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Improvement of seed germination of caper (*Capparis spinosa* L.) through magnetic fields

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Abstract

Caper is a sub-shrub mainly used for its flower buds, essentially in food, but also in the pharmacological industry. Low seed germination of caper due to dormancy results in low production of this crop. The exposure of seeds to stationary magnetic fields can improve germination and early seedling growth of horticultural crops. This experiment was designed to evaluate the effect of magnetic field with different intensities (0, 125 and 250 mT) and exposure times (1 h, 24 h and chronic exposure) on imbibition, germination and radicle growth of caper seed. Standard germination test was conducted and the growth of the seedlings was periodically determined using image analysis software. The exposure of caper seeds to magnetic fields increased imbibition, reaching values of seed moisture (39.5%) higher than the control (31%). Among different treatments, the highest final germination was obtained with both chronic exposure to 125 mT (82%) and 24 h exposure to magnetic fields of 250 mT (75%), while the germination of untreated seed was 57%. The positive effect of magnetic field exposure on seed germination only occurred with the addition of gibberellic acid to the substrate. As for radicle growth, the longest radicle was obtained by exposing the seeds 24 h to magnetic fields of 250 mT and with the chronic exposure of the seeds to both magnetic fields.

Keywords: Physical and physiological dormancy, imbibition, seedling growth, caper seed

INTRODUCTION

The caper plant (*Capparis spinosa* L.) is a perennial deciduous sub-shrub, woody at maturity, up to 80 cm high, with branches to 3 m long, and with deep roots. It is present in almost all the circum-Mediterranean countries. There are archaeological evidences of its consumption from 18,000 years ago in Ancient Egypt (Sozzi, 2001). It is mainly known for their floral buds, capers, however their fruits, caper berries and to a lesser extent vegetative shoots are also consumed. These products are usually consumed in pickles. Most parts of the plant have also been used in pharmacology. Furthermore, flowers provide a high ornamental value, thus, it is currently included in gardening, particularly in xeriscape.

Global demand for these products has increased, but poor field emergence of caper seeds greatly restricts the expansion of this crop. Main cause of this low emergence is dormancy (Baskin and Baskin, 2014), that may be physical and physiological types. Several studies have been carried out to improve its germination (e.g., Orphanos, 1983; Sozzi and Chiesa, 1995; Pascual et al., 2003, 2004, 2009; Pascual-Seva et al., 2008; Juan, 2017).

The biological effect of magnetic fields on plants has been explored in many studies, analysing seed germination, crop growth, photosynthetic activity, and biochemical changes under different magnetic conditions (García Reina et al., 2001; Martínez et al., 2002, 2009; Soltani, 2006 a,b; Afzal et al., 2012; Flórez et al., 2012). Therefore, one of the possible methods to improve caper seed germination is exposing the seeds to magnetic field, which has not been deeply analysed in this species. The main objective of the herein presented study is to analyse the effect of the magnetic field with varying exposure times, on water uptake, germination and seedling growth of caper seed.

MATERIALS AND METHODS

Two experiments were conducted at the *Universitat Politècnica de València* (Valencia, Spain) in order to evaluate the effect of magnetic fields on imbibition, germination capacity and radicle growth of caper seed. Fifty ripe fruits were collected from five adult plants of *Capparis spinosa* L. subsp. *rupestris* before dehiscence during the second week of September 2016. Seeds were extracted, rinsed in tap water and dried at room temperature for two days. Mature dark-brown seeds were selected, rejecting light seeds, which floated in tap water, as well as seeds measuring less than 2 mm in length. Selected seeds were stored in closed plastic cans at 7 ± 0.5°C until May 30th, 2017, when the tests started.

Magnetic fields were generated by ring magnets with magnetic induction values of 150 mT (B1) 250 mT (B2); the geometric characteristics are 35, 15 and 8 mm in external, internal diameter and high, respectively for B1, and 60, 23 and 12.5 mm for B2. The values of the magnetic field induction were provided by the manufacturers and correspond to the magnetic field strength at the core of the magnet.

