Biocatalysis and Agricultural Biotechnology 20 (2019) 101273

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Exposure of magnetic waves stimulates rapid germination of soybean seeds by enzymatic regulation in cotyledons and embryonic axis

Ramalingam Radhakrishnan

Department of Microbiology, Karpagam Academy of Higher Education, Coimbatore, Tamilnadu, India

ARTICLE INFO

Keywords:
Cotyledon
Embryonic axis
Enzyme
Magnetic field
Soybean

ABSTRACT

Magnetic field (MF) regulates several biological processes in living organisms including seed germination. This study was aimed to know the reason for rapid germination of MF pretreated soybean seeds by analyzing enzymatic changes in cotyledons and embryonic-axis. The fresh weight of cotyledons and embryonic-axis was gradually increased from 2 to 10 days, and their greater enhancement was observed in initial days of MF treated soybean. The activity of α-amylase was accelerated and nitrate-reductase was suppressed in both organs of seedlings exposed with MF. Acid and alkaline-phosphatase activities were initially decreased and later increased in embryonic-axis of MF treated seedlings, and nevertheless acid-phosphatase expression in MF cotyledons was declined. Hence, this is the first report of MF induced enzyme changes in germinating seeds, and the results of present study revealed that MF exposure regulates the expression of different enzymes, which play vital role to stimulate the rapid germination of soybean seeds.

1. Introduction

All living organisms are interacting with several physical, chemical and biological agents. The cell damage of magnetic radiation from mobile phones and electric lines is thrust area of research in animal and human. Some of the frequencies of radiations are helpful to diagnose and cure the diseases. Hence, the higher plants are sessile and continuously exposed to electromagnetic radiation from sun for food production. The invasive, economically inexpensive and non-toxic methods are favorable for improving the agricultural productions under current scenario. The particular range of magnetic field (MF) stimulated the seed viability, germination, early and late growth of plants (Radhakrishnan and Kumari, 2012). The different frequencies of MF promoted the growth of onion (Novitsky et al., 2001), cotton (Leelapriya et al., 2003), maize (Vashisth and Joshi, 2017), tomato (Dayal and Singh, 1986), rice (Carbonell et al., 2000), soybean (Radhakrishnan and Kumari, 2012) and several plants (da-Silva and Dobranszki, 2016). In addition, MF enhanced the survival of plants against drought (Sen and Alikamanoglu, 2014; Baghel et al., 2018), salinity (Kataria et al., 2019; Radhakrishnan et al., 2012; Radhakrishnan and Kumari, 2013a, 2013b), heavy metals (Chen et al., 2011) and temperature (Afzal et al., 2015) and also prevented the damage from several diseases (Abdollahi et al., 2012).

The study of MF induced bio-molecular changes in plants is very limited. Previously, we observed the MF pre-treated seeds showed a significant positive variation in morphology, biochemical and physiology of soybean plants and led to enhance the nutritional quality of harvested seeds (Radhakrishnan and Kumari, 2012; Radhakrishnan, 2018). MF treatments influenced the biochemical processes including the stimulation of proteins and enzymes in seeds to enhance the seed vigor (Vashisth and Nagaraja, 2010; Kataria et al., 2017; Baghel et al., 2019). The activity of cytochrome C oxidase and Ca^{2+}/calmodulin dependent cyclic nucleotide phosphodiesterase was changed in plants when exposed with MF (Nossol et al., 1993; Liboff et al., 2003). The seed germination and early growth of plants are regulated by number of enzymes, which solubilize the stored protein, oil and starch in seeds. The success of seed germination is essential for seedling establishment and crop yield (Han and Yang, 2015). The embryonic axis (radicle and plumule) development from seeds is depending on the cotyledons, which are major portion of seeds. The mobilization of nutrients from cotyledons is helpful to develop the embryonic axis. There is no report on enzymatic differences on cotyledons and embryonic axis during their growth under MF treatment. The present study was aimed to understand the seedling development of soybean cultured under MF field by analyzing the enzymatic changes in cotyledons and embryonic axis.

