RESEARCH ARTICLE

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Magnetopriming enhance germination and seedling growth parameters of onion and lettuce seeds

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Abstract
The main objective of this study was to improve seed quality by using magnetic field (MF) as a priming method to increase germination percentages (GP) and seedling emergence percentages (SEP) in onion and lettuce seeds. MF treatments on pre-hydrated seeds, significantly increased GP (up to 80% for onion, 87% for lettuce) and SEP (up to 76% for onion, 86% for lettuce) in both species. Magnetic treatments in other saying magnetopriming helped to increase germination and seedling emergence speed in treated seeds as well. The shortening of mean germination time allowed the treatments to establish uniform and well-developed seedlings. Our findings indicate that magnetopriming could be used as a pre-germination treatment before sowing.
Keywords: Magnetic field, Pre-germination treatment, Seed quality

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INTRODUCTION

Pre-sowing seed treatments are called "pre-germination" or "seed priming" help to eliminate the problems that arise during the period from seed sowing to seedling emergence. Seed priming treatments improved germination related parameters like water uptake, speed of germination, fresh/dry weight and length of seedlings and vigor indices (Shine et al., 2011). Priming is an approach that involves hydration of seeds adequately to allow metabolic events before germination, despite preventing radicle emergence to occur (Heydecker and Coolbear, 1977; Paparella et al., 2015). Seed priming is an easy, low-risk and low-cost method, which is successfully applied either to poor germinating seed lots or to seeds, which are sown under different stress conditions (Sung et al., 1998; Jisha et al., 2013).

The most common priming techniques include hydro-priming, where controlled water intake is provided with only water, osmopriming using osmotic solutions (PEG, KNO3, KH2PO4 etc.) and matrixpriming using solid media such as vermiculite. Today, although hydro-priming and pre-germination treatments using different chemicals are widely used commercially, the use of physical priming treatments (magnetic field, ultraviolet rays, ultrasound, ionizing radiation etc.) as a priming method, especially in vegetable seeds, is not common. Physical methods that protect plant health and improve storage properties can be a good alternative to increase plant production (Aladjadjyan, 2012; Arujo et al., 2016). Physical priming methods came into prominence as environment-friendly technologies, not requiring washing of seeds after treatment and not producing waste material compared to osmotic priming methods.

Magneticbiology is a new multidisciplinary science with biophysics at its center, covering various branches of science such as engineering, physics, chemistry and biology. This field studies the biological effects of electromagnetic (oscillating) or static low-intensity magnetic fields (MF) on tissues (without tissue heating) (Kataria, 2017a). MF is both a very old and a very recent area of plant research. MF is an unavoidable physical factor affecting living organisms. There is a MF of 50 microteslas (μT) naturally possessed by the earth around us (Belyavskaya, 2004). Some studies were conducted evaluating the response of plants to MF at different intensities, including near-zero (0-40 nT), low (up to 40 mT), and very high values (up to 30 T) (Teixeira da Silva and Dobranszki, 2015). Results show that biochemical, molecular, cellular and various effects on the whole plant occur in many plant species (Sen and Alikamanoğlu, 2016; Podlesna et al., 2019; Mohammadi and Roshandel, 2020; Jovičić-Petrović et al., 2021; Vashisth et al., 2021).

The biological effect of MF depends on the plant characteristics, such as species, variety, age of the material, number of chromosomes and structure of the target organ or tissue, the frequency of the current, the magnet poles, the MF intensity, the duration of the application and the correct dose of application. From previous studies, the most determining element for the MF effect for germination and seedling growth characteristics was the determination of a specific exposure period to the species (Aladjadjiyan and Ylieva, 2003; De Micco et al., 2014).

