DECIPHERING HIDDEN MECHANISMS IN THE BIOMAGNETIC RESPONSE IN PLANTS: A STUDY ON THE EFFECTS OF MAGNETIC FIELDS ON PLANT GROWTH, DEVELOPMENT, AND MOLECULAR RESPONSES

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LIST OF ABBREVIATIONS

GMF	**Geomagnetic Field**
MFE	**Magnetic Field Exposure**
HPLC	**High Performance Liquid Chromatography**
MS	**Mass Spectrometry**
EMF	**Electromagnetic Field**
DNA	**Deoxyribonucleic Acid**
RNA	**Ribonucleic Aci**
MHz	**Megahertz**
TMV	**Tobacco Mosaic Virus**
HR	**Hypersensitive Response**
NMR	**Nuclear Magnetic Resonance**
LC-MS	**Liquid Chromatography-Mass Spectrometry**
RFEF	**Radio Frequency Electromagnetic Fields**
MF	**Magnetic Field**
RNA-Seq	**RNA- Sequencing**
FPKM	**Fragments Per Kilobase per Million**
T	**Tesla**
OPLS-DA	**Orthogonal Partial Least Squares Analysis**
FC	**Fold Change**
MSEA	**Metabolite Set Enrichment Analysis**
QC	**Quality Control**
mRNA	**Messenger RNA**
coA	**Coenzyme A**

ABSTRACT

DECIPHERING HIDDEN MECHANISMS IN THE BIOMAGNETIC RESPONSE IN PLANTS: A STUDY ON THE EFFECTS OF MAGNETIC FIELDS ON PLANT GROWTH, DEVELOPMENT, AND MOLECULAR RESPONSES

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Plants sense and respond to environmental stimuli (light wavelengths, gravity, touch, electromagnetic stimulation) with alterations at the molecular level, which are expressed through physiological changes in growth and development. Earth's magnetic field also known as geomagnetic field (GMF) are consistent environmental stimuli. The GMF is generated by the rotation of Earth's iron core, which conducts electricity. Plants exposed to magnetic fields have shown a variety of physiological responses such as an increase in seed germination, plant growth, pigment synthesis, water and nutrients uptake, and alterations in the expression of proteins and enzymes. The effects of the Earth's magnetic fields on plants' molecular responses have not been well documented. Thus, a comprehensive understanding of magneto-reception dynamism in plants necessitates more in-depth approaches. In this study tomato (*S. lycopersicum*) and collard (*B. oleracea var. viridis*) seeds exposed to a magnetic field intensity of 4.7 Gauss were evaluated through phenotypic screenings of physio morphological changes, HPLC-LCMS analysis of metabolite expression and transcriptome profiling using RNA-sequencing. The objectives are: 1.) To screen plant growth responses to magnetic field exposure based on selected physio-morphological growth parameters; 2.) To screen plant metabolomics responses and the secondary metabolite expression in control versus exposed to magnetic fields through high-performance liquid chromatography-mass spectrometry (HPLC-MS). 3.) Identify the effects of magnetic fields in plants by transcriptome profiling to characterize genetic responses, and potential differential gene expression patterns in exposed plants. Seeds were sterilized and placed into for MFE. Seeds were exposed at 4.33 gauss plus the local GMF, for a total of 4.7 Gauss over a period of six days for two hours a day. The experiment followed a randomized block design with three replicants (petri dishes) per objective, repeated for two trials ***Physio Morphological-*** Following exposures, collards and tomatoes seeds were planted in potting soil under greenhouse conditions as growth parameters including stem diameter, chlorophyll concentrations, and plant height were recorded weekly for six weeks . The statistical results concluded that there was no significance in all growth parameters between control and exposed groups ($p<0.05$). ***Metabolomic Analysis-***Metabolomic extraction was completed immediately after exposures ended on the sixth day. Following a methanol-based metabolite extraction, metabolomics analysis was performed by HPLC-MS. The raw data received from HPLC-MS analysis was then analyzed using Metaboanalyst (https://www.metaboanalyst.ca/). Using Pathway and Enrichment analysis, 140 compounds were identified and grouped into metabolite pathways through hypergeometric analysis ($p < 0.05$). Linoleic acid metabolism was the highest expressed metabolic pathway $p < 0.00511$) in all trials for tomatoes and collards. The identified significant metabolites ($p < 0.05$) in both trials collards and tomatoes were C3 H N3 O3 P2 and pantothenic acid, respectively. ***Transcriptome Profiling-*** RNA extraction of control and exposed tomato samples was performed for RNA-sequencing analysis. Differential gene expression analysis was performed by the quantitation of mapped reads, dictated by FPKM

