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Full Length Research Paper

Stimulation of growth and some biochemical parameters by magnetic field in wheat (*Triticum aestivum* L.) tissue cultures

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In this study, various magnetic field intensities between 2.9 to 4.8 mT by 1ms^{-1} speed applied to mature embryos of Flamura-85 wheat variety and effects of magnetic field on different physiological (regeneration rate, average plant fresh weights, and average plant lengths) and biochemical [total protein, and chlorophyll amounts, superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX) enzyme activities] parameters were investigated. Compared to the control group, an increase was detected on physiological parameters of 2.2 and 19.8 s magnetic field applied Flamura-85 wheat variety. Total protein and chlorophyll amounts, SOD, CAT, POX and APX enzyme activities of 2.2 and 19.8 s magnetic field applied experimental groups presented increase compared to the control, depending on applied magnetic field intensity.

Key words: Antioxidant enzymes, chlorophyll amount, growth parameters, magnetic field, plant tissue culture, protein amount, wheat.

INTRODUCTION

Magnetic field is an inevitable environmental factor for all living organisms (Esitken and Turan, 2004). There are several hypotheses trying to explain cellular responses of magnetic and electromagnetic field on biological systems. The first one is the orientation of particles which present ferromagnetic properties in living systems, and the other one is the energy level alterations and changes on electron spin conditions of ionic formed atom and molecules which present paramagnetic properties (Van et al., 1998). Various researchers assume that magnetic fields between 10^{-3} to 10^{-2} Tesla can effect chemical reactions by changing electron spin locations and in this manner they have potential to cause biological effects (Fomicheva et al., 1992; Grundler et al., 1992; Belyavskaya et al., 1992).

Studies on the effects of magnetic field applications on plant enzyme activities are quite new. Various studies with different plants reported that magnetic field causes alterations on α- and β-amylase activities, glutathione S-transferase, superoxide dismutase, catalase and ascorbate peroxidase enzymes of the antioxidant defense system (Rochalska and Grabowska, 2007; Sahebjamei et al., 2007).

Reactive oxygen species (ROS) as superoxide radical ($O_2^{\cdot-}$), hydroxyl radicals ($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) are created in different cell compartments such as chloroplasts, mitochondria and peroxisomes in small amounts. In plants, balance between ROS production and scavenging is controlled by antioxidant defense system. When ROS production pass beyond the level that cellular antioxidant system can overcome, damages occur on cell components such as chlorophyll molecule, nucleic acids, membrane lipids and proteins. The first enzyme of the detoxification process is superoxide dismutase (SOD, EC 1.15.1.1) which is a metaloenzyme that uses trace elements like Zn, Cu, Mn and Fe as co-factor. SOD which has three different isoenzymes such

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Abbreviations: AL, average plant length; **2,4-D, 2,4**-dichlorophenoxyacetic acid; **EDTA,** ethylenediaminetetraacetic acid; **fw,** average plant fresh weight; **MF,** magnetic field; **MS,** Murashige and Skoog (1962); **NBT,** nitro-blue tetrazolium; **SD,** standard deviation; **SOD,** superoxide dismutase; **CAT,** catalase; **POX,** peroxidase; **APX,** ascorbate peroxidase.

