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Magnetic stimulation of marigold seed

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A b s t r a c t. The effects of magnetic field treatments of French marigold seeds on germination, early seedling growth and biochemical changes of seedlings were studied under controlled conditions. For this purpose, seeds were exposed to five different magnetic seed treatments for 3 min each. Most of seed treatments resulted in improved germination speed and spread, root and shoot length, seed soluble sugars and α-amylase activity. Magnetic seed treatment with 100 mT maximally improved germination, seedling vigour and starch metabolism as compared to control and other seed treatments. In emergence experiment, higher emergence percentage (4-fold), emergence index (5-fold) and vigorous seedling growth were obtained in seeds treated with 100 mT. Overall, the enhancement of marigold seeds by magnetic seed treatment with 100 mT could be related to enhanced starch metabolism. The results suggest that magnetic field treatments of French marigold seeds have the potential to enhance germination, early growth and biochemical parameters of seedlings.

K e y w o r d s: marigold, seed, stimulation, magnetic field

INTRODUCTION

Marigold is grown as an ornamental crop for loose flowers and as bedding plant in landscape. French marigold (*Tagetes patula* L.) is ideal for rockeries, edging, hanging baskets and window boxes. Both leaves and flowers are equally important from the medicinal point of view. Marigold petals are commercially valuable as a natural source of xanthophylls, pigments used primarily in poultry industry to intensify yellow colour of egg yolk. The essential oil present in different species of *Tagetes* is largely used in compounding of high-grade perfumes (Kaul *et al*., 2000). Crop stand establishment is always an important consideration in commercial field production. Similarly, rapid and uniform seedling emergence is the demand of every farmer as it is the foundation on which stand establishment is based and potential yield is determined. But, the emergence and stand establishment of marigold seeds are often slow and extremely erratic, particularly under hot or cool field conditions (Afzal *et al*., 2009). Currently, direct seeding is also popular among the vegetable and flower farming community in the world. However, it often results in uneven germination, slow emergence and poor stand establishment, but is less expensive initially and widely used in field operations. One of the greatest problems for marigold production is poor plant stand establishment. Magnetic seed pretreatments have been used successfully to improve germination rate and crop establishment, boost growth, development and ultimately yield of many horticultural and agronomic crops (Aladjadjiyan, 2010; Moon and Chung, 2000; Pietruszewski and Kania, 2010). Magnetic field pretreatment not only increased seed germination rate, seedling growth and yield (Balouchi and Sanavy, 2009) but also reduced the attack of pathogenic diseases (De Souza *et al*., 2006; Yinan *et al*., 2005). The beneficial effect of magnetic seed stimulation has been associated with various biochemical, cellular, and molecular events including enzyme activity (Rochalska and Grabowska, 2007) and synthesis of proteins. During magnetic seed treatment, an increase in ascorbic contents is also documented (Yinan *et al*., 2005). Magnetic treatments are assumed to enhance seed vigour by influencing the biochemical processes that involve free radicals and by stimulating the activity of proteins and enzymes. These effects were mainly attributed to the higher yield of crops under field conditions (Moon and Chung, 2000). Magnetic fields were used widely as pretreatment for seeds to increase seed vigour, seedling growth and yield, however, its physiological and biochemical mechanism are still poorly understood. Only a few studies relating to the biochemical and physiological aspects of magnetic seed stimulation have been reported so far and concentrated mainly on cereals and root crops.

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The aim of this study is to investigate the effects of magnetic field treatment on germination, early seedling growth and biochemical aspects of French marigold seeds under controlled conditions.

MATERIALS AND METHODS

The healthy seeds of French marigold were obtained from Evergreen Nursery, Faisalabad, Pakistan.

The presowing magnetic treatments were done by using a magnetic seed stimulator in the Department of Physics, University of Agriculture, Faisalabad, Pakistan. The instrument consisted of two pairs of cylindrical coils and each pair was wound on an iron bar. The two bars were placed one above the other through metallic supports. The coils were connected in series and attached to the power source. Thus, when a rectified electric current without smoothing passed through the electromagnet coils a full wave rectified non-uniform pulsating magnetic field was generated in the air space between the two bars (poles) (De Souza *et al.*, 2006).