Studies investigating the effects of MF on plants have increased considerably. Magnetopriming has become a popular method among physical seed treatments before planting due to its economic and environment-friendly properties and proven benefits. The results of this non-destructive seed priming method increases seed germination and vigor, shoot development, fresh weight, plant height, normal seedling rate and ultimately increasing yield without harming the environment have been published for many plant species (Aladjadjiyan, 2002; Vasilevski, 2003; De Souza et al., 2006; Carbonell et al., 2008; Shine et al., 2011; Bhardwaj et al., 2012; Bilalis et al., 2013; Efthimiadou et al., 2014; Baghel et al., 2016; Kataria et al., 2017b; Razmjoo and Alinian, 2017; Ivankov et al., 2021). According to the previous studies, magnetopriming treatment not only enhances the germination percentages of seeds with low viability, but also gives good outcomes even under abiotic stress conditions (Rochalska and Orzeszko-Rywka, 2005; Thomas et al., 2013; Hozayn et al., 2018). For instance, Kataria et al. (2017c) found that the negative effect of salinity on germination and seedling vigour of maize and soybean can be alleviated by magnetopriming with static magnetic field of 200 mT for 1 h. MF treatment enhanced water uptake and improve activity of hydrolytic enzymes (α amylase and protease) in treated seeds as compared to untreated seeds under both non-saline and saline conditions. Ultimately, magnetopriming increased the percentages of germination and seedling vigour.

Onion and lettuce are widely consumed vegetables worldwide. These species are also important for Turkey in terms of seed trade, planting and production amounts and economic value. In 2021, approximately 2,625,000 tons of onion and 540,000 tons of lettuce were produced in 77,000 and 21,000 hectares, respectively, in Turkey (TUIK, 2022). However, seeds of these species are sensitive and have relatively short storage life. In order to reduce seed consumption and production cost physcial pre-sowing treatments can be used to improve germination and emergening traits. So, quantifying to possible effects of magnetic treatment on the germination and seedling emergence of onion and lettuce could have important implications for the seed sector and gene banks.

The aim of this study was to improve seed quality utilizing magnetopriming. In the present study, we tried to increase the germination and seedling emergence percentages and speed of lettuce and onion seeds, which have emergence and germination problems in plant production. Seeds of chosen species are sensitive to low-high temperature or salinity stress during germination and emergence period, and can lose their viability rapidly during the storage period. Although in recent years the studies on vegetable seed priming have increased in Turkey, not enough research has been performed on physical seed priming treatments.

MATERIALS AND METHODS

Seed material

Lettuce (*Lactuca sativa* L.) seeds were obtained from Ataturk Central Horticultural Research Institute-Yalova and onion (*Allium cepa* L.) seeds were provided by a private company (Beta Ziraat). Initial germination percentage (included abnormal seedlings) and moisture content of the seed lots were determined according to the ISTA (2016) rules (Table 1). Afterwards seeds were hermetically sealed and stored at 4°C until used.

Table 1. Germination percentages (%) and moisture contents (%) of seed lots.

Species	Variety	Germination rate (%)	Moisture content (%)
Onion	Panko	75	8.0
Lettuce	Grise Maraichere	88	5.0

Hydro-priming of seeds

Before hydro-priming treatment seeds were first moistened. Moistening was carried out by placing seeds in petri dishes containing two Whatman filter papers and spray moistening with water until seeds reached 30% moisture. Hydro-priming of lettuce and onion seeds was done by humidifying seeds in hermetic packages at 15^{0}C, for 8 and 16 hours in dark conditions. Magnetopriming was rapidly done after the hydro-priming treatment.

Magnetic field generation and treatment

The applied static magnetic field was created by hollow rectangle magnets with a maximum and permanent strength of 800 militesla (mT) (Figure 1). Before the treatments, the magnetic field strength between the poles was measured and checked with a gaussmeter model Lakeshore 460-3.

(a) (b)

Figure 1. (a) Gaussmeter and static magnetic field generator (b) Seed sample between the poles.

