterol, and salvigenin have been identified in S. nemorosa (Ulubelen et al. 1994). Hence, the present study aimed to investigate the possible effects of electromagnetic fields on S. nemorosa to clarify some responsible physiological mechanisms, and to provide an alternative method, in sustainable agriculture regard.

Materials and Methods

Electromagnetic field treatment

The seeds of *S. nemorosa* were provided by a reliable company (Pakan Bazr, Isfahan, Iran). A solenoid with 50 cm length and 10 cm diameter was used to create an electromagnetic field. The intensity of the electric current and magnetic field magnitude were controlled by an ammeter and a tesla meter, respectively. For an electric current generated in the solenoid, the power supply (ED-345BM, China) with 60 Hz input current and 10 A output current was applied. To evaluate the possible role of water soaking before seed priming with electromagnetic fields, dry or 24-hour soaked seeds were subjected to electromagnetic fields at different intensities (0, 2, 4, and 6 mT), for three continuous days and each day for 30 min. The treated seeds were cultured in the pots containing coco peat, perlite, and garden soil in the ratio of 1:1:1 and maintained in the greenhouse conditions (Ps mention the photoperiod and light intensity. One month later, the seedlings were harvested for further growth and biochemical analysis in leaves.

Measurements of K, Ca, Fe, and Mg

Concentrations of elements were analyzed by a flame photometer (410 Sherwood, England) (K) and atomic absorption spectroscopy (Varian SPECTRAA-200, USA) approach (Ca, Fe, and Mg).

Protein Determination

Protein extraction was carried out using potassium phosphate buffer 50 mM (pH 7.5) containing 1% polyvinyl pyrrolidine and 1 mM EDTA. The resulted extracts were centrifuged (12000 rpm) at 4 °C for 20 minutes. Protein contents were quantified using Bradford reagent (Bradford 1976).

Estimation of phenylalanine ammonia-lyase (PAL) activity and flavonoid concentration

PAL was extracted using 50 mM Tris-HCl buffer (pH 8.8) containing 15 mM β-mercaptoethanol. PAL activity was determined according to the method represented by Ochoa-Alejo and Gómez-Peralta (1993). The reaction mixture containing extraction buffer, phenylalanine, distilled water, and enzyme extract was incubated at 37 °C for 1 h. The reaction was terminated with the addition of 6 M HCl. The samples were washed with ethyl acetate and the extracting solvent removed by evaporation. The solid residue was suspended in 3 mL of 0.05 M NaOH. The absorbance was spectrophotometrically measured at 290 nm.

For the determination of flavonoids, 0.05 g of fresh leaves were homogenized using 10 ml of methanol. Next, 1 mL of a 2 % aluminum trichloride solution was added to 1 ml of the extracted essence and reached 25 mL with ethanol. Then, the specimens were centrifuged at 3000 × g for 10 minutes. After that, the absorbance of the samples was analyzed at 330 nm using a spectrophotometer (T90 + UV / VIS spectrometer Karaltay (China)). The total flavonoid concentration was assessed using 33000 mMcm-1 coefficients (Zhishen et al.1999).

Estimation of ascorbate peroxidase (APX) activity

APX activity was determined spectrophotometrically by monitoring the absorbance decrease at 290 nm as described by Nakani and Asada (1981). Reaction mixture of this enzyme contained 50 mM phosphate buffer (pH 7), 0.5 mM ascorbate, 0.1% (v/v), hydrogen peroxide, and enzyme extract (the extinction coefficient of 2.8 mM^{-1} cm^{-1}).

Estimaion of peroxidase (POX) activity

To determine peroxidase activity, 100 µL of enzyme extract was added to the assay mixture containing 2 mL of 0.2 mM acetate buffer (pH 4.8), 200 µL of 0.04 M benzidine solution, and 200 µL hydrogen peroxide. To determine enzyme activity, the absorbance differences were monitored at 530 nm (Koroi 1989).

Estimation of superoxide dismutase (SOD) activity

The SOD activity was measured according to the method of Giannopolitis and Ries (1977). K-phosphate buffer (pH 7.8), 13 mM methionine, 75 mM nitro blue tetrazolium chloride, 20 µM riboflavin, 0.1 mM EDTA were applied as the enzyme reaction mixture. The absorbance of each sample was recorded at 560 nm.

Estimation of catalase (CAT) activity

The CAT activity was determined as described by (Pereira et al. 2002). The assay mixture consisted of 1 mL of 100 mM K-phosphate buffer (pH 7.5) and 2.5 µL hydrogen peroxide (30% solution). The reaction was started by adding enzyme extract and activity was measured by monitoring the degradation of hydrogen peroxide at 240 nm over 1 min.

