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Research Article

Seed Conditioning Effects on Germination Performance, Seedling Vigor and Flower Production of *Zinnia elegans*

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Abstract | Various seed conditioning techniques were assessed for uniform germination, seedling vigor, and flower production in zinnia (*Zinnia elegans* Jacq.). Two initial laboratory experiments evaluated the timing and frequency of seed magnetic stimulation to select the most promising treatments for field production testing and flowering. In the first experiment, a 3% moringa leaf extract (MLE) showed the earliest (2.8 d) and the highest germination rate (90%) with an average root length of 4.2 cm. Hydro-priming resulted in the largest seedling fresh weight (0.8 g), seedling shoot length (5.8 cm), and seedling vigor index (980). The seedling vigor index was determined by multiplying the percent germination with the seedling length (cm). In the second experiment, seeds treated at 25 mT for 5 min. showed the highest (65%) and earliest (3.2 d) germination (T50) although not significantly different from treatments with 50 mT for 5 or 10 min. or 75 mT for 5 min. In field trials, seeds primed with MLE alone or in combination with magnetically treated water (MTW) produced the tallest plants (104 cm) with longer internodes and the largest canopy diameter (42.4 cm). Using MLE along with MTW produced greater plant fresh weight (137 g), more flowers (27.5), and larger flower diameter (9.7 cm) similar to the results of using MTW alone. In summary, seeds primed with MLE in combination with untreated water or MTW resulted in improved plant growth and yield characteristics and may be recommended to commercial growers of zinnia or other annual flowers.

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Introduction

Zinnia (*Zinnia elegans* Jacq.) belongs to family Asteraceae and is a very popular annual flowering bedding plant with variable petal shapes and bright flower colors, which exhibits considerable phenotypic diversity among cultivars for morphological characters, plant habit and flower color. Zinnias are among the three leading specialty cut annual species being grown in USA (Dole and Wilkins, 2005). In Pakistan, zinnia is a leading bedding plant with continuously increasing demand on account of its high temperature tolerance and long lasting flowers of variable colors (Sharif *et al*., 2019). It is also a leading bedding ornamental species all over the world. Today's competitive cut flower and bedding plant markets demand seedling trays with 100% germination. Therefore, growers are keen to provide maximum seed germination for economic production and delivering uniform sized seedlings of high quality.

Links Researchers

Commercial field crop production needs early and uniform seedling emergence and crop stand establishment (Afzal *et al.*, 2012). However, seeds of zinnias exhibit erratic and slow germination, particularly when seeds are sown in soil under variable environmental conditions (Ahmad *et al.*, 2017). Seed emergence takes 4-7 days or more, which may cause problems of having non-uniform crop stand, ultimately affecting quality production. Zinnias are also directly seeded, which leads to slow and variable germination resulting in poor crop stand establishment. To overcome these issues, different seed priming techniques are being used, *viz.* hydropriming, halopriming, osmopriming, priming with growth hormones, matripriming etc. Magnetic treatment of seeds has also been reported to improve germination percentage and uniformity, growth, and agronomic yield of many traditional agronomic and non-traditional horticultural crops, either bedding or cut flower (Pietruszewski and Kania, 2010; Ziaf *et al.*, 2015).

Seed priming, a controlled seed hydration process, facilitates the initiation of various metabolic processes (Bradford, 1986) to increase germination rates and improve crop stand and yield (Khan, 1992). Priming seeds with MLE decreased germination time to enhance seedling growth due to increased chlorophyll, sugar content and amylase activity (Rochalska and Grabowska, 2007).

Pre-treatment of seeds with varying magnetic field frequencies increased the germination rate, growth and yield of annual medics and dodder (Balouchi and Sanavy, 2009), and *Zea mays* seeds when exposed to 100 mT h^{-1} in a comparison to non-treated seeds (Subber *et al.*, 2012). It also reduced the disease incidence (De Souza *et al.*, 2006). During magnetic stimulation of seeds, increase in ascorbic acid contents and proteins synthesis (Yinan *et al.*, 2005; Rochalska and Grabowska, 2007), scavenging of free radicals, stimulation of enzymes (Moon and Chung, 2000), are considered to enhance germination rate and crop stand establishment, which subsequently lead to elevated crops yield under open field conditions.