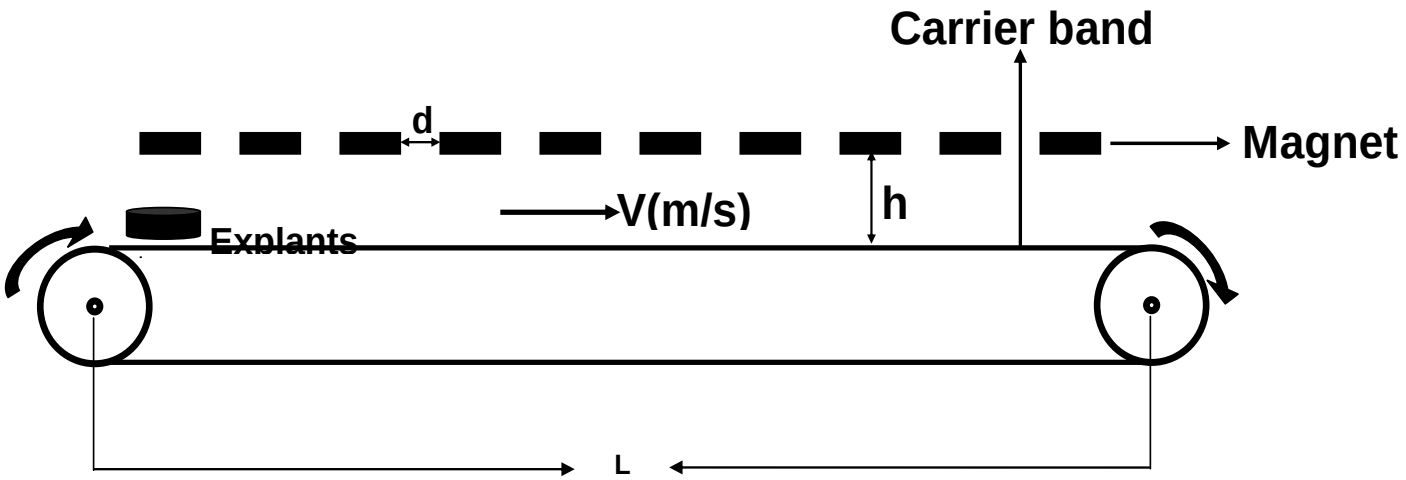

Figure 1. Magnetic field mechanism (Yaycili and Alikamanoglu, 2005).L= 2.2 m (Carrier band length), h= 0.060 m (distance between explants and magnets), d= 0.15 m (distance between magnets), V= 1 ms^{-1} (speed of explants passing under magnetic field).

as Mn-SOD, Cu/Zn-SOD and Fe-SOD scavenges superoxide radicals and also reduces production of hydroxyl radicals by Haber-Weiss reaction (Bowler et al., 1992; Arora et al., 2002).

Catalase (CAT, EC 1. 11.1.6), peroxidase (POX, EC 1. 11.1.7) and ascorbate peroxidase (APX, EC 1.11.1.11); which form enzymatic defense system against free radicals, protect plants against toxicity by transforming hydrogen peroxide (H_2O_2) to water (H_2O) and molecular oxygene (O_2) by dismutation reaction (Bowler et al., 1992; Asada, 2000).

These days, plant tissue culture techniques and artificially created magnetic fields are applied to plants combined, to develop plants that have high economical value, in shorter time. In this study, magnetic field was applied to mature embryo cultures of wheat for 0, 2.2, and 19.8 s periods to determine optimal magnetic field intensity for further investigations on *in vitro* magnetic field studies applied to wheat which has high agricultural importance. The aim of the study was to determine the effects of magnetic field on biochemical and growth parameters.

MATERIALS AND METHODS

Plant material

In this study, wheat (*Triticum aestivum* L.) seeds belonging to the Flamura-85 variety were used. This wheat variety was obtained from Thrace Agricultural Research Institute in Edirne, Turkey.

Maintaining cultures

Wheat seeds were surface sterilized according to the methods of Ozgen et al. (1998). After surface sterilization, seeds were incubated in 35°C for 2 h in sterile distilled water for swelling. Embryos of swelled seed were seperated by using sterile forceps \ and scalpel under aseptic conditions, then embryos were planted on MS (Murashige and Skoog, 1962) media containing 0.1 mg/l 2.4-D and 20 g/l sucrose.

Magnetic field application

In our study, we used 10 magnets which had 0.45 x 0.065 x 0.22 m size prepared by Joint institute for nuclear research, Dubna, Russia (J.I.N.R.) Laboratories. These magnets had height adjustment and were placed on a spinning band which turns by 1 m/s speed (Figure 1). Mature embryos belonging to the Flamura-85 variety were exposed to magnetic field by running them under magnetic field which has 2.9 to 4.8 mT flux intensity for 1 and 9 times. Therefore, mature embryos were exposed to magnetic field at 1m/s speed, 2.2 and 19.8 s. Mature embryos that were run under MF were transferred to fresh media immediately under aseptic conditions. Cultures were placed into the growth chamber; 26°C, and 16 h day / 8h dark conditions to observe their development. The control groups were transferred to fresh media at the same time with the same conditions and then were placed into the growth chamber (Yaycili and Alikamanoglu, 2005).

Determination of growth parameters

Explants, which were regenerated under culture conditions, were observed for 28 days and in the end of this period regeneration rate, average plant weight and average plant height were determined.

