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Effect of presowing magnetic treatment on properties of pea

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A b s t r a c t. The pea seeds were exposed to full-wave rectified sinusoidal magnetic fields. The effects of electromagnetic treatment on seedling growth and chlorophyll contents and have been investigated. Seed were sown after magnetic field treatment according to ISTA under controlled laboratory conditions. The magnetic filed treatment of seeds increased the growth significantly (*P*<0.05), while the increment in contents of chlorophyll have been found non significant (*P*<0.05). The shoot length, root length, root dry mass, shoot dry mass, fresh root mass and fresh shoot mass increased up to 140.5, 218.2, and 104, 263.6, 74.5, 91.3%, respectively. The result suggested that magnetic field could be used to enhance the growth in pea plant.

K e y w o r d s: pea, seed, magnetic treatment, growth, chlorophyll content

INTRODUCTION

Seed germination is a prerequisite for normal growth, increased development and yields of crops. There are various natural factors which oppose the germination and yield of crops (Achakzai *et al.,* 2010; Fujimaki and Kikuchi, 2010). In this regard, the agricultural scientists are trying to explore the technique, which must be proficient, eco-friendly, clean and affordable. Biological, chemical and physical presowing seed treatments are being used for better seed germination and growth (Perveen *et al.,* 2010). Chemical concerts were found to be effective for enhancing the germination, but might be detrimental at lateral stages of development (Dao-liang *et al.,* 2009). The effect of magnetic field (MF) treatment on biological systems, particularly in plant has been studied by various researchers since 19th century (Florez *et al.,* 2007), for enhancing germination rate, seedling vigor as well as growth at lateral stages of development physically (Marks and Szecówka, 2010; Podleśny, 2004). Magnetic field changes the free radicals, concentration of ions and electrical charges without any degradation/alteration in the chemical profile of seed and resultantly makes the membranes more permeable, free movement of ions activates the metabolic pathways by enhancing the biochemical and physiological feedback (Labes, 1993; Pietruszewski, 2007; Wards, 1978).

Various researchers have studied and reported that maize, wheat, sunflower, barley, corn, beans, tomato, fruits seed *etc*. treated with magnetic field showed high performance in terms of plant growth, height, yield, seed mass per spike as well as shoot and root length and total fresh and dry masses (Fischer *et al.,* 2004; Florez *et al.,* 2007; Martinez *et al.,* 2009; Pietruszewski and Kania, 2010; Zepeda-Bautista *et al.,* 2010). Further more, the MF strength, exposure duration and modulation are also important in this regard (Tkalec *et al.,* 2009). Harichand *et al.* (2002) observed that the treatment of MF at 10 mT for 40 hours boosted up the plant height, spike mass and crop yield. Similarly, Vashisth and Nagarajan (2008, 2010) reported positive results in the growth of maize, chickpea and sunflower seeds exposed to static magnetic field.

Pea (*Pisum sativum L.*) belongs to the family *Papilionaceae* and is a versatile crop cultivated as an essential part of cropping systems of the world. It is used in rotation with cereals and oil seeds. Pea is an important winter pulse crop of Western Europe, America, India, Australia and Pakistan. Pea is among the four important cultivated legumes next to soybean, groundnut and beans. In Pakistan it is cultivated under a wide range of agroecological regions in plains during winter and in highlands during summer. The average cultivated area of this crop during past few years has been 135 600 ha with 81 900 t production of dry peas (Anonymous,

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2000; Nisar *et al.,* 2008). So far, despite its importance, little attention has been given for the improvement of pea crop native to Pakistani land using physical methods.

The aim of this paper is to obtain the effect of magnetic treatment on the shoot and root length, root and shoot dry mass, fresh root and shoot mass and chlorophyll contents of pea plant.

MATERIALS AND METHODS

The pea seeds were obtained from Ayyub Agricultural Research Institute, Faisalabad, Pakistan. The seeds of uniform size and shape were exposed to controlled magnetic field in the Department of Physics and sown in Vegetable Seed Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad.

