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ENHANCEMENT OF ZINNIA SEED GERMINATION AND SEEDLING EMERGENCE THROUGH MAGNETIC SEED STIMULATION

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Abstract. Poor seed germination is a main hindrance to the commercial cultivation of Zinnia (*Zinnia elegans* Jacq.). Seed enhancement is a useful strategy to improve germination of major agronomic and horticultural crops. A lab study was conducted to investigate the potential of magnetic seed stimulation as a seed enhancement tool and its influence on germination and emergence capacity of zinnia. Magnetic seed treatment with 50, 100 and 150 mT strength each for 5, 10 and 15 min was compared with control (untreated). Magnetic seed stimulation reduced time to 50% germination and mean germination time and increased final germination percentage, germination energy and germination index. Root length, shoot length, seedling fresh and dry weight was also increased as a result of magnetic seed stimulation. Furthermore, magnetic field treatment also enhanced α-amylase activity, total soluble sugars and reducing sugars levels. Among all seed treatments, magnetic field with strength of 100 mT for 15 min was the most effective physical treatment for improving seed germination and seedling growth of zinnia.

Key words: *Zinnia elegans*, magnetic seed stimulation, germination, biochemical analysis

INTRODUCTION

Zinnia (*Zinnia elegans* Jacq.) is a multipurpose annual, cultivated worldwide as cut flowers and is suitable for flower beds, boarders and pots. The delicate and colorful

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flower on solitary stem can add glow to any garden in an elegant way [Cumo 2013]. Seed germination is a critical step to achieve economic success in a transplant operation. Zinnia has few problems relating to its germination and seedling establishment. Also there is a problem of stand establishment due to delayed emergence as it takes up to ten days to complete emergence. Reason for this poor germination is the presence of germination inhibitors like phenolic compounds and alkaloids in the pericarp and seed coat [Tao and Buta 1986, Bhattacharyya et al. 1999]. Furthermore, seed quality (viability and vigor) decreases during periods of long storage due to aging [Soltani et al. 2006] which is the main reason for poor germination.

Many shotgun approaches are being used for revival of seed germination in different crops such as seed coating and pelleting [Taylor et al. 1998], seed priming [Bradford 1986] and magnetic seed stimulation [Afzal et al. 2012]. Stationary magnetic field increased germination rate, root shoot lengths and growth of sunflower seeds, activities of enzymes like amylase, dehydrogenase and protease as compared to control [Vashisth and Nagarajan 2010]. Magnetic stimulation of marigold seed resulted in higher emergence percentage, increased emergence index, germination speed, vigorous seedling with increased root and shoot length, seed soluble sugars and α-amylase activity [Afzal et al. 2012]. Seed germination and initial development of maize and faba bean seedlings were improved when seeds were exposed to permanently still magnetic field [Florez et al. 2007, Podlesny and Pietruszewski 2007]. High-voltage electrostatic field (HVEF) elevated aged rice seeds vigor, decreased leakage conductivity and improve membrane system of aged rice seedlings through improvement in the activity of superoxide dismutase, peroxidase and catalase [Wang et al. 2009].

In view of previous work done on horticultural crops like cucumber [Yinan et al. 2005], tomato [Moon and Chung 2000] and flowers including marigold [Afzal et al. 2012], it can be assumed that magnetic seed stimulation can provide early establishment of vigorous, healthy and uniform zinnia seedlings. Therefore, this study was carried out to study the effect of magnetic seed stimulation on germination and seedling vigor of zinnia and to investigate the biochemical and physiological basis of magnetic seed stimulation.

MATERIALS AND METHODS

Seed material and experimental details. Experiment was conducted in Seed Physiology Laboratory, University of Agriculture Faisalabad, during 2015. Seed of Zinnia (*Zinnia elegans* Jacq.) cv. giant Dhalia flowered, were obtained from Evergreen Nursery, Faisalabad, Pakistan. Seeds were exposed to nine different magnetic seed treatments with varying intensity of magnetic field and duration of exposure. Treatment combinations included 50, 100 and 150 mT for 5, 10 and 15 minutes (min.). The untreated seeds were considered as control treatment. After magnetic seed stimulation, germination and emergence tests along with biochemical analysis were conducted.