values. For trial 1,2 control and 1,2 exposed 509, 434, 409, and 524 genes were differentially expressed, respectively. Exposure resulted in the down regulation of five novel genes, Solyc07g065840.2, a heat shock gene, Solyc09g010630.3, Solyc01g099770.3, Solyc01g101060.3, and Solyc03g119080.3. The proposed study can contribute to the fundamental understanding of magnetic field effects on plant development through a comprehensive approach, and the development of specific MFE protocols may alter the expression of important biosynthetic pathways and products.

Keywords: biomagnetism, plants, molecular and transcriptome profiling, magnetic fields

CHAPTER 1

INTRODUCTION

Plants sense and respond to various environmental stimuli (light wavelengths, gravity, touch, electromagnetic stimulation, etc.) which may be expressed through physiological changes in growth and development. One constant environmental stimulus that plants are exposed to is Earth's magnetic fields also known as geomagnetic fields (GMFs). All living things have encountered the effects of a natural geomagnetic field during their evolution. The GMF is continuously affecting living things and is recognized to have an impact on a variety of biological functions, hence studies of the impact of magnetic fields on organisms have drawn increasing attention from scientists.

One of the first studies on the effects of MF on plants was conducted by Krylov and Tarakonova (1960). By terming this action as magnetotropism, they suggested that the MF had an auxin-like influence on seeds that were germinated. It was also proposed that the auxin-like impact of MF explains how tomato fruits mature. (Boe and Salunkhe, 1963). Other plants' roots were examined, and it was concluded that some innate characteristic of a species, or possibly a group of species, may have been necessary before the tropism manifested. (Pittman, 1962). The impact of MF intensities greater than GMF levels has been discussed in an adequate number of articles. Intensities greater than GMF often correspond to values greater than 100 microT. Experimental values can reach very high MF levels, ranging from 500 microT to 15 T, as shown in **Table 1**'s summary. Most of the research has been on the germination of seeds from significant crops including wheat, rice, and legumes. However, numerous other physiological impacts of high MF on plants were identified, and they included changes in redox state, photosynthesis, growth, and development.

Although magnetic field exposure effects to stimulate growth and development are well-documented, the molecular impact is not well-understood. Thus, a comprehensive understanding of magneto-reception dynamisms in plants necessitates more in-depth approaches.

The proposed study can contribute to the fundamental understanding of magnetic field effects on plant development through a comprehensive molecular approach. As well as developing specific Electromagnetic Field (EMF) protocols that may enhance plant growth and development in various plant species. The objectives of this study are:

1.) To screen plant growth responses to magnetic field exposure based on overall biomass production and selected morph-physiological growth parameters.
2.) To screen plant metabolomics responses and the secondary metabolite expression in control versus exposed to magnetic fields through high-performance liquid chromatography (HPLC)-electrospray ionization mass spectrometry (MS)
3.) Identify the effects of magnetic fields in plants by transcriptome profiling to characterize genetic responses, and potential differential gene expression patterns in exposed plants.

CHAPTER 2

LITERATURE REVIEW

Magneto-reception in Plants

Plants are extremely reactive to their environment and the various stimuli present around them (Maffei, 2014). Due to the reactive nature of plants, it is no question that they are also affected by the GMFs of the environment, furthermore these GMFs have undoubtedly affected plant evolution and their biological processes as well (Occhipinti, 2014). Because of this, investigations of the influence of magnetic fields on organisms have been of increasing interest to researchers (Maffei, 2019). The natural GMF from the Earth extends far into space and is produced due to the thermal convection of the liquid iron inside the Earth.

