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Germination parameters and peroxidase activity of lettuce seed under stationary magnetic field

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Abstract

Abstract

The magnetic fields influence the biological systems by several mechanisms. The magnetic field induces the translation and rotation movements of the electric charges. The effects of a stationary magnetic field on the germination and initial stages of growth of Lettuce seeds (*Lactuca sativa* var.*longifolia*) have been studied. The seeds were exposed to a magnetic field strength (125 and 250mT) for different time including 0, 1, 6, 12 and 24 hour. The results revealed a significant difference ($P<0.01$) of Lettuce seeds traits exposed to stationary magnetic field. By conducting the results, germination parameters in 0 and 24 h were underneath the 1, 6 and 12 h exposed to both 125 and 250mT of magnetic field. Germination rate at both 125 and 250mT were increased. Root length at 250mT was rapidly raised and then rapidly reduced than 125mT. Peroxidase activity rose with increasing to exposed magnetic field. The decline in peroxidase activity above 12 h is seen for 250mT. Maximum peroxidase activity at 125mT was recorded incubated 24 h exposed to 125mT magnetic field. Finally, magnetic treatment produces an increase in the germination and first stages of growth of lettuce. So, the magnetic field as nondestructive growth factors to accelerate germination and early growth of lettuce can be used.

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Introduction

Magnetic fields abound in our natural environment, in industrial factories and in medical diagnostics as well as medical therapeutics. Knowledge of the mechanisms of the action of the magnetic field on various biological systems like cells, tissues and organs, may be effectively used as a means regulating biological activity and removing undesirable substances from the system. Magnetic fields were used widely as pre sowing seed treatments to increase seed vigor, seedling growth and yield (Phirke et al., 1996; Ahmet, 2003; Soltani et al. 2006). The magnetic field interacts with ionic currents in the embryo cell membrane. This interaction induces changes in the ionic concentrations and in the osmotic pressure on both sides of the membrane, and therefore in the water relations in seeds. (Garcia and Arza, 2001). The range for magnetic field strength from 0.072 to 0.128 tesla was experimented for seed germination of soybean, cotton and wheat by Phirke et al (1996). The cucumber seed germination rate was significantly enhanced by magnetic field pretreatment (Yinan et al. 2005). Podlesny et al (2005) carried out proved a desirable influence of the magnetic treatment on the emergence, growth and development of pea. Also, Martinez et al (2000) were showed the positive effect of 125mT stationary magnetic field on the first stages of growth in barley. Pretreatment of magnetic field (0.45 tesla) could accelerate seedling development and increase total leaf numbers (Yinan et al. 2005). Effect of 125 and 250mT magnetic field on seed germination of two wheat cultivars was investigated by Gholami and Sharafi (2010). Lentil (*Lens culinaris* Medik) seeds were exposed to magnetic field from 0 to 1.5T for time periods of 5 to 20 min for the testing of germination and early growth (Asgharipour and Razavi, 2011). Chronic exposure to 125 and 250mT had a significant stimulatory effect on the initial stages of growth of wheat plants, Martinez et al. (2002).

Lettuce (*Lactuca sativa*) is an annual plant of the Asteraceae family. It is most often grown as a leafy vegetable (Prohens and Nuez, 2008). The lettuces imbibed in darkness stimulate a form of secondary dormancy named skotodormancy (Bewley, 1980). Carpita and Nabors (1981) found that the action of the red light on lettuce seeds alters the ionic concentrations in the cells of the embryo via the action of the phytochrome, using dry seeds. There is no other choice than to suppose that the excess of ionic concentration that was found in the cells of the treated seeds emigrated from the cotyledons by means of the ionic flow through the cellular membrane, assisted in this case by the phytochrome, using dry seeds. On the other hand, previous researches cleared that the magnetic field sometimes changes the structural organization of cells, involving enzymes and membranes, and enhanced seed germination. The application of a magnetic field creates a stress condition for growth of plants just as environmental stresses such as drought, salinity, mineral deficiency, UV light, heat and chilling cause. All those conditions cause the production of reactive oxygen species (ROS) in the cells (Celik et al. 2009). So, the main objective of this study is to quantify the possible effects of magnetic treatment on the germination characteristics and peroxidase activity of Lettuce seeds subjected to *in vitro* stationary magnetic field (125 and 250mT) and exposure time (0, 1, 6, 12 and 24 hour).