The presowing magnetic treatments were administered using an electromagnet consisting of two pairs of cylindrical coils, each formed by 4,000 turns of 0.42 mm enamelled copper wire. Each pair of coils was wound 10 cm apart on an iron bar (dimensions 40 ×3.5 cm).The two bars were placed one above the other, their ends held by metallic supports. The coils were connected in series and fed through a power source using a variable transformer. A 50 Hz full wave rectified sinusoidal voltage was fed to the coils. When electric current passed through the coils magnetic field was generated in the air space between the two bars. Magnetic field treatment was applied according to De Souza *et al.* (2006) with some modification. Before treatment the seeds were disinfected with fungicide solution, washed thoroughly under tap water and in the end with distilled water. Seed were spread on moist filter paper in 15 cm Petri dishes for 16 h. The soaked pea seed were applied to the MF doses (strength and exposure duration) as follow:

MF, s	5 min	10 min	15 min
60 mT	D_1	D_2	D_3
120 mT	D_4	D_5	D_6
180 mT	D_7	D_8	D_9

Non-exposed seed were used as control (D_0). A rectangular glass dish having pea seed placed between the poles of electromagnetic having non-uniform magnetic strength for the required duration. The strength of the MF was controlled by regulating the current in the coil of electromagnet. A magnetic flux meter (ELWE, 8533996, Cremlingen, Germany) was used to measure the strength of MF between the poles. After respective treatment for specific period and strength the seeds were kept in germination incubator. All treatments in the experiment were run simultaneously along with controls under same laboratory conditions. Distilled water was given daily to each Petri dish to such extent that the filter papers remained wet. Seed germination was measured by following the method of ISTA (2004). The number of germinated seed was counted sharply after germination and the process was repeated for 7 days. After germination the seeds were sown in laboratory soil and the temperature was kept constant (22 ± 2°C). The growth parameters were determined according to De Souza *et al.* (2006) and Vashisth *et al.* (2010). The contents of chlorophyll (a) and (b) were measured by following the method of Yinan *et al.* (2005). The details of the methods are as follows. The weighed samples were put separately in 50 ml of *N*, *N*-dimethylformamide and extracted overnight in dark at 5°C, homogenized with the B-Brawn type homogenizer at 1 000 r.p.m. for 1 min. The homogenate was filtered through two layer cheese cloths, and was centrifuged at 2 500 r.p.m. for 10 min. The supernatant was separated and the absorbances were taken from 400-700 nm by using UV-Visible double beam spectrophotometer (Cecil, 7200). The absorbance was measured and following relations were used for the determination of chlorophyll (a) and (b) contents:

$$\text{Chl a} = 12.70A_{664.5} - 2.79A_{647}$$

$$\text{Chl b} = 20.70A_{647} - 4.62A_{664.5}$$

The data were analyzed using SPSS-16 software. For the laboratory experiment, two factor analysis of variance (ANOVA) was performed on a factorial experiment keeping the MF strength as a first factor and exposure duration second one. The significant levels ($P<0.05$) of difference for all measured trails; magnetic field, exposure time and interactions were estimated.

RESULTS AND DISCUSION

After germination, the seedling growth of pea was observed as compared to control. Presowing magnetic treatment significantly enhanced the growth characteristics such as shoot and root length, root and shoot dry and fresh masses. The chlorophyll (a) and (b) contents were found non significant at 95% confidence interval of mean after magnetic treatment. Table 1 depicts the mean values of shoot length, showing the 95% confidence interval of mean. Significant difference between magnetically treated and untreated seeds was observed for the doses of D_7 followed by D_2 treatment. However, significant differences were obtained for all other applied doses as compared to control. The lowest increment in shoot length was observed for D_9 treatment (14.1±0.85 cm), but it was higher than control (9.25±0.96 cm). So, a highly significant difference was observed for all MF treatments as compared to control for root length. The mean root length of treated sample was 17±1.29 cm for dose D_7 followed by D_2 (16±0.82 cm), while that of control attained the length of 5± 1.29 cm. Both shoot and root length showed significant difference at same doses of MF treatment (Table 1). The fresh root and shoot mass assimilation has found to be remarkably significant ($P<0.05$), pretreatment enhanced the fresh root and shoot mass as compared to untreated samples. The maximum values of fresh root and shoot masses were (1.78±0.05 g)

T a b l e 1. Effect of different MF strengths and exposure duration of pea seed on shoots length (SL), root length (RL), root dry mass (RDM) and shoot dry mass (SDM)

