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OBSERVATION OF THE EFFECT OF STATIC MAGNETIC FIELD 0.1 MT ON α-AMYLASE ACTIVITY IN LEGUME GERMINATION

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Abstract-*Studies on the influence of magnetic fields on plant growth and development provide a promising prospect in the field of agricultural engineering. But there are still many obstacles encountered, because the plant response to the magnetic field treatment varies depending on the strength, intensity, and duration of exposure. Type, age, as well as parts of the plant are used also gives a different response to magnetic field treatment. Previous studies showed that a magnetic field can increase the germination of some seeds of agricultural crops. In this study, The entire treatment duration of exposure 0.1 mT magnetic field causes an increase in α-amylase activity in all legume seedlings observed. Fluctuations in enzyme activity during the germination of kidney bean seedlings are relatively the same as the black turtle bean, as well as enzyme activity in soybean and mung bean. But between the two groups of seedling legumes (kidney beans and black turtle beans vs. Soybeans and green beans) show a very clear difference fluctuations enzyme activity during germination.*

Keywords: *α-amylase, seedling*

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Introduction

Plants are organisms that cannot move so that the growth is strongly influenced by local environmental factors. Most people assume that the factors responsible for affecting the growth and development of plants are: temperature, humidity, available nutrients in soil, and light. However, it has long been observed the magnetic field effect on plant growth and developent because the earth is actually a source of natural magnetic fields. Telford (1990) explains that according to Karl Frederick Gauss, magnetic field of the Earth has a close relationship with the rotation of the earth because the earth's magnetic poles close to the Earth's spin axis

Study the influence of magnetic fields on various types of plants have been done long ago. However, the results vary depending on the treatment of the magnetic field (strength, frequency, duration of exposure) (Criveanu, H.R. dan G. Taralunga. 2006 dan *Atak et al., 2003*) as well as the plant species, age and plant organ treated. As a result, a lot of things about the mechanism and the role of magnetic field in affecting the cell metabolism in plant tissues then expressing its influence on the plant growth and development cannot be fully understood (Atak et al., 2003.). This fact attracts many researchers to continue to study it, mainly due to some of the results of the previous studies provide promising prospects in the use of magnetic fields to improve the plant growth and production (De Souza et al., 2005 and Esitken et al., 2004).

Treatment of the magnetic field is known to be able to increase the germination percentage of various types of plants (Mausavizadeh et al., 2013; Pourakbar and Hatami, 2012**;** Farashas et all, 2011; Aladjadjiyan et al., 2003; Novitsky et al., 2001), germination rate (Mausavizadeh et al., 2013; Vashist and Nagarajan, 2010; Criveanu, and Taralunga. 2006 and Podlesny et al., 2005) growth rate of seedling (Mausavizadeh et al., 2013 and Agustrina et al., 2012), enzyme activities including α- amylase (Mausavizadeh et al., 2013; Pourakbar and Hatami, 2012**;** Reddy et al, 2012; Vashist, A., and S. Nagarajan. 2010; Atak et al., 2007, and Pintilie et al, 2006), chlorophyll content (Alikamanoglu et al., 2007; Atak et al., 2007; Pintilie et al, 2006; and Novitsky et al., 2001), and the size of parenchyma cells, vascular bundle cells as well as stomata size of young plants (Agustrina et al. 2011).

All of the results of the studies above indicate that treatment of the magnetic field is able to improve the quality of germination. One of the important enzymes in the germination process is α-amylase as instrumental in deciphering the food reserves in the cotyledons polysaccharide into simpler compounds. α-amylase activity during germination induced by an increase in water content at the beginning of seed germination (Vashisth and Nagarajan, 2010). The enzyme α-amylase activity in seeds were germinated breaks the glycoside bonds in the middle of the polysaccharide chain. Their activity creates a mixture of glucose, maltose, isomaltose and oligo-saccharides. Beta-amylase then tears off the rest of the maltose from the non-reductive end of the polysaccharide chain (Rochalska and Orzeszko-Rywka, 2005). The beginning process of decomposition of food reserves in the seed source is vital, because the availability of carbon compounds is essential for simple germination metabolic processes so that individuals eventually grow new plants.