E-mail address: ramradhakrish@gmail.com.

https://doi.org/10.1016/j.bcab.2019.101273
Received 10 April 2019; Received in revised form 3 July 2019; Accepted 29 July 2019
Available online 30 July 2019
1878-8181/

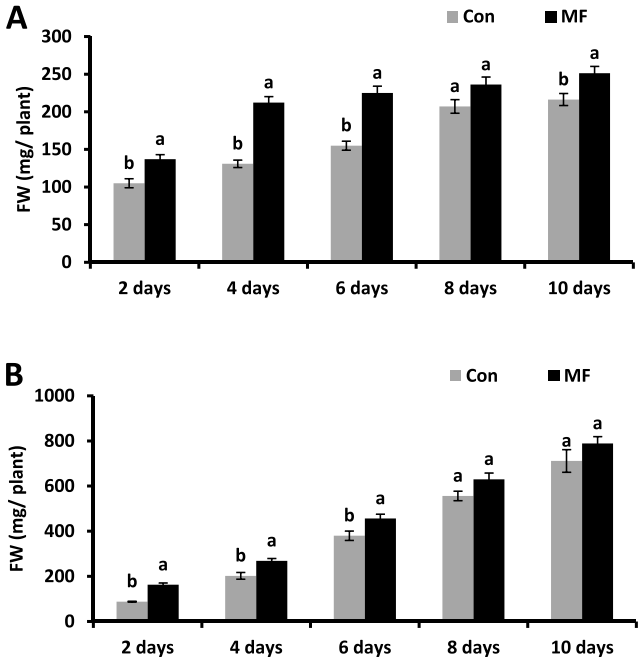

Fig. 1. Effect of magnetic field on fresh weight (FW) of cotyledons (A) and developed embryonic axis (B) during soybean seed germination.

2. Materials and methods

2.1. Seed pretreatment with pulsed magnetic field (PMF)

Soybean seeds were collected from Tamilnadu Agricultural University, Coimbatore, India and exposed to PMF as per the method described by Radhakrishnan and Kumari (2012). One hundred gram of seeds were exposed to 1500 nT at 10 Hz for 5 h per day and continue the PMF treatment up to 20 days. Seeds kept under similar condition were considered as control.

2.2. Seedling development

The MF treated and non-treated control seeds were cleaned in running tap water and 0.1% mercuric chloride for 5 min. The chemical deposition on seeds was washed with sterile distilled water. The surface sterilized seeds (30 numbers) were placed in pots containing clay and sand mixture (3:1). The water was sprayed at periodic intervals and maintained under natural photoperiod. The germination of seeds was monitored each day. The fresh weight of cotyledons and embryonic axis of germinated seedlings was recorded as two days intervals, up to 10 days.

2.3. Enzyme activity

The cotyledons and developed-embryonic axis were separated from control and MF treated seedlings. The enzyme activities such as α-amylase (EC 3.2.1.1), nitrate reductase (EC 1.6.6.2), acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) were determined to know a reason for rapid germination of soybean during MF exposure.

The cotyledons and embryonic axis were separately ground in distilled water and the crude enzyme extract was harvested after centrifugation for 15 min at 4 °C. α-amylase was assayed in above extract as per Dure (1960) and expressed as optical density (OD) g^{-1} fwt^{-1}. Nitrate reductase activity was examined according to the protocol of Hageman and Hucklesby, (1971). However, acid and alkaline phosphatase activities were tested in enzyme extract obtained from 0.1 M acetate buffer as per the method followed by Ikawa et al. (1964).