Most seed enhancement studies have focused on cereal and vegetable crops. Only limited research reports are available for ornamentals. Therefore, a study was carried out to evaluate the efficacy of magnetic seed stimulation and MTW or MLE priming on crop stand and flower yield of zinnia both under laboratory and field conditions. Seed priming and magnetic stimulation were hypothesized to escalate zinnia crop stand establishment through more vigorous and uniform seedlings.

Materials and Methods

Expt. 1. Seed priming effects on germination performance and seedling vigor

Seeds of zinnia cultivar 'Giant Dahlia Flowered' were obtained from Chanan Din and Sons, Lahore, Pakistan. On arrival, seeds were graded for its uniform size and grouped according to treatments. Seeds were primed at Seed Physiology Laboratory, Department of Agronomy, University of Agriculture, Faisalabad, and germinated in an incubator having set temperature and humidity conditions. For MLE, fresh fully matured leaves were harvested, washed with tap water, and then distilled water, and frozen for 24 h. Afterwards, extract stock solution was prepared using locally fabricated machine available at Department of Agronomy, University of Agriculture, Faisalabad, Pakistan, using 1:1 (w/v) ratio of leaves and distilled water without using any additive and finally diluted using distilled water for preparing working solutions. The stock solution was stored in refrigerator at 4 °C until use and was used within a week after preparation. Treatments included control (no priming), seed priming with (i) distilled water, (ii) 3% MLE (using distilled water; 3% MLE), (iii) MTW (methodology is mentioned in expt. 2), or (iv) 3% MLE solution (prepared by mixing MLE in MTW). Seeds were primed for 24 hours and air dried at room temperature to gain their original weight.

There were three replications of 20 treated or untreated seeds, which were placed on double sheets of Whatman No. 1 filter paper for germination, wetted using 3 mL of distilled water, in 9 cm diameter Petri dishes, at 25±2°C and 50±10% relative humidity (R.H.), in incubator for seven days under continuous florescent light. Petri dishes and filter papers were sterilized in autoclave for one hour prior to placing seeds for test. Seed was considered germinated when radicle protrusion was visible. Time to 50% germination (T_{50}) was counted in days until 50% of sown seeds germinated, while final germination percentage, seedling shoot length (cm), root length (cm), and fresh weight (g) of seedling were recorded after 30 days of sowing (Afzal *et al.*, 2009). Vigor index of seedlings was calculated according to following equation: Vigor index = Germination (%) × seedling length (cm) (Abdul-Baki and Anderson, 1973).

Expt. 2. Magnetic seed enhancement effects on germination performance and seedling vigor

Seeds were pretreated at Department of Physics, University of Agriculture, Faisalabad, using magnetic seed stimulator, which comprised of two coupling cylindrical coils both fixed on iron bars, placed above one another using metallic supports and attached to power source. MTW was obtained by passing it through magnetic field (10 mT) for ten minutes. When an electric current was supplied through the electromagnet coils, a full wave magnetic field was triggered in the space between the magnetic bars (De Souza *et al.*, 2006; Afzal *et al.*, 2012). Zinnia seeds were placed in a glass Petri dish between two magnetic bars and subjected to magnetic field of various strengths (25, 50 and 75 mT) and durations (5, 10 and 15 minutes). Seeds without any exposure to magnetic field were considered as a control group. The magnetic frequencies were induced by calibrating the electric current applied to the coils, unless the desired working magnetic frequency was established, which was assessed by using magnetometer. Data were recorded on same parameters using same procedures as described for expt. 1.