Magnetic seed stimulation. The pre-sowing magnetic treatments were applied by using a magnetic seed stimulator in the Department of Physics, University of Agriculture Faisalabad, Pakistan. When an electric current is passed through the electromagnet coils of magnetic seed stimulator a non-uniform magnetic field is generated in the air space (poles) between the two bars. Dry seeds of zinnia, packed in a transparent plastic bag were placed on the pole of electromagnet [Afzal et al. 2012]. The magnetic fields generated were adjusted by varying the voltage applied to the coils until the required working strength was achieved [De Souza et al. 2006].

Germination test. Three replicates of 25 treated or non-treated seeds were placed in 9 cm diameter Petri dishes on Whatman No. I filter paper wetted with 4 ml distilled water. Petri dishes were then shifted in a seed incubator set at 20°C for first 16 hours and then adjusted to 30°C for next 8 hours after that set at constant temperature of 20°C [ISTA 2015] and provided with light facility. Time to 50% germination (T_{50}) was calculated according to following formula of Coolebear et al. [1990].

$$T_{50} = t_i + \frac{(\frac{N}{2} - n_i)(t_j - t_i)}{n_j - n_i}$$

Where N is the number of final seeds germinated and ni and nj are cumulative number of seeds germinated adjacent seed count at times (Days) ti and tj when ni < N/2 < nj.

Mean germination time (MGT) was calculated according to the equation of Ellis and Robert [1981].

$$MGT = \frac{\sum Dn}{\sum n}$$

Where n is the number of seeds, which were germinated on the day D and D is the number of days counted from the beginning of germination. Final germination percentage (FGP) was determined on 10th day after sowing. Germination index (GI) was calculated as described by the AOSA [1983]. Energy of germination (%) was calculated on 4th day from the beginning of germination test by dividing number of seeds germinated on fourth day with final germination percentage.

Emergence assay. Emergence assay was performed in a wire house at Floriculture Research Area of Institute of Horticultural Sciences, University of agriculture Faisalabad at ambient environmental conditions. Control and treated seeds were sown in plastic trays (25 seeds in each) containing moist sand, replicated three times. Emergence was recorded daily for consecutive 10 days after sowing on the basis of appearance of cotyledons on the surface. Emergence attributes including time to 50% emergence (E_{50}), mean emergence time (MET), final emergence percentage (FEP) and emergence index (EI) were recorded according to procedure described in germination test. Energy of emergence (%) was calculated by dividing the number of seeds emerged on 4th day from the beginning of germination test over final emergence percentage. Root and shoot lengths of five randomly selected seedlings from each replicate were measured after 15 days of sowing and then average was taken. Fresh weight (mg) of these selected seedlings was taken after 15 days of sowing with the help of an electric balance. Seedlings were dried at 60°C for 48 hours in microwave oven for determination of seedling dry weight.

Seed biochemical analysis. The activity of α-amylase was measured in potassium phosphate (pH 7.0) extracted samples of zinnia seeds (0.1 g) following the modified DNS method [Varavinit et al. 2002]. Total soluble sugars were quantified in (0.1 g**)** seed sample after the grinding with the help of mortal pistal followed by hydrolysis with 2.5N HCl and then neutralized by sodium carbonate. The distilled water was used to make final volume 10 mL, centrifuged at 10000 xg and supernatant was used for measurement of total sugars following the phenol-sulphuric acid method [Thimmaiah 2004]. The reducing sugars were measured by DNS method reported by Sadasivam and Manickam [1992].