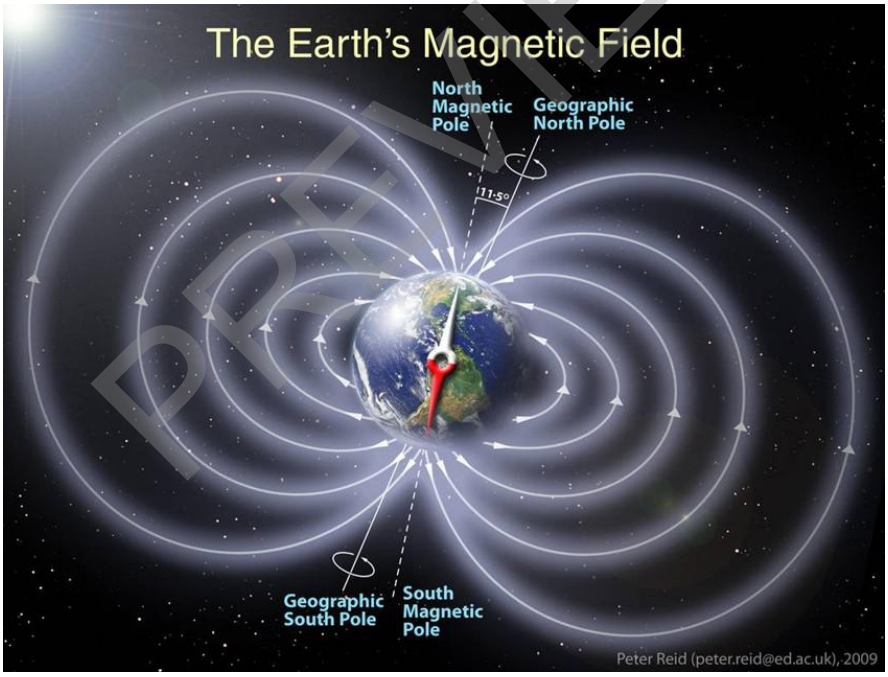

Figure 1. Schematic Illustration of Earth's Magnetic Field
(Reid, The University of Edinburgh)

To generate a uniform magnetic field a machine named the Helmholtz coil is necessary. Hermann von Helmholtz, a German scientist, and philosopher invented Helmholtz coils in 1849.

A Helmholtz coil is a pair of specially designed coils mounted on a common base at a fixed distance apart. The space between the coils is filled with a uniform magnetic field created by currents flowing through them. To measure the magnets strength, the coils are used in a different way. They are attached to an integrating fluxmeter, which provides a precise indication of the magnet's overall strength when it is rotated by a half-turn or full turn. A Helmholtz coil quickly, accurately, and simply measures the entire magnet at once. Helmholtz coils are therefore ideal for inspecting the quality of magnet parts after they have been magnetized.

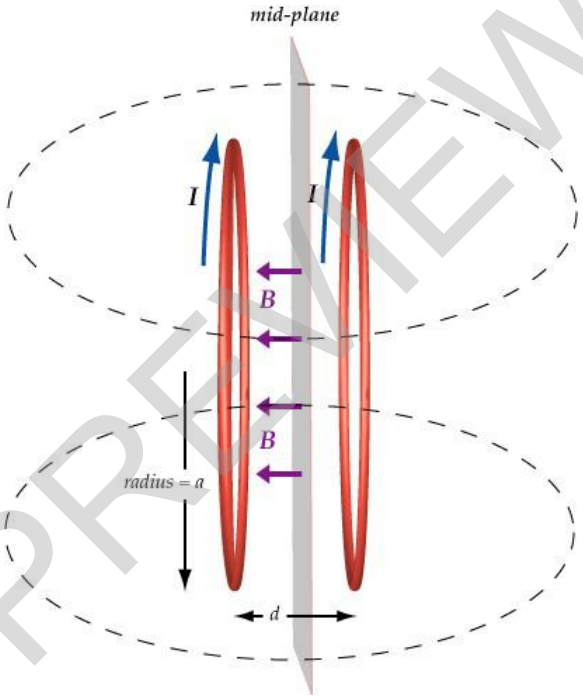

Figure 2. Helmholtz Coil Magnetic Field Illustration (Wire, 2012)

A moving charge in space is referred to as a "current" (denoted by the symbol I) and is measured in coulombs per second or amperes. A magnetic field is formed whenever a charge is in motion, whether it is moving through space or spinning around itself. The strength of a magnetic field is measured at a specific location in space (often called the field point). The field points of interest

in the Helmholtz coils are located within the mid-plane between the two coils. The strength of the magnetic field is determined by three variables, as shown in the equation above: the current **I**, the number of turns **N** in each coil, and the radius **a** of the coil. The total current in each coil is **NI**.

Researchers ponder whether plants can respond to magnetic fields (MF) and how it affects their short- and long-term physiological development. This could give insight into plant evolution as it is today and in the future.

