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ORIGINAL ARTICLES

Effect of magnetic fields on growth and anatomical structure of *Vicia sativa* L.

[1]Ahmad Majd, [2]Sara Farzpourmachiani

[1]Department of Biology, Faculty of Biolobical Sciences, North-Tehran Branch, Islamic Azad University, Tehran, Iran
[2]Department of Biology, Science and Research Branch, Islamic Azad University,Tehran, Iran

ABSTRACT

In this research, dry and wet vetch seeds (*Vicia sativa* L.) were exposed to three magnetic field strengths 100, 1700 and 3600G for 5, 10 and 20 min. Then seeds were transferred to MS culture medium. Growth and development of seedlings were studied on 14th day. The results showed that length of the wet seeds that were treated with a strength of 1700G for 20min were higher than the others. Cross sections of hypocotyl, root and leaf and longitudinal sections of shoot apical meristem were prepared and investigated by light microscope. The results of hypocotyl sections showed that treated seedlings had more vascular bundles, more diameter of xylem and more xylem tissue than control samples. Root sections showed that they had more vascular bundles and more secondary structure than control sample. Leaf sections showed more compressed palisade parenchyma than control. Longitudinal sections of shoot apical meristem showed an increase in tunica layers compare to control.

Key words: ontogeny of seedling, cross and longitudinal sections, magnetic field treatment, *Vicia sativa* L.

Introduction

Microorganisms, plants and animals on the earth live under a geo-magnetic field (GMF) as a natural magnet (Van *et al*., 2011; Moon and Chung, 2000; Pingping *et al*., 2007). External magnetic fields are more effective than GMF on biological systems (Van *et al*., 2011; Majd *et al*., 2010). Many studies showed that magnetic fields (MFs) affect the rate and percentage of seeds germination, seedlings development, reproduction and growth of meristem cells (Atak *et al*., 2007; Moon and Chung, 2000; Carbonell *et al*., 2002; Selim and El-Nady, 2011; Shabrangi and Majd, 2009; Majd *et al*., 2010) and cause higher yield (Rochalska, 2005). MFs have effect on DNA, RNA and protein synthesis (Shabrangi *et al*., 2011; Alikamanoglu *et al*., 2007). Nucleic acids biosynthesis inhibit by magnetic energy dose increasing (Racuciu *et al*., 2007). MFs induce α-Amylase, dehydrogenase, protease activity (Vashisth *et al*., 2010) and also increase nitrogen content (Rochalska, 2005). S pole of MF causes more increase in proliferation of protocorm-like bodies in *Phalaenopsis* than N pole and enhance the production of regular factors in plants (Van *et al*., 2011). Low frequency electric and magnetic fields have effects on enzyme activities such as superoxide dismutase, catalase and glutathione reductase. So they delay senescence and sings of decay (Piacentini *et al*., 2001). It also induces lipid peroxidation, generation of reactive oxygen species (Luciana and Abbre, 2005) and releases the peripheral proteins from membranes (Aksyanov *et al*., 2007). It induces chromosomal abnormalities such as lagging, fragments, micronuleous and chromosomal bridge in pollen mother cells (Pingping *et al*., 2007; Hanafy *et al*., 2006; Shabrangi *et al*., 2011), reduces in pollen grains number and mail sterility (Amjad and Shafighi, 2010), causes compression or tension of grain particular layers (Sumorek *et al*., 1999). It causes to delay in flowering specially in wet pretreated samples (Shabrangi *et al*., 2011), improves in seeds' sowing quality (Azharonok *et al*., 2009), affects on genetic diversity (El-bakatoushi, 2010) and induces cytological and ultrastructural changes (Selga & Selga, 1999). MF protects plants from drought and heat stress (Selim and El-Nady, 2011; Roman and Igor, 2002), stimulates secondary shoot production and rhizogenesis (Corneanu *et al*., 1994; Atak *et al*., 2007), affects on chlorophyll a, chlorophyll b and carotenoids and protects plant through scavenging reactive oxygen (Strzalka, 2003). MF changes water properities, magnetized water is the best treatments for improving the bad effects of drought stress (Selim and El-nady, 2011; Tian *et al*., 1989). MFs affect the ionic charges within pollen tube and cause abnormalities in it (Dattilo *et al*., 2005). Radio frequency radiations cause to mitotic abnormalities in root meristemic cells (Tkalec *et al*., 2009). Weak magnetic field causes to decrease in phytoferritin in plastids and increases the size and volume of cells in mitochondria (Belyavskaya, 2004), enhances formation of somatic embryos from mesophyll protoplast (Dijak *et al* 1986) and delays G_1 phase in root meristem cells (Belyavskaya,