Determination of chlorophyll

Leaves of 28 days old cultures were extracted in cold mortar by using cold 80% acetone (1/20 w/v). Homogenates were centrifuged at 3000 rpm for 20 min. Supernatant was taken and absorption rates of 645 nm for chlorophyll *a* and 663 nm for chlorophyll *b* were measured. Values were determined by Arnon (1949) formula.

Total protein and enzyme assay

Leaf samples of the control and different intensities of MF applied

Table 1. 3rd, 5th, 7th and 14th days regenerated plant numbers and regeneration percentages of the control and different intensities of MF exposed *T. aestivum* L. Flamura-85 variety.

Periods exposed to 2.9-4.8 mT MF (s)	Number of Eks.	Regeneration							
		3rd day		5th day		7th day		14th day	
		Number	%	Number	%	Number	%	Number	%
Control	30	7	23.33	15	50.00	27	90.00	27	90.00
2.2	30	10	33.33	23	76.67	30	100.00	30	100.00
19.8	30	11	36.67	24	80.00	30	100.00	30	100.00

plants which were regenerated under culture conditions were taken and homogenized by using 1/10 (w/v) phosphate buffer (pH 7). Supernatant was used for total protein amount, SOD, CAT, POX and APX activity measurements.

Total protein amount

Soluble protein amount was determined by Bradford method (Bradford, 1976).

SOD enzyme activity

Superoxide dismutase activity was determined by the photo-chemical method of Dhindsa et al. (1981). The reaction was started by adding 60 µM riboflavin to 50 µl of the extraction supernatant, 1.5 M Na_2CO_3, 200 mM L-methionine, 2.25 mM nitro-blue tetrazolium (NBT), 3 mM ethylenediaminetetraacetic acid (EDTA), 0.1 M phosphate buffer (pH 7.5) and dH_2O containing 2.9 ml reaction mix. Experimental tubes were incubated under 15 W lights for 10 min and then placed in the dark to stop the reaction. SOD isoenzymes were classified by using potassium cyanide (KCN) and H_2O_2 as inhibitors. Mn-SOD isoenzymes are resistant to both inhibitors, Fe-SOD isoenzymes are resistant to KCN and inhibited by H_2O_2, and Cu/Zn-SOD isoenzymes are inhibited by both inhibitors. 5 mM H_2O_2 and 5 mM KCN were added to the reaction mixture for spectral measurements of SOD isoenzymes. Measurements were taken at 560 nm wavelength.

Catalase enzyme activity

Catalase activity assay was performed by Aebi (1984) method. 1800 µl 0.05 M phosphate buffer (pH 7.0) and 40 mM H_2O_2 containing substrate buffer added to 200 µl extraction supernatant and spectrophotometric measurements were taken at 240 nm wavelength.

Peroxidase enzyme activity

Peroxidase activity assay was performed by Panda et al. (2003). 25 µl of the extraction supernatant was added to 1.95 ml 0.1 M phosphate buffer (pH 7.0), 40 mM H_2O_2 and 1.6% guaiacol containing the substrate buffer and spectrophotometric measurements were taken at 470 nm wavelength.

Ascorbate peroxidase enzyme activity

Ascorbate peroxidase activity assay was performed according to the methods of Nakano and Asada (1981). 50 µl of extraction supernatant was added to 1.95 ml 0.05 M phosphate buffer (pH 7.0), 0.1mM H_2O_2 and 0.5 mM ascorbate containing the substrate buffer and spectrophotometric measurements were taken at 290 nm wavelength.

Statistical analysis

Variance analysis was performed as statistical analysis of plant fresh weight for the control and the different intensities of magnetic field applied cultures, which were regenerated for 28 days. Dunnett's test was applied to the statistically significant plant fresh weight and plant length values (Zar, 1984). All physiological and biochemical parameters were investigated for correlation between them by using SPSS 11.5 statistical program with Pearson's correlation coefficient.

RESULT

Effects of MF on growth parameters

Regeneration percentages of mature embryo explants of Flamura-85 variety which were exposed to different MF intensities that were presented increased after the 3rd day when compared to the control group. As it can be seen from Table 1, MF application induced regeneration of cultures for a short period.

Average fresh weights of 28 days old mature embryo cultures of Flamura-85 variety were determined and depending on the applied magnetic field, average fresh plant weights increased for 2.2 s (25.18%) and 19.8 s (34.41%) exposed groups. Also average length of plants exposed to MF presented increase of 20.88% for 2.2 s and 30.41% for 19.8 s exposed groups (Table 2). The statistical analysis showed that the effect of MF exposure duration on plant fresh weight and plant length were significant at $P<0.05$.