Material and methods

Plant materials

Germination tests were carried out at laboratory conditions with lettuce cultivar (*Lactuca sativa* var.*longifolia*) seeds named Romain.

Seeds and Magnetic Treatment

Germination tests were applied according to the guidelines issued by the International Seed Testing Association (ISTA, 2004). Each filter paper with seeds was rolled and placed in a vessel containing distilled water. 24 hours later, when seeds were soaked, each roll was subjected to a magnetic treatment. The procedure of study was conducted according to Florez et al. (2007). The magnetic fields generated by ring magnets with magnetic induction values 125mT and 250mT. The geometric characteristics are 70mm external diameter, 30mm internal diameter, 10mm height for 125mT and 15mm height for 250mT. The magnet was placed at the top of the vessel to generate each magnetic field, and each roll was placed into hole of the magnet for the time established for each magnetic field. The 125mT and 250mT applied were obtained by exposing the seeds to each magnetic field induction for different time, as follows: h0 (without exposure), h1 (1 hour), h2 (6 hour), h3 (12 hour) and h4 (24 hour). The distance between any two vessels was at least 25 cm, to avoid the influence of each magnet on the other vessels around (Florez et al. 2007).

Germination Test

All experiments were carried out at 20±1ºC and 24 h photoperiod in incubator condition. The number of germinated seeds was recorded 4 times per day for the time necessary to achieve the final number or percentage of germinated seeds. Seeds were observed daily for up to 5 days and considered germinated when the radicle was approximately 1 mm long or more. The rate of germination was assessed by determining the mean germinating time. The percentage germination and the germination rate coefficient were calculated as follows:

$$\text{GP}=100\,\frac{n}{N}$$

Where: GP = germination percentage n = number of seeds germinated, N = total number of seeds.

$$GR = 100 \times \frac{\sum n_i}{\sum D_i n_i}$$

Where: GR = germination rate, ni = number of seeds germinated at day Di, Di = number of days after starting the test (Florez et al. 2007; Soltani et al. 2006).

Root length was measured for all seedlings on the 9th day of the germination test. At the end of the germination test, fresh weight of ten seedlings as well as dry weight after oven drying at 70°C for 10 h were determined (Balouchi and Modarres, 2009).

Peroxidase assay

For peroxidase assay, 1 gram of germinated seeds was grinded and then 3ml of distilled water added during homogenation. The slurry was centrifuged (SIGMA-3K30) at 10000g for 15min at 4°C. The supernatant, which contained peroxidase activity, was used as the enzyme source for the experiment. The substrate mixture contained 10 ml of 1% guaiacol, 10 ml of 0.3% hydrogen peroxide and 100 ml of 0.05M sodium phosphate (pH 6.5) buffer. The reaction cuvette contained 2.87 ml substrate mixture, 0.1 ml crude extract, and 0.03ml water in a total volume of 3 ml. Peroxidase activity was determined at 25°C with a spectrometer (PD-303UV) at 470 nm using guaiacol as the substrate and H_2O_2 as the hydrogen donor. One unit of activity is defined as a change in absorbance of 0.001 min^{-1} (Mousavizadeh et al., 2011).

Statistical analysis

Magnetic field was defined in two levels (125mT and 250mT) and time was supplemented in five levels (0, 1, 6, 12 and 24 hour). Data analyzed by SAS software (SAS Institute Inc., 2001). The ANOVA was performed for analysis of the data obtained for each experiment (P<0.01). The regression model was fitted to the data of each magnetic field using the Proc Reg of SAS. The changes of traits over long-term simulation versus times of magnetic field were describable using polynomial regression model ($y = a + bx + cx^2$). In one case it was impossible to fit model to the data (for the peroxidase activity of 125mT; see Fig. 5); hence, a simple, linear regression model ($y = a + bx$) was used (Soltani, 2007).