Corresponding Author: Ahmad Majd, Department of Biology, Faculty of Biolobical Sciences, North-Tehran Branch, Islamic Azad University, Tehran, Iran
E-mail: sfarzpourmachiani@yahoo.com

2004). MF is a useful tool for production of pharmaceutical plant, agriculture and industrial purpose (Ichim *et al*., 2007; Nagy *et al*., 2005).

In this study we have investigated the effect of magnetic fields on growth and anatomical structure of *Vicia sativa* L. This study would help us to know that how plants change their anatomical structure against magnetic field.

Material And Methods

Vicia sativa L. seeds were provided from Fereydunshahr, Esfehan, Iran. The seeds were divided in three groups: control, wet and dry seeds. In case of wet treatment, the seeds were soaked in distilled water for 30 minutes. Then wet and dry seeds were separately placed into test tube and exposed to magnetic field by zimman apparatus (Figure 1). Zimman apparatus was consist of different parts: two coils with several number of wire turns, pole pieces made by iron and cadmium lamp with a holder that test tube was placed instead of it. Magnetic field produced between two poles. Magnetic field strengths were measured by Axial B-probe that connected to a gauss meter. DC magnetic current was provided by power supply.

Fig. 1: Zimman apparatus

The seeds were exposed to 100, 1700 and 3600 Gauss (G) for 5, 10 and 20 min. Three replicates were used in the experiment with 18 seeds in each treatment. Treated and untreated seeds were strilled separately. They were surface washed with dish washing liquid for 5 to 6 min, then transferred to laminar air flow. The seeds were soaked in 20% sodium hypochlorite solution for 10 min, then sterilized in benomyl solution (0/2 gr in 50ml) for 10 min and merck ethanol for 30s. After each level, seeds were rinsed with distilled water and transferred on solid MS (Murashig and Skoog, 1962) basal medium containing 3% sucrose and 0/7% agar. MS medium cultures were maintained in incubator under a photoperiod of 16h day/8h dark at 23□ C. Germination and development of seedlings were investigated on 14th day. Anatomical studies were evaluated after 14 days. On 14th day, seedlings were sent out of MS medium culture. They were washed with water and cutted in 1cm pieces. The pieces of leaf, shoot and root were fixed in alcohol and glycin (80:20) for 1 week, then cross sections were prepared. Sections were soaked in 5% sodium hypochlorite solution, then transferred to 3% Acetic acid for 10 min. Sections were stained in methylen blue for 30s and then in carmine for 30 min. After each part, they were rinsed 3 to 4 times with sterile distilled water. Shoot apical meristems were prepared and fixed in FAA (37% Formaldehyde, 96% ethanol and 100% Acetic acid). Samples were dehydrated in 70% to 100% ethanol. The dehydrated specimens were impregnated and embeded in paraffin wax, then sectioned by microtome at a thickness of 6 mm. Sections were placed on slides and deparaffinized, then hydrated and stained in hematoxyline and eosin, cleared in Xylol and mounted in Enthalen. Cross and longitudinal sections were examined by light microscope (Olympus).

Data analysis:

Hypocotyl, epicotyl and root length, number of roots and wet and dry weight in treated and control samples were measured and determined by Analysis of Variance test (ANOVA). Post hoc multiple comparisons between treated and control samples were made using Tukey's test.

Results and Discussion

Growth study:

The highest and lowest root and shoot length was observed in wet condition (Table 1, Table 2). Wet condition may increase the macromolecules activity in treated samples so it may increase the effects of magnetic fields on treated samples. MFs cause to increase in root and shoot length compare to control (Majd *et al*., 2010). The most changes were observed in treated samples with 1700G for 20min. MFs cause to increase in cell membrane permeability and alanine absorption (Stange *et al*., 2002). Root and shoot length was decreased in MF intensity of 3600G. It may have effect on auxin activity and also decrease cellular reproductive (Majd *et al*., 2010). MFs increased wet and dry weight. These results confirmed the conclusion of other studies (Shabrangi *et al*., 2011; Florez *et al*., 2007).

