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Exposure of Greengram Seeds (*Vigna radiate* var. *radiata*) to Static Magnetic Fields: Effects on Germination and α -amylase Activity

¹K. Venkateswar Reddy, ²S. Raisa Reshma, ²S. Jareena and ²M. Nagaraju

¹Department of Biotechnology and Environmental Science, Jawaharlal Nehru Technological University, Hyderabad, A.P., 500083, India

²Department of Microbiology, National P.G. College, Nandyal, A.P., 518502, India

Corresponding Author: M. Nagaraju, Department of Microbiology, National P.G. College, Nandyal, A.P., 518502, India

ABSTRACT

The effects of static magnetic field on germination and α -amylase activity of greengram (mung beans) seeds were investigated under laboratory conditions. Experimental results indicated that seeds exposed for 45 min to the static magnetic field have shown the highest percentage of germination and α -amylase activity, improvements of +2.1 and +1.8 times, respectively. Furthermore, α -amylase activity of seeds treated for 45 min was significantly higher at pH 6.0 and 29°C temperature. Nevertheless, data illustrate that the seeds exposed to static magnetic field kept their germination speed and capacity levels compared to the unexposed control.

Key words: Static magnetic field, greengram, germination, α -amylase

INTRODUCTION

Beans of *Vigna*, important food crops both economically and nutritionally, are cultivated and consumed worldwide. With ever-rising costs of meats and fresh fruits and vegetables, dry beans are expected to add more to the human nutrition in coming years. Traditionally, they have been referred to as “poor man’s meat” and have contributed much to the diets of many people of several countries in Asia, Africa, Middle East and South America. By using some traditional methods, speed of seed germination has successfully been improved in many incidences (Kaur *et al.*, 2009; Pradhan and Badola, 2010; Adebisi *et al.*, 2011; Alderete-Chavez *et al.*, 2010, 2011). Similarly, the effects of seed priming (Rajpar *et al.*, 2006; Berchie *et al.*, 2010), soaking condition and temperature (Rahman *et al.*, 2011), heat (Travlos and Karamanos, 2007) and light, temperature and stratification (Raboteaux and Anderson, 2010) on seed germination have been reported widely in the past couple of years. However, much current research focuses on the irradiation of seeds in optimizing crops in terms of maximization of yield, promotion of plant growth and protection against diseases. In recent years, a renewed interest in bean research in Western European countries and the US is evident.

In this direction, investigations into electromagnetic effects on plants have already been carried out with some remarkable results. The optimal external electromagnetic field could accelerate and activation of seed germination (Maeda, 1993; Oomori, 1992) but the mechanism of these actions is till poorly understood (Morar *et al.*, 1988; Xiyao *et al.*, 1988). Electric and/or magnetic treatments are assumed to enhance seed vigor by influencing the biochemical processes that involve free radicals and by stimulating the activity of proteins and enzymes (Kurinobu and Okazaki, 1995). A study was added to the evidence that the seeds treated by magnetic stimulation seem to show

higher enzymatic activities which control the particular stages of seed germination (Aksyonov *et al.*, 2000). Perhaps, more alarmingly, the main advantage of using electromagnetic stimulation methods over traditional chemical processes is the absence of toxic residues. Likewise, several researchers reported on the stimulation effects that affects seed germination (Savostin, 1930; Murphy, 1942; Aladjajjiyan, 2002) and enzymatic activities (Akoyunoglou, 1964; Bhatnagar and Deb, 1977; Recuciui *et al.*, 2008) when using static magnetic field treatments. However, the impact of such techniques on germination and α -amylase activity of greengram seeds is scanty.

On the other hand, amylases are of commercial interest in traditional areas of applications such as starch processing in the food industry, in baking, beverage production, animal nutrition, leather, paper and pulp, textile and detergents (Pandey *et al.*, 2000; Dey *et al.*, 2001; Gigras *et al.*, 2002). In fact, germinating seeds are one of the imperative sources for amylase production. Hence physical parameters such as static magnetic field treatments considered for optimizing the production of enzymes. Nonetheless, enzyme-electromagnetic field activation is currently a fast growing field and a large number of studies have been produced currently.

An attempt, has therefore been made to evaluate the effects of static magnetic field on germination of greengram seeds. The specific objectives of this study were to determine the effects of static magnetic field on percent seed germination and to assess the influence of different levels of exposure periods, pH and temperature on α -amylase activity of treated seeds.

