International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Influence of Plant Growth Regulators and Magnetic Field on Development of Nutritionally Important Tomato Plant

Sandeepa Singh[1], Poonam Juneja[2]

[1]Department of Botany, Maitreyi College, University of Delhi
[2]Department of Physics, Maitreyi College, University of Delhi

Abstract
Plant hormones are signaling molecules and growth substances that occur naturally in plants. Growth regulators such as auxin and cytokinins play significant roles by controlling plant growth, development, flowering, fruiting, aging, leaf fall, etc. Alongside, the geomagnetic field is a natural element of earth's environment which influences the response of plants to gravity, different wavelengths of light, electrical signals, etc. Based on the type of plant used, growth and proliferation of plant organs heavily depends on the presence of nutrients and growth regulators in the growth medium, and by magnetic influence in the environment. In our present study, we have found a definite positive correlation between the two external factors i.e., magnetic field and phytohormones (NAA and BAP) upon plant growth. It was clearly observed that the growth of the experimental crop - tomato (*Solanum lycopersicum*) was positively influenced by phytohormones and magnetic field when the seedlings were grown in presence of 0.5 mg NAA and 1.0 mg BAP and kept under magnetic field of 100 and 150 Gauss. Vegetative growth of tomato seedlings was significantly increased upon treatment with magnetic field in presence of plant hormones, as compared to the control plants.
The current research is a **pioneer endeavour** in studying the role of plant growth regulators together with magnetic fields by evaluating the seedling growth responses to MF values higher than those of earth's magnetic field.

Keywords: MF (magnetic field), phytohormones, NAA (naphthalene acetic acid), BAP (6-benzylaminopurine), PGRs (Plant growth regulators)

1. Introduction

With the increase in global population, methods to increase yield have constantly been researched. Worldwide, agriculture faces the serious challenge of achieving production goals to meet the requirements of increasing population; scientists and farmers are constantly making significant improvements to enhance productivity while protecting the environment. Under natural conditions, food production is seriously affected by challenges posed by soil salinization and drought intensity, particularly in arid and semi-arid regions. High salinity soils are difficult for agriculture due to adverse effects on plant growth and crop yield [1, 2]. In addition, drought and salinity inhibit the activities of certain enzymes and disrupt cellular metabolism, which leads to plant death [3, 4].

Solanum lycopersicum (tomato) is a major vegetable crop, which is known commonly, only from the last century, used both as fresh and processed food. Tomato is a nutritionally important and economically strategic crop and consumed by major civilizations all over the world. Nowadays, increasing demand of the crop by the growing population is still a challenge in many countries especially in Singapore, Maldives, Bhutan etc.; therefore, the need to address the increasing gap between production and consumption of tomato. During the last two decades many biotechnological approaches have been focused on the improvement of tomato crop, which can grow in different agro-climatic zones to meet the demands [5]. Although, there are many reports on tomato culture, but only very few studies have been reported by Indian cultivators. In spite of the best efforts for improving tomato varieties, the yield of this crop remains low. Several studies have been made to understand their performance which mainly include the contribution of various components towards yield [6]. Hence, tomato has been chosen for our research investigation, to develop a better and reliable protocol for its growth.

The growth of plants is known to be affected by various internal and external factors including climatic changes and environmental stress [7]. Major factors include application of magnetic field and another, phytohormones to increase plant yield. Emerging evidence strongly suggests that a mixture of different auxins, cytokinins, and gibberellins plays an active role in governing different aspects of plant development in its life cycle.

The earth is a giant magnet and its geomagnetic field (GMF) influences productivity of crops tremendously by regulating the most important physiological processes, including photosynthesis, transpiration, respiration, and cell division in plants [8]. The presence of magnetic field (MF) is an inescapable environmental factor for plant life on the planet Earth. In fact, all living organisms have experienced the effect of the Earth's MF during the course of evolution. The GMF influences many biological processes and acts steadily on living systems. The magnetic field affects various biological activities of the plant such as germination, absorption of blue light by cryptochrome and overall growth.

The Earth has its own magnetic field called the geomagnetic field (GMF), whose magnitude is around 25 to 65 μT at the surface. Hence, all the living organisms on the Earth are subjected to a weak magnetic field (around 1.5 mT) constantly, which affects their biological activities. There are substantial variations in the strength and direction of the earth's magnetic (geomagnetic) field. On earth's surface, the vertical component is maximum at the magnetic pole, measuring about 67μT and at the magnetic equator it is zero. Conversely, the horizontal component is maximum at the magnetic equator, amounting to about 33μT, and is zero at the magnetic poles [9].