Table 1: Effect of magnetic field on roots, epicotyls, hypocotyls length, number of roots, wet and dry weight of dry seeds of *Vicia sativa* L.

Treatments		Root length(cm)	Number of roots	Epicotyl length(cm)	Hypocotyl length(cm)	Fresh weight(gr)	Dry weight(gr)
MF intensity(G)	Time(min)						
100	5	5.17±0.22	22.38±1.58	5.05±0.46	4.54±0.3*	0.35±0.04	0.03±0.005
	10	7.58±0.44*	22.83±1.36	5.67±0.1*	3.96±0.2*	0.43±0.03	0.029±0.001
	20	7.08±0.92	24.23±4.56	5.64±0.35*	3.84±0.21*	0.42±0.03*	0.027±0.003
1700	5	8.24±0.92*	16.22±0.89	4.64±0.21	4.3±0.11*	0.38±0.04	0.032±0.004
	10	9.45±0.22*	18.44±2.09	6.51±0.21*	4.51±0.12	0.58±0.03	0.043±0.003
	20	9.36±0.54*	14.77±2.78*	4.79±0.36	4.39±0.17	0.49±0.01	0.036±0.007
3600	5	7.67±0.74*	19.22±1.5	4.58±0.34	4.04±0.01*	0.41±0.02*	0.038±0.004
	10	5.24±0.28	18.11±0.43	4.73±0.1*	4.04±0.1	0.34±0.02	0.027±0.003
	20	5.29±0.46	14.77±2.78	5.24±0.41	5.09±0.19	0.3±0.04*	0.025±0.002
Control		5.24±0.35	19.92±2	5.71±0.68*	4.14±0.11*	0.51±0.02*	0.033±0.003

Data are the means ± SEM(n=3). *$P \square 0.05$, compared to control and the other treatments

Table 2: Effect of magnetic field on roots, epicotyls, hypocotyls length, number of roots, wet and dry weight of wet seeds of *Vicia sativa* L.

Treatments		Root length(cm)	Number of roots	Epicotyl length(cm)	Hypocotyl length(cm)	Fresh weight(gr)	Dry weight(gr)
MF intensity(G)	Time(min)						
100	5	5.21±1.09*	15.09±1.11	4.81±0.72	3.23±0.19	0.32±0.007	0.027±0.003
	10	4.29±0.26*	14.61±0.96	5.01±0.23	3.29±0.22*	0.3±0.01*	0.026±0.004
	20	6.08±0.16*	15.05±2.4	3.87±0.19*	4.08±0.2*	0.4±0.02*	0.026±0.001
1700	5	4.79±0.35	14.77±0.47	4.31±0.15	2.78±0.33	0.29±0.02*	0.026±0.001
	10	10.21±0.2*	18.11±0.64	6.79±0.34	4.5±0.06*	0.06±0.008	0.045±0.002
	20	13.36±0.52*	25.02±2.46*	7.7±0.06*	4.33±0.37	0.75±0.02*	0.05±0.005
3600	5	8.47±0.88*	17±2.92	3.03±0.73	3.78±0.14	0.45±0.04	0.04±0.005
	10	8.82±0.69*	18.77±2.94	4.13±0.37*	4.06±0.12	0.46±0.07	0.044±0.008
	20	8.19±0.24*	19.94±0.86	4.3±1.05	3.68±0.43*	0.51±0.07*	0.043±0.003
Control		5.54±0.29	18.61±1.72	5.47±0.57	4.44±0.15	0.47±0.02	0.029±0.001

Data are the means ± SEM (n=3). *$P \leq 0.05$, compared to control and the other treatments

Anatomical structure of hypocotyl:

The longest and lowest shoot length was observed in plant grown from wet seeds pretreated with 1700G intensities for 20 min and 3600G for 5 min, respectively (Majd *et al*., 2010). Epidermis, Cortical parenchyma, 9 vascular bundles and Pith were observed in hypocotyl cross section of control sample (Figure1-1). Treated samples with 1700G intensity had 4 vascular bundles but hypocotyl diameter and Cortical parenchyma was as the same as control (Figure1-2). Treated samples with 3600G had larger Cortical parenchyma than the others. They showed increase in number of hairs. They had 14 vascular bundles and a dwindle had accrued in Pith (Figure1-3).

