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Research Article

Effect of magnetic field on germination and early growth of soybean (*Glycine max*) seed.

Ghafoor Ahmad[1], Muhammad Ali[2], Muhammad Usman[3] and Samreena Rasheed[*1]

[1]Department of Chemistry, Bahauddin Zakariya University, Pakistan
[*1]Department of Physics, University of Agriculture Faisalabad, Pakistan
[2,3]Department of Physics, Bahauddin Zakariya University, Pakistan

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Abstract

Magnetic field is beneficial and essential tool in modern research. Magnetic field is a force that can be made by moving electric charges and magnetic dipoles which apply a power on other close-by moving charges and magnetic dipoles. Magnetic field has numerous applications in our daily life. Nowadays it is used in agriculture to enhance the seed germination, plant growth and yield because it has no toxic effect on environment as need us on soil. In this research work effect of magnetic field on soybean seed (Glycine max) was investigated. The soybean seed was taken from institute of Horticulture science. Non treated seeds were served as control while treated seeds were served as magnetically treated seeds. Seeds before sowing were treated with magnetic field stimulator which is fabricated in department of Physics University of agriculture Faisalabad, by varying magnetic field for different time exposure. The seed was exposed for different time duration and different magnetic field strength. The seeds were grown in three replicates in Petri dishes as well as in pots. The different parameters like germination, seedling growth, seedling fresh weight, seedling dry weight, root length, shoot length, root dry weight, shoot dry weight, leaf area, enzymes activity, proteins content, chlorophyll content and antioxidant activities were measured. It was noticed that the magnetic field effected the seed germination and other parameters. Difference between treated and non-treated plant seeds was checked by using different statistical tests namely RCBD, CRD, and ANOVA.

Keywords: *Magnetic field, Glycine max, RCBD, CRD, ANOVA*

1. Introduction

On the earth the magnetic field (MF) is the factor of inescapable. The natural component of the environment is Earth's MF (geomagnetic, GMF), which is experienced by all living organisms during the evolution process. Many biological processes influenced by the GMF which is regularly acting on living systems. The earth's magnetic field (geomagnetic, GMF), has significant differences in the direction and strength. The value of the vertical component is about 67μT which is maximum at magnetic pole and is zero at the magnetic equator at the surface of earth. The value of horizontal component is about 33μT, which is maximum at the magnetic equator, and is zero at the magnetic poles. The magnetic induction of the magnetic field is important to measure its intensity and its unit is Tesla (T) (Maffei et al., 2014). Soybean is considering as an important source of food for humans and animals. In pharmaceutical and industrial applications, various parts of soybeans can be used. Soybeans could also be considered as a major part of poultry feed in poultry industry. Protein, oil and carbohydrates are considered as primary seed storage compounds, soybean has numerous types of minerals, vitamins, and secondary metabolites, which are important for human health. The environmental and genetic factors affect the above-described seed components. However, the soil salinity plays an important role in the reduction of the yield of the soybean (Radhakrishnan *et al*., 2012).

The magnetic field is used to treat the seeds which has been developed as a valuable physical method to increase the post-germination of plant responses. The parameters like magnetic induction "B" and exposure time "t_E" are considered as the pre-sowing exposure system and they are related with post-sowing physiological effects. The mechanisms of interaction between biological material and the physical agent are not completely appreciated nor the effect of other factors that vary its action. The treatment of seed with magnetic field has minimum risk to damage it and its manipulation is very easy as compared to other parts such as leaves, stems or roots. The process of treatment of seeds with magnetic field has been

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discovered due to its beneficial effects on the quality of the seed. There are many hypotheses which have been introduced which could explain, how magnetic field affected the yield relating traits due to the modifications in morpho-physiological traits (Garcia1 *et al*., 2013).

Nowadays, the changes determined at the living systems which were exposed to different magnetic field strength and periods of magnetic field (MF) and electromagnetic field (EMF) with the lowest frequency have also been drawn the attention of the biologists, molecular biologists and chemists as physicists. For recent 30 years, studies have been done concerning the effects of magnetic field on microorganism, tissue, cell and subcellular structures of the plants and animals (Polk *et al*., 1995).

