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Role of pulsed electromagnetic field on enzyme activity, germination, plant growth and yield of durum wheat

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ABSTRACT

Researchers have focused their efforts on the use of magnetic field as a pre-sowing method, as it is an inexpensive, environmentally friendly technique. This study provides a holistic approach of an agricultural cultivation that can lead to the comprehension of the exact mechanism of magnetic field effect on plant and lead to the appropriate application of magnetic fields. Pulsed electromagnetic field was used for 0, 15, 30 and 45 min as a pre-sowing treatment in durum wheat seeds in a field experiment for three years. The experiment followed a completely randomized design, with four treatments (Control, MF-15, MF-30 and MF-45), two cultivars and three replications. The aim of this study was to determine the effect of magnetic field exposure on durum wheat seeds, covering a complete range of agronomic characteristics. The results obtained in this three-year experiment showed a positive impact of pulsed electromagnetic field, in durum wheat cultivation. Magnetic field has been found to enhance germination, tillering, dry weight, leaf area, chlorophyll content, photosynthetic rate, transpiration rate, stomatal conductance and yield of two durum wheat cultivars. Duration of 30 and 45 min of pre-sowing magnetic field treatment gave the best results. Regarding yield measurement, all magnetic field treatments (MF-15, MF-30 and MF-45) gave statistically significantly higher values compared to control. In addition, α-amylase activity measurements showed that magnetic field affects enzymes and this could possibly explain the improved germination.

1. Introduction

Investigation of magnetic field's influence on plant growth has started to develop rapidly during the last decades. Encouraging results indicated that magnetic field could enhance some plant functions. The use of magnetic field on seeds as a priming technique is getting more and more familiar among researchers (Bhardwaj et al., 2012).

The existence of earth's magnetic field is known to mankind since ancient times, through the discovery of the ability of the mineral magnetite to be oriented in the direction North – South when freely suspended above the earth (Stavroulakis, 2003). But despite the fact that it has always been one of the key features of the environment, until recently the study of the effect of the magnetic field in agronomic science was absent. Each region is characterized by a number of abiotic factors (water, air, soil, temperature) including the magnetic field. However, for many years, the magnetic field of earth constituted as an invariable parameter of the environment that was not taken into account in plant studies (Katsenios, 2013).

The father of bio-electromagnetism is Hippocrates, who first tried to cure breast cancer by exposure of the sun's electromagnetic radiation (therapy by the sun's rays). About 2000 years later, during the 18th century, Luigi Galvani tried to treat tumors, aneurysms and hemorrhages by applying electricity to tissues. In 1840, Recamier and Pravaz provided a method of destroying the cancer cells in the uterus through the use of electricity, which soon became common practice (Stavroulakis, 2003).

Different types of plant materials (seeds, seedlings, young plants and cuttings) have been used. These plant materials have been treated with different types of magnetic field. (Florez et al., 2007; Hajnorouzi et al., 2011). More particularly, in order to study the effects on plants, various types of magnetic fields have been used (static, electromagnetic, pulsed electromagnetic), in different

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intensities and exposure duration (Poinapen et al., 2013; Aguilar et al., 2009; Bilalis et al., 2012a, Radhakrishnan and Kumari, 2012). Regarding exposure time, it varies from 15 s (Muszynski et al., 2009) to 24 h (Martínez et al., 2009). Many experiments have been done with cereal grains (Torres et al., 2008; Vashisth and Nagarajan, 2008; 2010), legumes (Podlesny et al., 2004; 2005; De Souza et al. 2006; Odhiambo et al., 2009;), and perennials (Çelik et al., 2008; Dardeniz et al., 2006; Dhawi and Al-Khayri, 2009).

Investigations in various plant species showed a positive effect of the magnetic field on seed germination (Bhardwaj et al., 2012), plant development in the early stages (De Souza et al., 2014) and ultimately yield (Vashisth et al., 2013). Magnetic field has been found to improve the growth of plants, such as tomatoes (De Souza et al., 2006), the germination and the early stages of growth in plants such as sunflower (Vashisth and Nagarajan, 2010) and soybean (Radhakrishnan and Kumari, 2012). Moreover, researchers report the increase of the root system in young maize seedlings (Muraji et al., 1998). Animal experiments have shown that short duration PEMF seem to facilitate and improve the quality of skin wound healing in rats (Athanasiou et al., 2007).

The enhancement of plant growth parameters is absolutely desirable in modern and organic agriculture, since it can be achieved through the use of an environmentally friendly method, which is also inexpensive. It is worth noting that many researchers have focused their efforts on the use of magnetic field as a pre-sowing technique, as it is an inexpensive, environmentally friendly technique, which can be applied with relative ease (Vashisth and Nagarajan, 2010).

Magnetic field treatment on seeds has been found to increase the activity of hydrolytic enzymes such as amylases in sunflower (Vashisth and Nagarajan, 2010). In cucumber, magnetic field treated seeds showed higher activity of β-amylase and finally increased the rate of germination compared to control (Bhardwaj et al., 2012). The increase of enzyme activity could be a primary positive effect of magnetic field treatment that subsequently leads to higher germination percentage, plant growth and yield.