This paper report the study study done on the observation of α-amylase activity in the 4 types of 3 legume species: kidney bean and turtle black bean (Phaseolus vulgari), soybean (Glycine max), and mung beans (Vigna radiata) treated with 0.1 mT magnetic field with different long exposure long exposure to determine which treatment is most

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appropriate for improving quality (vigor) seedlings

Materials and Methods

1**.** Seed Germination

Legume seeds used in this study were kidney bean and black bean (*Phaseolus vulgaris*), soy bean (*Glycine max*), and mung bean (*Vigna radiata*) were collected from several plant breeding institutions. Seeds were selected for uniform size and shape. They were placed in petridish, given a sufficient water before treated by 0.1 mT magnetic field with exposure duration of: 7'48 "; 11'44", and 15'36 ". As controls were magnetically unexposed-seeds. The petridishes then were placed in germinator until the seedlings are ready to be used for measurements of α-amylase enzyme activity.

2**.** α-**amylase** activity

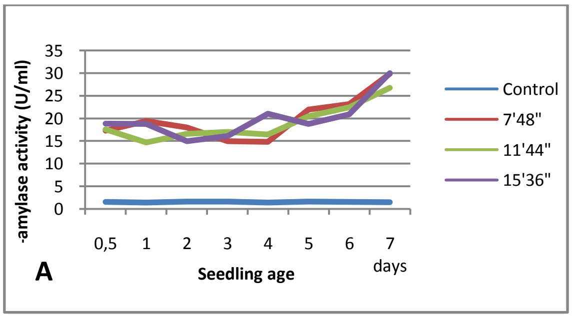
Observation α-amylase activity was done on the basis of seedling age (0.5 to 7 days) and length of hypocotyl (1, 3, 5, 7, and 9 cm). In order to measure the activity of α-amylase, the following reaction was set up.
a) Sample analysis: 250 μ l 250 μ l enzyme and substrate μ l (0.1% starch) were incubated in 30 ° C for 10 minutes. The reaction was stoped by adding 250 μl HCl, 250 μ l iodine solution, and 4 ml of distilled water.
b**)** Control analysis**:** 250 μ l enzyme activity was inactivated by adding 250 μl of 1 N HCl before incubation at 30 ° C for 10 minutes. Into the tube then the substrate (0.1% starch) 25 μ l, 250 μ l iodine solution, and 4 ml of distilled water were added.

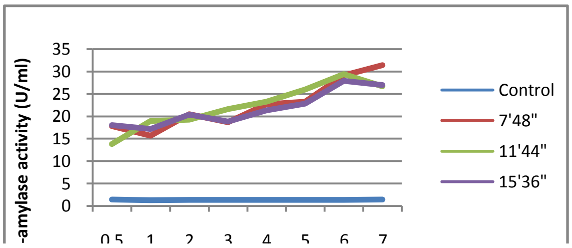
The α-amylase enzyme activity wasas then measured at Abs575

Results

Water plays a key role in the germination process. Germination is the process of growth of the zygote which begins with the process of water imbibition. Water status changes in enzyme activity in seeds stimulate germination, increased uptake of oxygen as a sign of an increase in seed respiration and metabolism. Germination process is completed with radicle protrusion through the seed coat and adsorption of water and oxygen rapidly increases (Vashisth and Nagarajan, 2010). Alpha amylase plays an important role in hydrolyzing the endosperm starch into sugars, which provide the energy for the growth of roots and shoots (Kaneko et al., 2002).

Studies on seed germination of various crops showed that magnetic field treatment can accelerate the hydration of seeds and improve seedling fresh weight, germination percentage (Bautista, 2013 and Reddy et al 2013) seedling dry weight (Pourakbar and Hatami, 2012), and the germination rate (De Souza et al., 2006).





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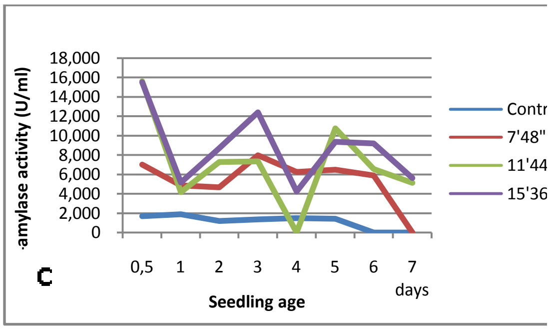