Seeds of two different species were preliminarily hydro-primed at two different periods of time (8 and 16 hours), before magnetic exposure. Subsequently seeds were exposed to the static magnetic field of stationary 800 mT for four different time periods (1, 2, 5, and 10 mins). 350 healthy seeds were put in the petri dishes, 150 seeds for germination and seedling tests and 50 as substitute. Before magnetic field exposure, the seeds were placed at equal distances from each other in petri dishes without overlapping (Yinan et al., 2005; Aladjadjiyan, 2010; Feizi et al., 2012). The seeds were kept at room temperature throughout the experiment (22±1°C). When the magnetic field treatment was completed, seeds were dried at 25°C for 24 hours, control seeds were kept under similar conditions but in the absence of static magnetic field (local geomagnetic field only).

Germination test

Germination of the seeds was determined by using "between papers" method (ISTA, 2016). One hundred fifty seeds in three replications of 50 seeds each were placed between two layers of moist germination papers using with distilled water in controls and all other treatments. The germination papers with seeds were rolled and placed in a plastic bag to avoid surface evaporation of moisture. Plastic bags were placed in the germination incubator in an upright position. Germination papers were checked and moistened when necessary.

For lettuce seeds germination test performed at 20°C for 7 days and onion seeds 20°C for 12 days. For each treatment, the number of germinated seeds was scored two times per day and considered germinated when the radicle was approximately 2 mm long or more. On the final day of each practice, seedlings with normal and abnormal growth were determined and expressed as a percentages (%).

Mean germination time (MGT) was calculated based on the equation of Ellis and Roberts (1980).

$$MGT = (\Sigma n \times D) / \Sigma n$$

n: The number of seeds, which were newly germinated on that day

D: Number of days counted from the beginning of germination

Seedling emergence test

In the seedling emergence experiment, 50 seeds with 3 replicates from each seed lot were sown at a depth of approximately 2-2.5 cm in the seedling pots in which perlite:peat was used at a ratio of 1:2.

Seedling growth pots were placed in the growth chamber, with temperature adjusted to 22±2°C. The seedlings were counted daily. The tests were carried out with 16 hours of lighting and 8 hours of darkness. The relative humidity was at 60-65% and the light intensity was measured as 6500 lux. Seedling emergence tests were executed for 20 days. The occurence of cotyledon leaves on the surface was the criterion for emergence. At the end of the experiment, the seedlings were classified as: normal, or abnormal (no roots or shoots formed, glassy, spiral rootlets, necrosis detected in the cotyledon leaves or not developed at all, inseparable from the seed coat). Seedling emergence time (SET) was calculated using the same method used to determine MGT.

Statistical analysis

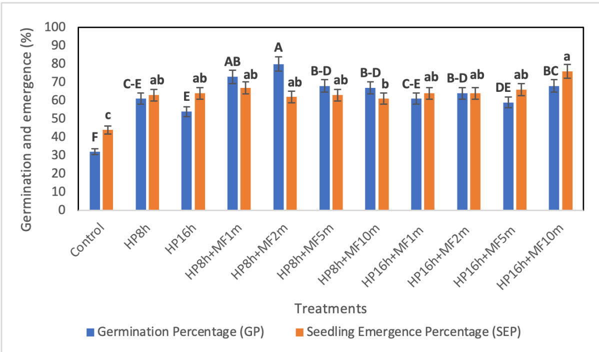
The data obtained at the end of the study was subjected to analysis of variance, and the differences between the means was compared using the LSD test at the 5% level. Obtained percentage values were subjected to $\sqrt{n}$ transformation. All statistical calculations were made using the JMP 8.0 package program.

RESULTS AND DISCUSSION

Seed quality parameters

Variance analysis results of magnetic treatments of germination percentages (GP) ($P<0.01$), seedling emergence percentages (SEP) ($P<0.05$), MGT ($P<0.01$) and SET ($P<0.01$) showed statistically significant differences in onion seeds (Fig. 2). All treatments including sole hydro-priming treatments, significantly improved seed quality parameters (GP and SEP) compared to those of the control seeds. The highest GP result was observed in the hydro-priming HP8h+MF2m treatment (80%), significantly higher compared with the lowest GP obtained in the untreated control seeds (32%) (Fig. 2). In other treatments, results ranging from 54% (HP16h) to 73% (HP8h+MF1m) were achieved. Similarly, SEP was positively affected by magnetic field treatments, the best result was obtained in HP16h+MF10m treatment (76%) compared to other treatments and control seeds (Fig. 2). As with the germination rate, the control group presented the lowest result (44%) in the SEP. In other treatments, close SEP values, 61% to 67% have been obtained.