The aim of this study was to determine the effect of magnetic field exposure on durum wheat seeds, covering a complete range of agronomic characteristics such as germination, tillering, dry weight, leaf area, chlorophyll content, photosynthetic rate, transpiration rate, stomatal conductance and yield. The activity of α-amylase was investigated to explain the increase of germination of durum wheat seeds. Our purpose was to have replicable results in a three-year period.

2. Materials and methods

2.1. Plant material

A field experiment was established at the Agricultural University of Athens (Greece), for three consecutive cultivation years (2009/10, 2010/11 and 2011/12). Two durum wheat (Simeto by Thrakiki Sporoparagogiki and Grecale by NAGREF) varieties were used. Simeto has a high yield potential with thousand grain weight (TGW) at 36–39 g and hectoliter weight at 78–82 kg/hl. Grecale has a medium to high yield potential with thousand grain weight (TGW) at 40 g or more and hectoliter weight at 80 kg/hl.

2.2. Treatments

Four different exposure times of pulsed electromagnetic field were applied in both cultivars. The seeds were treated using a PAPIMI electromagnetic field generator for 15, 30 and 45 min before planting. Non treated seeds were used as control. The PAPIMI device is a pulsed electromagnetic field (PEMF) generator (PAPIMI model 600; Pulse Dynamics, Athens, Greece. Manufacturer characteristics: 35–80 J/pulse energy, 1×10^{-6} s wave duration, 35–80 × 106 W wave power, amplitude of the order of 12.5 mT, rise time 0.1 ms, fall time 10 ms, repetitive frequency of 3 Hz). The same device has been used in medical and agricultural studies (Athanasiou et al., 2007; Bilalis et al., 2012b; Katsenios, 2013; Milgram et al., 2004).

2.3. Experimental design

The experiment followed a completely randomized design, with four treatments (Control, MF-15, MF-30 and MF-45), two cultivars and three replications for three years. Seeds were treated for 15 min (MF-15), 30 min (MF-30) and 45 min (MF-45). Non treated seeds were used as control (0 min). Every replication was consisted of an area of 6 m^2. The quantity of seeds used was 16 g/m^2 for all cultivars.

2.4. Measurements and observations

Germination (number of plants per 1 m row) measurement took place 20 DAS, while **tillering** (plants per 1 m row) took place 50 DAS. **Leaf area** (cm^2 per plant) and **stem dry weight** (g per plant) were destructive measurements and took place 150 DAS. Leaf area was measured by using an automatic leaf area meter (Delta-T Devices Ltd., Burrwell, Cambridge, UK). Stem dry weight was measured by a precision balance after the samples were oven dried at 70 °C for three days in order to measure the weight in grams per plant. For the **chlorophyll** (μg/cm^2) measurement, a portable chlorophyll meter (SPAD) was used. The measurement was taken 130 DAS. A calibration curve has been created in order to convert the SPAD measurement to μg/cm^2 (Lichtenthaler and Wellburn, 1983). Measurements of **photosynthetic rate** (μmol CO_2 m^{-2} s^{-1}), **transpiration rate** (mmol H_2O m^{-2} s^{-1}) and **stomatal conductance** (mol m^{-2} s^{-1}) were undertaken between the hours of 10.30 and 14.30 on fully expanded leaves, with five measurements per treatment. Measurements were made using an LCi Leaf Chamber Analysis System (ADC, Bioscientific, Hoddesdon, UK). Physiology measurements were taken 115 DAS.

The activity of **α-amylase** has been recorded on Simeto variety, for a single year. A reference curve has been produced, using standard solutions and then we took three measurements, the third, the fourth and the fifth day after sowing. The activity of α-amylase was determined using the method described by Guglielminetti et al. (1995). Samples (0.2–0.5 g fresh weight) were extracted in 100 mM Hepes-KOH, pH 7.5, containing 1 mM EDTA, 5 mM MgCl, 5 mM DTT, 10 mM NaHSO. Extracts were centrifuged (13,000 g, 15 min), the resulting pellets were washed with the extraction buffer and centrifuged again, and the resulting supernatants were combined and used for the enzymatic assays. Samples were assayed for the enzymatic activities at 25 °C in 0.5-mL reaction mixtures using the following method. α-amylase: samples pretreated at 70 °C in the presence of 3 mM CaC1, to eliminate interferences from β-amylase were incubated with 2.5% (w/v) soluble starch; activity of enzyme (1 unit) is defined as the amount of enzyme required to produce 1 pmol Glc min^{-1}.

2.5. Statistical analysis

The experimental data were analyzed using Statistica software (StatSoft, 1996), according to the completely randomized design. Analysis of variance (*ANOVA*) and comparisons of means were calculated using the least significant difference (*LSD*) test, at the *5%* level of significance.

3. Results

The use of pulsed electromagnetic field as a pre-sowing treatment was found to enhance durum wheat germination, tillering, dry weight, leaf area, chlorophyll content, photosynthetic rate, transpiration rate, stomatal conductance and yield. Moreover, it was found that magnetic field increased the activity of α-amylase, the third and the fourth day after sowing.