Treatments	Time (min)	SL (cm)	RL (cm)	RDM (g)	SDM (g)
Control		9.25 ± 0.96 f	5.50 ± 1.29 g	0.25 ± 0.00 i	0.11 ± 0.01 e
60 mT	5	17.50 ± 1.29 b	13.50 ± 1.29 c	0.38 ± 0.03 d	0.17 ± 0.02 c
	10	20.50 ± 1.29 a	16.00 ± 0.82 a	0.32 ± 0.01 f	0.15 ± 0.01 d
	15	17.25 ± 2.22 bc	13.00 ± 2.16 cd	0.31 ± 0.01 g	0.21 ± 0.02 b
120 mT	5	18.00 ± 2.16 b	14.25 ± 2.22 b	0.34 ± 0.01 e	0.17 ± 0.01 c
	10	15.50 ± 1.29 cde	11.50 ± 1.29 ef	0.44 ± 0.01 c	0.18 ± 0.01 c
	15	15.00 ± 0.82 de	12.25 ± 0.96 de	0.51 ± 0.02 b	0.18 ± 0.01 c
180 mT	5	22.25 ± 1.26 a	17.50 ± 1.29 a	0.29 ± 0.01 h	0.22 ± 0.02b
	10	16.75 ± 1.19 bcd	12.50 ± 0.58 cde	0.57 ± 0.02 a	0.18 ± 0.01 c
	15	14.13 ± 0.85 e	10.50 ± 1.29 f	0.50 ± 0.02 b	0.40 ± 0.01 a

Similar letters in columns correspond to statistically similar effects (mean values with 95% confidence level of interval are presented).

T a b l e 2. Effect of different MF strengths and exposure durations of pea seed on root fresh mass (RFM), shoot fresh mass (SFM) and chlorophyll (a) and (b) (mg g^{-1})

Treatments	Time (min)	RFM (g)	SFM (g)	Chl (a) (mg g^{-1})	Chl (b) (mg g^{-1})
Control		1.02 ± 0.01 e	2.30 ± 0.08 h	0.017 ± 0.004 a	0.006 ± 0.002 a
60 mT	5	1.31 ± 0.01 c	3.50 ± 0.08 d	0.028 ± 0.003 a	0.01 ± 0.005 a
	10	1.13 ± 0.01 d	2.68 ± 0.10 g	0.019 ± 0.007 a	0.008 ± 0.003 a
	15	1.28 ± 0.10 c	3.73 ± 0.10 c	0.020 ± 0.005 a	0.007 ± 0.003 a
120 mT	5	1.05 ± 0.01 e	3.38 ± 0.10 e	0.029 ± 0.006 a	0.01 ± 0.004 a
	10	1.33 ± 0.05 c	3.80 ± 0.00 bc	0.013 ± 0.003 ab	0.004 ± 0.002 ab
	15	1.45 ± 0.08 b	3.88 ± 0.05 b	0.009 ± 0.0006 b	0.003 ± 0.0004 a
180 mT	5	0.92 ± 0.01 f	3.23 ± 0.05 f	0.014 ± 0.0008 a	0.005 ± 0.003 a
	10	1.78 ± 0.05 a	4.40 ± 0.08 a	0.015 ± 0.001 a	0.005 ± 0.001 a
	15	1.13 ± 0.05 d	3.53 ± 0.10 d	0.008 ± 0.0001 c	0.003 ± 0.0006 c

Explanations as in Table 1.

and (4.4±0.08 g) over control (1.02±0.01 g) and (2.3±0.08 g), respectively (Table 2). This growth stimulation after MF pretreatment had a profound effect on lateral stages of growth. The mean value of pea plant height measured after 24 days of germination were as 20.5 cm (D_2), 19 cm (D_7), 18 cm (D_4 and D_5), 17 cm (D_3), 16 cm (D_1 and D_8) and 15 cm (D_6), while the untreated samples for three replicates attained the height of 9 cm. Table 1 demonstrates the mean values of dry root and shoot masses, respectively, showing the 95% confidence interval of mean. The greatest differences between treated and untreated samples were obtained for the doses of D_9 and D_6. However, significant differences were also obtained for all other applied doses then control.

The effect of MF treatment on chlorophyll (a) and (b) was found non significant at 95% confidence interval of mean. There was minute change in chlorophyll contents. Dose D_1, D_2, D_3, D_7 and D_8 enhanced the contents of chlorophyll (a) to some extent, while treatment D_5, D_6 and D_9 exerted negative impact on the contents of chlorophyll (a). Similarly, doses D_5, D_6, D_8 and D_9 showed the negative effect on chlorophyll (b), while other shows increasing trend (Table 2).