The researchers have shown that magnetic field changed the characteristics of cell membrane, effected the cell reproduction and caused some changes in cell metabolism. At the same time, it was put forward that magnetic field effected the growth characteristic and various cellular functions like mRNA quantity, gene expression, protein biosynthesis and enzyme activities and caused the changes concerning the various functions at the organ and tissue levels (Stein *et al*., 1992).

Electromagnetic fields (EMF) and Magnetic fields (MF) have been widely utilized as pre-sowing seed accomplish to enhance shoot growth and capitulate. In previous study's author have been establish that magnetic action generates Bio-stimulation of the premature growth stages and in the early hours creating about more than a small amount of seeds types. Stationary magnetic field acts have been establishing by different authors that as a non-invasive external germination and increase refreshment (Martinez *at al*., 2014).

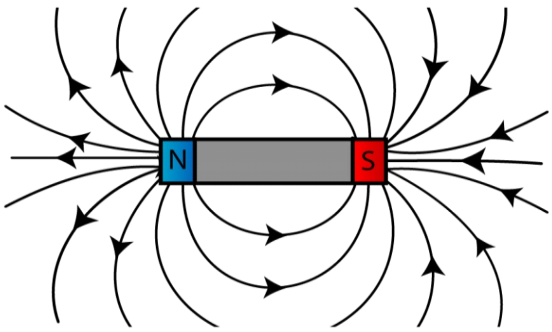

Figure 1.1: Representation of magnetic field lines around a bar magnet

Several researches have been originating on the pretreatment impact of EM field on plant's growth and seed germination. Different scientists have confirmed that when seeds are treated with MF their germination rate and vigor increase (Asgharipour and Omani, 2011).

Firstly, seeds must be treated by magnetic field and sowing. The bases of the future plants are, to increase the quality of seed and their properties of germination improved by magnetic field during their generative and vegetative period. Due to magnetic field certain type of biochemical and physiological variations in seeds occur which results as growth in breathing of seeds germination, water absorption becomes rapid and its feasibility increased (Lynikieneet*et al*., 2012).

Instead of these factors influences of magnetic and electric field also play an important role on germination of seed and early growth of seed.

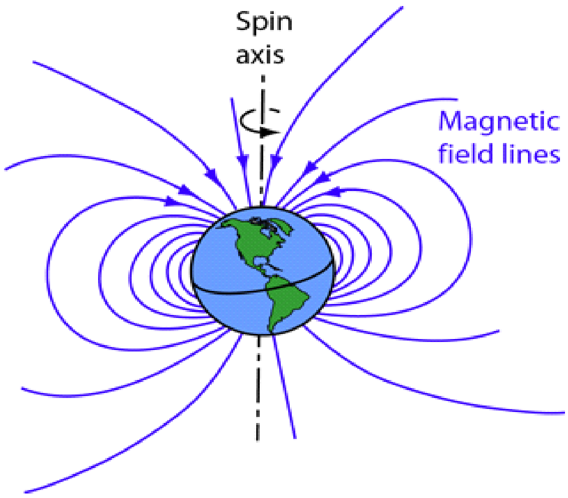

Figure 1.2: Earth Magnetic Fields

It is inferred plant improvement reaction might have been initiating and stimulating by low intensity magnetic field (Pittman *et al*., 2016). Magnetic fields are generated by electric currents, which can be macroscopic currents in wires, or microscopic currents related with electrons in atomic orbits. The magnetic field B is defined in terms of force on moving charge in the Lorentz force law. Magnetic field sources are fundamentally dipolar in nature, having a north and south magnetic pole. The SI unit for magnetic field is the Tesla which can be seen from the magnetic part of the Lorentz force law.