3.1. Germination and tillering

Magnetic field improved germination (plants per row) of durum wheat seeds (Tables 1 and 2). An interaction of magnetic field and variety has been recorded, in the three-year field experiment. The highest number of plants per row has been measured at *Grecale-45* treatment (80.96), with statistically significant differences compared to all other treatments, except *Grecale-30* (80.55) and *Grecale-15* treatment (78.80). These treatments gave values with no statistically significant differences compared to *Simeto-45* (78.34). Moreover, *Simeto-30* (73.17) and *Grecale-0* (71.68) gave higher values with statistically significant differences compared to *Simeto-15* (69.10), which gave higher values with statistically significant differences compared to *Simeto-0* (60.05). Tillering measurement (plants per row) showed statistically significant differences among treatments (Tables 1 and 2). An interaction of magnetic field and variety has been found, in the three-year field experiment. The highest number of tillering has been found at *Grecale-45* (129.16), followed by *Grecale-30* (123.86). Both treatments gave values with statistically significant differences compared to all other treatments, with statistically significant differences. *Grecale-15* (112.81) gave higher values with statistically significant differences compared to *Grecale-0* (104.62). Moreover, *Simeto-45* (96.62), *Simeto-30* (95.73) and *Simeto-15* (92.04) gave values that were statistically significant higher compared to the control (78.17), for significant level of *0.05*. Regarding year, the *third one* (100.20) gave values that were statistically significant higher compared to *first* and *second* year.

3.2. Plant growth

Plant growth characteristics gave statistically significant differences (Table 3). An interaction of magnetic field and variety has been found, in the three-year field experiment (Table 1). The highest values in plant dry weight (g per plant) were measured at *Simeto-45* treatment (15.23) with statistically significant differences compared to all treatments except *Grecale-45* (15.11), *Grecale-30* (14.64), *Simeto-30* (14.05) and *Grecale-15* (14.02). Moreover, *Simeto-15* (13.81) gave values that were statistically significantly higher compared to *Simeto-0* (12.03) and *Grecale-0* (11.94), for significant level of *0.05*. Regarding year, the *second* (14.82) and the *first* (14.57) gave values that were statistically significantly higher compared to the *third* year (12.17). Leaf area measurement (cm^2 per plant) showed statistically significant differences among treatments (Table 3). An interaction of magnetic field and variety has been found, in the three-year field experiment (Table 1). The highest leaf area has been found at *Grecale-45* treatment (249.26), with statistically significant differences compared to all other treatments, followed by *Grecale-30* (240.13). This treatment gave values with statistically significant differences compared to *Grecale-15* (230.94), which also gave values with statistically significant differences compared to *Grecale-0* (219.30). Moreover, *Simeto-15* (181.58), *Simeto-30* (176.61) and *Simeto-45* (174.41) gave values that were statistically significantly higher compared to *Simeto-0* (159.82). Regarding year, the *third* (206.88) and the *second* (205.44) gave values that were statistically significantly higher compared to the *first* year (199.71).

3.3. Physiology measurements

The chlorophyll content (μg/cm^2) of durum wheat plants was higher at the magnetic field treatments (Table 4). An interaction of magnetic field and variety has been found, in the three-year field experiment (Table 1). The highest values of chlorophyll content were measured at *Simeto-15* treatment (49.27), *Simeto-30* (48.61), *Simeto-45* (48.51) and *Grecale-30* (47.90) with statistically significant differences compared to all other treatments except *Grecale-45* (47.04). Moreover, *Grecale-15* (45.50) gave values that were no statistically significantly higher compared to Grecale-45 and *Grecale-0* (43.94). *Grecale-0* gave values that were statistically significantly higher compared to *Simeto-0* (38.95). Regarding year, the *first* (54.06) and the *second* (52.74) gave values that were statistically significant higher compared to the *third* year (54.06).

Photosynthetic rate (μmol $CO_2\,m^{-2}\,s^{-1}$) of durum wheat plants was higher at the magnetic filed treatments (Table 5). An interaction of magnetic field and variety has been found, in the three-year field experiment (Table 1). The highest photosynthetic rate has been found at *Simeto-30* (19.52) and *Simeto-45* (19.31) treatments, with statistically significant differences compared to all other treatments, except *Simeto-15* (17.78) and *Grecale-30* treatment (16.98). *Simeto-15* and *Grecale-30* gave values with no

Table 1
Analysis of variance (F values).