Results

As shown in Table 1, the results revealed a significant difference (P<0.01) of Lettuce seed traits in different times exposed to stationary magnetic field. In Figs 1-6. the curves were plotted for both 125 and 250mT of magnetic field in different times. It shows that in all cases, the 0 and 24 h were underneath the 1, 6 and 12 h exposed to both 125 and 250mT of magnetic field (with the exception of peroxidase activity at 125mT).

Root length

As seen in Fig 1, the root length was increased until 12 h incubated at both 125 and 250mT magnetic field. Then root length decreased with time rising to 24 h. Based on X^2 slope, root length at 250mT was decreased rapidly than at 125mT. On the other hand, root length at 250mT was rapidly raised and then rapidly reduced than 125mT. Root length of control seeds (0 h) was below that compare to magnetic treatment applied especially for 125mT stationary magnetic field (Fig. 1).

Table 1. ANOVA of Lettuce seed parameters estimate for the regression model relating the percentage significant difference of the mean and variance of stationary magnetic field (125 and 250 mT).

mT	trait	RMSE	a ± SE	b ± SE	c ± SE	R^2	CV%
125	Root length	0.358	1.41±0.13 **	0.16±0.03 **	-0.004±0.001 **	0.797	17.4
	Fresh weight	0.018	0.106±0.006 **	0.007±0.001 **	-0.0002±0.00006 **	0.958	14.2
	Dry weight	0.002	0.005±0.0008**	0.001±0.0002**	-0.00004±0.000008**	0.89	22.8
	Germination rate	2.34	45.15±0.87 **	0.806±0.218 **	-0.035±0.0087 **	0.62	5.02
	Germination %	5.32	89.66±1.98 **	1.409±0.49 *	-0.056±0.019 *	0.536	5.7
	Peroxidase activity	2098.7	3091.03±656.1**	233.6±53.32**	-	0.968	31.1
250	Root length	0.322	1.43±0.12 **	0.226±0.03 **	-0.009±0.001 **	0.921	16.0
	Fresh weight	0.018	0.126±0.006 **	0.004±0.001 *	-0.0002±0.00006 *	0.65	13.9
	Dry weight	0.001	0.008±0006 **	0.001±0.0001**	-0.00005±0.000006**	0.983	15.8
	Germination rate	2.052	43.57±076 **	1.07±0.19 **	-0.042±0.007 **	0.837	4.4
	Germination %	5.14	87.81±1.91 **	1.833±0.47 **	-0.066±0.019 **	0.73	5.4
	Peroxidase activity	1756.2	2772.6±654.9**	400.15±163.58*	-12.311±6.58 ns	0.976	30.3

The parameter estimates are: *RMSE* = root mean square error, *a*= the intercept, *b* and *c*= the slope *X* and X^2, *SE* = standard error, R^2 = R Square, *CV*= coefficient of variation.
** ($P<0.01$), * ($P<0.05$), ns ($P>0.05$).

Phirke *et al.* (1996) revealed that 0.10 tesla was the optimal level for of soybean, cotton and wheat germination. Soltani and Kashi (2004) demonstrated that magnetic field increased root length of lettuce versus control. DeSouza et al (2006) explained pre-sowing magnetic treatments under 100 and 170mT would enhance the growth and yield of tomato crop.

Fresh and dry weight

Fresh and dry weight of untreated seeds is lower than the magnetic treatment seeds especially for 125mT stationary magnetic field (Fig 2 and 3). Dry weight increasing at both 125 and 250mT magnetic field began at 1 h, and followed to 12 h, then were declined to 24 h. Dry weight at 250mT was raised as 125mT, but then rapidly reduced than 125mT (Fig. 3).