Statistical analysis. Experiment was layed out in Completely Randomized Design (CRD) and each treatment was replicated three times. Data were analyzed by using Fisher's analysis of variance technique at 5% level of probability while Least Significant Difference test was used to test the differences among mean values.

RESULTS

Seed germination. Magnetically treated seeds reached to 50% germination earlier than non-treated seeds (tab. 1). The shortest time to 50% germination (T_{50}) was noted in seeds exposed to field strength of 100 mT of non-uniform magnetic field for 15 min which is statistically similar to 150 mT for 5 and 15 min. Maximum T_{50} was taken by the untreated seeds and the seeds exposed to 50 mT for 10 min. Mean germination time in case of magnetically stimulated seeds was significantly reduced as compared with non-treated seeds (tab. 1). The lowest mean germination time was observed in seeds exposed to 100 mT for 15 min followed by 150 mT for 15 min while maximum T_{50} was recorded for control treatment (Untreated seeds). Maximum final germination was recorded in magnetic seed treatment of 100 mT for 15 min while minimum final germination was recorded in untreated seeds (tab. 1). Among all treatments, maximum final germination percentage was found for seeds treated with 100 mT for 15 min followed by 100 mT for 5 min and 150 mT for 15 min while germination was lowest for untreated seeds (tab. 1). Highest values of germination index and energy of germination index were recorded for the seeds exposed to 100 mT magnetic field for 15 min while minimum values were observed in control treatment (tab. 1).

Seedling emergence. All the magnetic seed stimulation treatments improved seedling emergence potential of zinnia seeds as compared to control treatment. Minimum time to 50% emergence (E_{50}) and mean emergence time (MET) were recorded in seeds exposed to magnetic field of strength 100 mT for 15 min while maximum E_{50} and MET were recorded for non-treated seeds (tab. 2). The highest final emergence percentage (FEP), emergence index (EI) and energy of emergence (EE) were noted in seeds exposed to magnetic field of strength 100 mT for 15 min while the lowest values of these parameters were found in non-treated seeds.

Table 1. Effect of magnetic seed stimulation on germination performance of zinnia seeds

Treatment		Time to 50% germination (days)	Mean germination time (days)	Energy of germination (%)	Final germination percentage (%)	Germination index
Intensity	duration					
Control	no treatment	3.37 a	24.00 e	5.10 a	32.67 f	3.80 f
50 mT	5 min	3.09 b	26.00 e	4.50 bc	38.89 e	4.04 ef
	10 min	3.20 ab	37.67 d	4.35 c	48.78 d	4.15 e
	15 min	2.80 c	45.00 c	4.67 b	54.22 c	4.21 e
100 mT	5 min	2.51 d	50.00 abc	3.80 d	62.89 ab	5.41 d
	10 min	2.33 d	47.33 bc	3.93 d	60.33 b	5.97 c
	15 min	1.66 f	55.33 a	2.99 f	67.67 a	8.40 a
150 mT	5 min	1.83 f	49.44 abc	3.78 d	60.78 b	5.40 d
	10 min	2.11 e	48.00 bc	3.95 d	59.33 bc	6.03 c
	15 min	1.80 f	52.67 ab	3.31 e	64.33 ab	6.41 b
LSD at $P \geq 0.05$		0.2057	0.2892	6.8888	5.1898	0.3403

Means within a column followed by the same letters are not significantly different at $P \leq 0.05$

Table 2. Effect of magnetic seed stimulation on emergence attributes of zinnia seeds