Statistical analysis

A factorial experiment based on a completely randomized design with three replicates was performed. Mean comparisons were carried out using Duncan's multiple range test at a probability level of 0.05.

Results

The exposure to electromagnetic filed increased biomass by a mean of 64.8 % and 42.5 % for wet and dry-treated seeds, respectively (Fig. I).

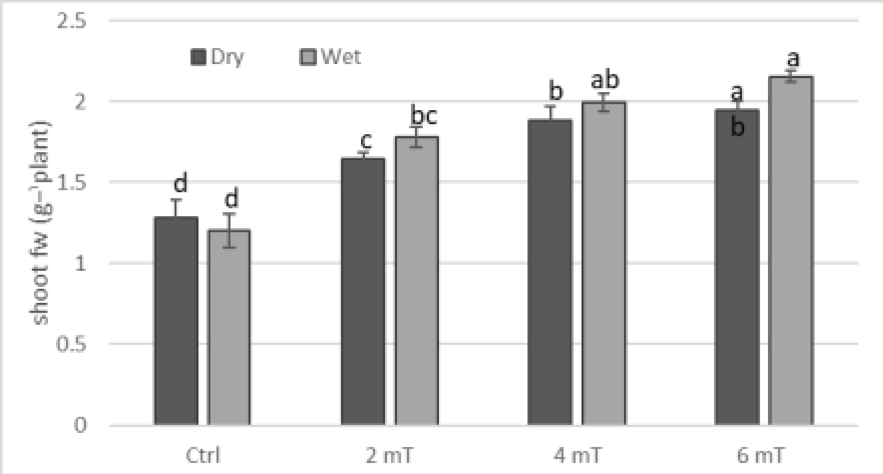

Fig. I. The electromagnetic field- associated changes in shoot fresh mass. Mean values ± SE are presented (n = 3 replicates), Different letters indicate significant differences according to the Duncan test ($P < 0.05$).

In all applied intensities, electromagnetic filed treatments significantly ($p \leq 0.05$) increased K concentration by averages of 26.4 % and 10.8 % for the wet and dry groups, respectively (Fig. II A). Similarly, the electromagnetic filed-treated seedlings of the wet and dry groups had significantly higher concentrations of Ca by mean of 48.3 % and 42.3%, respectively, than the control (Fig. II B). The electromagnetic field also caused significant ($p \leq 0.05$) rises in Mg levels by a mean of 43 % and 31 %, respectively for the wet and dry groups (Fig. II C). Besides, the Fe contents in electromagnetic filed-treated seedlings were higher than the control (mean=58 %) (Fig. IID).

The electromagnetic filed treatments at all applied intensities were found to increase both Chla and carotenoid concentrations, while these treatments did not make significant ($p \leq 0.05$) changes in Chl b (except for 4 mT at the wet condition) (Fig. III). Moreover, there was no considerable difference between the dry and wet groups. The electromagnetic filed- treated seedlings had significantly ($p \leq 0.05$) higher protein levels (means of 38.4 % and 55.6 %, respectively for wet and dry groups) when compared to the untreated controls (Fig. III).

In comparison with the control, the applications of electromagnetic filed treatments induced peroxidase activities by averages of 25.3 % and 43.8 %, respectively for the wet and dry groups (Fig. IV). With a similar trend, the electromagnetic filed treatments made slight enhancements in APX activities by mean of 15.8 % (Fig. IV). While, exposures to electromagnetic fields led to reductions in activities of SOD enzyme by averages of 27.5 % and 53 %, respectively for the wet and dry groups (Fig. IV). Except for 2 MT treatments, the electromagnetic filed treatments of 4 and 6 mT (especially in wet condition) resulted in the significant ($p \leq 0.05$) slight decreases in catalase activities (Fig. IV). The considerable higher activities of PAL enzyme were observed in the electromagnetic filed-treated seedlings among which 4 mT groups in both dry and wet conditions had the highest (Fig. IV). Similarly, the electromagnetic filed treatments caused significant ($p \leq 0.05$) stimulations in flavonoid concentrations in both wet and dry groups (Fig. IV).