	Germination (plants per row) 20 DAS	Tillering (plants per row) 50 DAS	Dry weight (g per plant) 150 DAS	Leaf area (cm^2 per plant) 150 DAS
Year	6.39 ^{ns}	57.26^c	493.97^c	9.13^a
Variety	348.36^c	586.31^c	19.37^a	1017.86^c
Magnetic	178.08^c	71.51^c	249.00^c	88.10^c
Variety*Magnetic	18.46^c	9.05^c	3.63^a	13.33^c
Year*Variety*Magnetic	1.27^{ns}	2.75^{ns}	4.39^{ns}	2.76^{ns}

	Chlorophyll content (μg per cm^2) 130 DAS	Photosynthetic rate (μmol CO_2 $m^{-2}\,s^{-1}$) 115 DAS	Transpiration rate (mmol H_2O $m^{-2}\,s^{-1}$) 115 DAS	Stomatal conductance (mol $m^{-2}\,s^{-1}$) 115 DAS	Yield (kg per hectare)
Year	1399.29^c	262.28^c	41.17^b	5.71 ^{ns}	172.35^c
Variety	0.51 ns	233.72^c	17.53^a	70.08^b	98.26^c
Magnetic	77.05^c	120.14^c	76.94^c	129.12^c	38.24^c
Variety*Magnetic	30.19^c	3.63^a	0.95^{ns}	14.67^c	2.63^{ns}
Year*Variety*Magnetic	1.60^{ns}	1.65^{ns}	1.18^{ns}	0.81^{ns}	1.48^{ns}

ns = not significant.

[a] Significance at 0.05.
[b] Significance at 0.01.
[c] Significance at 0.001.

Table 2
Interaction of magnetic field and variety on germination (30 DAS) and tillering (50 DAS) of durum wheat for three years.

Germination (plants per row) 20 DAS				Tillering (plants per row) 50 DAS			
*Magnetic*Variety*		*Year*		*Magnetic*Variety*		*Year*	
Simeto 0	60.05 e	First	74.30 a	Simeto 0	78.17 e	First	106.37 a
Simeto 15	69.10 d	Second	74.84 a	Simeto 15	92.04 d	Second	105.81 a
Simeto 30	73.17c	Third	73.10 a	Simeto 30	95.73 d	Third	100.20 b
Simeto 45	78.34 b			Simeto 45	96.62 d		
Grecale 0	71.68c			Grecale 0	104.62c		
Grecale 15	78.80 ab			Grecale 15	112.81 b		
Grecale 30	80.55 ab			Grecale 30	123.86 a		
Grecale 45	80.96 a			Grecale 45	129.16 a		

Means followed by the same letter for treatments are not significantly different according to the least significant difference (LSD) test.

Table 3
Interaction of magnetic field and variety on dry weight and leaf area (150 DAS) of durum wheat for three years.

Dry weight (g per plant) 150 DAS				Leaf area (cm^2 per plant) 150 DAS			
*Magnetic*variety*		*Year*		*Magnetic*variety*		*Year*	
Simeto 0	12.03c	First	14.57 a	Simeto 0	159.82 f	First	199.71 b
Simeto 15	13.81 b	Second	14.82 a	Simeto 15	181.58 e	Second	205.44 a
Simeto 30	14.05 ab	Third	12,17 b	Simeto 30	176.61 e	Third	206.88 a
Simeto 45	15.23 a			Simeto 45	174.41 e		
Grecale 0	11.94c			Grecale 0	219.30 d		
Grecale 15	14.02 ab			Grecale 15	230.94c		
Grecale 30	14.64 ab			Grecale 30	240.13 b		
Grecale 45	15.11 ab			Grecale 45	249.26 a		

Means followed by the same letter for treatments are not significantly different according to the LSD test.

Table 4
Interaction of magnetic field and variety on chlorophyll (130 DAS) of durum wheat for three years.

Chlorophyll content (μg per cm^2) 130 DAS			
*Magnetic*variety*		*Year*	
Simeto 0	38.95 d	First	54.06 a
Simeto 15	49.27 a	Second	52.74 a
Simeto 30	48.61 a	Third	31.85 b
Simeto 45	48.51 a		
Grecale 0	43.94c		
Grecale 15	45.50 BCE		
Grecale 30	47.90 a		
Grecale 45	47.04 ab		

Means followed by the same letter for treatments are not significantly different according to the LSD test.

statistically significant differences compared to *Grecale-15* (15.89), Grecale-45 (16.70) and *Simeto-0* (15.52). However, *Simeto-15* and *Grecale-30* gave values with statistically significant differences compared to *Grecale-0* (11.66). Regarding year, the *third* (19.14) gave values that were statistically significantly higher compared to the *second* year (17.07), which also gave values that were statistically significantly higher compared to the *first* year (13.79). Transpiration rate (mmol $H_2O\ m^{-2}\ s^{-1}$) of durum wheat plants showed some statistically significant differences regarding magnetic field treatment, variety and year. Treatments of *30* (4.06) and 45 min (3.90) of magnetic field exposure gave values that were statistically significantly higher compared to 15 min (3.38). All magnetic field treatments gave values that were statistically significant higher compared to *control* (2.24). Regarding year, the *third* (3.88) and the *second* (3.41) gave values that were statistically significantly higher compared to the *first* year (2.89). Finally, the variety *Simeto* (3.55) gave values that were statistically significant higher compared to *Grecale* (3.24). Stomatal conductance (mol $m^{-2}\ s^{-1}$) of durum wheat plants was higher at the magnetic field treatments (Table 5). An interaction of magnetic field and variety has been found, in the three-year field experiment (Table 1). The highest stomatal conductance has been found at

Table 5
Interaction of magnetic field and variety on photosynthetic rate (115 DAS), effect of magnetic field, variety and year on transpiration rate (115 DAS) and interaction of magnetic field and variety on stomatal conductance (115 DAS) of durum wheat for three years. Means followed by the same letter for treatments are not significantly different according to the LSD test.