Shoot diameter, number of vascular bundle and volume of cells of cortical parenchyma increased by magnetic field increasing. This results confirmed the conclusion of other studies in which *Lens orientalis* L. had

more vascular xylem and cortical parenchyma compare to control when exposed to magnetic field (Shabrangi & Majd, 1384). Magnetic field may induce the cambium differentiation to xylem and phloem and improve the translocation of photoassimilate (Selim and El-nady, 2011).

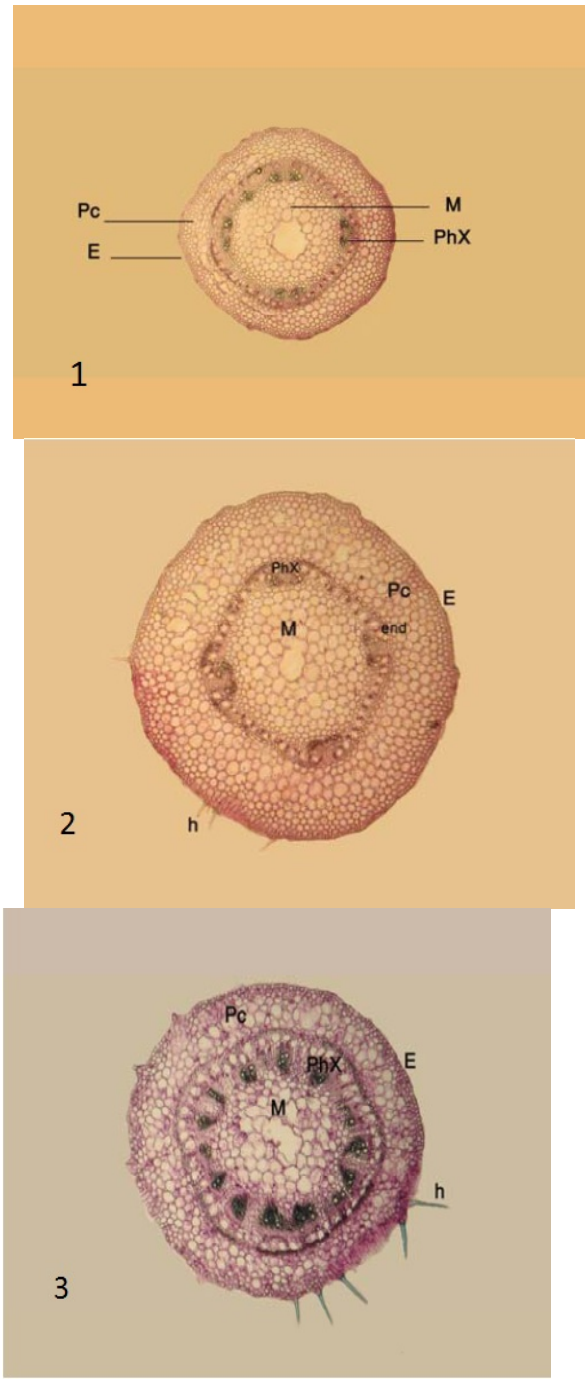

Fig. 2: cross section of stem hypocotyls (4×). 1- Control, 2- 1700G- 20min-wet, 2- 3600G- 5min- wet E- Epidermis, Pc- Parenchyma of cortex, end- endodermis, PhX- a group of Phloem and Xylem, M- Medulla , h- hair

Anatomical structure of root:

The highest and lowest root length was observed in plant grown from wet seeds pretreated with 100G for 10 min and 1700G for 20 min respectively (Majd *et al*., 2010). Cortical parenchyma, Pericycle, Phloem tissue and

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Xylem tissue was observed in root cross section of control sample (Figure 2-1). Treated samples with 100G had more xylem and xylem tissue. They entered to secondary structure faster than control (Figure 2-2). Treated samples with 1700G for 20min had more diameter and vascular bundle compare to control but they decreased compare to with 100G treated samples control (Figure 2-3).