Discussion

The electromagnetic filed treatments not only increased biomass accumulation, but also improved nutritional status, photosynthetic pigments, and protein concentrations. Exposure of mung bean seed to extremely low-frequency magnetic fields (155mG) enhanced plant early growth (Tien and Wang 2009). The growth-

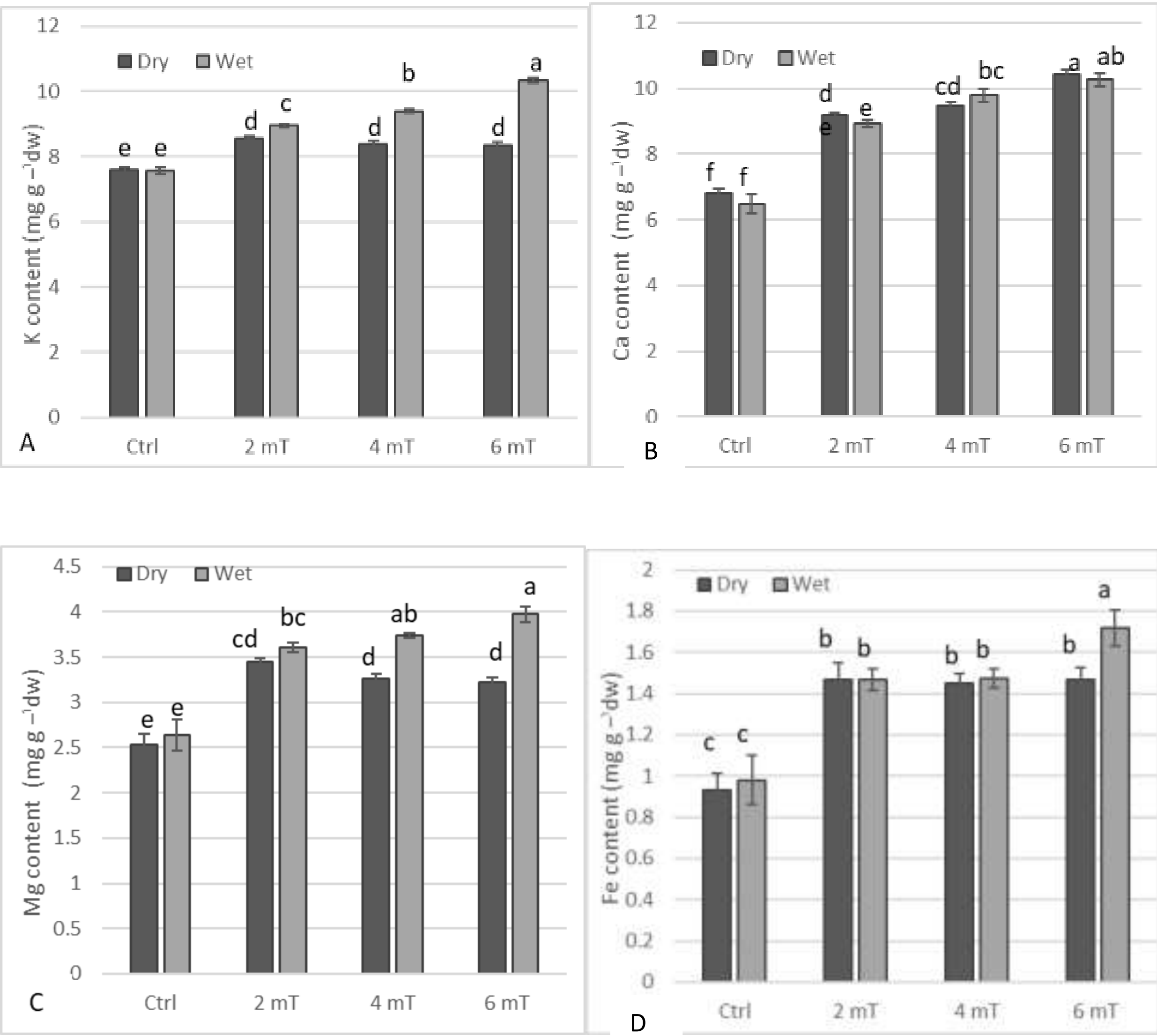

Fig.II. The electromagnetic field- associated changes in nutritional status in leaves. Mean values ± SE are presented (n = 3 replicates), Different letters indicate significant differences according to the Duncan test (P < 0.05).

promoting roles of magnetic field applications have been reported in different plant species, including sunflower (Vashisth and Nagarajan 2010; Mildažienė et al. 2019), cotton (Leelapriya et al. 2003), and soybean (Shine et al. 2011)