Photosynthetic rate (μmol $CO_2\ m^{-2}\ s^{-1}$) 115 DAS				Transpiration rate (mmol $H_2O\ m^{-2}\ s^{-1}$) 115 DAS						Stomatal conductance (mol $m^{-2}\ s^{-1}$) 115 DAS			
*Magnetic*Variety*		*Year*		*Magnetic*		*Year*		*Variety*		*Magnetic*Variety*		*Year*	
Simeto 0	15.52 b	First	13.79c	0	2.24c	First	2.89 b	Simeto	3.55 a	Simeto 0	0.17c	First	0.19 a
Simeto 15	17.78 ab	Second	17.07 b	15	3.38 b	Second	3.41 a	Grecale	3.24 b	Simeto 15	0.20 b	Second	0.18 a
Simeto 30	19.52 a	Third	19.14 a	30	4.06 a	Third	3.88 a			Simeto 30	0.24 a	Third	0.19 a
Simeto 45	19.31 a			45	3.90 a					Simeto 45	0.24 a		
Grecale 0	11.66c									Grecale 0	0.13 d		
Grecale 15	15.89 b									Grecale 15	0.17c		
Grecale 30	16.98 ab									Grecale 30	0.18c		
Grecale 45	16.70 b									Grecale 45	0.17c		

Table 6
Effect of magnetic field, variety and year on yield of durum wheat. Means followed by the same letter for treatments are not significantly different according to the LSD test.

Yield					
Magnetic		*Year*		*Variety*	
0	2428.5 b	First	2406.3 c	Simeto	2729.8 b
15	2941.8 a	Second	3332.8 a	Grecale	3126.7 a
30	3113.4 a	Third	3045.5 b		
45	3229.2 a				

Simeto-30 (0.24) and *Simeto-45* (0.24) treatments, with statistically significant differences compared to *Simeto-15* (0.20) treatment. *Simeto-15* gave values that were statistically significantly higher compared to *Grecale-30* (0.18), *Grecale-45* (0.17), *Grecale-15* (0.17) and *Simeto-0* (0.17). *Grecale-0* (0.13) gave the lowest values of all treatments with statistically significant differences.

3.4. Yield

The pre-sowing application of magnetic field increased yield of durum wheat (Table 6). Yield (kg per ha) of durum wheat plants showed some statistically significant differences regarding magnetic field treatment, variety and year. The treatments of *45* (3229.2), 30 (3113.4) and 15 min (2941.8) of magnetic field exposure gave values that were statistically significantly higher compared to 0 min (2428.5). That means that all magnetic field treatments gave values that were statistically significantly higher compared to *control*. Regarding year, the *second* (3332.8) gave values that were statistically significantly higher compared to the *third* year (3045.5). The *third* year gave values that were statistically significantly higher compared to the *first* year (2406.3). Finally, the variety *Grecale* (3126.7) gave values that were statistically significantly higher compared to *Simeto* (2729.8).

3.5. Activity of α-amylase

The activity of α-amylase has been conducted on Simeto variety. The results showed that pre-sowing application of magnetic field on durum wheat seeds leads to increased activity of α-amylase at 30 min of exposure, the third day after sowing (Fig. 1). Moreover, at the fourth day after sowing, in all magnetic field measurements (15, 30 and 45 min) the observed enzyme activity was statistically significantly higher compared to the untreated seeds (*control*). At the fifth day, all treatments gave similar results, with no statistically significant differences.

4. Discussion

The results obtained in this three-year experiment showed a positive impact of pulsed electromagnetic field, in durum wheat cultivation. Magnetic field has been found to enhance germination, tillering, dry weight, leaf area, chlorophyll content, photosynthetic rate, transpiration rate, stomatal conductance and yield of two durum wheat cultivars. In addition, α-amylase activity measurements showed that magnetic field affects enzymes and this could possibly explain the improved germination.

In 1999 Pietruszewski found that the presowing application of magnetic field increased the germination of two wheat varieties, while similar are the findings of Aksenov in 1997. Increase of the percentage of germination rate as a result of exposure to a magnetic field states also Cakmak et al. (2010) for wheat grain. The positive influence of the magnetic field in the germination of corn seeds has also been expressed and through the reduction of time of germination. Florez et al. (2007) indicates that this reduction eventually led to an increase of 16–25% germination rate compared to the control. It should be noted that the emergence of the capacity improvement is recorded in controlled conditions (Petri dishes) and field conditions (Bilalis et al., 2012b).

Recently, Javed et al. (2011) found that pretreated corn seeds with different electromagnetic treatments particularly 100 and 150 mT for 10 min significantly alleviated the drought-induced adverse effects on growth by improving photosynthesis, transpiration rate and stomatal conductance. Pulsed Electromagnetic Field (PEMF) improved main physiology measurements such transpiration rate, photosynthetic rate and stomatal conductance at the early stages of cotton plants (Bilalis et al., 2013).

