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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 presowing 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.