***Review Paper:***

# An assessment of the application of magnetic fields in the study of *in vitro* plant cell and tissue cultivation

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## Abstract

*The magnetic field can affect the structure, physiology and biochemistry of plants. The use of magnetic fields on cultured plant cells and tissues is a proven fact. The system to deliver magnetic flux is very important. Experiments have been performed by directly placing cultures in the vicinity of the magnetic field from a permanent magnetic source. Such a system delivered a fixed magnetic strength. Electric magnets are a better mode to deliver magnetic field to plants because it is easy to deliver tailor-made magnetic exposure. Majority of such studies utilized a magnetic field in the order of milliteslas, some of them utilized a high magnetic field upto 17.8 Tesla. The experiments also utilized varying exposure times and differential exposure to the north or south pole.*

*Such studies reported either an increase or decrease of many parameters such as cell size, shape, metabolite content, chlorophyll content and enzyme activity. Most of the studies discussing magnetotropism on plants utilized seeds or intact plants to find out the effect of magnetic fields on plants. Such studies also give some understanding of the mechanism of action. Using a plant cell culture system, it is possible to grow single cells and small cell aggregates. Therefore, in vitro systems offer an excellent tool to understand the mechanism of action at the cellular level.*

**Keywords:** Callus, Magnetic field, Magnetotropism, Plant tissue culture.

## Introduction

Living things on earth are exposed to light, temperature changes, physical forces, radiations, electric and magnetic fields. Living things including plants were evolved and diversified under this natural geomagnetic field. The intensity of the earth's geomagnetic field can vary from around 30 μT to 70 μT [17]. This means living things on earth are designed to work in this range of magnetic fields. However, there exist scenarios where living things are exposed to a higher magnetic field.

In humans, an occupational exposure of 0.6 μT or above is related to the incidence of brain tumours[23]. Plants also express altered metabolic properties in varied magnetic field intensities[15]. As in the case of futuristic interplanetary missions, the space shuttle grown plants will be exposed to a weaker magnetic field intensity ranging from 0.1 to 1 nT[4]. Therefore, studies utilizing the magnetic field as a tool for regulating plant growth, enriching the nutritional value and unravelling the cellular response draw more attention.

In the case of plants, *in vitro* culture technology offers a promising tool to investigate the effect and mechanism of magnetic field exposure to plant cells. There have been attempts to use a magnetic field in modulating the growth, metabolism and differentiation if plant cells are cultured under *in vitro* conditions.

For such a purpose, the experimental system that deliver magnetism is of high importance. The current paper discusses the magnetic field experimental system used for such a study and its effects on cultured plant cells.

## Experimental set up to expose cultures to magnetic field

Cultured plant cells are grown under aseptic conditions inside a sterile glass containing growth media. In an experimental set up to determine magnetic effect on cultured plant cells, it is very important that the cells are properly experiencing pre-determined magnetic field intensity. Therefore, design of such a system attracts much attention.

To generate magnetic fields, both permanent magnets and electromagnets were used. The mode of exposure varied and can be classified into (i) Direct mounting, (ii) Belt system (iii) Magnetic coils.

**(i) Direct mounting of permanent magnets:** Use of permanent magnets involves a simple system using permanent magnets of intensities 0.1, 0.15 and 0.2 Tesla. The group placed the *in vitro* cultures in conical flasks directly on the top of the permanent magnet. To prevent interaction of magnetic fields, they used plastic tray and thick wood pieces[25]. In an simpler model, the magnets were adhered to the wall of the culture vessel using adhesive tapes[10]. In such a system, the exposure duration can be controlled only by physically taking away the magnets from the proximity of the cultures.

**(ii) Belt system involving permanent magnets:** The belt system involves permanent magnets fixed to a moving belt. The magnets were at a distance of 6 cm from the *in vitro*

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cultures. Belt was moving with a constant speed of 1m/s and exposure was given multiple times with varying exposing time[26]. The advantage of such a system is that the cultures are exposed to magnetic field at a predetermined duration.

**(iii) Magnetic coil:** The new technology of producing magnetic field has also been utilized by some researchers in plant tissue culture. An electromagnetic cylindrical coil of 40 cm height and 12 cm diameter produced an electromagnetic field[14]. A similar model has also been used in *Matricaria chamomilla* cell suspension cultures[9]. The advantage of electric coil is that the intensity and duration can be easily adjusted without physically removing the magnets. In an another electromagnetic prototype model, C shaped steel sections and coils were connected in series which produced a static and uniform flux density field at the airgap[2]. Similar C configuration was also used using rare earth magnets[11]. The reports indicate the exposure of cultured cells at a continuous mode throughout the culture duration or fixed duration multiple times.