Effect of MF on chlorophyll amount

Chlorophyll amounts of the regenerated control and different intensities of MF applied regenerated mature embryo explants of Flamura-85 variety were detected by measuring the absorbance values of 645 and 663 nm wavelength using a spectrophotometer. Total chlorophyll amount of 2.2 s MF and 19.8 s applied groups showed increase by 32.04% and 34.95% compared to the control,

Table 2. Regenerated plant average fresh weights, average fresh weight percentages, average lengths and average length percentages of 28 days old control and different intensities of MF exposed group of *T. aestivum* L. Flamura-85 variety.

Periods exposed to 2.9 to 4.8 mT MF (s)	Average fresh weights		Average plant lengths	
	Mg	%	Cm	%
Control	183.22 ± 48 a*	100	14.8 ± 1.58 a*	100
2.2	229.36 ± 32 b	125.18	17.2 ± 2.25 b	120.88
19.8	246.26 ± 31 b	134.41	19.3 ± 3.10 b	130.41

* Different letters indicate significant differences relative to controls at P<0.05 (Dunnett's test). Data are mean ±SD.

Table 3. Total chlorophyll, chlorophyll *a* and chlorophyll *b* amounts of 28 days old regenerated control and different intensities of MF exposed group of *T. aestivum* L. Flamura-85 variety embryo cultures. Data are mean ±SD.

Periods exposed to 2.9-4.8 mT MF (s)	Chlorophyll amount (mg g^{-1}fw)		
	Total Chlorophyll	Chlorophyll a	Chlorophyll b
Control	0.721 ± 0.079	0.504 ± 0.027	0.217 ± 0.069
2.2	0.952 ± 0.093	0.665 ± 0.033	0.287 ± 0.029
19.8	0.973 ± 0.096	0.693 ± 0.023	0.280 ± 0.039

Table 4. Total protein amount of regenerated control and different intensities of MF exposed group of Flamura-85 embryo cultures. Data are mean ±SD.

Periods exposed to 2.9 to 4.8 mT MF (s)	Total protein amounts (mg g^{-1}fw)
Control	0.78±0.006
2.2	0.87±0.005
19.8	0.88±0.006

respectively (Table 3).

Effect of MF on total protein amount

Total protein amount of the control and MF applied groups of the regenerated mature embryo explant cultures of Flamura-85 variety was calculated by taking measurement at 595 nm wavelength and using BSA as the standard. Total protein amount of 2.2 and 19.8 s MF applied regenerated plant explants showed increase by 12.09% and 13.51% compared to the control, respectively (Table 4).

Effect of MF on SOD enzyme activity

Total-SOD, Mn-SOD, Fe-SOD and Cu/Zn-SOD enzyme activities of the regenerated control and different intensities MF exposed groups of Flamura-85 variety mature embryo explants were calculated by measuring absorbance values at 560 nm wavelength. Total-SOD enzyme activities of 2.2 and 19.8 s MF applied regenerated plant leaves showed increase of 62.27% and 88.49%, Fe-SOD activities presented increase of 77.91% and 99.59%, Mn-SOD activities presented increase of 42.55% and 78.72%, Cu/Zn-SOD activities presented increase of 29% and 54.55% compared to the control, respectively (Table 5).

Effect of MF on POX enzyme activity

Peroxidase enzyme activities of the regenerated control and different intensities MF exposed groups of Flamura-85 variety mature embryo explants were calculated by measuring absorbance values at 470 nm wavelength. POX activity of the control and 19.8 s magnetic field exposed regenerated cultures were found as 41.11 ± 5.59 and 74.16 ± 6.04 ΔA_{470} g^{-1}(fw) min^{-1}, respectively (Table 6).

Effect of MF on CAT enzyme activity

CAT enzyme activities of the regenerated control and

Table 5. Total-SOD, Fe-SOD, Mn-SOD (B) and Cu/Zn-SOD enzyme activities (U mg^{-1} protein) of regenerated control and different intensities of MF exposed group of Flamura-85 embryo cultures. Data are mean ±SD.