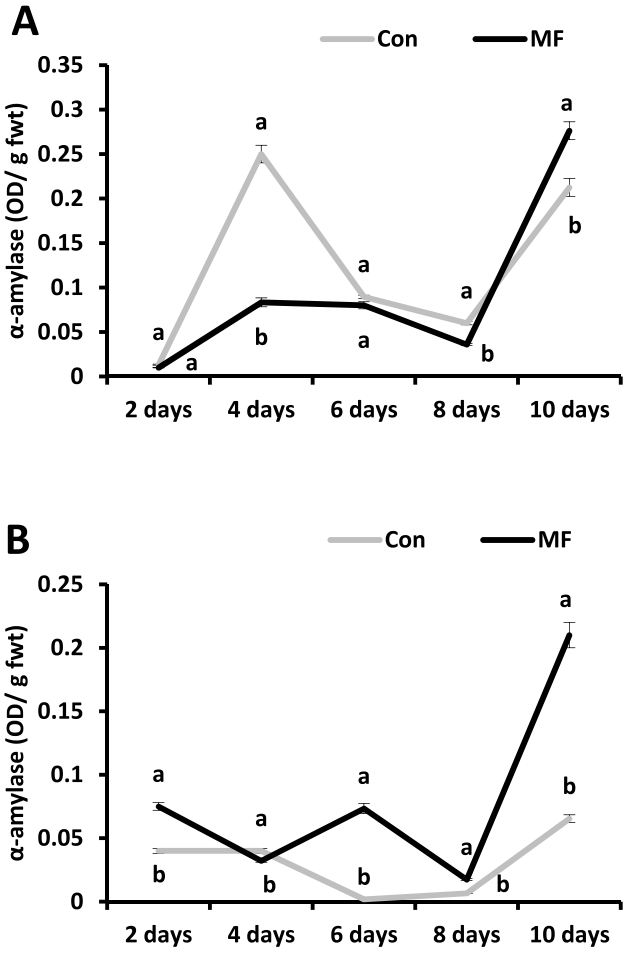

Fig. 2. Effect of magnetic field on α-amylase activity of cotyledons (A) and developed embryonic axis (B) during soybean seed germination.

2.4. Statistical analysis

The biomass and enzyme activities of cotyledons and embryonic axis of soybean seedlings after MF treatment were compared with their controls by statistical software, SPSS 11. The calculation of mean ± SE (standard error) and one-way analysis of variance (ANOVA) of each sample were used to find out the significance difference among control and treatment.

3. Results and discussion

Seed germination is a first step to develop an entire plant. The regular events during embryo development are controlled by several genetic and environmental factors. An improvement of crop production is possibility achieved by application of diversified plant growth promoting chemicals, plant beneficial micro-organisms and physical stimulants, which are accelerating seed germination and plant growth (Radhakrishnan and Lee, 2013; Radhakrishnan et al., 2014; Radhakrishnan, 2018). Previously, we have reported that 10 Hz MF significantly enhanced seed germination of soybean (Radhakrishnan and Kumari, 2013a, 2013b). Current study showed that the fresh weight of cotyledons and embryonic axis was significantly changed during the initial phase of vegetative growth of soybean at MF treatment (Fig. 1). At fourth day, cotyledon growth (biomass) was twofold