In line with our results, exposure of soybean seeds to the pulsed magnetic field (1.5 mT at 10.0 Hz) enhanced plant growth, biomass accumulation, and protein concentration (Radhakrishnan and Ranjitha-Kumari 2012). Likewise, seed treatment with electromagnetic field resulted in an enhancement in plant early growth in Echinacea purpurea (Mildaziene et al. 2018). Seed priming with the electromagnetic field was found to change phytohormones and leaf proteome (Mildažienė et al. 2019). Analysis of leaf proteome revealed that electromagnetic field treatment stimulated expression patterns of photosynthesis-related genes (Mildažienė et al. 2019). In maize and soybean, the growth-promoting roles of the electromagnetic field have been attributed to the triggered signaling of reactive oxygen species (ROS). As is well known, ROS are considered as signaling molecules that contributed to the mobilization of materials, regulation of Ca+2 signaling, hormone signaling, cell wall characteristics, redox regulation, and modulation of gene expression (Kataria et al. 2017).

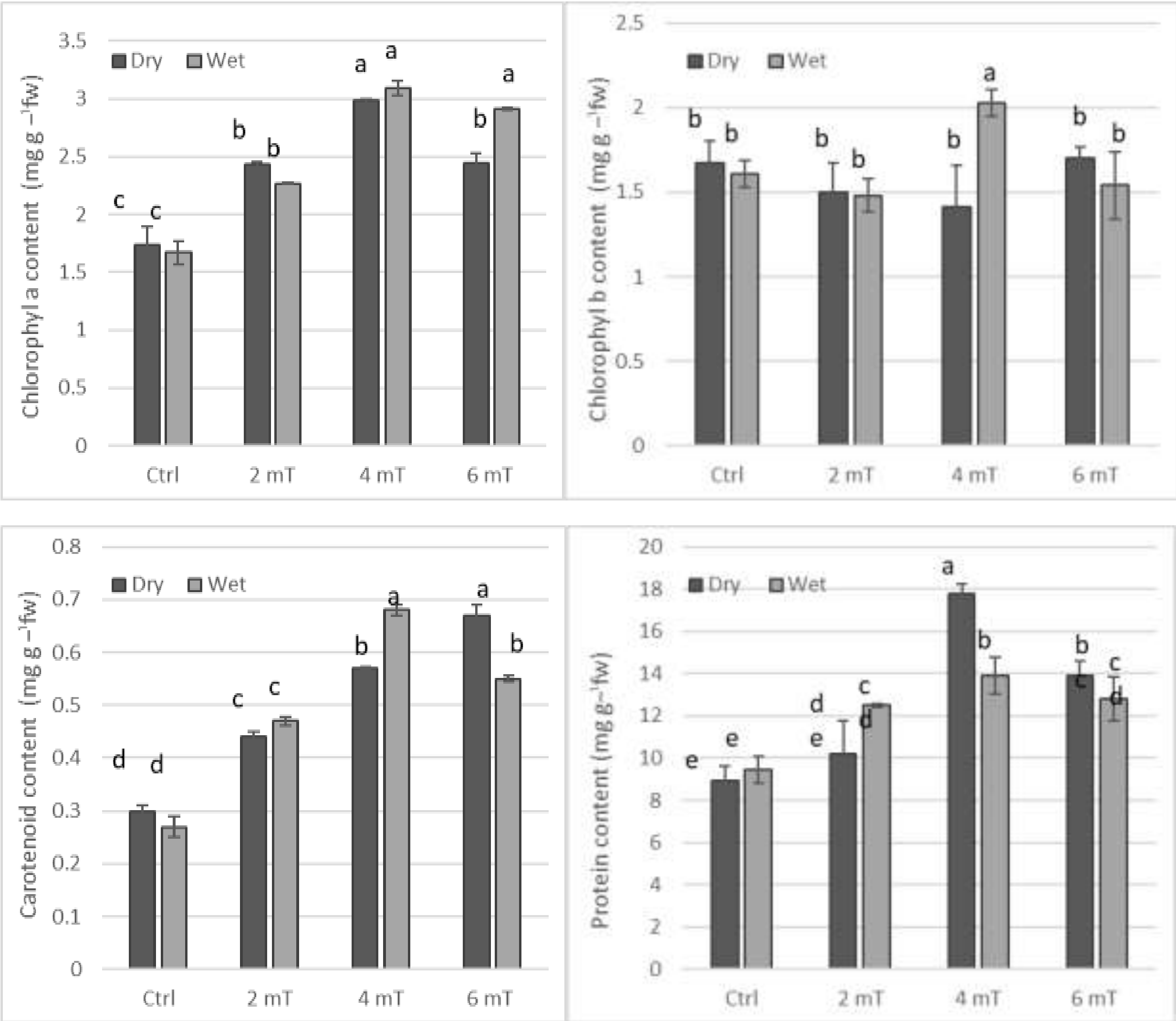

Fig.III. The electromagnetic filed- associated changes of photosynthetic pigments and protein content. Mean values ± SE are presented (n = 3 replicates), Different letters indicate significant differences according to the Duncan test ($P < 0.05$).