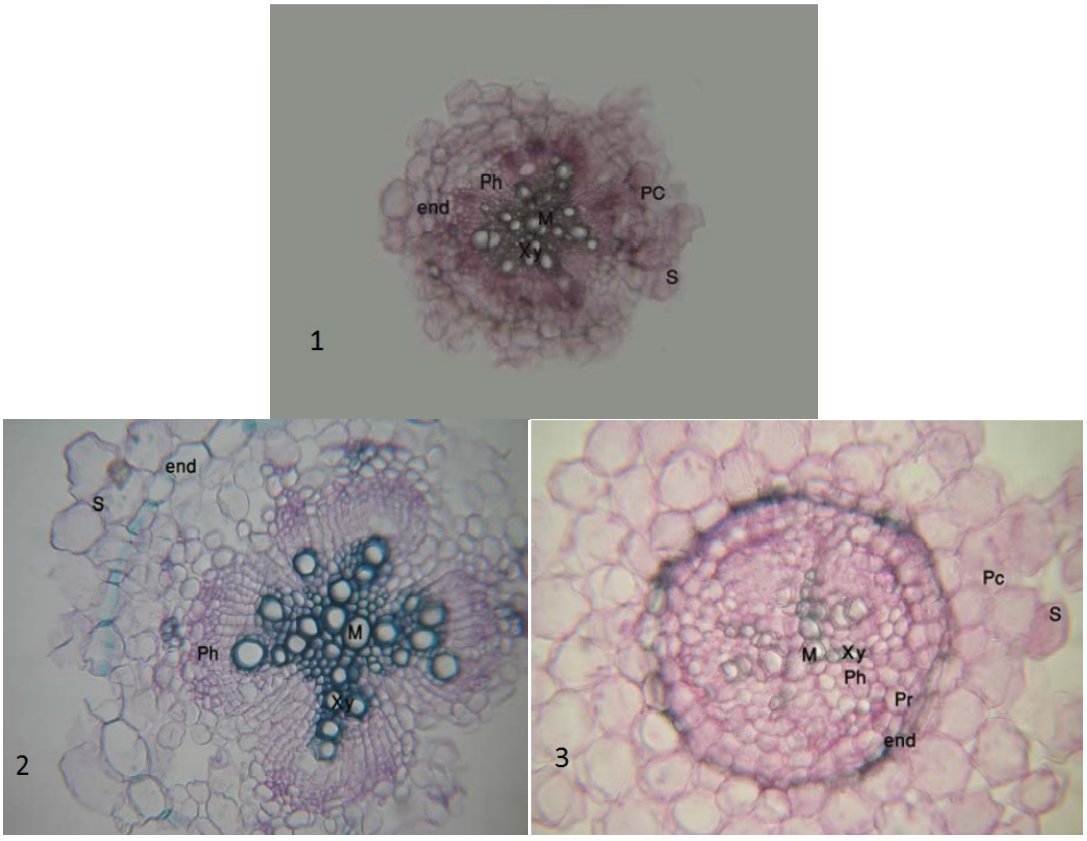

Fig. 2: cross sections of root, 1- control (10×), 2- 100G- 5min- wet, 3- 1700G- 20min- wet S- Subereous, Pc- Parenchyma of cortex, end- endodermis, Ph- Phloem, Xy- Xylem, M- Medula

This results confirmed the conclusion of effect of magnetic field on *Lens orientalis* L., *Arachis hypogaea* L.. They showed that xylem tissue and secondary structure of root was increased by magnetic field (Shabrangi & Majd, 1384) (Arbabian & Majd, 1384). MFs may increase peroxidase activity and peroxidase has a role in lignifications, suberization and auxin catabolism (Atak *et al*., 2007). So MFs may increase xylem tissue by this way.

Anatomical structure of leaf:

Maximum and minimum of leaf area was observed in treated samples with 100G for 10 min and 1700G for 20 min (Majd *et al*., 2010). Adaxial parenchyma, palisade parenchyma, spongy parenchyma, vascular bundle, Collenchyma and Abaxial parenchyma was observed in leaf cross section of control sample (Figure 3-1). Treated samples with 100G intensity had smaller and more compressed spongy parenchyma cells than control (Figure 3-2). Treated samples with 1700G had larger spongy parenchyma cells than treated samples with 100G. They had more compressed palisade parenchyma than control (Figure 3-3).