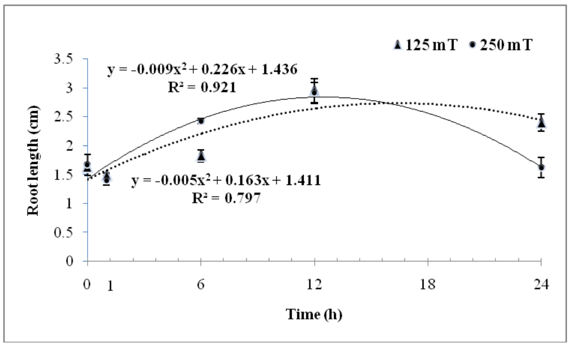

Fig. 1. Root length of Lettuce germinated seeds subjected to 125 and 250mT stationary magnetic field for diferrent times. The vertical bars represent the SE of the mean.

Magnetic field can have different effects on plant metabolism (Cakmak *et al.,* 2010). Magnetic treatment increased the absorption and assimilation of nutrients (Kavi, 1977), and ameliorated photosynthetic activities (Lebedev *et al.,* 1977). Aladjadjiyan (2002) detected that seed exposure to a 150mT magnetic field stimulated shoot development and led to increase of the fresh weight and shoot length of maize plants. Cakmak *et al.* (2010) investigated the effects of low static magnetic field (4 or 7mT) on seedling growth of bean or wheat seeds in different osmotic conditions. They reported that the application of magnetic field (4 or 7mT) significantly enhanced of early growth, and dry biomass accumulation of bean and wheat seeds. Also, treatment of lentil seeds with magnetic fields increased mean germination time, seedling length and seedling dry weight (Asgharipour and Razavi, 2011). Soltani and Kashi (2004) revealed that magnetic field increased fresh weight of lettuce root and shoot versus control.

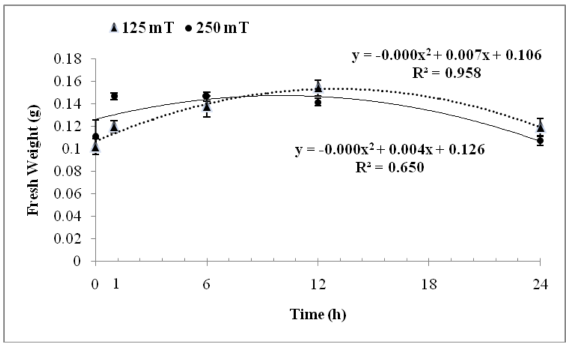

Fig. 2. Fresh weight of Lettuce germinated seeds subjected to 125 and 250mT stationary magnetic field for diferrent times. The vertical bars represent the SE of the mean.

germination rate

As seen in Fig 4, germination rate at both 125 and 250mT were raised. The 250mT (X=1.072) increased rapidly than the 125mT (X=0.806). Germination rate of 0 as control and 24 h exposed to both 125 and 250 mT magnetic field were lower than the other times. Germination rate was improved in to both 125 and 250mT magnetic field for 1, 6 and 12 h incubated (Fig. 4).

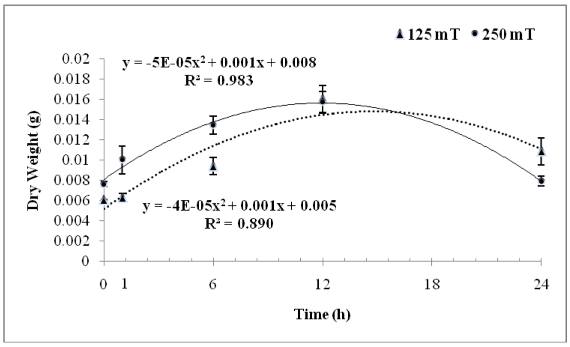

Fig. 3. Dry weight of Lettuce germinated seeds subjected to 125 and 250mT stationary magnetic field for diferrent times. The vertical bars represent the SE of the mean.