Water uptake

Imbibition test was performed collocating 50 seeds in a volume of 25 ml of high-purity water obtained in a water purification system (Wasserlab Ecomatic Type II. Analytical Grade Water) between two Whatman no.1 filter papers in 9-cm Petri dishes, following the method presented by García Reina et al. (2001). These dishes were placed above the ring magnets with the respective magnetic field inductions (B1, B2) for 24 h at room temperature. Untreated seeds were taken as control. After magnetic field treatments, imbibition test was maintained for another 8 days, when the maximum water imbibition was reached (Fernández, 2016). During this period, seed moisture content (ISTA, 2016) and water uptake (imbibition) were determined on daily basis. For this purpose, seeds were taken out from the Petri dish every day, blotted with a paper towel, immediately weighed and returned to the Petri dish (Orozco-Segovia et al., 2007). Seed dry mass was determined at 103 °C for 24 h (ISTA, 2016) in a forced-air oven (Selecta 297; Barcelona, Spain). Seed moisture content was calculated on a dry mass basis [(fresh mass – dry mass)/dry mass]*100 (ISTA, 2016), and water uptake as [(final mass – initial mass)/initial mass]*100 (Orozco-Segovia et al., 2007).

Seed germination

Germination tests were performed in Petri dishes, as the ones used in the water uptake experiment, between two layers of Whatman No.1 filter paper (ISTA, 2016) moistened with distilled water, or 500 mg L^{-1} of gibberellic acid (GA) solution to saturate the substrate, to which 2 g L^{-1} thiram (THIRAM-80) was added to prevent fungal development. Treatments comprised of 200 seeds (four replications of 50 seeds each). Treated and untreated seeds were placed in a growth chamber (Climatronic; Barcelona, Spain) under alternating temperature and light: 12 h at 20 ± 1°C in the dark and 12 h at 30 ± 1°C under a photosynthetic photon flux density of 324 $\mu mol\ m^{-2}\ s^{-1}$ for a maximum of 60 days. Seeds were considered germinated when the primary root protruded from the seed coat (ISTA, 2016). Magnetic treatments were established in terms of magnetic induction (B1 and B2) and the time of exposure (0, 1, 24 h and chronic exposure (∞)).

Germination data of each replicate were fitted to the logistic function $G = A\ [1 + \exp^{(\beta-kt)}]^{-1}$ (Torres and Frutos, 1989; Pascual et al., 2003) defined as a special case of the Richards' function (Causton and Venus, 1981), where G = cumulative germination (%); t =germination time (days); A = final germination percentage; β and k are function parameters. Derived quantities with biological significance were calculated, as were the time in days to reach 50% of final germination percentage ($Gt_{50} = \beta/k$) and the mean relative cumulative germination rate (k/2, days^{-1}).

Radicle growth

Once the seed was germinated, radicle growth was measured on daily basis up to 7 days after the radicle protruded from the seed coat, using Digimizer V. 5.3.3 (MedCalc Software, 2018).

Data analysis

Results were analysed by analysis of variance using Statgraphics Centurion XVII (Statistical Graphics Corporation, 2014). Percentage data were arcsin transformed before analysis. A probability of ≤ 5% was considered significant. Mean separations were performed, when appropriate, using the least significant difference (LSD) at $P \leq 0.05$.

RESULTS AND DISCUSSION

Water uptake

Treated seeds reached greater water uptakes and moisture contents as compared to untreated seeds (Figure 1). Magnetic field induction increased ($P \leq 0.01$) both water uptake and moisture content in relation to the untreated seeds (2nd and 9th day; Table 1). Both water uptake and moisture content of control seeds were similar to that obtained in previous studies (Juan, 2017). On the other hand, moisture content of seeds subjected to the magnetic fields was similar to that obtained in scarified seeds (39%; Juan, 2017), which seems to indicate that the permeability of seed coat (including the hilum) was increased by magnetic field.

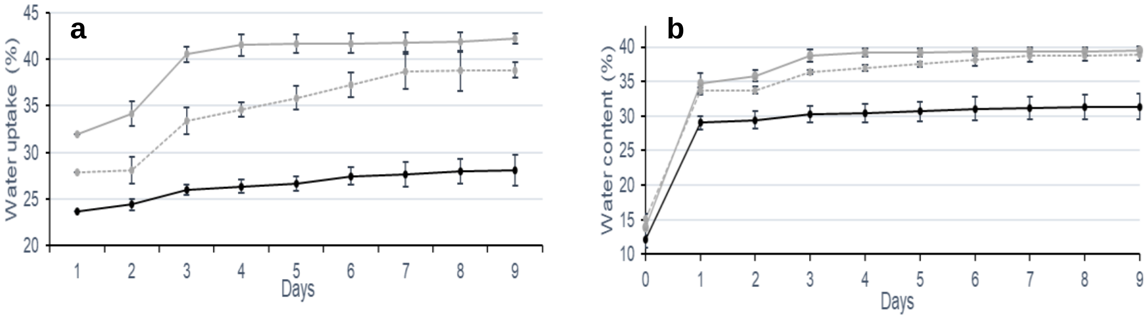

Figure 1. Water uptake (a; %) and water content (b; %) of the seeds subjected to magnetic field inductions (--125 mT; — 250 mT; — control) for 24 hours and soaked in distilled water up to 9 days. Average values for 4 replicates. Vertical bars represent the standard error.