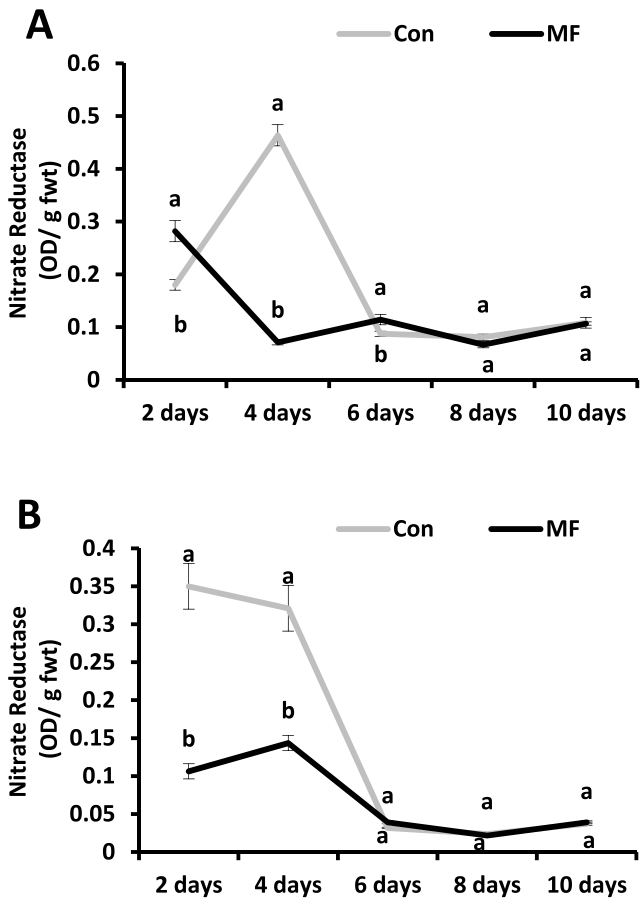

Fig. 3. Effect of magnetic field on nitrate reductase activity of cotyledons (A) and developed embryonic axis (B) during soybean seed germination.

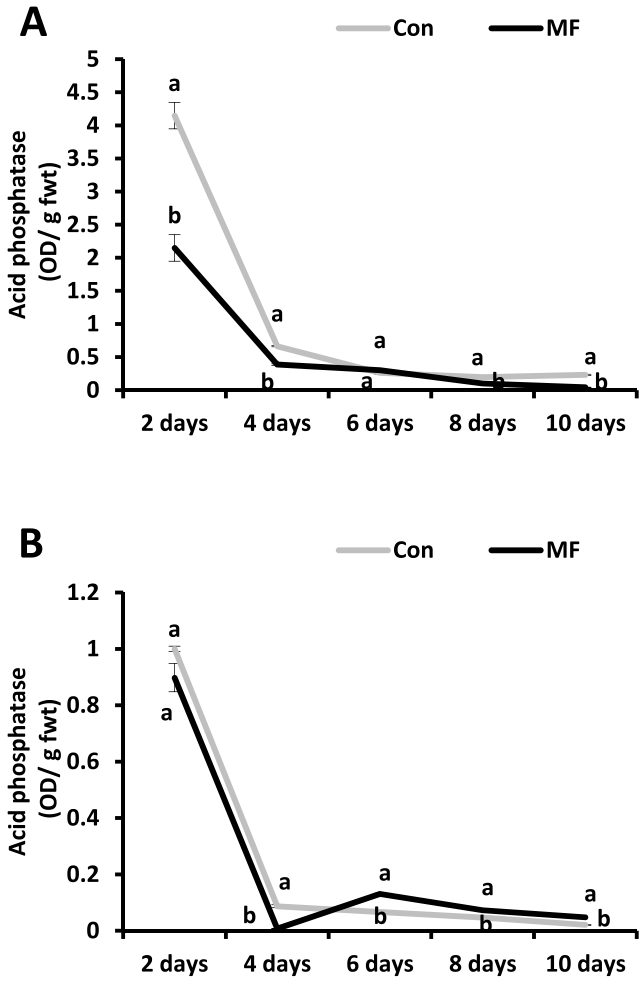

Fig. 4. Effect of magnetic field on acid phosphatase activity of cotyledons (A) and developed embryonic axis (B) during soybean seed germination.

increased and than their control. Similarly, embryonic axis and hypocotyle development (biomass) was significantly higher in early days. Later, both organelles growth were slowdown in MF treatment. After 10 days, the biomass of cotyledons was higher due to the effect of MF and embryonic axis growth was not significantly increased than MF non-treated seeds. An increase of seed germination and seedling weight due to effect of MF exposure was reported in several crop plants such as rice, wheat, maize, sunflower, tomato, soybean and cotton (Carbonell et al., 2000; Florez et al., 2007; Fischer et al., 2004; Radhakrishnan and Kumari, 2013a, 2013b; Leelapriya et al., 2003).

Florez et al. (2007) suggested that changes of enzyme expression in seeds might be triggered the seed germination. α-amylase, nitrate reductase, acid and alkaline phosphatase were studied in this experiments. The activity of α-amylase was significantly declined in MF exposed cotyledons from 2 days to 8 days, but it was enhanced at 10 days (Fig. 2). Embryonic axis of MF treated seeds showed a higher rate of α-amylase activity than their controls, excluding 4 days. Starch hydrolyzing procesrs in seedlings is conducted by amylase enzymes, which convert starch in to simple sugars and provide the energy for developing seedlings (Piacentini et al., 2001).