$F_{magnetic} = qvB$

To be composed of (Newton x second)/ (Coulomb x meter). A smaller magnetic field unit is the Gauss (1 Tesla= 10,000 Gauss). Both magnetic field and electric field can be characterized from the Lorentz force law. The Earth magnetic field is approximately a magnetic dipole, with the magnetic field S pole closer to the earth's geographic north pole and the other magnetic field N pole near the Earth's geographic south pole. The cause of the field can be explained by dynamo theory. The earth's magnetic field, which efficiently extends few many tens of thousands of kilometers into space, is called the magnetosphere. It was observed that seed lots of flax (Linumusitatissimum L.), buckwheat (Fagopyrum esculentum Moench.), sunflower (Helianthus annus L.), and field pea (Pisum sativum L.) exposed to magnetic fields produced earlier and more vigorous seedlings and improved seedling vigor due to pre-germination (Florez*et al*., 2007)

It has been shown that both low frequency electromagnetic field (EMF) and magnetic fields (MF) influence on microorganisms, plants and animals. There are many studies showings that magnetic field flux and exposure time affects different features of plants positively or negatively. Today we know that magnetic fields have a positive effect on plant characteristics such as seed germination, seedling growth, agronomic traits and seed yield. In addition, shoot and root regeneration was increased in cultured explants exposed to magnetic fields (Emiroglu*et al*., 2003).

In vitro research has shown that EMF changes cell membrane characteristics, cellular functions and growth. Magnetic field experiments showed that gene expression, protein biosynthesis, enzyme activity, cell reproduction and cellular metabolism increased relative to controls. In other studies, which were made in order to show the effects of the magnetic field on plants, researchers used the conditions of the geomagnetic field (gmf) and on its 105- 106-fold screening. Significant changes in the duration of the G1 phase of the cells of the root meristem were observed and during this period, RNA and protein synthesis under the gmf conditions were intensive (Rowland *et al.,* 2002).

Biochemical reactions that have more than one unpaired electron were affected by magnetic fields. More than 50 enzymes (like heme enzymes), which produce free radical products during catalysis were affected by magnetic field treatment. Peroxidases (POX) are heme-containing glycoproteins encoded by a large multigene family in plants. Studies have suggested that POX plays a role in lignification, suberization, auxin catabolism and self-defense against pathogens, salt tolerance and senescence. It is known that auxin has a role in root formation. There is a relationship between an increase in IAA and root induction. Peroxidase has a role in the formation of the connection between auxin metabolism (IAA-oxidation) and cell wall complex (Sasaki *et al*., 2001).

Many studies have also shown that peroxidases play a role in the plant growth process. Cytokinin's play an important role in axillary bud growth, chloroplast development and shoot formation and the delay of senescence. Cytokinin's also regulate the expression of plant peroxidase genes. Peroxidases have several very important roles in plants. They are known as good physiological markers of rooting in many species (Kim *et al*.,2002).

POX plays a role in the formation of cofactors, which are necessary for root initiation. Lignification in the cell wall, a process that is catalyzed by peroxidases, may occur during rooting. The tomato gene TPX1, which encodes a basic peroxidase product, is involved in the synthesis of lignin and suberin. Exposure to a magnetic field has been shown to stimulate shoot and root regeneration in explanted soybean tissue cultures (Emiroglu*et al*., 2003).

2. Experimental

A field study was designed to assess the growth response of seeds as affected by a magnetic field. The soybean seeds selected as experimental material. Well ripened and uniform sized seeds of soybean were obtained from the institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan. Healthy, well ripened and uniform seeds of soybean were selected and cleaned to make them free debris.

2.1 Plant Material

The research material used for this study was consisted of soybean seeds. The seeds were genetically uniform and only healthy seeds were selected. These seeds were divided into two groups.

I) Control seeds (without treatment)
2) Seeds were exposed to magnetic field

Seven polythene bags were filled with seeds. One bag was considered as control and other six bags were exposed to magnetic field.

2.1.1 Magnetic Seeds Stimulator

Seeds of soybean were treated with magnetic field before sowing by using, the Magnetic seed stimulator also known as electromagnet, which was fabricated in Bio Electromagnetic lab in department of Physics, University of Agriculture Faisalabad. Bio simulator having model No 85396 was constructed and placed in Bio Electromagnetic research lab, department of physics, University of Agriculture Faisalabad. This simulator consisted of a wire which is used to generate magnetic field around the conductor. The thickness of wire which was determined with the help of screw gauge is about 0.0004 m. Each coil has number of turn around it. The generation of magnetic field mostly depends on no. of turn on the coil. Greater is the no. of turns around the coil, greater will be the generated magnetic field.