Treatment		Time to 50% emergence (days)	Mean emergence time (days)	Energy of emergence (%)	Final emergence percentage (%)	Emergence index
Intensity	duration					
Control	no treatment	3.20 a	28.56 e	5.300 a	32.00 f	4.76 e
50 mT	5 min	3.13 ab	33.22 e	4.70 bc	38.56 e	5.10 e
	10 min	3.40 a	43 d	4.51 c	49.11 d	5.22 e
	15 min	2.77 bc	48.89 cd	4.98 ab	54.89 c	5.29 e
100 mT	5 min	2.50 cd	52.33 bc	4.03 d	58.33 bc	6.27 d
	10 min	2.30 d	53.50 bc	4.10 d	60.00 bc	7.37 b
	15 min	1.65 f	61.50 a	3.17 e	66.33 a	8.48 a
150 mT	5 min	1.81 ef	50.22 bc	3.94 d	58.67 bc	6.81 c
	10 min	2.13 de	52 bc	4.06 d	57.67 bc	7.90 b
	15 min	1.81 ef	55.83 ab	3.40 e	61.17 ab	7.90 b
LSD at $P \geq 0.05$		0.3693	0.3584	6.2619	5.5647	0.5357

Means within a column followed by the same letters are not significantly different at $P \leq 0.05$

Maximum root and shoot lengths were recorded in magnetically treated seeds at 100 mT for 15 min while seeds exposed to 150 mT for 10 min also gave similar results for root length alone (figs 1a–b). Untreated seeds produced seedlings with minimum root and shoot length. Overall magnetically treated seeds gave better results over control treatments for root and shoot length. Fresh and dry weights of seedlings produced from magnetically stimulated seeds were significantly higher than seedlings from non-treated seeds (fig. 2). Seeds magnetically treated with 100 mT of non-uniform magnetic field for 15 min followed by 150 mT for 15 min gave highest seedling fresh weight. Similarly highest seedling dry weight was given by the seeds treated with 100 mT 15 min followed by 150 mT for 10. Seedlings from control treatment gave the lowest values of fresh and dry weight.