In the early stage of growth, hydrolysis of endosperm storage protein is regulated by nitrate reductase, which is catalyzing the nitrate into nitrite (Sivasankar and Oaks, 1995). The action of nitrate reductase in cotyledons and embryonic axis was significantly changed before 6 days during the effect of MF (Fig. 3), and while it was neutralized in both organs. Four days old cotyledones and embryonic axis had higher activity of nitrate reductase at controlled conditions and, while MF treatment suppressed that activity. The transport and accumulation of calcium content in MF treated plants correlated the growth and nitrate reductase activity of soybean seedlings (Radhakrishnan and Kumari, 2013a, 2013b). The up and down regulation of nitrate reductase activity in cotyledons and embryonic axis are regular process in seedling development and concentration of calcium in MF treated seeds might be one of the reason for changing their activity.

The function of phosphatase is known to mobilize the phosphate reserves in the cotyledons to embryonic axis. Organic phosphate was hydrolyzed by acid phosphatase to involve in tissue differentiation (Kaneko et al., 2002). In our study showed that acid phosphatase was higher in cotyledons than embryonic axis during the seedling development (Fig. 4). Initially (2 days), the expression level of acid phosphatase was greatly enhanced and later, it was declined in both organs of seedlings. Nadir et al. (2012) observed that acid phosphate activity was declined in *Vigna radiata* L. seedling after 4 days. MF treated seedlings demonstrated a low activity of acid phosphatase, but no significant difference in their expression at 6 and 8 days old cotyledons when compared to their control. In contrast, acid phosphatase activity was enhanced after 2 days in embryonic axis exposed with MF. Previously, we reported MF treatment enhanced the acid phosphatase and alkaline phosphatase in 10 days old soybean seedlings (Radhakrishnan and Kumari, 2013a, 2013b). In current study, MF treated cotyledons showed that alkaline phosphatase expression was lower than control cotyledons at 2 and 4 days and their activity was higher during the continues growth of cotyledons (Fig. 5). At the development of

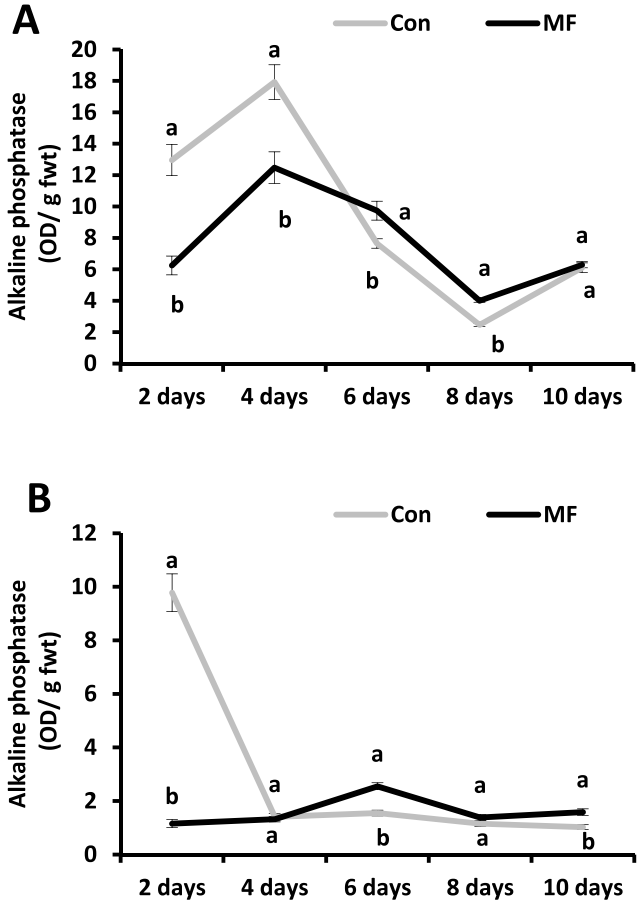

Fig. 5. Effect of magnetic field on alkaline phosphatase activity of cotyledons (A) and developed embryonic axis (B) during soybean seed germination.

embryonic axis, alkaline phosphatase activity was differed due to the exposure of MF. Four days later, their activity was higher in developed embryonic axis than controls.