Expt. 3. Priming and magnetic seed enhancement effects on plant growth

Priming and magnetic seed stimulation treatments, which performed better in expt. 1 and 2, were selected for comparison in a field experiment, conducted at Floriculture Research Area, Institute of Horticultural Sciences, University of Agriculture, Faisalabad (31.4336° N, 73.0683° E), Pakistan. Seeds were pretreated according to the treatments and sown in plastic plug trays of 128 cells, containing coconut coir and leaf manure substrate (1:1; v/v). Trays were kept in a shade house until seedlings developed 3-4 true leaves and were transplanted after three weeks of sowing. Land was rigorously ploughed, leveled and treatments were laid out according to randomized complete block design having three replicates, containing 20 plants each. Plants were spaced at 30 cm between plants and rows in flat beds. Data were recorded at harvest for plant height (cm), plant canopy diameter (cm), inter-nodal distance (cm), fresh weight of a plant (g), flower diameter (mm), and number of flowers per plant. Plants were harvested when terminal flowers had opened, taken to the laboratory within 30 min. of harvest and above data were recorded.

Statistical analysis

Laboratory experiments were laid out individually in completely randomized design, while field experiment was organized in a randomized complete block design and treatments were replicated thrice. Fisher's analysis of variance technique was used for data analysis and treatment means were compared using LSD test at $P<0.05$ (Steel *et al.*, 1997).

Results and Discussion

Expt. 1: Seed priming effects on germination performance and seedling vigor

Seeds primed with 3% MLE germinated earliest with 2.8 d for 50% germination, while untreated seeds (control) took 5.2 d (Figure 1A). All primed seeds had more than double the germination percentage of untreated seeds (Figure 1B). No differences were observed among hyrdopriming, 3% MLE priming, priming with MTW alone or with MLE (MLE+MTW). Seed priming using MLE (alone) or as 3% solution with MTW reduced mean germination time. Effective seed priming components are quite helpful in enhancing germination capacity of grasses (Hardegree and Emmerich, 1992). Moreover, water exposed to the action of a magnetic field usually indicate reduced surface tension, which induce more water uptake in plant (Galland and Pazur, 2005), and same might have happened with seeds primed in MTW or MLE+MTW.

Greatest shoot length (5.7 cm) were recorded for hydro-primed seeds followed by priming with 3% MLE (4.8 cm), whereas seeds primed with 3% MLE +MTW produced shortest shoot length (4.4 cm) (Figure 1C). Provision of liquid medium through priming solution, crucial for cell enlargement and division as well as other metabolic processes, may increase shoot length, particularly due to elevated tensile strength of cell wall and other metabolic reactions taking place inside the cell (Haghpanah *et al.*, 2009).

Priming of seeds with 3% MLE produced longer roots (4.3 cm) as compared with control (no priming) (3.3 cm), however, statistically similar to hydropriming (4.1 cm; Figure 1D). Parallel results were also revealed by Basra *et al.* (2011), where MLE priming increased seedling root length of sunflower. Hydro-primed seeds produced higher seedling fresh weight (0.8 g) followed by MTW (0.7 g), whereas seeds primed with 3% MLE alone or along with MTW had lowest seedling fresh weight (0.6 g; Figure 1E). Seeds primed with water had highest vigor index (981.7) followed by MLE plus MTW, while no priming or priming with MTW had lowest vigor index (Figure 1F). Seed priming with water or 3% MLE proved best option for germinating zinnia seeds, which has depicted its efficiency by influencing metabolic activities and triggering germination process due to availability of water and growth regulators, as reported earlier in maize (Basra *et al*., 2011).