Parameter	Control	2.2 s	19.8 s
T-SOD	5.91±1.89	9.59±2.63	11.14±2.98
Fe-SOD	4.89±0.54	8.7±1.45	9.76±1.36
Mn-SOD	0.47±0.08	0.67±0.09	0.84±0.09
Cu/Zn-SOD	0.55±0.05	0.66±0.06	0.85±0.07

Table 6. POX, CAT and APX enzyme activities of regenerated control and different intensities of MF exposed *T. aestivum* L. Flamura-85 variety embryo culture leaves. Data are mean ±SD (n=10).

Parameter	Control	2.2 s	19.8 s
POX ($\Delta A_{470}\, g^{-1}$(fw) min^{-1})	41.11±5.59	62.63±6.16	74.16±6.04
CAT (U mg^{-1} protein)	3.88±0.51	5.14±0.38	6.73±0.94
APX (U mg^{-1} protein)	0.69±0.12	1.03±0.14	1.12±0.17

different intensities MF exposed groups of Flamura-85 variety mature embryo explants were calculated by measuring absorbance values at 240 nm wavelength. Catalase enzyme activities of the control and 19.8 s magnetic field exposed regenerated explants were 3.88 ± 0.51 U mg^{-1} protein and 6.73 ± 0.94 U mg^{-1} protein, respectively (Table 6).

Effect of MF on APX enzyme activity

APX enzyme activities of the regenerated control and different intensities MF exposed groups of Flamura-85 variety mature embryo explants were calculated by measuring absorbance values at 290 nm wavelength. Ascorbate peroxidase enzyme activities of the control and 19.8 s magnetic field exposed regenerated explants were 0.69 ± 0.12 U mg^{-1} protein and 1.12 ± 0.17 U mg^{-1} protein, respectively (Table 6).

Investigation of effect of MF on physiological and biochemical parameters by correlation method

As it can be seen in Table 7, high leveled correlation was calculated between biochemical and physiological parameters depending on MF application. According to the Pearson's correlation coefficient; correlation of 1 was detected between total protein and total chlorophyll, chlorophyll *a* and average fresh weight, total protein and average fresh weight, total SOD and average plant length at $P<0.05$. Likewise, correlation of 1 was detected between APX and Fe-SOD at $P<0.01$. Also correlation of 0.999 was detected between total protein and chlorophyll *a*, total chlorophyll and average fresh weight, POX and average plant height at $P<0.05$ and correlation of 0.998 was detected between POX and total SOD, APX and chlorophyll a, Fe-SOD and chlorophyll *a* at $P<0.05$.

DISCUSSION

It has been presented by different studies that MF is a factor which effects cell metabolism of meristem cells, it has significant effect on mitosis and it changes G_1 phase of plant cell cycle. Changes on metabolic reactions, cell signaling systems, cell cycle, transcription and protein synthesis cause different biological responses on plant systems (Dul'binskaya, 1973; Lebedev et al., 1975; Belyavskaya et al., 1992; Fomicheva et al., 1992; Paul et al., 2006). According to many researcher's results on studies with different plants, it was reported that MF affects plant growth positively and regeneration rate, plant fresh and dry weight, leaf number, length, shoot number, rooting rate increased compared to the control (Lucchesini et al., 1992; Corneanu et al., 1994; Yaycili and Alikamanoglu, 2005).

In our study, in the 3rd day after we applied magnetic field to the cultures regeneration, rates of all MF intensities were increased compared to the control. In this study, regeneration difference between the control and MF exposed explants started to decrease after the 7th day. According to the established cultures, MF induced regeneration rapidly and at the 28th day, average plant weight and average plant height increased compared to the control. The highest effect of magnetic field on average fresh weight and height were detected on 19.8 s MF exposed experimental group (34.41% and 30.41%, respectively).

In our study, different time and intensity applications on

Table 7. Relationship between physiological and biochemical parameters of wheat seedlings under the magnetic field conditions. Significant levels at P<0.05 are presented by* and at P<0.01 are presented by ** using Pearson's correlation coefficients.