The wounding wire was separated based on no. of turns. First layer has two thousand and three hundred no. of turns on the coil while second layer of wire has three thousand no of turns on the coil respectively. Wire offered resistance which was measured with the help of AVO meter. The resistance of that wire was about to be seventy-four ohm.

When electric current is changed with the help of voltage regulator, a magnetic field is generated in the simulator which can be adjusted by voltage regulator. A change of 5V of potential difference induces magnetic field of 10 mT in the coils. No type of magnetic field other than that of earth own magnetic field was sensed inside the experimental electromagnet as soon as switched off.

2.2 Methodology

The methodology adopted to treat the soybean seeds for different intensities of magnetic field for different time intervals is given in table below.

Table 2.1: Scheme about magnetic treatment

Treatment	Magnetic Field Strength(mT)	Time of Exposure (Seconds)
T_0	Control	--
T_1	75	30
T_2	125	30
T_3	175	30
T_4	75	60
T_5	125	60
T_6	175	60

Table 2.2: Scheme about plantation

T_0R_1	T_1R_1	T_2R_1	T_3R_1	T_4R_1	T_5R_1	T_6R_1
T_0R_2	T_1R_2	T_2R_2	T_3R_2	T_4R_2	T_5R_2	T_6R_2
T_0R_3	T_1R_3	T_2R_3	T_3R_3	T_4R_3	T_5R_3	T_6R_3

2.3 Germination of soybean seeds

2.3.1 In vitro studies

The *in vitro* experiment was conducted in the Bio Electromagnetic research Laboratory of Department of Physics, University of Agriculture, Faisalabad in Petri dishes (10 cm diameter Pyrex Band). As there were seven treatments with three replications of each treatment, so 21 Petri dishes was used. Each Petri dish carried 5 seeds. Firstly, Petri dishes were sterilized by keeping in autoclave machine at the temperature of 121^0C and pressure 15 pascal for 20 minutes.The number of germinated seeds in all the Petri dishes was calculated after 24, 33, 48 and 96 hours of sowing. The germination data was recorded on daily basis. Seeds as untreated (control) were also tested. Data were recorded till no more seed germination. During germination parameters data such as germination percentage, Mean Emergence time, Seed Germination index, percentage effects were noted and calculated accurately. For %Germination number of emerged seeds was recorded daily according to the seedling evolution.

2.3.2 *In Vivo* studies

In order to see the effect of varying degrees of magnetic field treatment and exposure time the soybean seeds were sown in pots. For this, soybean seeds were sown in soil which is same in all pots. 10 seeds were sown with uniform interval at a uniform depth of half inch in every pot with the help of marked stick. A measured volume of 240 mL water per pot was applied in pots. Soon after sowing as the experiment was conducted in triplicate, 30 seeds of every treatment were soon in 3 pots with three replications. Untreated seeds were kept as control for the purpose of comparison. All plant groups were irrigated daily only with water during experiment, 150 mL water per pot. However, Experiment was continued for another 12 days to observe differences in seedlings growth and weight. The brief description of these parameters is presented below.

2.3.3 Analysis of Growth and Related Parameters

To analyze the growth, six plants were selected randomly from each replication, 2 small, 2 medium and 2 larges. Then all physical parameters of these six plants was calculated after 12 days of grown with the help of measuring tape and weight balance. Similarly, after 24 days of grown for second reading plants were extracted from each replication of all treatments. Similarly, the third reading was taken after 36 days of germination. For finding significant difference of treated plants with controls one, analysis of variance technique (ANOVA) was applied.