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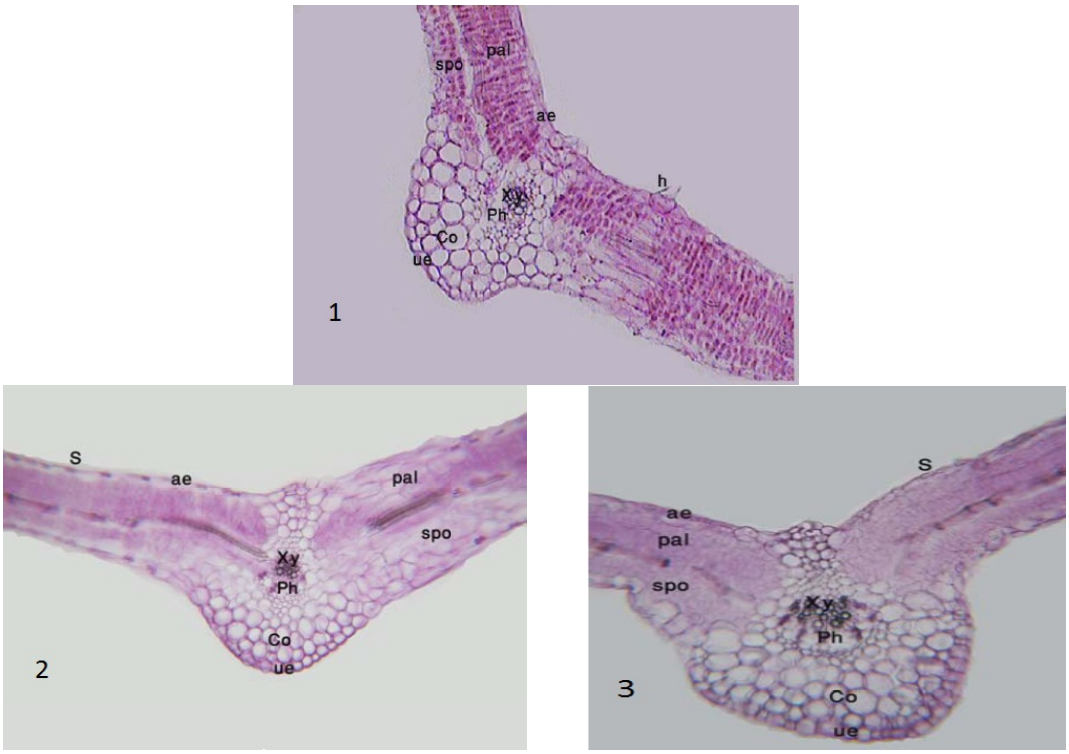

Fig. 3: cross section of leaf, 1- control (10×), 2- 100G-5min-wet (10×), 3- 1700G-20min-wet (40×) ae-adaxial parenchyma, h- hair, S- Stomata, pal- palisade parenchyma, spo- spongy parenchyma, Ph- Phloem, Xy- Xylem, Co- Collenchyma, ue-

These results comfirmed the conclusion of other investigations (Shabrangi & Majd, 1384) (Arbabian & Majd, 1378) (Selim & El-nady, 2011). Leaf chlorophyll content was increased when plants were irrigating with magnetic water. Magnetic field increased chlorophyll a, chlorophyll b and total chlorophyll levels (Atak, 2000). The increase in photosynthetic contents caused to increase in mesophyll tissue thickness (Selim & El-nady, 2011). Treated seeds with 1700G may compressed palisade parenchyma for improving water transportation to leaf area. This MF intensity may decreased water in leaf so plantlet may want to improve this stress with compress in palisade parenchyma.

Anatomical structure of shoot apical meristem:

Treated samples with 1700G for 20min and 3600G for 5min had highest and lowest shoot length (Majd *et al*., 2010). Tunica, Curpus, Initial ring and Eboush foliare was observed in shoot apical meristem longitudinal sections of control sample (Figure 4-1).

Tunica, Curpus, Initial ring, Medular meristem, procambium and Primordium foliare was observed in treated samples with 1700G for 20min. Tunica layers increased compare to control (4-2). Tunica, Curpus, Initial ring, Medular meristem, procambium and Eboush foliare was observed in longitudinal section of treated samples with 3600G for 5min. Tunica layers increased compare to control (4-3).