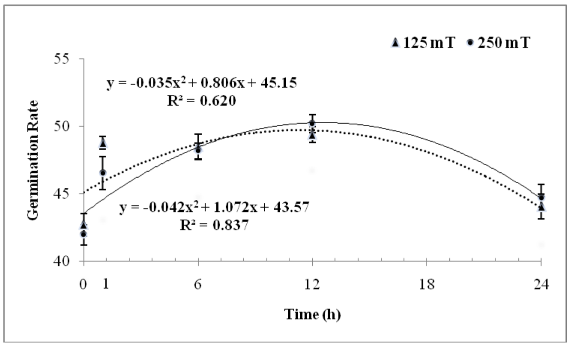

Fig. 4. Germination rate of Lettuce seeds subjected to 125 and 250mT stationary magnetic field for diferrent times. The vertical bars represent the SE of the mean.

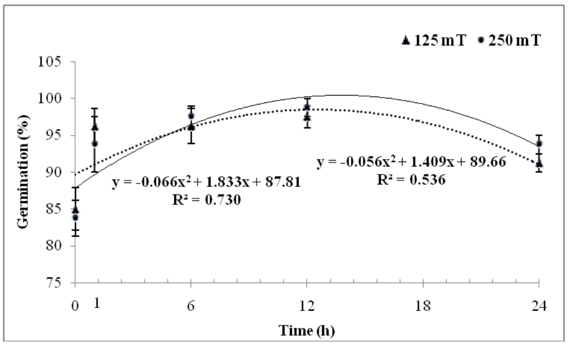

Fig. 5. Germination percantage of Lettuce seeds subjected to 125 and 250mT stationary magnetic field for diferrent times. The vertical bars represent the SE of the mean.

In treated seeds with magnetic field, plant emergence was more regular and it was occurred earlier in comparison to the control Podlesny et al (2005) confirmed the positive effect of the magnetic treatment on the germination and emergence of broad bean. Mean germination time in wheat was significantly increased with increasing the time of seed exposed at magnetic field (Gholami and Sharafi, 2010). Magnetically treated lettuce seeds absorb more water and absorb it faster than untreated seeds (Garcia *et al.,* 2001). Experimented on water absorption by lettuce seeds previously treated in a stationary magnetic field of 0-10mT. The results provides that the magnetic field alters the water relations in seeds, and this effect may be the explanation of the reported alterations in germination rate of seeds by the magnetic field (Garcia *et al.,* 2001). Yinan et al (2005) showed that sprouting and growth of the seedlings were accelerated in the cucumber seeds treated with magnetic field for a short time. Furthermore, they reported an increase in plant growth, lipid oxidation and ascorbic acid contents under magnetic conditions. Florez *et al.* (2007) observed an increase for initial growth stages and an early sprouting of rice and maize seeds exposed to 125 and 250mT stationary magnetic fields. Martinez *et al.* (2000 and 2002) observed similar effects on wheat and barley seeds magnetically treated. The greatest increases of the various treatments were obtained for plants exposed to 0.3 and 1.2 T for 10 min (Asgharipour and Razavi, 2011). Martinez et al. (2009) reported that germination rate of tomato treated seeds to 125 and 250mT magnetic field was higher than the control. Florez et al. (2007) realized that germination and growth of maize seeds treated by magnetic field had grew higher and heavier than control, the greatest increased were obtained for plants exposed for 24 h.

germination percantage

The germination percantage of Lettuce seeds that was exposed for 0 as control and 24 h to both 125 and 250mT magnetic fields were below to compare of other time treatments achieved (Fig 5). magnetic field of 125 and 250mT for 1, 6 h and especially 12 h were successful in improving the germination percantage (Fig 5). Germination percantage increasing at 125 and 250mT began in 1 h subjected to magnetic field, and the germination percantage at 250mT (X=1.833) increased rapidly than 125mT (X=1.409). After 12 h followed to 24 h, germination percantage was decreased at both 125 and 250mT (Fig. 5).