Table 1. Effect of the magnetic field induction (24 hours) on the water uptake and the moisture content of seeds soaked in distilled water at 2 and 9 days.

	Water uptake (%)		Moisture content (%)	
	2d	9d	2d	9d
Magnetic induction (MI)				
0	24.41 c	28.13 b	29.40 b	31.38 b
150 mT	28.12 b	38.85 a	33.72 a	38.82 a
250 mT	34.16 a	42.21 a	35.84 a	39.48 a
Analysis of variance				
Factors (degrees of freedom)	% Sum of squares			
MI (2)	84.17**	80.03**	73.30**	61.89**
Residual (9)	15.82	19.95	26.69	38.11
Standard deviation	2.01	3.46	1.86	2.46

Mean values followed by different lower-case letters in each column indicate significant differences at $P \leq 0.05$ using the LSD test. ** Indicates significant differences at $P \leq 0.01$.

This increase in water uptake is in agreement with the findings of Soltani et al. (2006 a) in seeds of *Asparagus officinalis* L., in which the magnetic field increased water uptake and germination of seeds. García Reina et al. (2001) also reported an increase in the total mass of absorbed water with magnetic treatments, which were related with variations in the ionic currents across the cellular membrane induced by magnetic fields. The fields originate changes in the ionic concentration and therefore in the osmotic pressure, regulating the water entrance into the seeds.

Seed germination

The germination obtained in the seeds placed in a substrate moistened with distilled water was practically very less (on average 2.5%), both in the seeds subjected or not to the magnetic field. The data did not adjust to the logistic model, and was therefore not included in the analysis of variance.

The coefficients of determination (R^2) for the 28 curves corresponding to the substrate moistened with GA [4 replicates from 2 magnetic fields strengths (B1 and B2) and 4 exposing times (0, 1, 24 h and ∞)] were higher than 0.85, and the model was statistically significant ($P \leq 0.01$; data not included). Thus, it can be stated that the logistic function is adequate to analyze the caper seed germination in this experiment, allowing the utilization of the variable A as well as other variables such as β and k, as it was in previous studies on the germination of caper seeds (Pascual et al., 2009; Juan, 2017).

Figure 2 (a and b) show the fitted curves corresponding to the average cumulative germination of the two factors (magnetic field induction and exposure time).

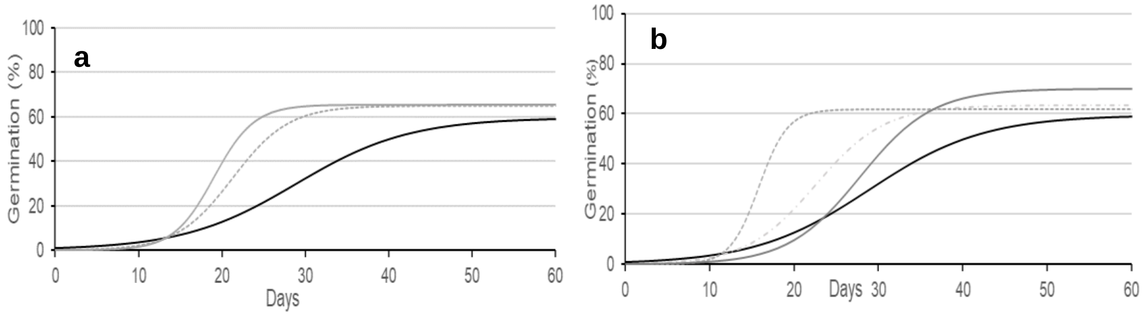

Figure 2. Logistic model adjusted to the cumulative germination obtained with caper seeds subjected to different magnetic field inductions (a: --125 mT; — 250 mT; — control) exposure times (b: — 0 h; - · - · 1h; ------ 24h; ——— ∞). Average values.