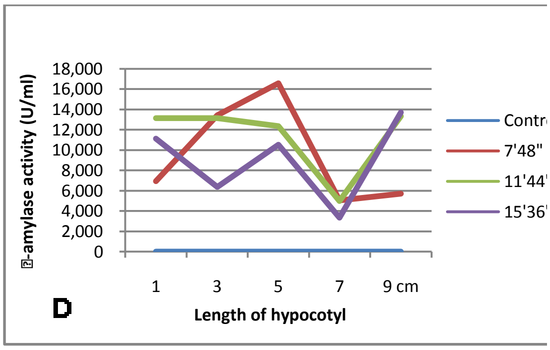
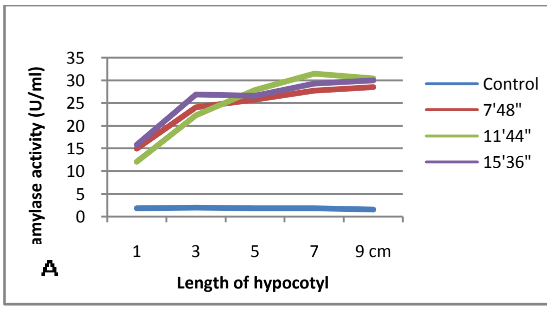
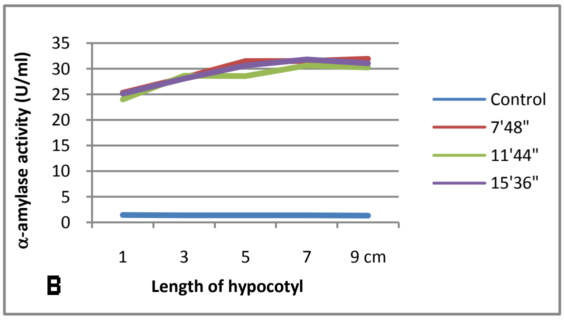
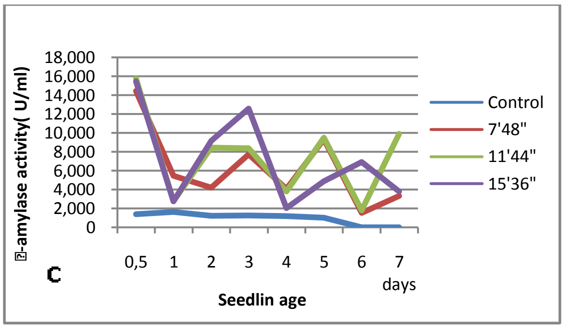


Figure 1. Changes in the activity of α-amylase in legum seedling of *Phaseolus vulgaris*: (A) kidney bean and (B) black turtle bean, (C) *Glycine max*: white soybean, and (D) *Vigna radiata*: mung bean within7 days of germination







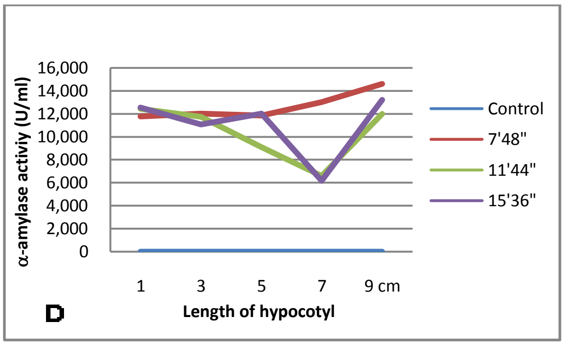


Figure 2. Changes in the activity of α-amylase in legum seedling of *Phaseolus vulgaris*: (A) kidney bean and (B) black turtle bean, (C) *Glycine max*: white soybean, and (D) *Vigna radiata*: mung bean measuerd based on length of hypocotyl

Based on several other studies Morejon et al. (2007) explains in his paper that magnetic field exposure to normal water (water+ions) changes some of its physical and chemical properties such as: surface tension, conductivity,

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solubility of salts, refractive index and pH. By application of magnetically treated water (MTW) in pine seed germination, he showed that it improves germination percentage. The result leads to a hypothesis that MTW properties as mwntioned above make it much easier to can penetrate inner parts of the seed. Several other theories have also been proposed to explain the mechanism of magnetic field effect in plant germination metabolisma, including biochemical changes or altered enzyme activities (Majd and Shabrangi, 2009). Garcia and Arza (2001) carried out an experiment study on water absorption by lettuce seeds previously treated in a stationary magnetic field of 1 to 10 mT. They reported an increase in water uptake rate due to the applied magnetic field, which may be the explanation for the increase in the germination seed of treated lettuce seeds. It seems changes in intracellular levels of ca^{2+} and in other ionic current density across cellular membrane (Lyle et al., 1991) cause alteration in osmotic pressure and changes in capacity of cellular tissues to absorb water (Garcia and Arza, 2001)