The effect of pretreatment of magnetic field strength, duration of exposure and their interactions are given in Table 3. Of the magnetic field strength and exposure time, 60 and 180 mT for 10 min exposure increased the shoot length and that of 180 mT (5 min) significantly enhanced the growth of root length followed by 60 mT for 10 min ($P<0.05$). The root dry mass was found higher for irradiation of 120 mT for 15 min, while 180 mT increased the shoot dry mass significantly ($P<0.05$) (Fig. 1 and Table 2). The assimilation of fresh mass of root and shoot was observed at MF treatment of 120 mT (15 min) and 180 mT (10 min), respectively (Fig. 1, Table 2). On the other hand, non significant ($p<0.05$) change was noted for 60 mT (10 min) and 120 mT (5 min) for chlorophyll (a) and 60 mT (5 min) and 120 mT (5 min) for chlorophyll (b) (Fig. 2, Table 2).

Table 4 shows the percentage effect (positive/negative) of MF treatment over untreated samples. The percent increment was found 140.5 and 218.2% for shoot and root length, respectively and that of root and shoot dry masses percent gain was observed from 8-104 and 36-263.6%, respectively. The assimilation of fresh root and shoot mass was observed 74.51 and 91.3%. The minimum positive effect of MF treatment was 81.1, 90.91, 8, 36.4, and 16.5% for shoot length, root length, root dry mass, shoot dry mass and fresh shoot mass, respectively and negative effect of MF treatment on root fresh mass, chlorophyll (a) and (b) was observed -9.80, -51, and -51.4%, respectively (Table 2). This effect was found conspicuous at lateral stages of development. Figures 3 and 4 show the photographs of pea root and shoot length grown from exposed and control seeds. In summary, the growth of pea samples treated with magnetic field were significantly higher and superior then the control. It is found that the MF treatments had acceleratory effect on root and shoot length, root and shoot dry and fresh masses, while change for chlorophyll (a) and (b) was marginal.

After MF treatment, fast and more uniform seedling growth, assimilation of masses in various morphological and physiological characteristic of pea plant was observed as compared to control. Our results are in accordance with various researchers, which have been reported for verities of crops. Florez *et al.* (2007), Vashista and Nagarajan (2008, 2010) noted a considerable enhancement in germination characteristics such as seedling vigor, shoot and root growth in maize, chickpea and sunflower seeds when treated magnetically. Similarly, Fischer *et al.* (2004) observed higher germination and growth of sunflower as compared to untreated

Table 3. Effect of magnetic field strength, duration of exposure and their interaction by analysis of variance (ANOVA) for variables

Parameters	MF		Time		MF x Time	
	F	*P*	*F*	*P*	*F*	*P*
Shoot length (cm)	116.72	0.001	17.95	0.001	10.99	0.001
Root length (cm)	100.42	0.001	11.73	0.001	8.25	0.001
Root dry mass (g)	1652.9	0.001	492.03	0.05	455.65	0.001
Shoot dry mass (g)	526.62	0.001	240.48	0.001	140.51	0.001
Root fresh mass (g)	104.83	0.05	135.76	0.001	152.08	0.001
Shoot fresh mass (g)	838.76	0.001	45.7	0.001	144.99	0.001
Chlorophyll a (mg g^{-1})	4.05	0.05	2.64	0.05	1.38	>0.05
Chlorophyll b (mg g^{-1})	3.68	0.05	4.24	0.05	1.07	>0.05

Statistical significance level ($P<0.05$), the values $P>0.05$ are non significant.