Our results point out that electromagnetic filed can be utilized to improve the uptake of nutrients, thereby enhancing plant growth. The reports on electromagnetic filed-mediated changes in the nutritional status of the plants are rare. In soybean, the electromagnetic field-treated seedlings had higher amounts of macronutrients (K and Mg) and micronutrients (Fe, Zn, Cu, and Mn) when compared to the control (Radhakrishnan and Ranjitha-Kumari 2012). In strawberry, the exposure to the electromagnetic field also promoted concentrations of N, Mg, Ca, K, Zn, Mn, and Fe (Eşitken and Turan 2004). Electromagnetic filed-associated increase in the growth of the root system has been regarded as a key mechanism by which plant nutrition can be modified (Bilalis et al. 2013). Modifications in hormonal balances and photosynthesis are another potential major mechanism by which electromagnetic field treatment can enhance growth and nutrition (Mildažienė et al. 2019).

The recorded augmentation in carotenoid levels in response to electromagnetic filed treatment may be considered as a key mechanism by which this treatment may improve plant resistance against stress conditions. Electromagnetic field treatment may trigger specific signaling, thereby activating some defense-related responses (Mildažienė et al.

2019). In plants, the reception of electromagnetic field associate with particular intracellular signaling, especially Ca2+ signaling (Galland and Pazur 2005). The modification in water uptake (Reina et al. 2001; Sudsiri et al. 2017), plant nutrition (Duarte Diaz et al. 1997; Eşitken and Turan 2004; Radhakrishnan and Kumari 2012), protein profile (Novitsky et al. 2001; Radhakrishnan and Kumari 2012; Mildažienė et al. 2019); antioxidant system (Radhakrishnan and Kumari 2012), photosynthesis (Shine et al. 2011; Mildažienė et al. 2019), respiration (Nossol et al. 1993), secondary metabolism (Mildaziene et al. 2018) have been proposed as key mechanisms through which electromagnetic filed treatment may improve plant growth, metabolite production, and yield.

The electromagnetic field treatments were found to differentially affect the activities of antioxidant enzymes. The treatments induced peroxidase activities, while declined the activities of catalase and SOD enzymes. Therefore, it seems that the electromagnetic filed receptions and following signaling may lead to a change in cellular redox status. It is believed that alterations in cellular redox status regulate the expression patterns of genes, especially stress-responsive genes (Foyer and Noctor 2013). However, molecular evidence on this filed is rare and the exact molecular involving mechanisms remain elusive. Induction in the activity of catalase and polyphenol oxidase (two important antioxidant enzymes) occurred in response to the magnetic field (Radhakrishnan and Ranjitha-Kumari 2012). Catalase is a major enzymatic antioxidant which contributed to the detoxification of H2O2 and regulation of polyphenol oxidase (Sahebjamei et al. 2007). It appears that since catalase is not