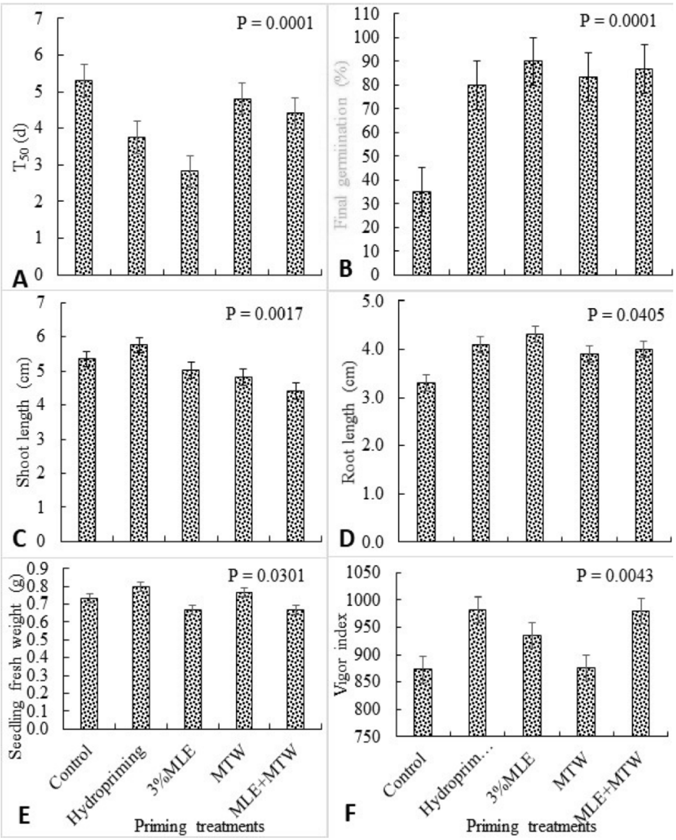

Figure 1: *Effect of seed priming on time to get 50% germination (A), final germination (B), shoot length (C), root length (D), seedling fresh weight (E) and vigor index (F) (calculated according to following equation: Vigor index = Germination (%) × seedling length (cm) of 'Giant Dahlia Flowered' zinnia.*

Expt. 2: Magnetic seed enhancement effects on germination performance and seedling vigor

Seeds pretreated with 25 mT magnetic field for 5 min. took less time to 50% germination (3.3 d) compared to rest of treatments, while seeds treated with 75 mT for 15 min. delayed germination up to 6.0 d (Figure 2A). Application of 25 mT for 5 min. produced higher final germination percentage, which was similar to 50 mT for 5 min. and 75 mT for 5 min (Figure 2B). However, all magnetic treatments performed better than untreated seeds except 50 mT for 15 min., which was similar to untreated seeds. Magnetic seed stimulation increases metabolites and protein synthesis in the seeds, which enhances germination and provides energy to emerging seedling (Rochalska and Grabowska, 2007). Magnetic seed stimulation is now being used successfully as a tool to increase seed vigor of many horticultural crops (Moon and Chung, 2000; Ziaf *et al*., 2015). Exposure of marigold seeds to 100 mT magnetic field treatment resulted in increased germination speed and germination percentage up to four times compared to untreated (control) seeds (Afzal *et al*., 2012).

Magnetic seed stimulation had no statistically significant impact on seedling shoot or root length, seedling fresh weight and vigor index of zinnia (Figure 2C-F). There were only numeric differences among treated and untreated seeds, which are contradictory to previous sequel of Afzal *et al*. (2012), who documented an increased seedling shoot length when seeds were pretreated with 100 mT magnetic strength.