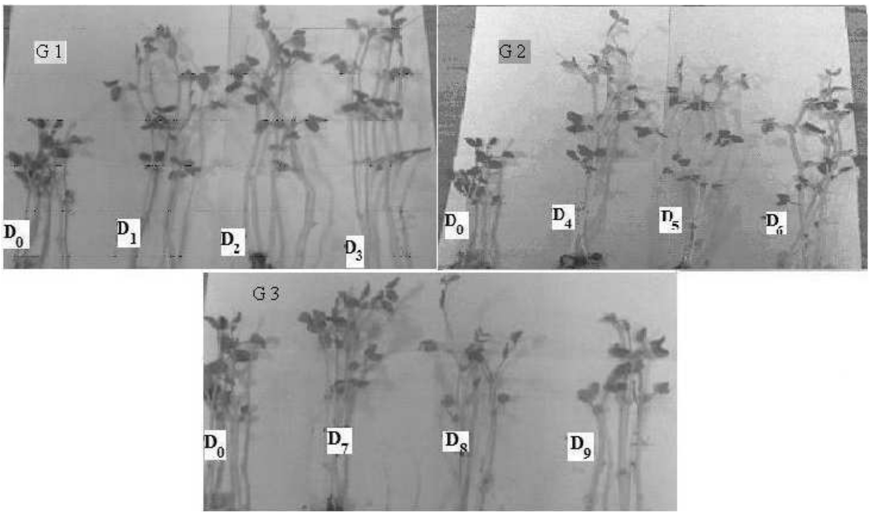

Fig. 1. Shoot length after MF treatment: G1: D_0 – control, D_1, D_2, D_3; G2: D_0 – control, D_4, D_5, D_6; G3: D_0 – control, D_7, D_8, D_9.

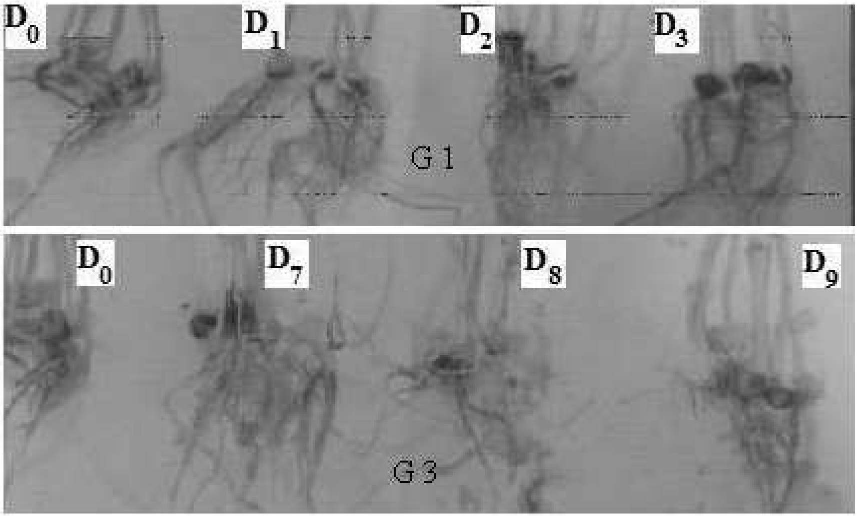

Fig 2. Root length after MF treatment: G1: D_0 – control, D_1, D_2, D_3; G3: D_0 – control, D_7, D_8, D_9.

T a b l e 4. Percentage change (%) (positive/negative) on various parameters of pea plant after MF irradiation

Treatments mT (min)	SL* (cm)	RL (cm)	RDM (g)	SDM (g)	RFM (g)	SFM (g)	Chl (a) (mg g^{-1})	Chl (b) (mg g^{-1})
Control	0.00	0.00	0	0.00	0.00	0.00	0.00	0.00
60 (5)	89.19	145.45	52	54.55	28.43	52.17	84.19	103.19
60 (10)	121.62	190.91	28	36.36	10.78	16.52	140.43	47.17
60 (15)	86.49	136.36	24	90.91	25.49	62.17	47.10	40.18
120 (5)	94.59	159.09	36	54.55	2.94	46.96	136.28	101.40
120 (10)	67.57	109.09	76	63.64	30.39	65.22	-0.11	-16.14
120 (15)	62.16	122.73	104	63.64	42.16	68.70	-29.85	-35.85
180 (5)	140.54	218.18	16	100.00	-9.80	40.43	1.65	2.14
180 (10)	81.08	127.27	8	63.64	74.51	91.30	4.45	-6.68
180 (15)	52.76	90.91	100	263.64	10.78	53.48	-50.99	-51.39

*SL – shoot length, RL – root length, RDM – root dry mass, SDM – shoot dry mass, RFM – root fresh mass, SFM – shoot fresh mass, Chl – chlorophyll (a) and (b).