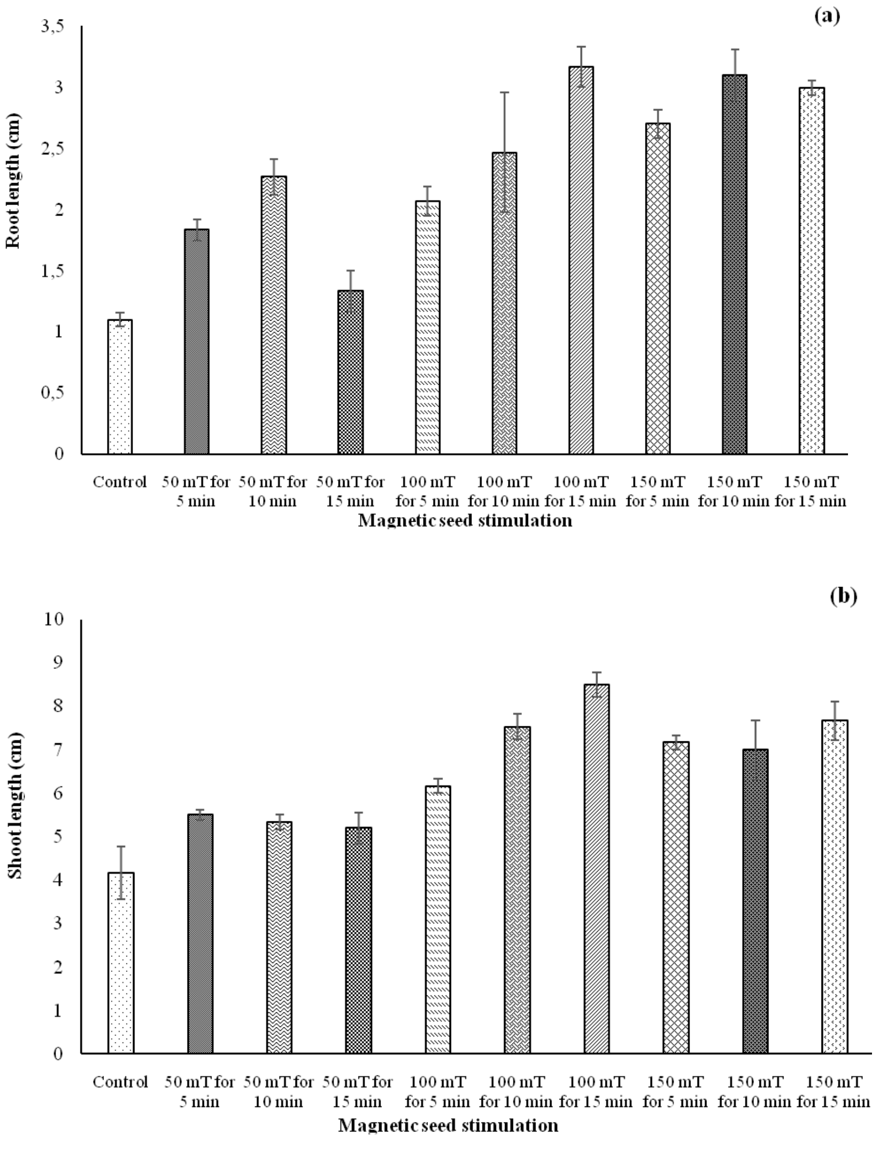

Fig. 1. Effect of magnetic seed stimulation on root (a) and shoot length (b) of zinnia seedlings during emergence test