Higher germination occured in first 8 hours HP and after magnetic treatments than HP treatments (8-16h) alone and 16 hours HP treatment before magnetic exposure. Seeds obtained higher germination percentages when exposure time reduced in HP 8h treatments.



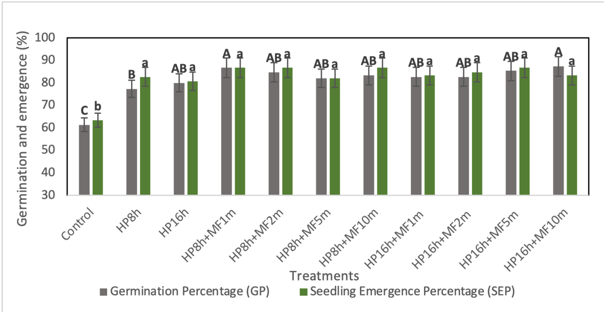
*The difference between the values shown in the similar columns with the same letter was not significant ($p < 0.05$).

Figure 2. Germination and seedling percentages of onion seeds. Bars in the same column represent SEM value.

A statistically significant difference was achieved between the effects of hydro-priming and magnetopriming on GP ($p < 0.01$), SEP ($p < 0.01$), MGT ($p < 0.01$) and SET ($p < 0.01$) in lettuce seeds (Fig. 3).

According to the results of the GP, the lowest result was 61% in the control group and the highest rate was 87% in the HP16h+MF10m treatment. In all treatments, germination values varying between 77% and 85% were significantly higher than the control group (Fig. 3). The lowest SEP (63%) was obtained by the control group, while the highest seedling emergence rate was 86% in 4 different treatments (HP8h+MF1m, HP8h+MF2m, HP8h+MF10m, HP16h+MF5m). In other treatments were included in the same group with the treatments that gave the highest statistical value (Fig. 3).

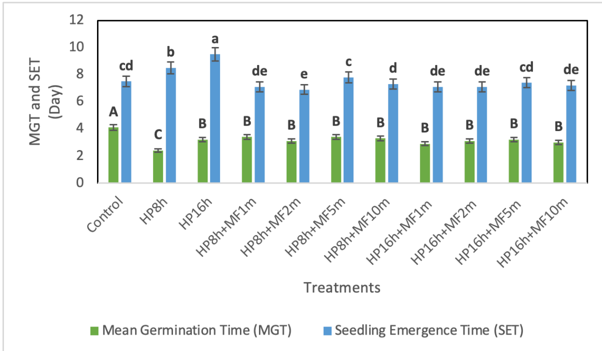
Observations were also made regarding the effect of magnetopriming on germination and seedling emergence speed. The MGT of all exposed seeds was quite lower than control seeds in other saying exposed seed showed better performance and nearly same results were observed for SET measurements (Fig. 4). As seen in Figure 4 which presents MGT of treated onion seeds; HP8h was the fastest treatment regarding the germination speed (2.4 day), and the slowest germination was in the control seeds (4.1 day). All other treatments attained similar results and were not statistically significanlty different.



*The difference between the values shown in the same columns with the same letter was not significant ($p < 0.05$).

Figure 3. Germination and seedling percentages of lettuce seeds. Bars in the same column represent SEM value.

According to the SET results, HP16h showed the slowest emergence (9.5 day), while the fastest emergence was 6.9 day in HP8h+MF2m treatment, which also provided the highest GP (Fig. 4). Overall, shorter seed exposures (1-2 minutes) resulted in higher germination and faster seedling emergence time.