Results showed that treated samples with 1700G were in primordium foliare level but treated samples with 3600G and control samples were in Eboush foliare. Treated samples had more tunica layers than control. The other investigations showed that MFs are effective on RNA, protein synthesis and enzyme activity (Goodman *et al*., 1995). It increased reproduction of cell meristem. But investigations of effect of weak magnetic field on root and shoot meristematic cells showed that meristem cells of treated samples decreased compare to control (Nanushyan, 2001). EMF affect the key cellular process such as gene transcription (George, 1996). MFs intensities of 1700G and 3600G may increase in DNA synthesis so they cause to increase in cells in Tunica zone.

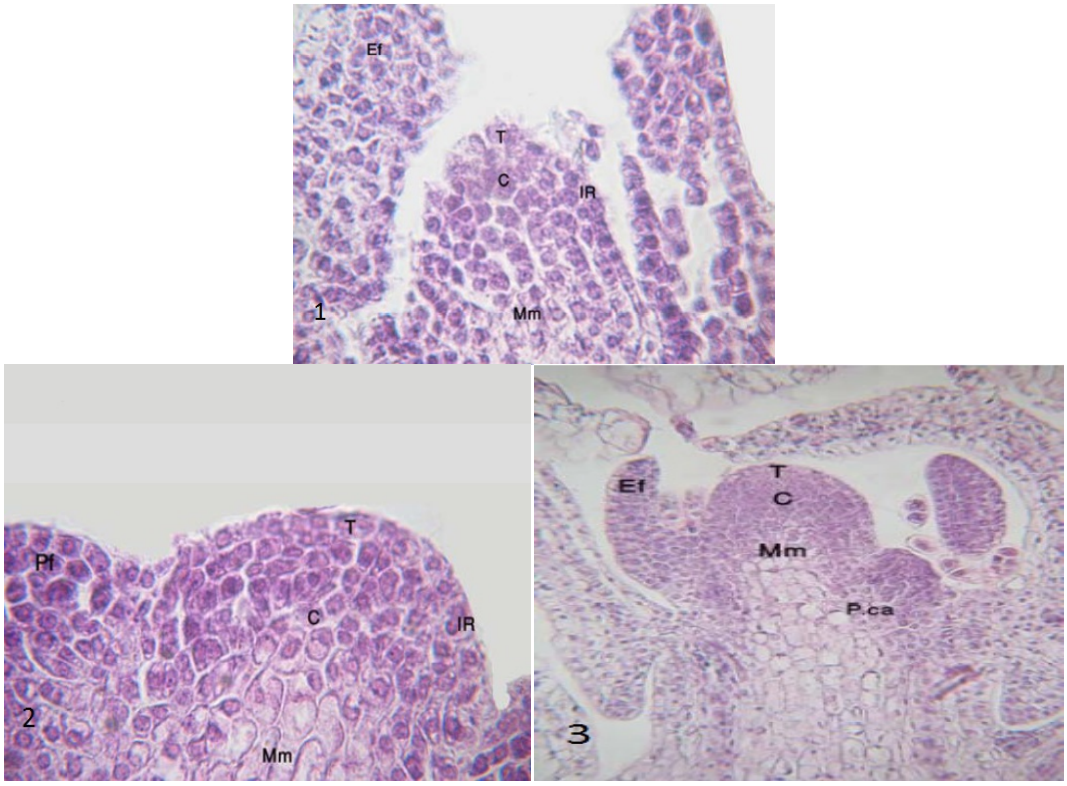

Fig. 4: longitudinal section of shoot apical meristem, 1- control, 2- 1700G-20 min- wet, 3- 3600G- 5min-wet T- Tunica, C- Curpus, Mm- Medular meristem, IR-Initial Ring, Pca- Procambium, Ef- Eboush foliare, Pf- Primordium foliare

Conclusion:

Results showed that treated samples had more diameter and more vascular bundles in root and shoot and more xylem tissue in root. They had compressed spongy and palisade parenchyma in leaf and more Tunica layers in shoot apical meristem. It has been known that different magnetic field intensities cause various reactions in plants. These reactions have no linear effect on strength and period of exposed to magnetic field (Atak *et al*., 2003).

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