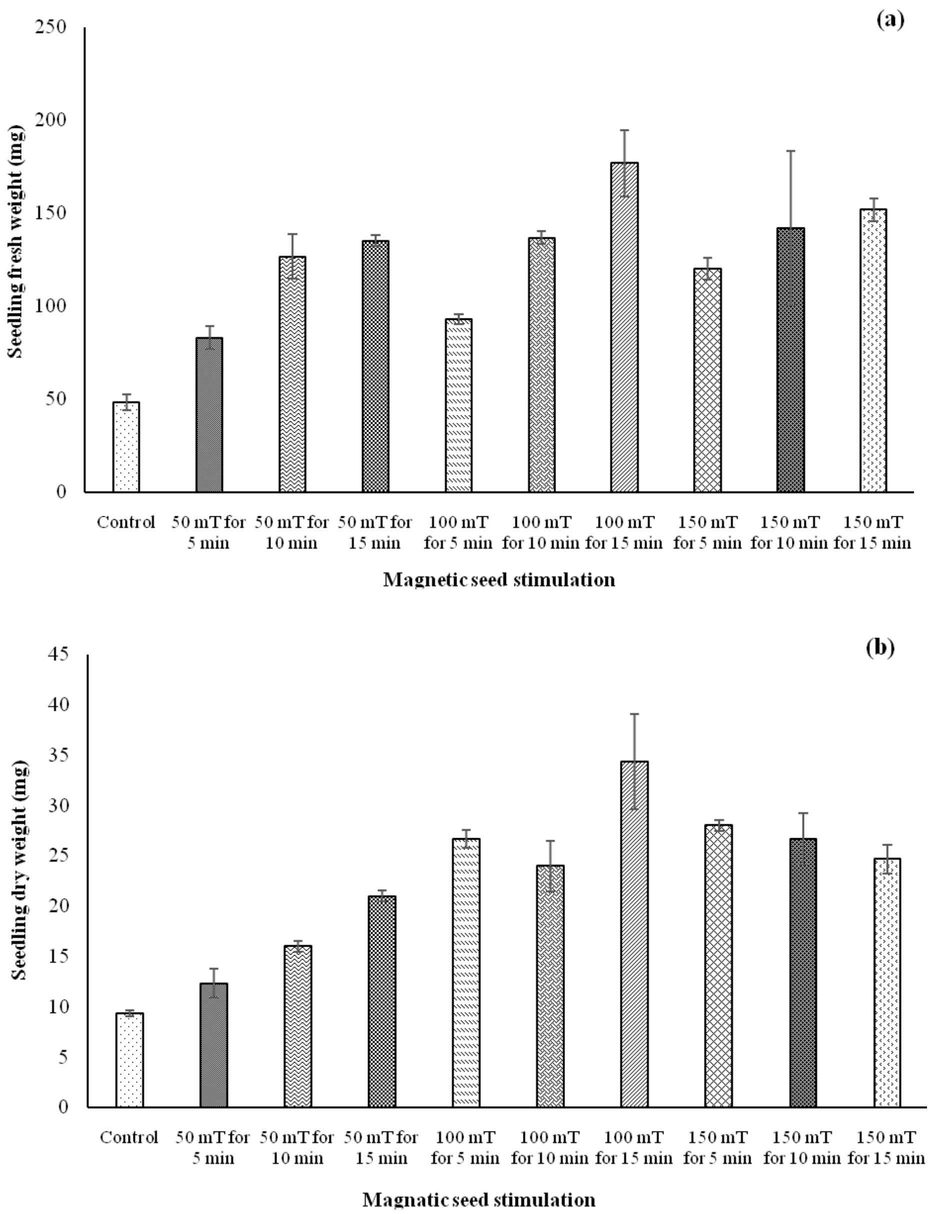

Fig. 2. Effect of magnetic seed stimulation on seedling fresh and dry weight of zinnia during emergence assay