Different species of plants respond distinctively to magnetic fields based on the duration of exposure and intensity of MF. By observing and studying the positive physiological responses of different species of plants towards the MF, magnetic fields can be employed as potential growth enhancers and this knowledge can be utilized in agriculture development research. Podlesny et al. (2005) [10] demonstrated that enhancement in agriculture production by physical methods like magnetic field can be considered harmless for the environment and effective in modifying the physiological and anatomical characteristics in plants. Further, the magnetic field not only alters the chemical pathways in the plant, but also affects various physical properties of solutes inside the cytoplasm of plant cells [11]. Numerous studies have demonstrated that such treatments are effective for enhancing agronomic seed quality and have potential to be used for seed decontamination, activation of germination, and seedling growth.

Magnetic fields can either have stimulatory effects on the growth of plants or may not have any significant effects. A low-frequency magnetic field (MF) of 20 mT / 40 mT / 60 mT when applied on the seeds and young seedlings of tomato with exposure time 20 minutes a day, resulted in an increase in the rate of germination, size of tomato fruit and an increase in the quality as well as quantity of tomato fruits [12]. The effect of short-term exposure (20 seconds and 40 seconds) to magnetic field (MF) of 50 Hz, 0.5 mT on wheat (*Triticum* spp.) seeds was studied and the fresh weight and height of the seedlings were observed, which gave varying results depending on the time of exposure [13]. Hence, there is a relationship between pre-treatment exposure time and physiological response of the plant. Another study showed that when Mung seeds (*Vigna radiata*) were exposed to 5mT, 10mT, 30mT and 60mT of MF, prior to germination with an exposure time of 15, 30, 45 and 60 minutes, there was an increase in the rate of germination till 10mT of MF, after which the rate of germination decreased with an increase in the strength of MF. The speed of germination increased significantly up to 45 minutes of exposure time and decreased as the exposure time increased [14]. Studies also showed better root formation in maize as a result of magnetic treatment [15]. Further study shows biostimulation in tomato when exposed to magnetic field [16]. Negative effects of electromagnetic field on plants have also been recorded such as decrease in fresh weight in *Hordeum vulgare* seedlings when exposed to 10 mT [17] and delayed flowering in *Arabidopsis thaliana* at near null MF [18, 19].

Apart from magnetic field, plant hormones play a significant and key role in overall development of plants. Plant growth regulators (PGRs) or phytohormones (PHs) are internally secreted signal molecules produced in very low concentrations that regulate plant growth. There are two types of phytohormones - inhibitors and promoters. Plant hormones tend to promote plant growth at lower concentrations, while at higher concentrations they may have inhibitory effects [20]. They play a crucial role in regulating different biochemical and physiological processes governing plant growth under optimum and stressful conditions. Further, the interaction between PHs is important for survival of plants as they stimulate signaling pathways [21].

Various plant growth regulators (PGR) have varied anatomical and physiological effects on the plant. Although macro and micro nutrients of in vitro culture media may not vary greatly from species to species, for successful plant regeneration the concentrations of growth regulators (both auxin and cytokinin) are critical and are specific to genotype, explant type and need of the project. It has also been reported that each species required a particular hormone concentration for optimum growth and differentiation [22].

The PGRs under consideration here are of classes auxin (namely NAA) and cytokinin (namely BAP). Auxins are a group of endogenous PGRs responsible for mainly growth and formation of root and shoot in the plant body along with other cellular or physiological processes like cell expansion, root initiation, embryonic development, fruit setting, apical dominance and inhibition of abscission [23, 24]. Under normal conditions, auxin is produced from the apical meristem of plants shoots, buds and tips of root. The effect of auxins in plants is dependent on their concentration. It has been proven experimentally that auxins work together with CKs during various physiological processes such as cell cycle progression, leaf development and seed maturation [24] and influence the plant growth responses under environmental stresses. The in vitro assays have demonstrated that there is one tryptophan (Trp)- independent auxin biosynthetic pathway whereas four Trp-dependent pathways, namely indole3-acetamide (IAM) pathway, tryptamine (TAM) pathway, indole-3-acetaldoxime (IAOx) pathway, and indole-3-pyruvic acid (IPA) pathway [25]. Several studies support the important role of auxin in controlling the physiological and metabolic processes during drought and salinity stresses [26].