Parameter	F W	AL	Chla	Chlb	T-Chl	Protein	Total-SOD	Mn-SOD	Fe-SOD	Zn/Cu-SOD	CAT	POX	APX
FW	1	-	-	-	-	-	-	-	-	-	-	-	-
AL	0.975	1	-	-	-	-	-	-	-	-	-	-	-
Chl*a*	0.983	0.940	1	-	-	-	-	-	-	-	-	-	-
Chl*b*	0.992	0.839	0.974	1	-	-	-	-	-	-	-	-	-
T-Chl	0.938	0.917	0.998*	0.983	1	-	-	-	-	-	-	-	-
Protein	0.986	0.925	0.999*	0.927	0.999*	1	-	-	-	-	-	-	-
Total-SOD	1*	0.982	0.988	0.957	0.976	0.981	1	-	-	-	-	-	-
Mn-SOD	0.977	1*	0.943	0.843	0.920	0.928	0.983	1	-	-	-	-	-
Fe-SOD	0.999*	0.962	0.997*	0.956	0.991	0.994	0.996	0.964	1	-	-	-	-
Zn/Cu-SOD	0.915	0.982	0.858	0.720	0.825	0.836	0.927	0.980	0.893	1	-	-	-
CAT	0.847	0.994	0.898	0.777	0.870	0.880	0.956	0.994	0.928	0.996	1	-	-
POX	0.996	0.991	0.977	0.904	0.962	0.968	0.998*	0.992	0.990	0.947	0.971	1	-
APX	0.998	0.960	0.998*	0.958	0.992	0.995	0.996	0.962	1**	0.889	0.925	0.989	1

the experimental groups showed effects on the total chlorophyll, chlorophyll a and chlorophyll b amounts compared to the control. The highest effect was at 19.8 s MF exposed group. Also, the applied MF increased total protein amount of the experimental groups from 12.09% to 13.51% compared to the control.

Free radicals play important role on electron transport chain and chemical reaction kinetics. An orientation on free radicals' spin conditions by external MF and the energy increase as a result of this orientation accelerate chemical reactions in cell (Aladjanjiyan and Ylieva, 2003). Reactive half life of free radicals which occur during metabolic activity of organism increase by the effect of MF and antioxidant defense mechanism act as a protection from deleterious effects of these radicals (Sahebjamei et al., 2007; Wang et al., 2008).

In our study, spectroscopic measurements were conducted to determine the effect of MF on antioxidant enzymes such as SOD, CAT, POX and APX which generate antioxidant defense system of plants.

In this research, as a result of spectroscopic measurements of total-SOD and its isoenzymes (Mn-SOD, Cu/Zn-SOD and Fe-SOD), an increase was detected depending on MF application. The highest increase was detected for Fe-SOD.

As we determined the effect of MF on POX, CAT and APX, we observed increase for all the three enzyme activities. These increases were 80.39%, 73.45% and 62.32% for 19.8 s magnetic field applied experimental groups compared to the control, respectively. Various studies reported that MF increase enzyme activities of CAT, POX, and polyphenol oxidase (Lebedev et al., 1975; Pintilie et al., 2006; Wang et al., 2008).

We calculated high leveled correlation between all the parameters as a result of the conducted correlation analysis. Correlation rate between average fresh weight and Fe-SOD and T-SOD were 0.999 and 1 at P<0.05, respectively. Correlation rate between Fe-SOD and APX was 1 at P<0.01. This result shows that MF affects plant fresh weight over free radicals. Correlation rates between total chlorophyll, total protein and chlorophyll a were 1 and 0.999 at P<0.05, therefore we considered that increase in protein synthesis as a result of magnetic field, increased total chlorophyll and chlorophyll a amount. Furthermore, correlation between Fe-SOD and chlorophyll a, chlorophyll a and APX were 0.998 at P<0.05, correlation between Fe-SOD and APX was 1 at P<0.01. These measurements show that chlorophyll a biosynthesis was affected by free radicals that occurred as a result of the magnetic field.

As a result, we found that MF affected physiological parameters, soluble protein amount and chlorophyll amount positively. Also it increased antioxidant enzyme activities such as SOD, POX, CAT and APX. Positive effect of MF on antioxidant defense system parameters made us consider that free radicals which occur as a result of increased metabolic activity and/or increased free radical lifetime induces antioxidant defense system. Thus, results of the different researches with various plants support our thoughts by reporting that MF reduces negative effects of stress factors such as gamma radiation, UV-B radiation, salt stress and heat stress on plants (Ruzic and Jerman, 2002; Yinan et al., 2005; Alikamanoglu et al., 2007; Maheshwari and Grewal, 2009). We may conclude that the reducing effect of MF on the negative effects of various abiotic stress factors on

plant growth and productivity will increase the use of MF applications in the future.

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