The results of our study conclude that the enhancement of embryonic axis development (root and shoot) of MF treated soybean was achieved by increasing the starch hydrolyzing enzyme, α-amylase in both organs. In addition, the up and down regulation of nitrate reductase, acid and alkaline phosphatase helped to promote the seedling growth of soybean. The detailed future study of reserve metabolites mobilization from cotyledons and embryonic development would be results to understand more about the MF induced changes in seedling development.

References

Abdollahi, F., Niknam, V., Ghanati, F., Masroor, F., Noorbakhsh, S.N., 2012. Biological effects ofweak electromagnetic field on healthy and infected lime (*Citrus aurantifolia*) trees with phytoplasma. Sci. World J. 2012, 1–6.
Afzal, I., Noor, M.A., Bakhtavar, M.A., Ahmad, A., Haq, Z., 2015. Improvement of spring maize (*Zea mays*) performance through physical and physiological seed enhancements. Seed Sci. Technol. 43, 1–12.
Baghel, L., Kataria, S., Guruprasad, K.N., 2018. Effect of static magnetic field pretreatment on growth, photosynthetic performance and yield of soybean under water stress. Photosynthetica 56 (2), 718–730.
Baghel, L., Kataria, S., Jain, M., 2019. Mitigation of adverse efects of salt stress on germination, growth, photosynthetic efciency and yield in maize (*Zea mays* L.) through magnetopriming. Acta Agrobot. 72 (1), 1757.
Carbonell, M.V., Martynez, E., Amaya, J.M., 2000. Stimulation of germination in rice (*Oryza sativa* L.) by a static magnetic field. Electro. Magneto. Boil. 19 (1), 121–128.
Chen, Y.P., Li, R., He, J.M., 2011. Magnetic field can alleviate toxicological effect induced by cadmium in mungbean seedlings. Ecotoxicology 20, 760–769.
da-Silva, J.A., Dobranszki, J., 2016. Magnetic fields: how is plant growth and development impacted? Protoplasma 253 (2), 231–248.
Dayal, S., Singh, R.P., 1986. Effect of seed exposure to magnetic field on the height of tomato plants. Indian J. Agric. Sci. 56, 483–486.
Dure, L.S., 1960. Site of origin and extent of activity of amylases in maize germination. Plant Physiol. 35, 924–936.
Fischer, G., Tausz, M., Kock, M., Grill, D., 2004. Effects of weak 16 2/3 Hz magneticfields on growth parameters of young sunflower and wheat seedlings. Bioelectromagnetics 25, 638–641.
Florez, M., Carbonell, M.V., Martinez, E., 2007. Exposure of maize seeds to stationary magnetic fields: effects on germination and early growth. Environ. Exp. Bot. 59 (2007), 68–75.
Hageman, R.H.G., Hucklesby, D.P., 1971. Nitrate reductase in higher plants. Methods Enzymol. 17, 491–503.
Han, C., Yang, P., 2015. Studies on the molecular mechanisms of seed germination. Proteomics 15 (10), 1671–1679.
Ikawa, T., Nisizawa, K., Miwa, T., 1964. Specificities of several acid phosphatases from plant sources. Nature 203, 939–940.
Kaneko, M., Itoh, H., Ueguchi-Tanaka, M., Ashikari, M., Matsuoka, M., 2002. Plant Physiol. 168, 1264–1270.
Kataria, S., Baghel, L., Jain, M., Guruprasad, K.N., 2019. Magnetopriming regulates antioxidant defense system in soybean against salt stress. Biocatal. Agricult. Biotechnol. 18, 101090.
Kataria, S., Baghel, L., Jain, M., Guruprasad, K.N., 2017. Pre-treatment of seeds with static magnetic field improves germination and early growth characteristics under salt stress in maize and soybean. Biocatal. Agricult. Biotechnol. 10, 83–90.