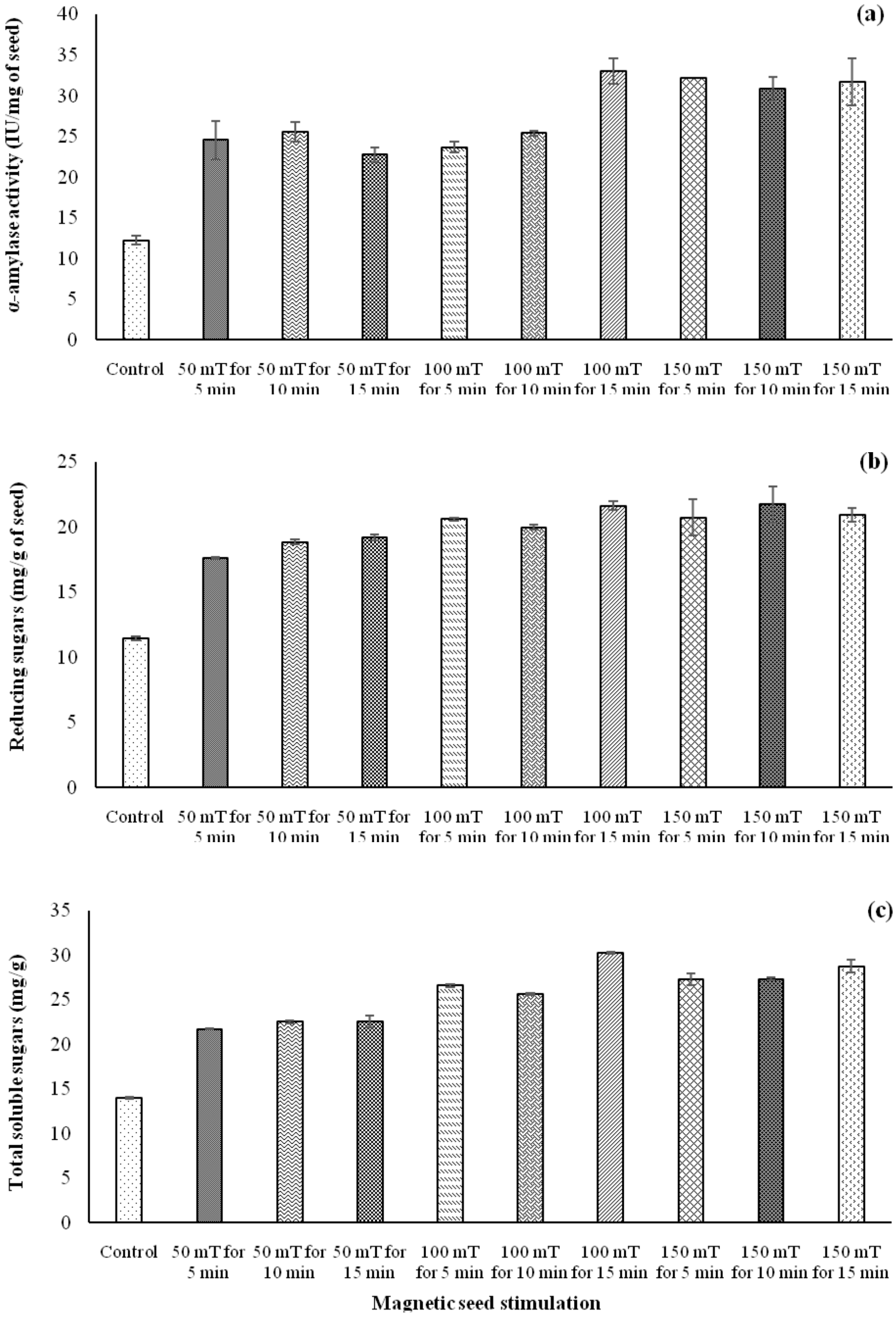

Fig. 3. Effect of magnetic seed stimulation on biochemical attributes of zinnia seeds

Seed biochemical analysis. A significant increase in α-amylase activity in magnetically stimulated seeds was observed as compared to the non-treated seeds (fig. 3a). Maximum α-amylase activity was observed in seeds treated with magnetic field of strength 100 mT for 15 min. Similarly magnetic seed stimulation at 150 mT for 15 min, 150 mT for 5 min and 150 mT for 10 min were also effective in increasing seed α-amylase activity. The highest reducing sugars were measured in seeds exposed to 150 mT for 10 min and highest total soluble sugars were measured in seeds exposed to 100 mT for 15 min while minimum values were recorded from untreated seeds (fig. 3b–c).

DISCUSSION

Importance of seed germination for commercial cultivation of flowering plants especially of those grown through nursery saplings is undeniable. Seed aging during storage results into poor and erratic germination with seedlings of low vigor [Al-Maskri et al. 2002]. In present study, magnetic seed stimulation significantly improved seed germination and seedling growth of zinnia. The effect of 100 mT for 15 min was more pronounced in synchronizing germination and emergence as depicted by lower T_{50}, MGT, E_{50}, MET and higher FGP, GI, GE, FEP, EI and EE in treated seeds compared with control and other magnetic treatments (tabs 1–2). Podlesny et al. [2005] also showed an acceleration of emergence in pea after seed exposure to magnetic field. Better improvement of seed germination and seedlings emergence in marigold seeds by magnetic seed treatment might be because of an energetic excitement of one or more parameters of the cellular substratum (proteins and carbohydrates) or water inside the dry seeds by the direct effect of magnetic field [De Souza et al. 2006]. An improvement in germination potential and seedling vigor due to influence of magnetic field in the seeds of cereals and vegetables were also observed by Hirota et al. [1999], Moon and Chung [2000], Florez et al. [2007] and De Souza et al. [2008]. Similarly, Carbonell et al. [2000] also reported that magnetic seed treatments significantly enhanced germination of rice seeds compared with non-treated seeds. Slow germination, emergence pattern and seedling establishment in seeds treated with low strength of magnetic field with short (tabs 1–2) might be due to unsuitable combination of magnetic field strength and exposure time [Kavi 1983].