Dry seeds of marigold were kept in an empty glass Petri dish and then Petri dish was exposed to magnetic field by placing it between two iron bars. The seeds were exposed to five different magnetic seed treatments: 25, 50, 75, 100 and 125 mT for 3 min. The unexposed seeds were considered as controls. The magnetic fields were generated by adjusting the voltage applied to the coils until the required working strength was achieved and measured with the help of magnetometer.

Four replicates of 25 treated or untreated seeds were germinated in 9 cm diameter Petri dishes on Whatman No.1 filter paper wetted with distilled water under continuous florescent light at 25°C in a growth chamber (Vindon, England) for seven days. Visible root protrusion was recognized as germination. The time to get 50% germination (T_{50}), mean germination time (MGT) and germination index (GI) were calculated according to Afzal *et al.* (2009). Germination energy (GE) was recorded on the 4th day after planting. It is the percentage of germinating seeds on the 4th day after planting relative to the total number of seeds tested.

Control and treated seeds were sown in plastic trays (25 in each) containing moist sand, replicated four times, and were placed in a growth chamber. Mean daily temperature in the growth chamber was 25°C with continuous fluorescent light during the course of investigation. Emergence was recorded daily on the basis of appearance of cotyledons on the surface (AOSA, 1983). Seedlings were harvested after two weeks and washed with deionized water after harvest. All emerged seedlings were washed and separated into root and shoot for the determination of their fresh and dry mass. Dry mass was determined after oven drying the samples at 65°C for three days.

A sample was prepared by using potassium phosphate buffer (pH 7.0), extracted sample of marigold (0.1 g), phenyl methyl sulphonyl fluoride (PMSF) (10 mmol) was used as proteases inhibitor, then it was centrifuged at 1 000 x g (gravity, m s^{-2}) at 4°C for 20 min. After preparation of sample, α-amylase activity (EC 3.2.1.1) was determined by following the modified DNS method (Varavinit *et al.,* 2002).

Total soluble sugars were quantified in (0.1 g) marigold sample after grinding with the help of mortal pestal followed by hydrolysis with 2.5N HCl and then neutralised by sodium carbonate. Distilled water was used to make final volume of 10 ml, centrifuged at 10 000 x g and the supernatant was used for measurement of total sugars following the phenol-sulphuric acid method (Thimmaiah, 2004).

The reducing sugars were measured by DNS method from the marigold sample (0.1 g) extracted in 80% ethanol twice using 5 ml volume each time.

All the experiments were repeated thrice and the experimental Petri dishes and trays were arranged in a completely randomized design. Data recorded each time were pooled for statistical analysis to determine the significance of variance ($p<0.05$). For comparison of treatment means, standard errors were computed using Microsoft Excel.

RESULTS AND DISCUSSION

Most of the magnetic field treatments significantly improved seed performance in terms of reduction of time to reach 50% germination and mean germination time with increasing germination energy, germination index, final germination percentage (FGP), seedling shoot and root lengths compared to the control seeds (Table 1). Magnetic seed treatment with 100 mT for 3 min resulted in maximum final germination, GE and GI compared with untreated and treated seeds (25, 50, 75 and 125 mT for 3 min). There was a marked reduction in T_{50} and MGT in seeds subjected to 100 mT. Although most of the magnetic seed treatments improved root and shoot length, the maximum response was obtained with seeds magnetically treated with 100 mT for 3 min.

Magnetic seed treatment significantly ($p<0.05$) influenced the seedling vigour of marigold (Table 2). Maximum invigoration as indicated by lower values of T_{50} and higher values of final emergence percentage (FEP), root and shoot length was noted in seeds exposed to 100 mT followed by 125, 50, 75, and 25 mT.

Seeds exposed to magnetic field having strength above 25 mT significantly increased α-amylase activity, total and reducing sugars, but maximum response was recorded in seeds treated with 100 mT (Fig. 1). Magnetic seed treatment with 25 mT failed to improve α-amylase activity, total and reducing sugars of French marigold.