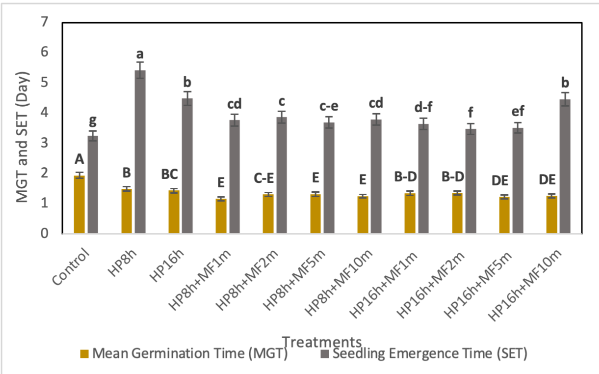


*The difference between the values shown in the same columns with the same letter was not significant ($p < 0.05$).
**The lettering in the mean germination time and seedling emergence time values indicates the "a" value which is the slowest.

Figure 4. Mean germination time (day) and seedling emergence time (day) of onion seeds.

The MGT of all exposed lettuce and onion seeds were quite lower than control (Fig. 5). While the slowest germination was 1.9 day in the control group, the fastest germinated seeds were observed in HP8h+MF1m treatment at 1.1 day. In other treatments, they presented values close to each other (1.2-1.4 days). Within the context of SET, the slowest (5.4 day) SET was in the treatment HP8h, the fastest seedling emergence was achieved in the control group with 3.2 day, contrary to all other parameters (Fig. 5).

Effects of hydro-priming on seed germination and seedling growth is well known for many years. Magnetopriming is successfully used for seed treatment, promoting higher germination ratio, well established seedling stage and ultimately increased crop yield (Shine et al., 2017; Ivankov et al., 2021; Ziaf et al., 2022). Although magnetopriming is known as a physical priming method applied to dry seeds, there are also few studies where it is applied to pre-soaked or hydrated seeds with successful results (Florez et al., 2007; Feizi et al., 2012; Kubisz et al., 2012; Mousavizadeh et al., 2013). According to Feizi et al. (2012), exposure of hydro-primed and dry tomato seeds to magnetic field significantly improved mean germination time, root, shoot and seedling length and vigor index compared to untreated seeds. Kubisz et al. (2012) found that 20 mT magnetic field treatment after 12 hours soaked onion seeds (cultivar Eureka) led to increased energy of germination (40 to 62%) and germination capacity (by about 6%). These results are in line with our findings, enhancement in the germination and seedling parameters were observed after hydro-priming, and also increased further by hydro-priming followed with magnetic field treatment. We presumed that wet seeds may respond better to magnetopriming since physiological activity was initiated. However, hydro-priming prior to magnetopriming did not provide any extra advantage to the treatment. Particularly, given wet seeds required drying after magnetic treatment causing additional operations and expenses.



*The difference between the values shown in the columns with the same letter was not significant ($p < 0.05$).
**The lettering in the mean germination time and seedling emergence time values indicates the "a" value which is the slowest.

Figure 5. Mean germination time (day) and seedling emergence (day) time of lettuce seeds.

Germination and seedling growth parameters in our results were in agreement with the observations reported for magnetopriming of onion and lettuce seeds (Soltani and Kashi, 2004; De Souza et al., 2008; Kubisz et al., 2012; Mousavizadeh et al., 2013; Hozayn et al., 2015; Zalama and Fathalla, 2020). In the present study, magnetopriming not only helped with seed germination but also promoted proper vigour and desired seedling growth. Alexander and Doijode (1995) demonstrated that, germination and seedling emergence rate, root and seedling lengths, fresh and dry weights were increased in low viability rice and onion seeds after magnetic field treatment. In onion seeds an increase in germination (36.6%) and emergence (127.3%) was recorded compared to low viability control seeds. This is valuable in getting faster and well-developed seedlings in field conditions. Capability of lettuce seed water uptake increased when treated with a stationary magnetic field ultimately increasing the germination rate. Latef et al. (2020) reported that, growth parameters were increased by static magnetic field (SMF) treatment. They stated that this positive impact of SMF on lettuce was due to the development of osmoregulation substances, secondary metabolites, stimulation of the reactive oxygen species scavenging system via the improvement of enzymatic and non-enzymatic antioxidants and hence, the mitigation of lipid peroxidation, thereby improving the quality of lettuce leaves.