Increased root and shoot lengths, fresh and dry weight of seedlings produced by magnetically treated seeds as compared to seedlings of non-treated seeds of zinnia might be the result of increased rate of cell division in the root tips and earlier start of emergence as indicated by lower values of MET [Afzal et al. 2012]. These results highlight usefulness of magnetic field in improving the sowing quality of *Zinnia elegans*. De Souza et al. [2008] also found that root and shoot lengths were increased in magnetically treated seeds as compared to non-treated seeds. Similar findings were found by Aladjadjiyan [2002] and Vashisth and Nagarajan [2010] who reported that magnetic field application enhanced seed performance in terms of speed of germination, root and shoot length and seedling dry weight significantly compared to unexposed control. Improvement in seed germination and seedling emergence attributes along with seedlings lengths, fresh and dry weight highlight the usefulness of magnetic stimulation in improving the seed vigor.

Magnetic treatments are assumed to enhance seed vigor by influencing the biochemical processes like stimulating the activity of proteins and enzymes [Kurinobu and Okazaki 1995]. An increased α-amylase activity along with contents of total and reducing sugars of high strength magnetically treated seeds was observed in the present study (fig. 4). It confirms the primary role of magnetic treatment in either stimulating protein synthesis or enhancing the activities of existing enzymes [Morar et al. 1993], thereby producing germination metabolites in requisite amounts. Afzal et al. [2012] reported that an increased α-amylase activity resulted into increased contents of total and reducing sugars in marigold seeds subjected to magnetic seed stimulation. Improvement in seedlings performance with 100 mT for 15 min magnetic treatment might be due to changes in intracellular Ca^{2+} levels that control numerous processes in plants. Increase in Ca^{2+} level after exposure to magnetic field suggest that Ca^{2+} entry into the cytosol can constitute primary magnetic field sensing mechanism in plants [Belyavskaya 2004]. The increased total soluble sugars, reducing sugars and thereby seedling dry weight suggests that magnetic seed treatments remarkably improved seedling vigor due to increased starch hydrolysis.

CONCLUSION

The present study suggests that exposure to magnetic fields produce biochemical and physiological changes in zinnia seeds. On the basis of results obtained from this investigation it may be concluded that most of magnetic seed treatments enhanced performance of zinnia. The influence of the magnetic field on seed germination, emergence and seedling growth depends on the strength of the magnetic field and the time of exposure. Magnetic seed stimulation with 100 mT for 15 min showed maximum potential as a beneficial seed physical enhancement technique for better stand establishment of *Z. elegans*.

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WZMOŻENIE KIEŁKOWANIA NASION CYNII I POJAWIANIA SIĘ SIEWEK POPRZEZ MAGNETYCZNĄ STYMULACJĘ NASION

Streszczenie. Słabe kiełkowanie nasion jest główną przeszkodą w komercyjnej uprawie cynii (*Zinnia elegans* Jacq.). Wzmocnienie nasion jest użyteczną strategią w celu poprawienia kiełkowania głównych upraw rolniczych i ogrodniczych. Doświadczenie przeprowadzono w celu zbadania potencjału magnetycznej stymulacji nasion jako narzędzia wzmocnienia nasion i jej wpływu na kiełkowanie i zdolności wschodów cynii. Z kontrolą (bez zabiegów) porównano zabiegi działania magnetycznego na nasionach z siłą 50, 100 i 150 mT przez 5, 10 i 15 min. Magnetyczna stymulacja nasion zmniejszyła do 50% czas kiełkowania i średni czas kiełkowania oraz zwiększyła ostateczny procent kiełkowania, energię kiełkowania i wskaźnik kiełkowania. W rezultacie magnetycznej stymulacji nasion zwiększyła się też długość korzenia, długość łodygi oraz świeża i sucha masa. Ponadto zabieg pola magnetycznego wzmógł aktywność α-amylazy oraz poziom całkowitej zawartości cukrów rozpuszczalnych i cukrów redukujących. Wśród wszystkich zabiegów na nasionach, pole magnetyczne o sile 100 mT działające przez 15 min było najbardziej skutecznym zabiegiem poprawiającym kiełkowanie nasion i wzrost siewek cynii.

Słowa kluczowe: *Zinnia elegans*, magnetyczna stymulacja nasion, kiełkowanie, analiza biochemiczna

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