Aladjadjiyan (2002) detected that Maize seeds exposure to a 150mT magnetic field stimulated shoot development and led to increase of the germination. The positive effect of magnetic field on Lettuce seeds germination percentage had published by Soltani and Kashi (2004). The results indicated that the effects of treatment depended on strength and period of exposure to magnetic field (Asgharipour and Razavi, 2011). Soltani et al. (2006) have explained the positive effect of magnetic field on *Asparagus officinalis* seed germination and seedling growth.

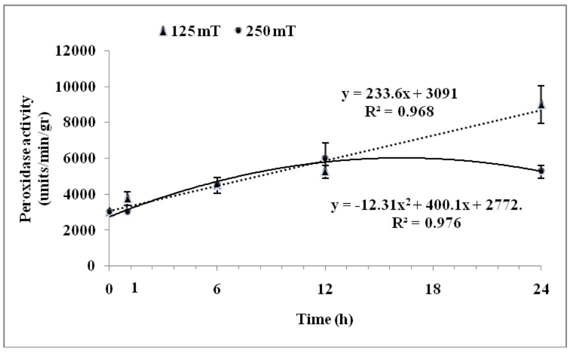

Fig. 6. Peroxidase activity of Lettuce seeds subjected to 125 and 250mT stationary magnetic field for diferrent times. The vertical bars represent the SE of the mean.

peroxidase activity

As seen in Fig 6, the first part of the 250mT graph is suggested that peroxidase activity rose with increasing to exposed magnetic field. The slightly decline in peroxidase activity was seen above 12 h for 250mT. It suggests that high oxidative stress of Lettuce seedling at 250mT seen when magnetic field gets to 12 h. Also, peroxidase activity was raised at 125mT. Peroxidase activity at 125mT was increased straightly (X=233.6) to 24 h. Maximum peroxidase activity at 125mT was recorded in 24 h exposed to magnetic field. Peroxidase activity of 0 as control exposed to both 125 and 250mT magnetic field were lower than the other times.

Racuciu et al. (2008) reported that the activities of some enzymes were increased by exposure to magnetic field. Previous studies showed that Magnetic fields induced lipid peroxidation and generation of reactive oxygen species (ROS) (Ishisaka et al. 2000). Amara et al. (2007) revealed that static magnetic field exposure (250mT, during 3 h) did not significant effect on enzymes as glutathione peroxidase and did not cause oxidative stress and DNA damage in cells. The mechanism of stimulating effect of magnetic field pretreatment on seed germination and seedling growth was unknown (Yinan et al. 2005), although most seemed to involve changes in intracellular levels of Ca^{2+} and in other ionic current density across cellular membrane (Lyle et al., 1991), which caused alterations in osmotic pressure and changes in capacity of cellular tissues to absorb water (Garcia and Arza, 2001). Cytochemical studies indicate that plant root cells exposed to weak magnetic field show Ca^{2+} over-saturation in all organelles and in cytoplasm unlike the control ones (Belyavskaya, 2004).

Conclusion

The use of magnetic fields to accelerate germination and early seedling growth stimulation of lettuce seed is possible. The impact of magnetic fields may be positive or negative on biological systems, depending upon the prevailing conditions where the event occurs. Magnetic field increased the rate and percentage of germination of lettuce seeds. Also, root length, fresh weight and dry weight of seedlings were increased with exposing to magnetic field until 12 hour at both 125 and 250mT treatments. On the others hands, our results cleared that the peroxidase activity of seeds increased under magnetic field. It seems that, the peroxidase activity in treated Lettuce seed with low to moderate magnetic field can be effective in triggering the fast germination and early vigor of seedling. At the time of exposure of seeds with magnetic field, the best time was 1 to 6 hours. The magnetic field as nondestructive growth factors to accelerate germination and early growth of lettuce can be used. It seems that high seed vigor leads to high yield by shortening the days from sowing to complete ground cover. Stimulation of lettuce plants with magnetic field in more advanced stages may have significant positive effects on plant growth and development. Further experiments are needed.

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