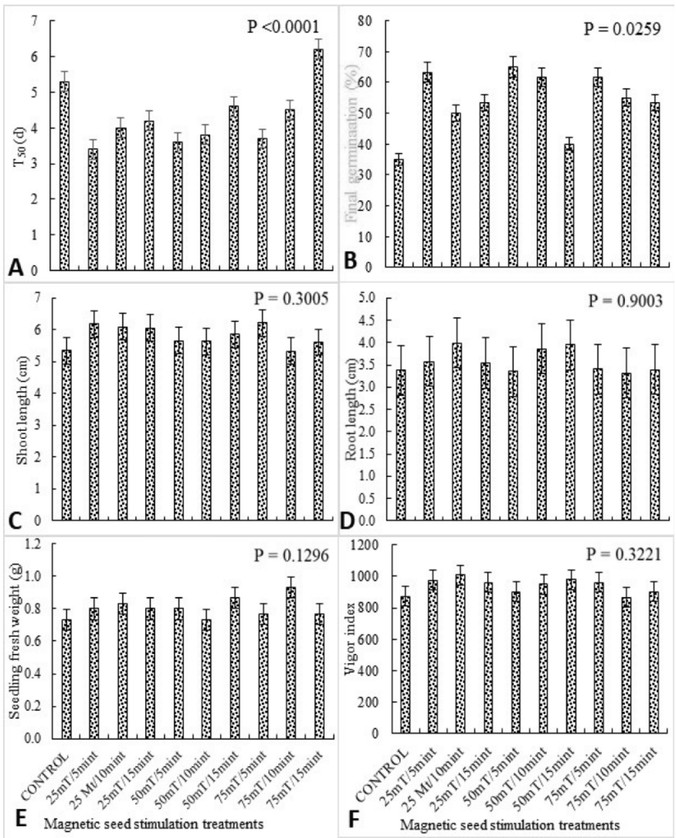

Figure 2: *Effect of magnetic seed stimulation on time taken to 50% germination (A), final germination (B), shoot length (C), root length (D), seedling fresh weight (E) and Vigor index (F) (calculated according to following equation: Vigor index = Germination (%) × seedling length (cm) of 'Giant Dahlia Flowered' zinnia.*

Expt. 3. Seed priming and magnetic seed enhancement effects on growth and yield

Seeds primed with 3% MLE alone or with MTW produced tallest plants (Figure 3A). Plants grown from untreated seeds produced shortest plants, while higher concentration of magnetic stimulation treatment, i.e. 75 mT for 5 min. also reduced plant height of zinnia. MLE being rich in amino acids, ascorbate, and several growth hormones particularly cytokinins may increase cell division and elongation and result in taller plants (Makkar and Becker, 1996). Seeds primed with 3% MLE plus MTW resulted in maximum plant canopy diameter (46.2 cm) followed by 3% MLE alone (42.3 cm), which were statistically similar (Figure 3B). Seeds treated with 75 mT magnetic seed stimulation for 5 min. produced plant canopy with the smallest diameter (30.6 cm). Cytokinins, being integral component of MLE, are best known plant growth regulator responsible for branching and increasing plant growth.

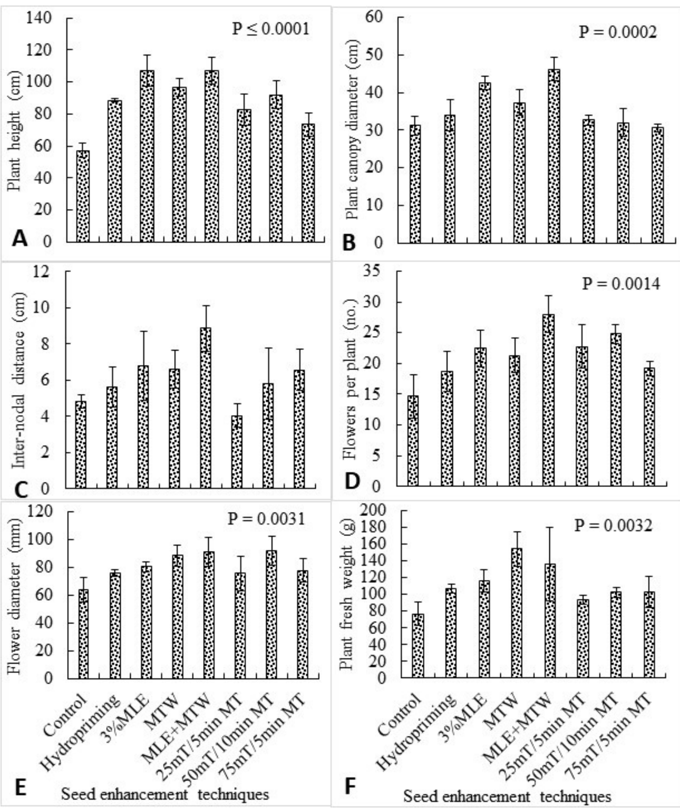

Figure 3: *Effect of seed priming and magnetic seed stimulation on plant height (A), plant canopy diameter (B), inter-nodal distance (C), number of flowers per plant (D), flower diameter (E) and plant fresh weight (F) of 'Giant Dahlia Flowered' zinnia.*