## Effect of magnetic field intensity

Cell suspension cultures of *Matricaria chamomilla* exhibited a higher accumulation of the flavonoid apigenin in presence of a static magnetic field of 4 mT. The magnetic field also influenced the DPPH activity as well as the total phenolic content[9]. Another important understanding from this study is the influence of magnetic field on cell shape. A 6 mT exposure resulted in a more round shape. However, there was no evidence of cell viability loss[9]. *In vitro* cultures of *Curcuma longa* exhibited a stimulatory effect on growth of shoots in the presence of a lower magnetic field of 200 mT[11]. Wheat cultures responded negatively to magnetic field, as it reduced the callus growth when compared to controls[14]. The reports of magnetic fields on their effect on cultured plant cells are summarized in table 1.

## Mechanism of action of magnetic field on plant cells

Data available indicates that magnetic field is able to decrease or increase effect on most of the plant growth as well as biochemistry aspects[24]. Such effects are termed as magnetotropic effects or magnetotropism[3]. Some examples of magnetotropic effects are reported with regard to shoot growth[7], antioxidant activity[7], root growth[13], seed yield[19], chlorophyll and protein content[16] secondary metabolites[22] and flowering[1].

Few attempts have been made to understand the basic mechanism behind the response of plants under a magnetic field. It is estimated that altered free radical movement under a magnetic field is one of the factors responsible for such changes[8,20]. Another mechanism may be changes in reactive oxygen and nitrogen[12]. The movement of paramagnetic ions found in the cell has also been influencing such changes[5]. Some studies even indicate that changes in the quantum properties of molecules are responsible for magnetotropism[6,27].

The magnetic field is proven to affect both the structural and functional processes of plants. The key physiological process of a plant cell is the differential permeability of the structural element, the cell membrane. The differential permeability and many of the cellular functions are made possible with the proper structural orientation of ions, enzymes and proteins attached to it. Therefore, the molecular order of the plasma membrane under a magnetic field is a key factor[21].

**Table 1**
**Effect of magnetic field on the *in vitro* plant cell and tissue cultures**

| S.N. | Strength of Magnetic field used | Duration | Outcome |
|---|---|---|---|
| 1 | 150 mT | 0-5 min for three weeks | 4 min induction was favorable for in vitro shoot multiplication in *Genista aetnensis*[2] |
| 2 | 8.8 and 17.8 Tesla | Cultures were grown under dark for 28 days | Magnetic field significantly influenced callus induction in the wheat vanities[14] |
| 3 | 200 mT static magnetic field for 20, 40, 60, 80, 100 and 120 min treatment | Cultures were analyzed after 7-15 days of treatment | 40 min duration treatment exhibited stimulatory effects on *in vitro* growth[11] |
| 4 | 2 mT, 4 mT and 6 mT, 60 minutes, three days | Chemical analysis on 13$^{th}$ day of in vitro growth | Accumulation of phenolics, flavonoids, apigenin and hydrogen peroxide was increased[9] |
| 5 | 0.1 Tesla, 0.15 T and 0.2 T, N and S pole plarities | After 60 days | The 0.2 T along with a N pole combination enhanced biomass of protocorm like bodies in *Phalaenopsis*[25] |
| 6 | 2.9 mT and 4.8 mT, belt system, 1ms$^{-1}$ rotation ; 2.2s, 6.6s and 19.8 s exposure | 28 days | Plant height, fresh weight, Leaf number, chlorophyll content varied wrt to magnetic field[26] |

**Selective advantages of plant tissue culture system:** Plant cells under *in vitro* cultural conditions are extremely suitable for magnetic field-driven molecular mechanisms. This is because of the six reasons that cultured plant cells have selective advantages over full plants in understanding the magnetic mechanism. They are (i) Cells are grown under controlled conditions (ii) Callus cells are undifferentiated (iii) Possible to induce shoot and root morphogenesis (iv) Response to light (v) Single cells or small cell aggregates can be grown under suspension culture - a unique opportunity to study cell size and shape parameters as well as cell permeability studies and (vi) Easy to regulate cell cycle stage parameters.

The studies on wheat tissue culture report genotype variations and exhibit significant differences in the growth rates under magnetic field[14]. This means that there is a possibility of such genotype-specific differences among individual cells also. In suspension cultures, all the cells are not of the same genotype and exhibit somaclonal variations leading to heterogeneity[18].

Therefore, it is possible to produce cultures that are more sensitive or less sensitive to magnetic fields through single-cell cloning. Such a system provides better opportunities for understanding and tagging the genes that respond to magnetic strength.

## Conclusion

Magnetic flux is able to interfere with cultured plant cells and tissues like any other living microbes, plants and animals. However, *in vitro* cultures provide a unique technique to grow only a few undifferentiated cells or even single cells. Therefore, *in vitro* cultured plant cells will exhibit a better response to magnetic fields. However, it is unlikely that a magnetic field can accelerate significant enhancement of a secondary metabolite, enzyme, growth or regeneration.

On the other hand, they provide a better and more reliable tool for unravelling the mechanism of action of living cells in response to magnetism. This will give potential insights into understanding plant behavior under a magnetic field or even in disease treatment. The data generated in this regard are only a few and hence require more research. Further, a better instrumental design is required in exposing cultured cells to tailor-made magnetic flux. Magnetic fields may also work as a novel means of intervention to achieve enhanced nutritional content also.

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