Cytokinins (CKs) are another group of PGR associated mainly with cell division (cytokinesis), delay in senescence, bushier branches, meristem differentiation, etc. BAP is a synthetic cytokinin that stimulates

cell division. Auxin and cytokinin exhibit synergistic effects of regulating cell division and antagonistic effects of controlling plant growth and development, lateral root formation, bud formation and tolerance against various environmental stresses such as drought, salinity, and temperature [27]. Deficiency of cytokinin results in smaller apical meristem and stunted shoot. CKs are activated when plants are exposed to salt stress, especially when they interact with other plant hormones like auxins and ABA [28].
NAA is also a synthetic hormone belonging to the auxin family which also has positive effects on plant growth at lower concentrations. A study conducted showed that fruit yield of tomato increases significantly on auxin application, specifically NAA [29], whereas, another study on tomato showed taller plants at lower concentrations [30].
To add a distinct dimension to the available research, our study was aimed to gain insight into the cumulative impact of phytohormone supplements and magnetic field on the growth and morphology of our experimental plant - tomato (*Solanum lycopersicum*).

2. Materials and Methods

2.1 Inoculation of tomato seeds

The seeds of tomato (*Solanum lycopersicum*) variety "Pusa Ruby" were procured from Indian Agricultural Research Institute, New Delhi, India.
Surface sterilization of the seeds was carried out and the seeds were sown in eggtrays containing a mixture of soilrite, vermiculite, agropeat in the proportion of 1:1:1. Four seeds of each crop were sown in each compartment of eggtray and enough seeds were planted to get 30 young seedings for experimentation.
The eggtrays were exposed to 16h photoperiod and kept in the culture room at 28^0C. The eggtrays were watered every day to keep the soil hydrated and the seeds began to germinate after about 8 days.
16-day old small seedlings were subjected to experimental procedures, when they were about two inches in height. All the experiments were performed in triplicates. Along with the experimental plants, a large number of control seedlings of tomato crop were grown in absence of magnetic field and phytohormones for efficient comparison and compilation of experimental results.

2.2 Phytohormones

NAA - A concentrated stock solution of NAA was made at 1 mg/ml in water and kept in the refrigerator.
BAP - In a similar manner, concentrated stock solution of BAP was prepared at 5 mg/ml and kept in the refrigerator.
Both the hormones were diluted to required concentration and supplied to the seedlings by adding them into the soil.

2.3 Experimental Design

Well grown and healthy seedlings of tomato were subjected to experimental procedures, wherein they were exposed to different magnetic field strength i.e., 100 Gauss and 150 Gauss. All the seedlings were provided similar phytohormone treatment of 0.5 mg NAA and 1.0 mg BAP. Control seedlings of tomato were raised devoid of phytohormones and in absence of geomagnetic field.
In our experiment, three seedlings each of tomato, growing in each compartment of eggtray were taken forward for exposure to MF. Compartments or sections of the tray bearing seedlings were kept under the influence of magnetic field (100 Gauss and 150 Gauss) for three hours, on alternate days. Sets of seedlings of tomato were kept under 100 Gauss and 150 Gauss, such that the entire set up had six different sets of results. The experiments were carried out for 15 days and the results were analyzed.

Figure1: Electromagnets employed in the experiment

An Electromagnet (EMU-75) from SES Instruments, Roorkee [Figure 1] was used to expose the seedlings to the desired magnetic field. The electromagnet can deliver field Intensity of 11,000 ± 5% gauss in an air-gap of 10 mm. The air-gap can be continuously varied upto 100 mm with a two-way knobbed wheel screw adjusting system. Flat-faced pole pieces of 75 mm diameter and two energizing coils, each wound on non-magnetic formers and having a resistance of 12 ohms approx., form part of the electromagnet.
A Constant Current Power Supply, DPS-175, designed to be used with the electromagnet, Model EMU-75 has six closely matched constant current sources in parallel, which allows a smooth adjustment from 0 to 3.5 A per coil, i.e. 7A.
The Digital Gaussmeter, Model DGM-102 operates on the principle of Hall Effect in semiconductors and can measure 0-2 K gauss with a resolution of 1 gauss and accuracy of ±0.5% and has been used to measure the applied field.