Leelapriya, T., Dilip, K.S., Sanker-Narayan, P.V., 2003. Effect of weak sinusoidal magnetic field on germination and yield of cotton (*Gossypium*sp.). Electromagn. Biol. Med. 22, 117–125.
Liboff, A.R., Cherng, S., Jenrow, K.A., Bull, A., 2003. Calmodulin dependent cyclic nucleotide phosphodiesterase activity is altered by 20 mT magnetostatic fields. Bioelectromagnetics 24, 2–38.
Nadir, S., Saeed, A., Naz, R., Siddiqua, A., Sherazi, M., Wazir, S.M., Saeed, A., 2012. Isolation, purification and characterization of acid phosphatase from germinating *vigna radiata* seeds. J. Chem. Soc. Pak. 34, 3.
Nossol, B., Buse, G., Silny, J., 1993. Influence of weak static and 50 Hz magnetic fields on the redox activity of cytochrome-C oxidase. Bioelectromagnetics 14, 361–372.
Novitsky, Y.I., Novitskaya, G.V., Kocheshkoiva, T.K., Nechiporenko, G.A., Dobrovolskii, M.V., 2001. Growth of green onions in a weak permanent magnetic field. Russ. J. Plant Physiol. 48, 709–715.
Piacentini, M.P., Fraternale, D., Piatti, E., Ricci, D., Vetrano, F., Dacha, M., Accorsi, A., 2001. Senescence delay and change of antioxidant enzyme levels in *Cucumis sativus* (L.) etiolated seedlings by ELF magnetic fields. Plant Sci. 161, 45–53.
Radhakrishnan, R., 2018. Seed pretreatment with magnetic field alters the storage proteins and lipid profiles in harvested soybean seeds. Physiol. Mol. Biol. Plants 24 (2), 343–347.
Radhakrishnan, R., Kang, S.M., Baek, I.Y., Lee, I.J., 2014. Characterization of plant growth-promoting traits of *Penicillium* species against the effects of high soil salinity and root disease. J. Plant Interact. 9 (1), 754–762.
Radhakrishnan, R., Kumari, B.D.R., 2012. Pulsed magnetic field: a contemporary approach offers to enhance plant growth and yield of soybean. Plant Physiol. Biochem. 51, 139–144.
Radhakrishnan, R., Kumari, B.D.R., 2013a. Influence of pulsed magnetic field on soybean seed germination, seedling growth and soil microbial population. Indian J. Biochem. Biophys. 50, 312–317.
Radhakrishnan, R., Kumari, B.D.R., 2013b. Protective role of pulsed magnetic field against salt stress effects in soybean organ culture. Plant Biosyst. 147 (1), 135–140.
Radhakrishnan, R., Lee, I.J., 2013. Regulation of salicylic acid, jasmonic acid and fatty acids in cucumber (*Cucumis sativus* L.) by spermidine promotes plant growth against salt stress. Acta Physiol. Plant. 35, 3315–3322.
Radhakrishnan, R., Leelapriya, T., Kumari, B.D.R., 2012. Effects of pulsed magnetic field treatment of soybean seeds on calli growth, cell damage, and biochemical changes under salt stress. Bioelectromagnetics 33, 670–681.
Sen, A., Alikamanoglu, S., 2014. Effects of static magnetic field pretreatment with and without PEG 6000 or NaCl exposure on wheat biochemical parameters. Russ. J. Plant Physiol. 61 (5), 646–655.
Sivasankar, S., Oaks, A., 1995. Regulation of nitrate reductase during early seedling growth. Plant Physiol. 107, 1225–1231.
Vashisth, A., Joshi, D.K., 2017. Growth characteristics of maize seeds exposed to magnetic field. Bioelectromagnetics 38 (2), 151–157.
Vashisth, A., Nagaraja, S., 2010. Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. J. Plant Physiol. 167, 149–156.