Low-intensity magnetic field has been used in strawberry plants and had a positive effect on the yields, in weight and in the number of fruit (Esitken and Turan, 2004). The pre treatment of lettuce seeds with magnetic field also led to an increase in final yield (De Souza et al., 2008). With presowing application of the magnetic field in cotton, it has been found that certain varieties can double their yields compared to control (Leelapriya et al., 2003). A recent study used two types of hybrid corn using three different durations of exposure to the magnetic field. The results showed that the ideal exposure to the magnetic field varies even among the groups of the same species (Bilalis et al., 2012c). A similar finding has been noted by other researchers who argue that it is necessary to find the exact parameters of radiation that cause positive biostimulation in seeds, which depends on the genotype (Aguilar et al., 2009). Pietruszewski (1996) has reported increase in crop yields after the influence of the magnetic field.

The exposure of seeds at magnetic field was found to increase the activity of α-amylase in the third and fourth day after sowing, thus giving an advantage during germination. On the fourth day after seeding, the magnetic field treatments exhibited increased activity of 50–100% compared with the control treatment. Amylases are enzymes that catalyze the hydrolysis of starch in the early stages of seed germination. The hydrolytic enzymes secreted by diktyosomata inside the endosperm and catalyze the hydrolysis of starch to glucose reserves. Glucose is the source of energy and carbon skeletons for growth of the young seedling, which in the early stages of life remains heterotrophic. Recent studies have reported higher activity of α-amylase in sunflower seeds having treated with magnetic field (Vashisth and Nagarajan, 2010). In contrast, in soybean seeds the pretreatment with PMF resulted to α-amylase activity reduction eight days after treatment. (Radhakrishnan and Kumari, 2012).

The multiple regression analysis indicates that there was a statistically significant relationship between Dry Weight (DW150), Leaf Area (LA150) and Yield (YIELD):

$$Yield = 32.024 + 7.048 \times DW150 + 0.8 \times LA150$$

	St. error	p-level
DW150	3.314	0.037
LA150	0.175	0.000

This equation explains that Leaf Area ($p < 0.001$) and Dry Weight ($p < 0.05$) affected statistically significant the Yield of durum wheat (Fig. 2).

In a similar field experiment conducted in tomato crop, magnetic field treatment, in certain times of exposure, improved shoot diameter, number of leaves per plant, fresh and dry weight,

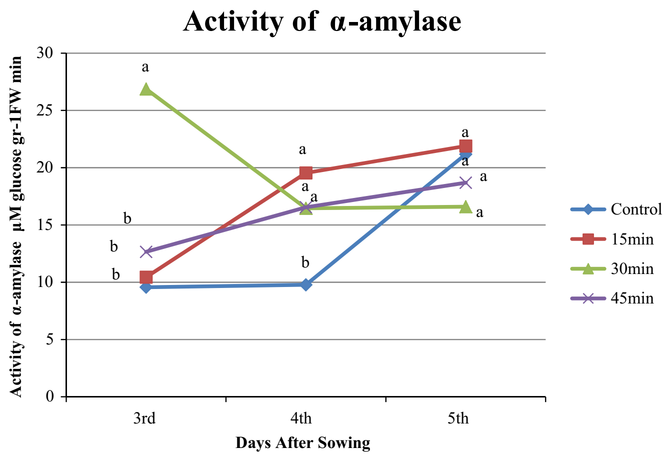

Fig. 1. Effect of magnetic field on α-amylase activity. Means followed by the same letter are not significantly different according to the LSD test.

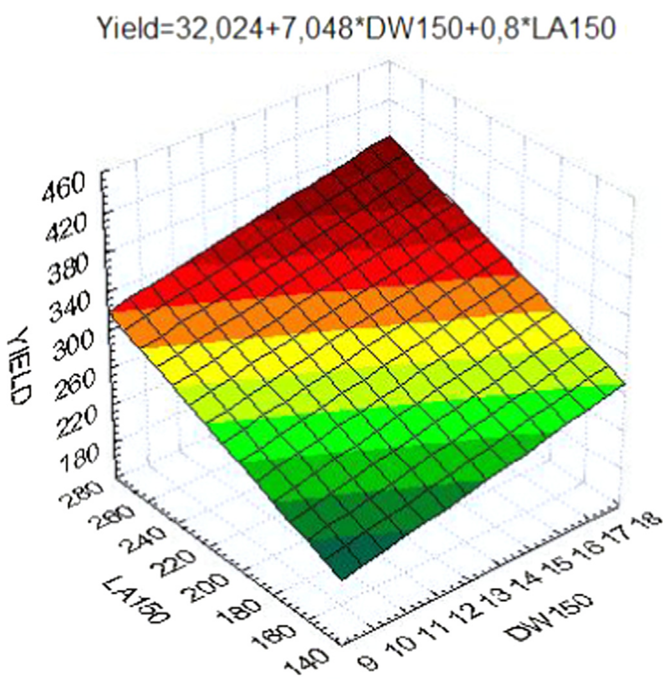

Fig. 2. Multiple regression analysis of yield, dry weight and leaf area.

number of flowers, and yield per plant.