3. Results and Discussion

The present investigation was carried out to demonstrate that treatments with magnetic field can have an important effect in increasing the growth of plants. In the past few decades, vast progress has been made in methods to explore regeneration potential and development in important plant species, but the efficiency is still low. To circumvent this bottleneck, a novel method was devised.
In our present study, 16-day old seedlings of tomato (*Solanum lycopersicum*) were grown in presence of phytohormones NAA (0.5 mg) and BAP (1 mg) in uniform manner and exposed to varying strengths of geomagnetic field and subsequently evaluated for the impact of these two external forces on plant growth and morphology. The parameters taken into consideration during the study were leaf length and shoot length of tomato seedlings. Apart from the experimental plants that were subjected to geomagnetic field and exogenous hormones, control seedlings were grown in absence of magnetic field and phytohormones to compare and analyze experimental data in unambiguous and accurate manner.
Data in Table 1A clearly indicates the marked increase in leaf length and shoot length of tomato seedlings, as compared to the control plants during the experimental period, when they were grown under influence of phytohormones and magnetic field.
It was clearly observed that there was a significant increase in the leaf and shoot length of seedlings in presence of a magnetic field of 100 Gauss. In tomato seedlings subjected to 100 Gauss field, the most significant increase in leaf length was 0.8 cm in leaf 1 of seedling 2 and shoot length was 1.4 cm in seedling 1 (Table 1-A).

Table 1-A: Tomato seedlings exposed to field strength of 100 Gauss

Day No.	Seedling	Length of Leaf 1 (cm)	Length of Leaf 2 (cm)	Shoot Length (cm)
Day 1	1	1.7	1.5	2.0
	2	1.3	1.2	2.2
	3	1.8	1.0	2.0
Day 3	1	1.9	1.6	2.1
	2	1.5	1.2	2.5
	3	1.9	1.0	2.3
Day 5	1	2.0	1.7	2.1
	2	1.6	1.2	2.6
	3	2.0	1.2	2.6
Day 7	1	2.1	1.9	2.2
	2	1.8	1.2	2.6
	3	2.0	1.2	2.6
Day 9	1	2.1	2.0	2.5
	2	1.9	1.5	2.8
	3	2.0	1.4	2.7
Day 11	1	2.3	2.0	2.8
	2	2.1	1.7	2.8
	3	2.2	1.5	2.8
Day 13	1	2.3	2.0	3.4
	2	2.1	1.7	3.0
	3	2.2	1.5	2.8

Table 1-B: Tomato seedlings exposed to field strength of 150 Gauss

Day No.	Seedling	Length of Leaf 1 (cm)	Length of Leaf 2 (cm)	Shoot Length (cm)
Day 1	1	1.5	1.4	2.7
	2	1.8	1.4	2.2
	3	1.8	1.4	2.0
Day 3	1	1.6	1.4	2.7
	2	1.8	1.4	2.4
	3	1.9	1.6	2.2
Day 5	1	1.7	1.4	2.7
	2	1.8	1.4	2.5
	3	1.9	1.7	2.4
Day 7	1	1.9	1.8	3.0
	2	2.0	1.8	2.7
	3	1.9	2.2	2.9
Day 9	1	2.2	2.1	3.5
	2	2.2	2.0	2.9
	3	2.3	2.4	2.9

Day 11	1	2.3	2.1	3.5
	2	2.3	2.1	2.9
	3	2.3	2.4	2.9
Day 13	1	Wilted	Wilted	Wilted
	2	Wilted	Wilted	Wilted
	3	Wilted	Wilted	Wilted

Further, at 150 Gauss it was seen that maximum growth occurred in both leaf and shoot in leaf 1 of seedling 1 at 0.8 cm and at 0.7 cm, respectively (Table 1-B). At 150 Gauss, out of the three seedlings exposed to novel experimental conditions, the seedlings did not survive beyond eleven days of treatment, hence were excluded from the experiment. This could be on account of possible toxicity induced on the juvenile seedlings due to high MF.

When compared with control data, here also it was observed that there existed a positive correlation between magnetic field and phytohormone-treated seedlings and plant growth. In control tomato seedlings, devoid of any exogenous auxin and cytokinin and magnetic field, highest growth in leaf length took place at 0.5 cm in leaf 1 of seedling 2, while most rapid shoot growth was at 0.6 cm of seedling 2 (Table 1-C).