Exposure of Plants to a Lower MF Intensity than GMF

The term "weak" or "low MF" typically refers to intensities between 100 nT and 0.5 mT, while "superweak" or "conditionally zero" (also known as the "magnetic vacuum") refers to MFs below 100 nT. For several reasons, studies of low MF effects on biological systems have caught the attention of biologists. Interplanetary navigation, for instance, will put people, animals, and plants in magnetic environments where the MF is close to 1 nT. It is understood that a galactic MF induction is less than 0.1 nT, 0.21 nT in the neighborhood of the Sun, and on the surface of Venus at 3 nT (Belov and Bochkarev, 1983). This sparked a renewed interest in the function of MFs in controlling plant growth and development. (Belyavskaya, 2004). Two techniques used in laboratories to produce low MFs are shielding (enclosing the experimental area in ferromagnetic metal plates with high magnetic permeability, which deviate MF and concentrate it in the metal) and compensating (by using Helmholtz coils). Generally speaking, studies on the growth of plant responses have been carried out with varying MF intensities. Early in 1963, it was discovered that an MF with a moderate intensity could be useful for triggering or promoting plant growth responses. (Pittman, 1963).

Since then, a few studies have compared the outcomes of using low-MF conditions to those of high-MF conditions in order to assess the effects of low-MF conditions. While dry weights and germination rates remained unaffected, sunflower (*Helianthus annuus*) seedlings exposed to 20 T vertical MF exhibited slight but significant increases in total fresh weights, shoot fresh weights, and root fresh weights. (Fischer et al., 2004). Low MF intensities of 10 and 100 T at 50 or 60 Hz were found to affect membrane transport processes in root tips in broad bean (Vicia faba) seedlings (Stange et al., 2002), whereas soybean (Glycine max) seeds exposed to pulsed MF of 1500 nT at 0.1, 1.0, 10.0, and 100.0 Hz for 5 h per day for 20 days, induced by enclosure coil systems, significantly increased the rate of seed (Radhakrishnan and Kumari, 2013). Under controlled laboratory circumstances, MF treatment enhanced germination-related characteristics of soybean seeds, such as water uptake, rate of germination, length of seedlings, fresh weight, dry weight, and vigor indices. (Shine et al., 2011). There have also been reports of controversial statistics. Various in vitro cultures of distinct species of the genus Solanum were either stimulating or inhibiting the growth of in vitro plants when exposed to near null MF. The impact was influenced by the species, genotype, initial explant type, duration of treatment, and even culture medium. (Rakosy-Tican et al., 2005). Barley (*Hordeum vulgare*) seedlings grown in Helmholtz coils with a 10 nT MF intensity revealed a reduction in fresh weight of shoots (by 12%) and roots (by 35%), as well as dry weight of shoots (by 19%) and roots (by 48%), compared to GMF controls. According to this groundbreaking study's findings, very low MF can delay the growth and formation of organs. (Lebedev et al., 1977).

Table 1. Summary of Magnetic Field Effects on Plants less than GMF (Source: Frontiersin.org, 2014)

Plant species	Plant organ	Effect	MF intensity	References
EXPOSURE TO MF VALUES LOWER THAN THOSE OF THE GMF				
Actinidia deliciosa	Pollen	Release of internal Ca^{2+}	10 μT	Betti et al., 2011
Allium cepa	Root and shoot	Decrease in the cell number with enhanced DNA content	<GMF	Nanushyan and Murashov, 2001; Belyavskaya, 2004 and references cited therein
Arabidopsis thaliana		Delayed flowering Reproductive growth	Near null	Xu et al., 2012, 2013
Glycine max	Protoplasts Seeds	Increased protoplasts fusion Seed germination	<GMF 1500 nT	Nedukha et al., 2007 Radhakrishnan and Kumari, 2013
Helianthus annuus	Seedlings	Increases in fresh weight	20 μT	Fischer et al., 2004
Hordeum volgare	Seedlings	Decrease in fresh weight	10 nT	Lebedev et al., 1977
Lepidium sativum	Roots	Negative gravitropism	<GMF	Kordyum et al., 2005
Nicotiana tabacum	Protoplasts	Increased protoplasts fusion	<GMF	Nedukha et al., 2007
Pisum sativum	Epicotyl	Promotion of cell elongation; ultrastructural peculiarities increase in the $[Ca^{2+}]_{cyt}$ level	<GMF	Negishi et al., 1999; Belyavskaya, 2001; Yamashita et al., 2004
Solanum spp.	*In vitro* cultures	Stimulation/inhibition of growth	<GMF	Rakosy-Tican et al., 2005
Triticum aestivum	Seeds and seedlings	Activation of esterases reduction of growth	from 20 nT to 0.1 mT	Bogatina et al., 1978; Aksenov et al., 2000
Vicia faba	Root tips	Alter membrane transport processes	10 and 100 μT	Stange et al., 2002