5. Conclusions

Pre-sowing pulsed electromagnetic field treatment enhanced germination, tillering, dry weight, leaf area, chlorophyll content, photosynthetic rate, transpiration rate and stomatal conductance of two durum wheat cultivars. Duration of 30 and 45 min of pre-sowing magnetic field treatment gave the best results. The exposure of seeds at magnetic field was found to increase the activity of α-amylase in the third and fourth day after sowing, thus giving an advantage during germination. Enhancement of germination, tillering, plant growth and physiology of durum wheat plants has led to increased yield in this three year experiment. Such studies provide a holistic approach of an agricultural cultivation that can lead to the comprehension of the exact mechanism of magnetic field effect on plant tissues and lead to the appropriate application of magnetic fields (Efthimiadou et al., 2014).

References

Çelik, Ö., Atak, Ç., Rzakoulieva, A., 2008. Stimulation of rapid regeneration by a magnetic field in Paulownia node cultures. J. Cent. Eur. Agric. 9 (2), 297–304.

Aguilar, C.H., Pacheco, A.D., Carballo, A.C., Orea, A.C., Ivanov, R., Bonilla, J.L.L., Montanez, J.P.V., 2009. Alternative magnetic field irradiation effects on three genotype maize seed field performance. Acta Agrophys. 14 (1), 7–17.

Athanasiou, A., Karkambounas, S., Batistatou, A., Lykoudis, E., Katsaraki, A., Kartsiouni, T., Papalois, A., Evangelou, A., 2007. The effect of pulsed electromagnetic fields on secondary skin wound healing: an experimental study. Bioelectromagnetics 28, 362–368.

Bhardwaj, J., Anand, A., Nagarajan, S., 2012. Biochemical and biophysical changes associated with magnetopriming in germinating cucumber seeds. Plant Physiol. Biochem. 57, 67–73.

Bilalis, D., Katsenios, N., Efthimiadou, A., Karkanis, A., 2012a. Pulsed electromagnetic fields effect in oregano rooting and vegetative propagation: a potential new organic method. Acta Agric. Scand. Sect. B – Soil Plants 62, 94–99.

Bilalis, D., Katsenios, N., Efthimiadou, A., Karkanis, A., 2012a. Investigation of pulsed electromagnetic field as a novel organic pre-sowing method on germination and initial growth stages of cotton. Electromagn. Biol. Med. 31 (2), 143–150.

Bilalis, D., Katsenios, N., Efthimiadou, A., Karkanis, A., 2012b. Pulsed electromagnetic field: an organic compatible method to promote plant growth and yield in two corn types. Electromagn. Biol. Med. 31 (4), 333–343.

Bilalis, D., Katsenios, N., Efthimiadou, A., Karkanis, A., Khah, M.E., Mitsis, T., 2013. Magnetic field pre-sowing treatment as an organic friendly technique to promote plant growth and chemical elements accumulation in early stages of cotton. Aust. J. Crop Sci. 7 (1), 46–50.

Cakmak, T., Dumlupinar, R., Erdal, S., 2010. Acceleration of germination and early growth of wheat and bean seedlings grown under various magnetic field and osmotic conditions. Bioelectromagnetics 31, 120–129.

Dardeniz, A., Tayyar, S., Yalcin, S., 2006. Influence of low-frequency electromagnetic field on the vegetative growth of grape cv. Uslu. J. Cent. Eur. Agric. 7 (3), 389–395.

De Souza, A., García, D., Sueiro, L., Gilart, F., 2014. Improvement of the seed germination, growth and yield of onion plants by extremely low frequency non-uniform magnetic fields. Sci. Hortic. 176, 63–69.

De Souza, A., García, D., Sueiro, L., Gilart, F., Porras, E., Licea, L., 2006. Pre-sowing magnetic treatments of tomato seeds increase the growth and yield of plants. Bioelectromagnetics 27 (4), 247–257.

De Souza, A., Sueiro, L., Gonzalez, L.M., Licea, L., Porras, E.P., Gilart, 2008. Improvement of the growth and yield of lettuce plants by non-uniform magnetic fields. Electromagn. Biol. Med. 27, 173–184.

Dhawi, F., Al-Khayri, J.M., 2009. The effect of magnetic resonance imaging on date palm (*Phoenix dactylifera* L.) elemental composition. Commun. Biometry Crop Sci. 4 (1), 14–20.

Efthimiadou, A., Katsenios, N., Karkanis, A., Papastylianou, P., Triantafyllidis, V., Travlos, I., Bilalis, J.D., 2014. Effects of presowing pulsed electromagnetic treatment of tomato seed on growth, yield, and lycopene content. Sci. World J. 369745, 6 (pages).

Esitken, A., Turan, M., 2004. Alternating magnetic field effects on yield and plant nutrient element composition of strawberry (*Fragaria ananassa* CV. camarosa). Acta Agric. Scand. Sect. B – Soil Plants 54, 135–139.