The positive effects of magnetopriming were also shown on accelerating seed germination and seedling growth (Martinez et al., 2009; Feizi et al., 2020; Ghanbarpouri et al., 2021; Alvarez et al., 2021). Our study displayed the potential of magnetic seed stimulation to decrease germination and seedling emergence time. Shorter seed exposures (1-2 minutes) resulted in higher germination and faster seedling emergence time in onion seeds. Coherent results were obtained in another study in which germination and seedling growth parameters and mean germination time were also measured. The study conducted with 2 different onion seed lots, MGT were decreased 30 minutes/60 mT treatment in high quality seeds and 60 minutes/30 mT treatment density in low quality seeds compared to the control group (Hozayn et al., 2015). Similar results were obtained in our study in lettuce seeds, while the slowest germination was 1.9 days in the untreated seeds, the fastest germinated seeds were monitored in HP8h+MF1m treatment at 1.1 days. Contrary to all other parameters, the fastest seedling emergence was achieved in the untreated lettuce seeds with 3.2 days (Fig. 5). Garcia et al. (2001) point out that, exposed lettuce seeds germinated earlier than the control seeds which were treated 1-10 mT stationary magnetic field, which could be due to increasing the water uptake into the cells. In the same way, Shine et al. (2011) also showed that, seeds treated with magnetic field of 250 and 300 mT for 30 min showed a significant increase in water uptake (54% and 90% respectively) after 40 minute of imbibition. This can be due to the small invisible perforations on the seed coat which allows water uptake faster so increase germination rate. However, despite the many studies conducted to understand the effect of magnetic field treatments on living organisms, the exact mechanism, especially in plants, remains unclear. According to the literature, several theories have been proposed including, influencing the permeability of cell membrane and ion transport, causing changes in osmotic pressure and capacity of cellular tissue and then accelerating seed water absorption process (Labes, 1996; Garcia et al., 2001; Soltani et al., 2006; Kataria et al., 2015), improving seed coat membrane integrity and reducing the cellular leakage and electrical conductivity (Vashisth and Nagarajan, 2010), increasing free Ca^{2+} concentration which possibly signals the cells to enter into an early mitotic cycle, enhancing enzymes activity in seed (Shine et al., 2011).

This study has shown that, sole hydro-priming and magnetopriming combinations with hydro-priming led to a considerable enhancement in germination and seedling emergence rate of both onion and lettuce seeds (Fig. 2 and Fig. 3). Also ensured increased speed in the germination and seedling emergence days, except for lettuce seedling emergence time (Fig. 4 and Fig. 5). Magnetopriming presumably stimulates the seeds and triggers the germination and seedling emergence processes. However, the effect of the treatment still needs to be confirmed with a larger number of seed lots and in the field environment.

CONCLUSION

Increasing the seed quality with pre-sowing treatments is considered an important step for plant production. Pre-sowing treatment of seeds with magnetic field has been proven to have beneficial effects on many crops. Our results suggest that magnetopriming of pre-hydrated onion and lettuce seeds could improve seed quality parameters compared to untreated seeds. Magnetopriming can also be used on dry seeds that have not been moistened, this provides more economical and faster results by bypassing the moistening and drying stages applied in conventional priming methods.

Determining the effectiveness of MF treatments in different species that have not been studied before will produce important results for seed science and technology. Since the exposure time and dose may vary according to the species and even varieties, the determination of the appropriate dose and time is crucial for magnetopriming.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

All authors declare that they have no conflicts of interest

Author contribution

Mustafa Emre Sarı (M.E.S), Ibrahim Demir (I.D) and Nurcan Memiş carried out experimental part of the study. M.E.S., I.D. and Kutay Coşkun Yıldırım (K.C.Y) reviewed the manuscript, K.C.Y done statistical analysis, M.E.S and I.D. designed the experiments and M.E.S conceived the principal idea and wrote the paper.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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