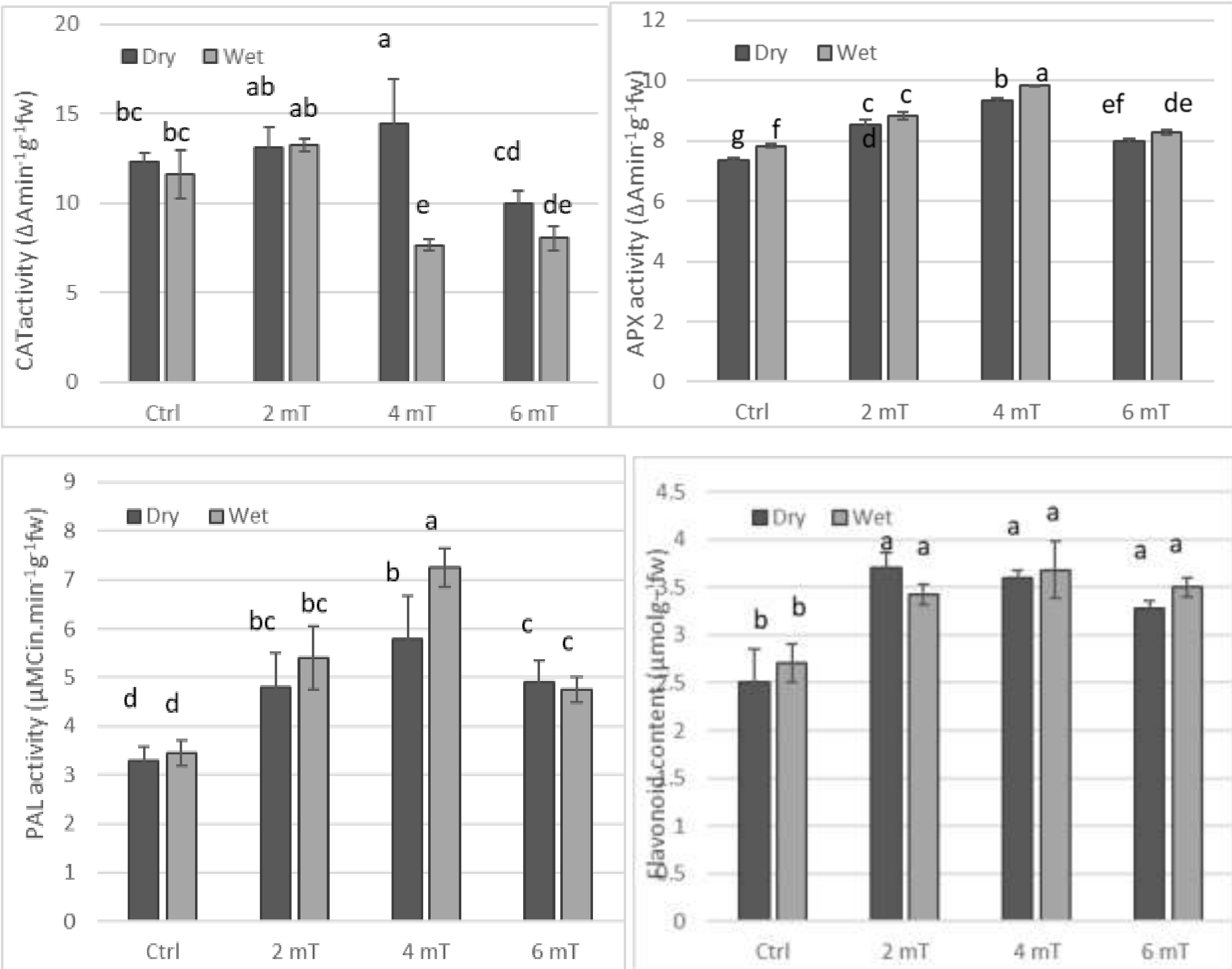

Fig. IV. The EMF- associated changes in activities of several antioxidant enzymes, PAL, and flavonoid concentrations. Mean values ± SE are presented (n = 3 replicates), Different letters indicate significant differences according to the Duncan test (P < 0.05).

significantly effective in scavenging of hydrogen peroxide, peroxidase increases to detoxify H2O2 (Sahebjamei et al. 2007).

Our results revealed that the electromagnetic field associated with inductions in secondary metabolism and increases in concentrations of phenolic secondary metabolites. As PAL is one of the most important enzymes involved in the production of vanities of phenylpropanoid derivatives, its activity is considered as a significant index of activation of secondary metabolism and defense system. These results are consistent with the findings of (Mildaziene et al. 2018). In line with our findings, seed treatment with magnetic field modified productions of storage proteins (globulin and prolamin) and fatty acids (eicosapentaenoic acid, lignoceric acid, caprylic acid, heptadecanoic acid, linoleic acid, and palmitic acid) in soybean (Radhakrishnan 2018)

Conclusion

Taken collectively, this experiment provides valuable evidence on the advantages of electromagnetic filed toward plant growth and physiology. The electromagnetic filed treatments at all applied intensities not only displayed growth-promoting effects, but also it caused modifications in the uptake of minerals, protein concentration, photosynthetic pigments, and activities of antioxidant enzymes. Furthermore, seed priming with electromagnetic field triggered PAL activity (the key enzyme in the production of phenylpropanoids) and the accumulation of phenols. These results support this hypothesis that the application of electromagnetic field treatment may improve plant primary and secondary metabolism, through which growth, physiology, and production of secondary metabolites would be modified. Further studies, especially at molecular levels, may help to elucidate the complicated involved mechanisms.

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