MATERIALS AND METHODS

Germination tests were carried out at laboratory conditions with greengram seeds. This experiment was conducted as wholly randomized design with three replications. Each plot contained 25 seeds. Germination tests were performed 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. Four hours later, when seeds were soaked, each roll was subjected to a static magnetic field.

Primarily, rolls containing seeds were placed in between the two pairs of South pole coils at 5 millitesla field strength, and were exposed for different periods, viz., 15, 30, 45 and 60 min individually. In this study, non exposed seeds were used as control. After exposure, all the vessels containing seed rolls were labeled, then, seeds were moistened. Eventually, vessels were placed in germinator at 23°C temperature. Germination was considered to take place once the tip of the radicle (1-2 mm) has emerged from the seed (Moon and Chung, 2000). The number of germinating seeds was counted and tabulated after 24 h. Thus, percentage of germination was observed in seeds treated for different exposure periods at the above mentioned conditions.

Additionally, α -amylase activity in germinated seeds treated for different exposure periods was determined according to the method described by Bernfeld (1955) and modified by Dure (1960). Tissues were blended in a Kenmix with unbuffered distilled water and then centrifuged between 0-4°C. Supernatant was heated to 70°C to inactivate β -amylase (Sun and Henson, 1991). Reaction mixture included 0.8 mL enzyme solution, 0.5 mL of 0.1 M citrate buffer (pH 6.0) and 0.5 mL of 1% starch. This was incubated for 3 min in a water bath at 30°C and the reaction was stopped by adding 2.0 mL of 1% dinitrosalicylic acid colour reagent. Maltose released was measured at A_{510} in a Spectronic-20D spectrophotometer. Finally, the reducing sugar formed by enzymatic activity was calculated (Guglielminetti *et al.*, 1995) using a standard curve obtained with glucose (0-5 μ M).

Furthermore, α -amylase activity in germinated seeds was tested at different pH (viz. pH 4, 5, 6, 7 and 8) and temperatures (viz. 0, 8, 29, 37 and 60°C) conditions. To study the effect of pH on α -amylase activity under EMF, the following reaction was setup. Substrate (1% starch) of 0.5 mL and 0.5 mL of enzyme solutions were added to 0.5 mL of 0.1 M citrate buffer of different pH values. Then, tubes were incubated in a water bath at 30°C for 3 min and the reaction was stopped by adding 0.5 mL of dinitrosalicylic acid reagent to all the tubes and the reducing sugar released was measured at 510 nm. Similarly, to determine the effect of temperature on α -amylase activity, the reaction mixture containing 0.5 mL of 0.1 M citrate buffer (pH 6.0), 0.25 mL of substrate and 0.25 mL of enzyme solution was subjected to different temperature conditions for 3 min. Finally, enzyme activity was determined by following the above mentioned procedure.

Meanwhile, for establishing the EMF, a locally designed MF generator (Sahebamei *et al.*, 2007) and a 220 volts Alternative Current power supply equipped with a variable transformer as well as a single-phase full-wave rectifier was used. The maximum power was 1 kilo watts, the passing current 50 amperes Direct Current. This system was designed to generate EMFs in the range of 0.5 microtesla (μ T) to 75 millitesla (mT). It consisted of two coils (each with 3000 turns of 3 mm copper wire) on a U-shaped laminated iron core (to prevent eddy current losses). Using two vertical connectors, the arms of the U-shaped iron core were terminated in four circular iron plates covered with this layer of nickel (each 23 mm thickness, 260 nm in diameter).

Statistical analysis: Data analysis was performed by using the SAS (Statistical Analysis System) statistical software package (SAS Institute, 1997), where a significance test based on ANOVA was done.

RESULTS

Results of ANOVA indicated that the seed germination percentage and α -amylase activity were notably influenced by static magnetic field treatment of seeds. In fact, seed germination percentage was appreciably increased by increasing the exposure period over control and later declined (Fig. 1). For instance, at 5 mT field strength, in South pole field, the germination percentage at 15 min was 43, it was increased by 1.5 times to 63% at 45 min, later declined by 1.9 times to 33% at 60 min. Thus, greatest percentage of germination was noticed at 45 min. Furthermore, α -amylase activity in germinated seeds was increased by increasing the exposure period over control and later declined (Fig. 2). For example, enzyme activity was 5 micromoles per milliliter at 15 min; it was drastically increased by 3.8 times to 19.2 μ M mL⁻¹ at 45 min and later decreased by 1.5 times to 12.5 μ M mL⁻¹ at 60 min.