2.6.6 Determination of total phenolic contents (TPC)

The total phenolic contents in sample extracts were determined by Folin-Ciocalteu method (Folin and Ciocalteu, 1927). 1mL of sample was mixed with 5mL of Folin-Ciocalteu (10%) and 4mL of sodium carbonate (20%) and incubated for 1 hour. The absorbance of the resulting blue color complex was measured at 765nm. Quantification was done with respect to the standard (gallic acid). The calibration curve was prepared with different concentrations of gallic acid. 1mL aliquots of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.10mg/mL gallic acid solution in methanol were mixed with 5mL of Folin-Ciocalteu reagent (diluted ten folds) and 4mL of sodium carbonate (20%). The absorbance was noted after 1 hour at 765nm and the calibration curve was plotted by taking absorbance as a function of concentration. Total content of phenolic compounds in plant extracts in gallic acid equivalents (GAE) were calculated by the following formula.

$$T = C \times V / M$$

Where

T = total contents of phenolic compound in mg GAE/g plant extract.
C = the concentration of gallic acid calculated from calibration curve in mg/mL.
V = the volume of extract in mL.
M = the weight of plant extract in grams.

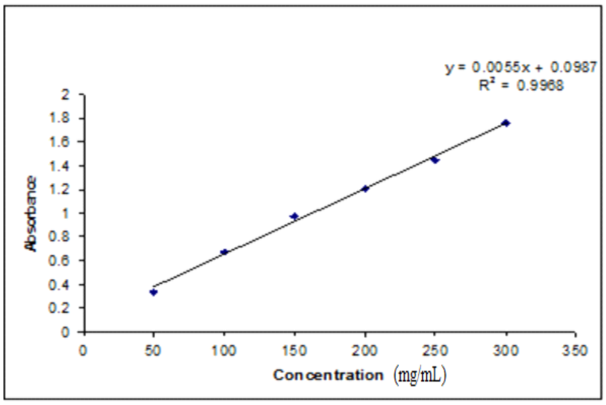

Figure 3.3: Standard curve of Gallic acid

2.6.7 Total flavonoids contents

The total flavonoid contents of plant extracts were determined according to the method given by (Chang *et al*., 2006). 0.5mL of plant extract was mixed with 2mL of distilled water and 0.15mL of 5% $NaNo_2$ solution and incubated for 6 minutes. After that 0.15mL of 10% $AlCl_3$ solution was added to that and again incubated for 6minutes followed by the addition of 4% NaOH solution to the mixture. Volume of the reaction mixture was made up to 5mL by the addition of methanol and mix well. Absorbance of the reaction mixture was taken at 510nm after incubation for 15minutes. Total flavonoid contents (TFC) of the extracts were expressed as catechin equivalents from the linear regression curve of catechin.

3. Results and discussion

It has been observed that electromagnetic (electric and magnetic) fields cause physiological and biochemical changes in seeds. Water assimilation becomes faster breathing and photosynthesis of germination seeds intensifies, and all this results in improved viability of a variable seeds.

Influence of an alternating magnetic field depends upon on the value of the magnetic induction. A magnetic field affects germination mainly during the initial 50h of the process. The highest positive influence of the field was observed in the field with magnetic field induction 50 and 80mT.

Conclusion

The aim of this research was to test the effect of different magnetic field strength on physical and biochemical Parameters of soybean seed. Two experiments were conducted in present research. In first experiment; the seeds of soybean were germinated on wet filtration paper. This paper is set in Petri dishes under well controlled Laboratory conditions in the Physics Department University of Agriculture FSD. Germination, seedling growth, seedling fresh and dry weights were calculated in this experiment. Seedlings were dried in electric oven of bio electromagnetic lab of Physics department. Fresh weight and dry weight of seedling were calculated with the help of electric balance.

In second experiment soybean seeds were germinated in plastic pots which were filled with the same soil in under well controlled laboratory conditions in Physics Department University of Agriculture Faisalabad. Seeds were sown into the pots near half inches of depth. Percentage of seed germinated, Mean emergence time, seed germinating index, shoot length, root length, root fresh weight, shoot fresh weight, root dry weight, shoot dry weight of soybean plants and were checked in this experiment. Both fresh and dry weight of soybean plants was measured with the help of electric balance. Plants height was calculated with the help of measuring tape. This research showed that magnetic field has positive effect on all measured parameters. Magnetic field enhances the germination of all parameters. The lowest value was obtained for controlled seeds.

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