The main problem for marigold production is poor plant stand establishment, and unavailability of high quality seeds of marigold is another main reason hindering its production. The enhancement of growth under magnetic conditions appears to have been reported in many crops (Moon and Chung, 2000). We also found that the exposure of French

T a b l e 1. Germination capacity and seedling vigour of marigold as influenced by magnetic seed treatment

Treatment (mT)	MGT	T_{50}	GI	GE	FGP	Root length	Shoot length
	(days)			(%)		(cm)	
0 (Control)	5.33 a	4.17 a	7.44 d	16 c	36 c	2.55 e	2.20 c
25	5.24 ab	3.83 b	7.80 d	18 c	38 c	3.15 c	2.60 bc
50	5.26 a	3.75 b	12.39 c	36 b	58 b	3.3 b	3.02 ab
75	5.11 b	3.63 c	5.75 d	12 c	26 d	3.12 c	2.79 b
100	4.50 d	1.50 d	31.28 a	54 a	80 a	4.92 a	3.27 a
125	4.82 c	3.58 c	16.38 b	40 ab	54 b	2.95 d	2.85 ab
LSD	0.15	0.11	2.99	16.04	4.48	0.13	0.45

Signs not sharing the same letters in a column differ significantly at $p \leq 0.05$; MGT– mean germination time, T_{50} – time taken to 50% germination, GE – germination energy, GI – germination index, FGP – final germination percentage.

T a b l e 2. Emergence capacity and seedling vigour of marigold as influenced by magnetic seed treatment

Treatment (mT)	MET	E_{50}	EI	FEP (%)	Root length	Shoot length	Fresh mass	Dry mass
	(days)				(cm)		(mg seedling^{-1})	
0 (Control)	5.40 b	4.08 b	2.75 f	14.67 e	1.98 b	1.79 b	14.07 f	0.83 c
25	5.67 a	4.50 a	3.10 e	22.67 d	1.99 b	1.56 e	14.67 e	0.86 c
50	5.34 b	3.51 c	7.03 c	32.00 c	1.54 d	1.69 c	15.40 d	0.91 bc
75	5.45 b	3.93 b	3.88 d	24.00 d	1.71 c	1.72 c	19.33 b	1.14 b
100	5.09 c	3.03 d	14.57 a	58.67 a	2.44 a	1.85 a	38.93 a	2.29 a
125	5.13 c	3.15 d	8.19 b	34.67 b	1.43 e	1.63 d	17.07 c	1.00 bc
LSD	0.18	0.21	0.21	2.65	7.82	3.84	0.50	0.26

Signs not sharing the same letters in a column differ significantly at $p \leq 0.05$; MET – mean emergence time, FEP – final emergence percentage, E_{50} – time taken to 50% emergence, EI – emergence index.

marigold seeds to almost all magnetic treatments (except for 25 and 75 mT for 3 min) was highly profitable in improving all the germination parameters such as mean germination time, time to reach 50% germination, germination energy, germination index and final germination percentage (Table 1). These findings suggest possible resonance as a phenomenon which enhances seed germination performance and vigour when a proper magnetic treatment is applied. The effect of 100 mT for 3 min was more pronounced in synchronizing germination and emergence as depicted by lower MET, T_{50} and MGT, and higher GI, GE, FEP, FGP, root and shoot lengths in treated seeds compared with untreated control and other magnetic treatments (Table 2). Podleśny *et al.* (2005) also showed an acceleration of emergence in pea seeds after exposure to magnetic field. Enhanced germination and emergence in marigold seeds by magnetic seed treatment might be due to 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 vigour due to the influence of magnetic field in the seeds of cereals and vegetables was also observed by Aladjadjiyan (2010); Moon and Chung (2000); and Nimmi and Madhu (2009). Similarly, Carbonell *et al.* (2000) also reported that magnetic seed treatments significantly enhanced germination of rice seeds compared with untreated seeds. Slow germination, emergence pattern and seedling establishment in seeds treated with high strength (125 mT) magnetic field (Tables 1, 2) might be due to unsuitable combination of magnetic field and exposure time.