seed samples. Marks and Szecówka, (2010) reported impact of variable magnetic field stimulation on growth of above-ground parts of potato plants, which increased as result of presowing magnetic treatment. Florez *et al.* (2004, 2007) reported enhancement in germination in rice when exposed to 125/250 mT MF for specific time intervals, which indicate that the better results are dependent to appropriate MF strength and exposure time combination. It is well understood from literature that the best outcome of seed germination are possible when optimal exposure doses are applied. The results of root and shoot length, root and shoot dry and fresh mass showed that specific combination of MF doses such as D_7 (180 mT, 5 min) for shoot and root length, D_6 (120 mT, 15 min) for root dry mass, D_9 (180 mT, 15 min) for shoot dry mass and D_8 (120 mT, 10 min) for root and shoot fresh mass gave highly significant response as compared to untreated samples (Table 2). This observation indicates that seed germination following growth and development take place at appropriate combination of MF exposure duration and strength (Bhatnagar and Deb, 1977). We found that magnetic field pretreatment could enhance plant growth, which is consistent with various previous studies (De Souza *et al.,* 2006; Martinez *et al.,* 2002). It is obvious from this study that appropriate MF treatment for specific time duration could accelerate the growth and enhance fresh and dry masses significantly in pea plant. Our results of pea plant growth in present study are in accordance with Yinan *et al.* (2005) for cucumber. Podleśny *et al.* (2004) reported the similar results for magnetically treated wheat, barley and various bean cultivar seeds. Similarly, the combined effect of MF and exposure duration accounted in this article are in agreement that reported by various other workers (Fischer *et al.,* 2004; Harichand *et al.,*2002; Martinez *et al.,*2009; Pietruszewski and Kania, 2010).

The magnetic field mechanisms of action on plants germination and seedling growth is not well known yet, however several theories have been proposed, including biochemical changes or altered enzyme activities. Seed germination stimulation might be attributed to a combined effect of biochemical, physiological, metabolic as well as enhanced enzymatic action. It is assumed that the magnetic field treatment influences the structure of cell membrane and in this way increase their permeability and ion transport in the ion channels, which as a result affects the metabolic pathways. The enzymes which are necessary for seed germination at particular stages of germination were found higher in magnetically treated seeds during seed germination. The magnetic field affects the biological objects by non-conventional spins, free radicals, liquids crystals or mobile electron charges. Chemically these free radicals are very active species, which take part in fast reactions and cause changes in the biochemical and physiological processes during seed germination and an increase in water uptake rate due to magnetic field treatment was found, which may be responsible for increased pea seed germination as well as seedling growth and at lateral stages of development (Labes, 1993; Podleśny *et al.,* 2004; Wards, 1978). Our findings of fresh and dry masses and increase in root and shoot lengths are not correlated with chlorophyll contents however the enhancement in seedling length and assimilation of dry and fresh masses might be attributed to the higher α-amylase, dehydrogenase and protease activities (Shantha and Nagarajan, 2010). This may be so because α-amylase is responsible for the degradation of food reserves of the seedling during germination. An increase in germination speed in magnetically treated seeds can be attributed to a consequence of increased activity of α-amylase compared with unexposed controls. These results are in accordance with the results of Bhatnagar and Deb (1978) that wheat seeds treated at 150 and 100 mT had significantly higher ($P<0.01$) α-amylase activity than the controls. An increase in dehydrogenase activity has been reported in primed soybean, carrot and tomato compared with unprimed seeds. Also, greater glucose-6-phosphate dehydrogenase activity has been reported in primed sweet corn seeds. Exposure of magnetic field appears to act like priming, with similar enhancement effects. One theory of the magnetic field interaction with biological systems is 'radical-pair mechanism' consisting of the modulation of single-triplet inter-conversion rates of a radical pair by magnetic fields. Magnetic field increased the average radical concentration, prolonging their life time and enhancing the probability of radical reaction with cellular components. When the exposure time of MF was increased, the effects of the MF on growth changed because of the increased peroxides activities, while root formation was increased; chlorophyll content of plantlet exposed to a MF for this exposure time was decreased with respect to controls (Atak *et al*., 2007).

CONCLUSIONS

1. The presowing magnetic field treatment significantly enhanced the seedling length, dry and fresh mass in comparison to controls.

2. Magnetic treatments had positive effect on root and shoot length, root, shoot dry and fresh mass significantly, while non effective to enhance the contents of chlorophyll (a) and (b).

3. Among the various combinations of field strength and duration, 120 mT for 15 min and 180 mT for 10 min exposure yielded superior results.

4. The improved root length suggests that magnetically treated seeds can be used in practical agriculture where better root growth will enable extraction of moisture from deeper soil layers.

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