Flórez, M., Carbonell, M.V., Martínez, E., 2007. Exposure of maize seeds to stationary magnetic fields: effects on germination and early growth. Env. Exp. Bot. 59 (1), 68–75.

Guglielminetti, L., Yamaguchi, J., Perata, P., Alpi, A., 1995. Amylolytic Activities in Cereal Seeds under Aerobic and Anaerobic Conditions. Plant Physiol 109, 1069–1076.

Hajnorouzi, A., Vaezzadeh, M., Ghanati, F., 2011. Growth promotion and a decrease of oxidative stress in maize seedlings by a combination of geomagnetic and weak electromagnetic fields. J. Plant Physiol. 168 (10), 1123–1128.

Javed, N., Ashraf, M., Akram, N.A., Al-Qurainy, F., 2011. Alleviation of adverse effects of drought stress on growth and some potential physiological attributes in maize (*Zea mays* L.) by seed electromagnetic treatment. Photochem. Photobiol. 87, 1354–1362.

Katsenios, N., 2013. Investigation of Pulsed Electromagnetic Field Influence on Plant Growth. Agricultural University of Athens, Greece.

Leelapriya, T., Dhilip, K.S., Sanker Narayan, P.V., 2003. Effect of weak sinusoidal magnetic field on germination and yield of cotton (*Gossypium* spp.). Electromagn. Biol. Med. 22, 117–125.

Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592.

Martínez, E., Flórez, M., Maqueda, R., Carbonell, M.V., Amaya, J.M., 2009. Pea (*Pisum sativum*, L.) and lentil (*Lens culinaris*, Med.) growth stimulation due to exposure to 125 and 250 mT stationary fields. Pol. J. Environ. Stud. 18 (4), 657–663.

Milgram, J., Shahar, R., Levin-Harrus, T., Kass, P., 2004. The effect of short high intensity magnetic field pulses on the healing of skin wounds in rat. Bioelectromagnetics 25, 271–277.

Muraji, M., Asai, T., Tatebe, W., 1998. Primary root growth rate of *Zea mays* seedlings grown in an alternating magnetic field of different frequencies. Bioelectrochem. Bioenerg. 44, 271–273.

Muszynski, S., Gagos, M., Pietruszewski, S., 2009. Short-term pre-germination exposure to ELF magnetic fields does not Influence seedling growth in durum wheat (*Triticum durum*). Pol. J. Environ. Stud. 18 (6), 1065–1072.

Odhiambo, J.O., Ndiritu, F.G., Wagara, I.N., 2009. Effects of static electromagnetic fields at 24 h incubation on the germination of rose coco beans (*Phaseolus vulgaris*). Romanin J. Biophys. 19 (2), 135–147.
Pietruszewski, S., 1996. Effects of magnetic biostimulation of wheat seeds on germination, yield and proteins. Int. Agrophys. 10 (1), 51–55.
Podlesny, J., Pietruszewski, S., Podlesna, A., 2004. Efficiency of the magnetic treatment of broad bean seeds cultivated under experimental plot conditions. Int. Agrophys. 18, 65–71.
Podlesny, J., Pietruszewski, S., Podlesna, A., 2005. Influence of magnetic stimulation of seeds on the formation of morphological features and yielding of the pea. Int. Agrophys. 19, 61–68.
Poinapen, D., Brown, D.C., Beeharry, G.K., 2013. Seed orientation and magnetic field strength have more influence on tomato seed performance than relative humidity and duration of exposure to non-uniform static magnetic fields. J. Plant Physiol. 170 (14), 1251–1258.
Radhakrishnan, R., Kumari, B.R., 2012. Pulsed magnetic field: A contemporary approach offers to enhance plant growth and yield of soybean. Plant Physiol. Bioch 51, 139–144.
Stavroulakis, P., 2003. Biological Effects of Electromagnetic Fields. Mechanisms, Modeling, Biological Effects, Therapeutic Effects, International Standards, Exposure Criteria, XVI, 793, p. 280.
StatSoft, Inc.,1996. STATISTICA for Windows (Computer program manual). Tulsa, OK: StatSoft, Inc. 2300 East 14th Street, Tulsa, OK 74104.
Torres, C., Diaz, J.E., Cabal, P.A., 2008. Magnetic fields effect over seeds germination of rice (*Oryza sativa* L.) and tomato (*Solanum lycopersicum* L.). Agron. Colomb. 26 (No. 2), 177–185.
Vashisth, A., Nagarajan, S., 2008. Exposure of seeds to static magnetic field enhances germination and early growth characteristics in chickpea (*Cicer arietinum* L.). Bioelectromagnetics 29 (7), 571–578.
Vashisth, A., Nagarajan, S., 2010. Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. J. Plant Physiol. 167, 149–156.
Vashisth, A., Meena, N., Joshi D, K., Singh, R., 2013. Study of biophysical and growth parameters in relation to yield in sunflower (*Helianthus annuus*). Indian J. Agric. Sci. 83 (2).