Increase in water status during seed imbibition in maieze seeds as a result of magnetic field treatment is detected early appearance of hydration water, greater amount of cytoplasmic bulk water, hydration water, molecular mobility of cytoplasmic bulk water, hydration water of macromolecules. This early hydration of macromolecules as well as greater activities of membranes and enzymes during germination were responsible for Quicker germination of seeds Vashisth and Nagarajan (2010). These results are consistent with the results of our study on the treatment of the magnetic field exposure to the amylase activity in germination of legume seeds. All of treatments of duration of exposure of magnetic field seems increased the activity of α-amylase in all legume seedling observed.

The activity of α-amylase in legume seedlings measured both by seedling age (Fig. 1) and length of hypocotyl (Fig.2) is greater in seedlings from treated seeds with magnetic fields exposure than from untreated seeds or controls. Moreover, in the figure above, it can be seen that the entire treatment of exposure duration of magnetic field in the early germination increased enzyme activity. The fact lead to the hypothesis that the entire treatment duration of exposure of magnetic field in the present study has the same effectiveness in increasing the activity of α-amylase during germination.

The enzyme α-amylase known has a substantial role in the degradation of food reserves of the seedling during germination. In the present study, increase in enzyme activity has been seen since the early germination: 0.5 days of germination (Fig. 1) or at the time of hypocotyl length only reaches about 1 cm (Fig.2) which is in accordance with the results of other studies on the germination enzyme activities in various magnetically-treated seeds. The increase in activity of germination enzymes activity is then followed with the improvement of seedling performance or vigor such as the early onset of germination, germination percentage, seedling growth rate, as well as fresh and dry weight (Mausavizadeh et al., 2013; Pourakbar and Hatami, 2012; Vashisth and Nagarajan, 2010, and Atak et al., 2007). These results strengthen the opinion Reddy et al (2012) that higher enzyme activity in magnetic field treated seeds could be triggering the fast germination and early vigor of seedlings.

The enzym α-amylase activity during germination fluctuates. Fluctuations in enzyme activity during

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germination appear to be relatively similar in kidney bean seedlings and black turtle bean seedlings, it is also the α-amylase activity between white soybean seedlings and mung bean seedlings. But when we compare the α-amylase enzyme activity among legume seelings of *Phaseolus* sp., *Glycine* sp., and Vigna sp., seems very different. This result proves that plants respond differently to the magnetic field treatment, among other things, depending on the plant species.

On Phaseolus, the increase in enzyme α-amylase activity in seedlings from magnetically-treated seeds of mung bean seems greater than those of from white soybean. This is clearly observed when the enzyme activity measurement in seedling done base on the length of hypocotyl. The comparison of α-amylase activity in seedling from magnetically-treated seeds of *Phaseolus* sp. to other seedlings from *Glycine* sp., and *Vigna* sp., showed that the fluctuation of α-amylase activity seedlings during germination from magnetically-treated seeds of *Glycine* sp., and *Vigna* sp. are greater (Fig. 1). The observation result of the present study indicates that the highest increase in α-amylase activity as a result of magnetic field exposure in early seed germination is detected in seedlings from magnetically-treated seeds of mung bean (*Phaseolus vulgaris*).

Conclusion

The present study suggest that the duration of exposure treatmen of magnetic field 0,1 mT from 7'48 "; 11'44", and 15'36 improve the activiy of enzyme α-amylase during germination. The discussion on the pereset study leads to the assumption: first, magnetic field change the chemically process in plant cell system or the enzymes properties (Morejon et al., 2007 and Lyle et al., 1991) and second, the magnetic field changes the properties of water so it become easier to penetrate and hydtrate the seed cells (Reddy et al., 2012; Vashisth dan Nagarajan, 2010). The magnetic field treatment in early germination of legume seeds increases in the activity of enzym α-amylase due to the high increase in water status of seedlings (Vashisth and Nagarajan, 2010) then is followed by improvement of seedling performance or vigor.

As a result, the application of magnetic field on the seed would not only useful as a methods to improve the seedling vigor which is important in agriculture engineering to enhance crop production but as suggested by Reddy et al., (2012) it may also provides and excelent sources of α-amylase, extensively used industrially.

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