Table 1-C. Tomato Control - No Magnetic Field and No Phytohormones

Day No.	Seedling	Length of Leaf 1 (cm)	Length of Leaf 2 (cm)	Shoot Length (cm)
Day 1	1	2.0	1.2	2.0
	2	1.8	1.6	0.6
	3	2.0	1.6	1.3
Day 3	1	2.0	1.2	2.0
	2	1.8	1.6	0.7
	3	2.0	1.7	1.5
Day 5	1	2.0	1.2	2.1
	2	1.9	1.7	0.7
	3	2.1	1.7	1.8
Day 7	1	2.0	1.2	2.2
	2	2.0	1.7	1.1
	3	2.1	1.8	1.8
Day 9	1	2.0	1.3	2.4
	2	1.8	1.8	1.1
	3	2.1	1.8	1.8
Day 11	1	2.0	1.3	2.6
	2	2.1	1.8	1.2
	3	2.2	1.9	1.8
Day 13	1	2.0	1.3	2.7
	2	2.3	1.9	1.2
	3	2.4	2.0	1.8

Overall results strongly suggest strong and definite correlation between the combined influence of geomagnetic field and plant hormones on plant growth. Importantly, the magnetic field of 100 Gauss continues to be a favorable factor in inducing positive response in the seedlings of tomato while at higher dosage of 150 Gauss, the growth response diminishes and they fail to survive till the end of experiment. As this is the first kind of study initiated to observe the simultaneous impact of two entirely different parameters (geomagnetic field and phytohormones) on plant growth, not reported before in literature, further research is required to evaluate diverse plant characteristics i.e., height of adult plant, flower and fruit morphology, fruit yield by ~~and~~ following the plants for a longer duration. Additionally, a detailed experimental investigation on other agronomically important crops would yield a better insight into the prospects of the technique.

Figure 2: Tomato seedlings being grown under geomagnetic field 100 Gauss.

It is a well-established fact that when exposed to natural MF, plants respond differently which might positively or negatively influence their development [31].

Application of magnetic field is known to promote increase in the germination rates by possible increase in membrane permeability, thereby facilitating water absorption by seeds and improving plant growth. However, in other studies multiple negative effects of MF on plants have been reported, which include inhibition of the cell growth, increase of the free radicals and increase in lignin and suberin on the cell walls [32].

In our study, loss of vigour and wilting of seedlings in tomato seedlings after 11 days of culture under the influence of a magnetic field of 150 Gauss was observed. In contrast, seedlings maintained at 100 Gauss and control seedlings continued to grow well throughout the experiment. Kordas [31] also reported a decrease in stem length of wheat in a similar experiment. Therefore, wilting of all seedlings at high magnetic strength can be attributed to adverse effects of the applied field, though further studies are under way to firmly establish this fact.

4. Conclusions

The present study deals with two distinct aspects of morphogenesis simultaneously: effect of phytohormones and effect of magnetic field on carefully chosen plant species - tomato (*Solanum lycopersicum*). Therefore, the prime objective of the present study was to standardize and discover the cumulative effect of plant growth regulators and magnetic field on nutritionally and economically significant crop plants and bring the protocol to common use.

The study focused on assessing certain growth characteristics such as the length of the shoot and leaves of tomato, when they were subjected to the magnetic field while being grown in soil supplemented with phytohormones auxin and cytokinin. The experimental crop plants exhibited similar and vigorous growth response in presence of plant hormones NAA (0.5 mg) and BAP (1.0 mg), at magnetic field of 100 Gauss, while at higher mf of 150 Gauss, the growth of leaves and shoot followed slowed down in tomato seedlings. This behavior unambiguously shows that the general development of plants is affected by the presence of exogenous phytohormones and mf applied and the performance of parameters evaluated was better than the control plants. The overall morphology of all the experimental plants remained normal throughout.

5. Acknowledgements

The Authors extend their sincere thanks to the Principal, Maitreyi College, for providing us with an excellent opportunity to investigate important factors affecting plant growth and development. We also thank Prof. M. V. Rajam, Department of Genetics, South Campus, University of Delhi, New Delhi for his kind help by providing important resources required for the project.

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