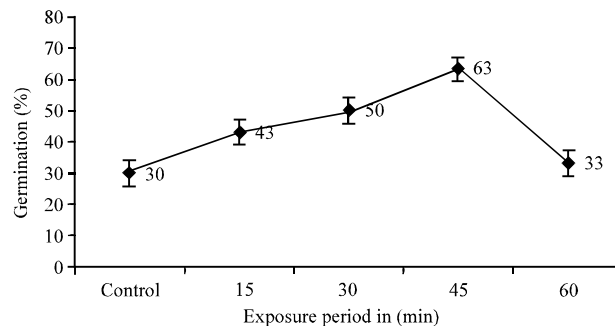


Fig. 1: Percent germination of greengram seeds as influenced by static magnetic treatment at 5 mT field strength in South pole field

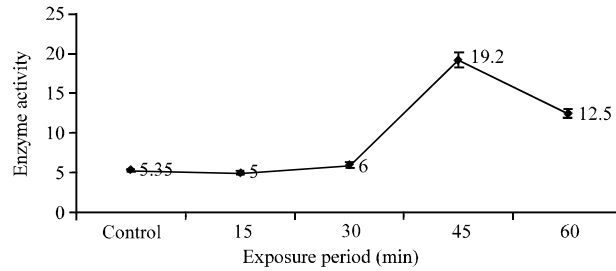


Fig. 2: Activity of α -amylase ($\mu\text{M mL}^{-1}$) in germinated greengram seeds as influenced by static magnetic treatment at 5 mT field strength in South pole field

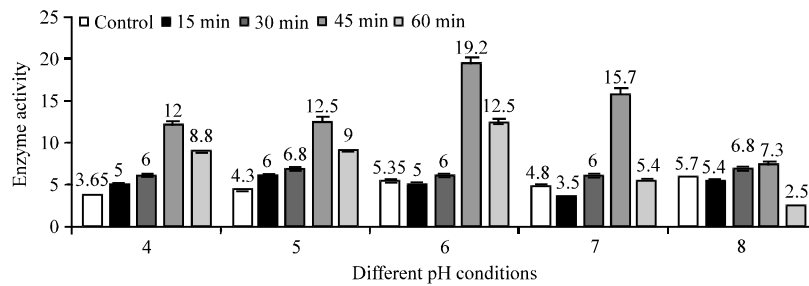


Fig. 3: Activity of α -amylase ($\mu\text{M mL}^{-1}$) in treated (5 mT field strength in South pole field) germinated greengram seeds as influenced by different pH conditions

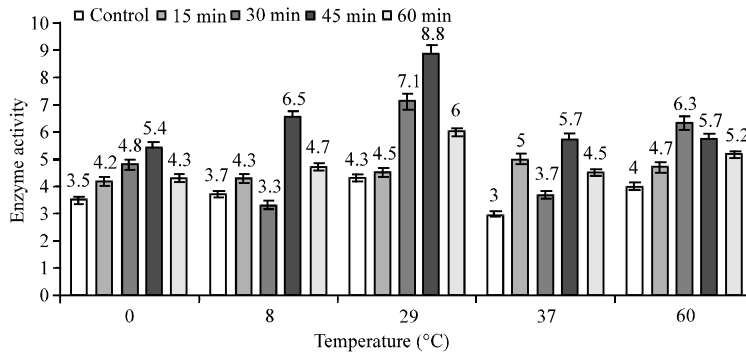


Fig. 4: Activity of α -amylase ($\mu\text{M mL}^{-1}$) in treated (5 mT field strength in South pole field) germinated greengram seeds as influenced by different temperature conditions

Additionally, α -amylase activity was increased by increasing the pH of the buffer and later declined (Fig. 3). For instance, enzyme activity of seeds exposed for 45 min was $12 \mu\text{M mL}^{-1}$ at pH 4.0, it was increased by 1.6 times to $19.2 \mu\text{M mL}^{-1}$ at pH 6.0 and later severely declined by 2.6 times to $7.3 \mu\text{M mL}^{-1}$ at pH 8.0. Same trend was observed in seeds exposed for different time periods. Similarly, α -amylase activity was increased by increasing the temperature and later decreased (Fig. 4). Indeed, the activity was increased gradually but not significantly. For example, enzyme activity of seeds exposed for 45 min was $5.35 \mu\text{M mL}^{-1}$ at 0°C , it was improved by 1.6 times to $8.8 \mu\text{M mL}^{-1}$ at 29°C and later decreased by 1.5 times to $5.7 \mu\text{M mL}^{-1}$ at 60°C . Similar tendency was also observed in seeds exposed for different time periods.