Improvement in shoot and root length, seedling fresh and dry mass of magnetic treated seeds as compared to control (Table 2) may be due to an increased rate of cell division in the root tips and earlier start of emergence as indicated by lower values of MET and E_{50} (Afzal *et al.*, 2009). This showed a great use of magnetic field in improving the sowing quality of marigold seeds. Aladjadjiyan (2010) also ascertained that root and shoot lengths were increased in magnetically treated seeds as compared to

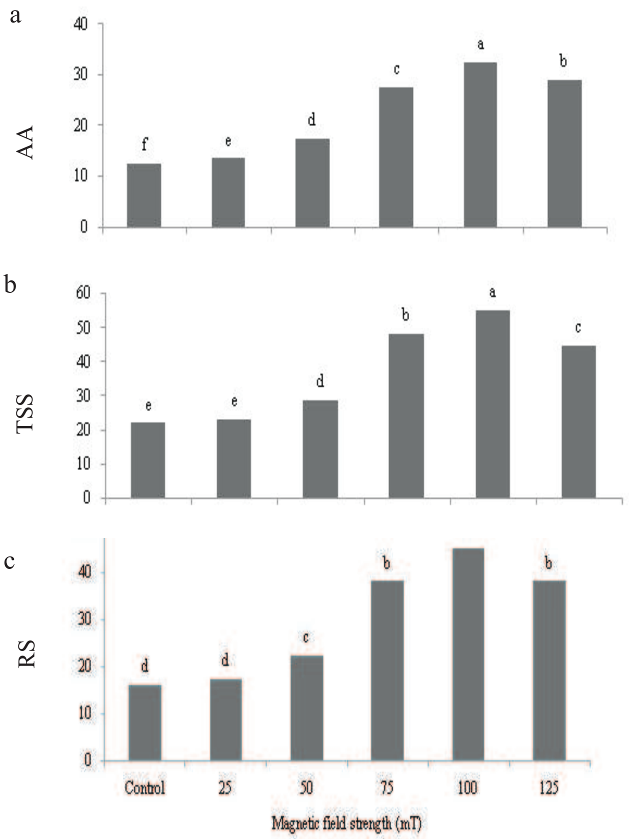

Fig. 1. Response of: a – α-amylase activity (AA), b – total soluble sugars (TSS) and, c – reducing sugars (RS, Ul mg of seeds^{-1}) of French marigold to magnetic seed stimulation. Signs not sharing the same letters in a column differ significantly at p$\leq$0.05.

untreated seeds. Similarly, Vashisth and Nagarajan (2008) reported that magnetic field application significantly enhanced seed performance in terms of speed of germination, root and shoot length and seedling dry mass compared to unexposed control.

Magnetic treatments are assumed to enhance seed vigour by influencing the biochemical processes that involve free radicals, and by 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 this present study (Fig. 1). It confirms the primary role of magnetic treatment in either stimulating protein synthesis or enhancing the activities of existing enzymes (Rochalska and Grabowska, 2007), thereby producing germination metabolites in requisite amounts. These results are consistent with the findings of Afzal *et al.* (2009) who reported that increased α-amylase activity resulted in increased contents of total and reducing sugars of African and French marigold seeds subjected to seed enhancement techniques. Improvement with 100 mT magnetic treatment might be due to changes in intracellular Ca levels that control numerous processes in plants. Increase in Ca level after exposure to magnetic field suggests that Ca entry into the cytosol can constitute the primary magnetic field sensing mechanism in plants (Belyavskaya, 2004).

The exposure of French marigold seeds to almost all magnetic treatments (except for 25 and 75 mT for 3 min each) was highly beneficial in improving all the germination parameters such as mean germination time, time to reach 50% germination, germination energy, germination index and final germination percentage (Table 1). These findings suggest possible resonance as the phenomenon which enhances seed germination performance and vigour when a proper magnetic treatment is applied. The influence of the magnetic field on germination, emergence and seedling growth depends on the energy of magnetic field and exposure time.

CONCLUSIONS

1. Most of the magnetic field treatments of French marigold seeds have the potential to enhance germination, early growth and biochemical parameters of seedlings.

2. Magnetic seed treatment with 100 mT for 3 min showed maximum seed invigoration and better performance in marigold seeds, thereby activating α-amylase and increasing reducing and total sugars.

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