Priming with 3% MLE plus MTW or MLE alone produced plants with longest internodes (Figure 3C) and highest number of flowers per plant (Figure 3D). Untreated seeds produced shortest internodes and the least number of flowers per plant. Flower quality of non-primed seeds, measured on a rating scale of 1-9 with 9 for best quality and 1 for inferior quality flowers, was also inferior to those plants treated with MLE. Higher sugar contents were recorded in sugar beet roots when subjected to magnetic seed stimulation (Pietruszewski and Wojcik, 2000), which might be the possible reason of increased number and quality of zinnia flowers per plant.

Magnetic seed stimulation at 50 mT for 10 min. and MLE priming using MLE+MTW produced largest flower diameter (91.9 mm each), while non-treated seeds produced smallest flower diameter (63.8 mm), as shown in Figure 1E. Seeds pre-treated with full-wave rectified sinusoidal non-uniform magnetic fields (MFs) induced by an electromagnet at 100 mT (rms) for 10 min. and at 170 mT (rms) for 3 min. improved growth and quality yield in tomato (De Souza *et al.*, 2006). MLE being rich in cytokinins and ascorbate, along with MTW, is also responsible for the cell division and protein synthesis (Zamiran *et al.*, 2013), might have contributed to increase plant growth. MLE increases protein synthesis, which lower water potential.

Seed treatment with MTW alone or in combination with MLE produced highest plant fresh weight (154 and 137 g, respectively), while plants grown from untreated seeds had lowest fresh weight (77 g) (Figure 3F). Due to protein synthesis, MTW endorsed more water uptake, which reduces water potential and water moves in the plant, thereby enhancing fresh weight per plant (Yinan *et al.*, 2005). On the other hand, MLE has high level of ascorbate, whose application as foliar spray or priming solution is considered to be a principle factor for improving plant growth and yield attributes. MLE has plenty of zeatin, which plays an influential part in cell division and elongation, hence enhancing growth parameters (Taiz and Zeiger, 2006). These properties might have boosted up the seed germination performance and plant growth characteristics.

Overall, use of 3% MLE priming with or without MTW produced high quality uniform size seedlings. Therefore, these organics such as MLE would be a cheaper and safe source to improve nursery quality and increase returns from marketing seedlings of ornamentals. The industry is looking for different crops and the use of organic and natural materials. MLE did not only improve germination and quality of zinnia nursery seedlings but also improved quality of freesia flowers and extended postharvest longevity of cut roses, gladiolus and tuberoses (Ahmad *et al*., 2019a, b).

Conclusions and Recommendations

Seeds primed with 3% MLE in combination with MTW proved to be the best priming protocol and may be used by growers to treat zinnia and other flower seeds for producing uniform crop stand and better flower production.

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Novelty Statement

Manuscript describes effects of various seed conditioning techniques using priming with different solutions and magnetically stimulation approaches on uniform germination, seedling vigor and flower production of zinnia, which has erratic seedling growth when grown without prior treatment. Seeds primed with 3% MLE or water with or without MTW performed the best and may be used by commercial growers for zinnia nursery production.

Author's Contribution

Principal author Shaiza Rasool conducted the trials and collected data. Iftikhar Ahmad designed the experiments and helped in conducting experiments, data analysis, write up and revision. Khurram Ziaf helped in research planning, data analysis and contributed during write up, Irfan Afzal helped in lab. analysis, and extraction of MLE, Muhammad Abdul Salam Khan developed graphs, M. Aashir Sajjad helped in data analysis, while M. Zain Ali reviewed the manuscript. All authors reviewed and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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