Physio Morphological changes in Plants under Magnetic Field Exposure

Abiotic and biotic stresses, as well as dynamic interactions between DNA, RNA, proteins, and metabolites, all contribute to the phenotype of an organism. Growing interest in "phenomics," or the application of extensive methods to ascertain the relationship between gene expression and the manifestation of physical traits, is the result of recent developments in sequencing and phenotypic technologies. Plants can experience physiological and molecular changes in growth and development due to environmental stimuli (such as different light wavelengths, gravity, touch, electromagnetic stimuli, etc.).

The effect of GMFs on plant behavior and development was first proposed by Krylov and Tarakanova. The pair suggested an auxin-stimulated growth response in germination seeds due to magneto tropism in the early 1960s (Krylov and Tarakonova, 1960). This phenomenon was used to characterize a tomato ripening effect three years later (Boe and Salunkhe, 1963). To understand how plants perceive gravity, Audus and Whish exposed plant organs to magnetic fields (Audus and Whish, 1964). Sweet pepper (*Capsicum annuum L.*) seeds subjected to 57–60 mT revealed increased germination rates, better fruit quality, quicker growth rates, and higher phosphorus and vitamin C concentrations (Ahamed et al., 2013). In the same way, Grewal and Maheshwari (2011) found a correlation between magnetic treatment of seeds (3.5-136 mT) and substantial increases in the emergence rate index, shoot dry weight, and nutrient content in chickpea (*Cicer arietinum*) and snow pea (*Pisum sativum var. saccharatum*).

Additionally, it has been proposed that magnetic field exposure (MFE) may affect a plant's ability to form proteins and develop roots (Fu, 2012; Aladjadjiyan, 2002; Rcuciu et al., 2006). In soybean (*Glycine max*), MFE was linked to higher production of moieties, chlorophyll contents, and reactive oxygen species-scavenging enzymes (Asghar et al., 2016). Low-intensity magnetic fields aided in the epicotyls' elongation, which is most noticeable in the central section (Negishi et al., 1999). According to more data, plants exposed to various magnetic field frequencies may alter the synthesis of macronutrients and fruits (Eşitken and Turan, 2004). Despite these findings, there has been controversy regarding how plants react to MFE at various intensities. According to Tkalec et al. (2005), plants exposed for two hours to a 23V/m electromagnetic field at 900MHz grew less than controls, whereas a similar electromagnetic field at 400MHz had no impact. Arabidopsis spp. plants grown in a near-null magnetic field (50nT) took longer to flower, and the removal of the local geomagnetic field had a detrimental impact

on reproductive growth (2012) (Xu et al.). After MFE at a comparatively low intensity, a discernible reduction in the fresh weight of shoots and roots as well as the desiccated weight of shoots and roots was seen in barley (Hordeum volgare) (Lebedev et al., 1997). MFE of *Solanum spp*. at near-null either stimulated or inhibited plant growth during in-vitro experiments. According to the results, the outcome was influenced by the species, genotype, original explant type, treatment duration, and culture medium (Rakosy-Tican et al., 2005). A lack of reaction from the reporter gene after MFE disproved the claim made by Sztafrowski et al. (2017) that CTCT sequences in plant promoter regions may function as electromagnetic field response elements. There have not been many thorough analyses that show the MFE's basic effects. It is possible to take a fresh approach to figuring out the fundamental effects of MFE in plants during growth and development by using conventional methods created for accurate phenotypic and molecular analyses.

Metabolomic Studies of Plants

The metabolome of an organism is a complex collection of thousands of molecules that differ in size, polarity, quantity, and stability (Kim et al., 2011; Viant et al., 2017). It reflects the organism's metabolic state and sheds light on the metabolic pathways that have been impacted by external stresses. A variety of environmental stress responses, such as high soil salinity (Zhang et al., 2016), drought (Michaletti et al., 2015), and the distinction between genetically related cultivars, can be detected molecularly in plants by metabolic profiling. Metabolomics is the branch of science concerned with the qualitative and quantitative study of the metabolites of integrated living systems and their dynamic responses to changes in the environment. The relationship between cell changes and phenotypes—which accurately depict the physiological