DISCUSSION

In many treatments applied in this study, seedlings grown from seeds treated with static electromagnetic fields developed longer hypocotyle and roots. In this direction, favorable effects of magnetic field on germination and emergence of seeds were shown for Oak (Celestino *et al.*, 2000), onion and rice (Alexander and Doijode, 1995), lettuce (Reina *et al.*, 2001), white mustard (Edmiston, 1972), potato (Pittman, 1972), tomato (De Souza *et al.*, 2006), barley (Mericle *et al.*, 1966), rice (Carbonell *et al.*, 2000), maize (Kato, 1988; Aladjadjiyan, 2003; Florez *et al.*, 2007), tobacco (Aladjadjiyan and Ylieva, 2003) and soya bean (Aladjadjiyan, 2003). More recently, Gholami and Sharafi (2010) reported that the percentage of germinated seeds was increased and simultaneously time required for germination was decreased upon electromagnetic field treatment of two wheat cultivars. In all, the previous studies have shown that the seed germination was faster after treatments with magnetic fields.

In this review, we found that the germination percentage of seeds was notably enhanced by 2.1 times over control when treated in South pole field, at 5 mT field strength for 45 min (Fig. 1). Same was reported by Martinez *et al.* (2000) in reference to the growth and development of barley seeds subjected to static magnetic field. Additionally, studies conducted by Florez *et al.* (2004), Florez *et al.* (2007) and Vashisth and Nagarajan (2008) indicate that magnetic field can be used as a method of seed vigor improvement in rice, maize and chick pea respectively. Like wise, numerous reports have indicated that the best effects of seeds stimulation with physical factors are possible to obtain when optimal exposure doses and exposure periods are applied.

Further, α -amylase activity was significantly higher in seeds exposed to static magnetic field for 45 min (Fig. 2). According to Vashisth and Nagarajan (2010), in germinating seeds, enzyme activities of α -amylase, dehydrogenase and protease were considerably higher in treated seeds in contrast to controls. In fact, the higher enzyme activity in magnetic field treated seeds could be triggering the fast germination and early vigor of seedlings. Several other studies have also demonstrated an improvement of amylase activity in germinated seeds by ultrasonic stimulation (Yaldagard *et al.*, 2008), gamma irradiation (Rao and Vakil, 1983; Kurobane *et al.*, 1979; Ananthaswamy *et al.*, 1970), selenium (Malik *et al.*, 2011) and static magnetic field (Bhatnagar and Deb, 1977). Prashanth *et al.* (2008) and Shaoyi *et al.* (2009) combinedly reported that static magnetic field treatment of α -amylase solution could enhance the activity over control. Moreover, many studies have found that evidence to support the static EMF stimulation of α -amylase that the starch and oligosaccharide levels gradually decreased, subsequently, the reducing sugar levels increased during early hours (48-96 h) in germinating seeds (Paul *et al.*, 1970; Jaya and Venkataraman, 1981; Shastry and John, 1991). Contrary to this, continuous and delayed exposure of power frequency electro magnetic fields could decrease the α -amylase activity in germinating seeds of *Vicia faba* L. (Rajendra *et al.*, 2005). Furthermore, utmost activity of α -amylase was recorded at pH 6.0 in seeds exposed for 45 min (Fig. 3). This is a basic property of all enzymes and is probably due to concomitant alteration in the confirmation of the enzyme protein caused by changes in the pH of its environment. Similarly, greatest activity of α -amylase was observed at temperature 29°C in seeds exposed for 45 min (Fig. 4). This may perhaps be accounted for by denaturation of the enzymes protein at temperature higher than this.

CONCLUSION

The results of the present investigation clearly indicated that magnetic field application noticeably enhanced seed performance in terms of laboratory germination, speed of germination,

seedling length and α -amylase activity compared to unexposed control. The greatest increase was obtained for seeds continuously exposed to 5 mT field strength for 45 min. Additionally, highest activity of α -amylase was noticed at pH 6.0 and temperature 29°C. Therefore, the present findings are significant in terms of determining the suitable combination of physical parameters to optimize the percentage of seed germination and α -amylase activity in electro magnetically treated seeds. As a result, this would not only useful in enhancing the seed germination percentage but also provides an excellent source of α -amylase, is extensively used industrially.

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