Seeds subjected to the magnetic field germinated earlier than control seeds [Figure 2; ($P \leq 0.01$) Table 2], and showed a slightly higher, but not significant ($P \leq 0.05$) cumulative germination. There were no significant differences ($P \leq 0.05$) between average values obtained with two magnetic field strengths, but cumulative germination (G), maximum germination percentage (A) and time taken to 50% germination (Gt50) were significantly ($P \leq 0.01$) affected by the magnetic field induction and exposure time interaction. Maximum A (80%) corresponded to the chronic exposure of seed to the B1, differing ($P \leq 0.05$) from all the other combinations, except from exposing the seeds during 24 h to B2 (72%), which in turn did not differ ($P \leq 0.05$) from the other exposure times to this magnetic regime (Figure 3a). Regarding Gt_{50}, the lowest value ($P \leq 0.05$) resulted from exposure of seeds to24 h at any of the two magnetic field inductions (Figure 3b). In relation to the mean relative cumulative germination rate, it is remarkable that the 24 h exposing time increased its value ($P \leq 0.01$) in relation to the other durations.

Figure 4 shows the logistic model adjusted for the different combinations of magnetic inductions and exposing times. The maximum germination was obtained with chronic exposure to B1, not differing from the 24 h exposure to B2, which furthermore considerably shortened the germination process. Similar results were obtained by Flórez et al. (2007) in maize seeds.

Table 2. Effect of the magnetic field induction and the exposure time on the cumulative germination (G), maximum germination percentage (A), number of days necessary to reach 50% final germination (Gt_{50}; days) and mean relative cumulative germination rate (k/2; days^{-1}).

		G (%)	A (%)	Gt_{50} (d)	k/2 (d^{-1})
Magnetic induction (MI)					
	150 mT	65.83	64.77	22.84	0.14
	250 mT	66.00	65.41	21.80	0.20
Exposure time (ET)					
	Control	57.00	59.43	29.01 a	0.07 b
	1 h	63.25	63.39	23.22 c	0.12 b
	24 h	64.00	61.98	16.64 d	0.29 a
	∞	70.50	69.91	27.11 b	0.11 b
Analysis of variance					
Factors (degrees of freedom)		% Sum of squares			
MI (1)		0.02 ns	0.06 ns	0.51 ns	2.24 ns
ET (3)		14.98 ns	11.31 ns	76.70 **	34.18 **
MI x ET (3)		41.59 **	38.61 **	14.52 **	6.07 ns
Residual (24)		43.41	50.02	8.27	57.48
Standard deviation		9.39	9.39	1.79	0.12

Mean values followed by different lower-case letters in each column indicate significant differences at $P \leq 0.05$ using the LSD test. ns: Indicates no significant difference. ** Indicates significant differences at $P \leq 0.01$.

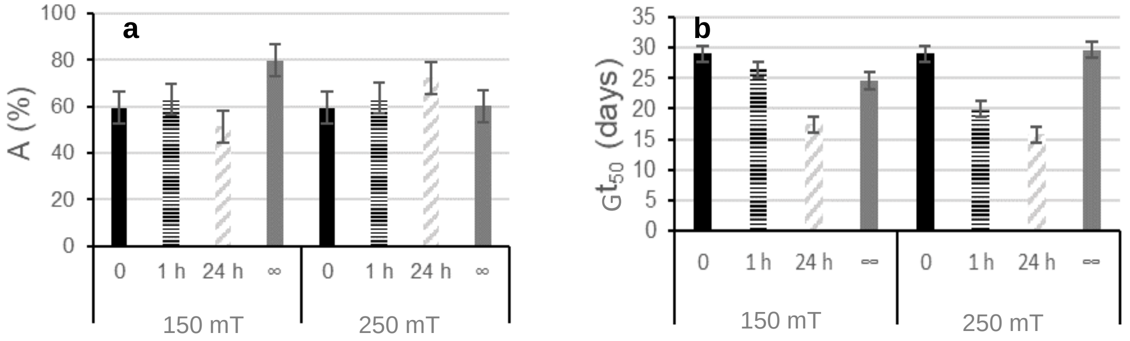

Figure 3. Magnetic induction and exposure time interaction for maximum germination percentage (a; A) and number of days necessary to reach 50% final germination (b; Gt_{50}). Average values of 4 replications. Vertical bars represent the standard error.

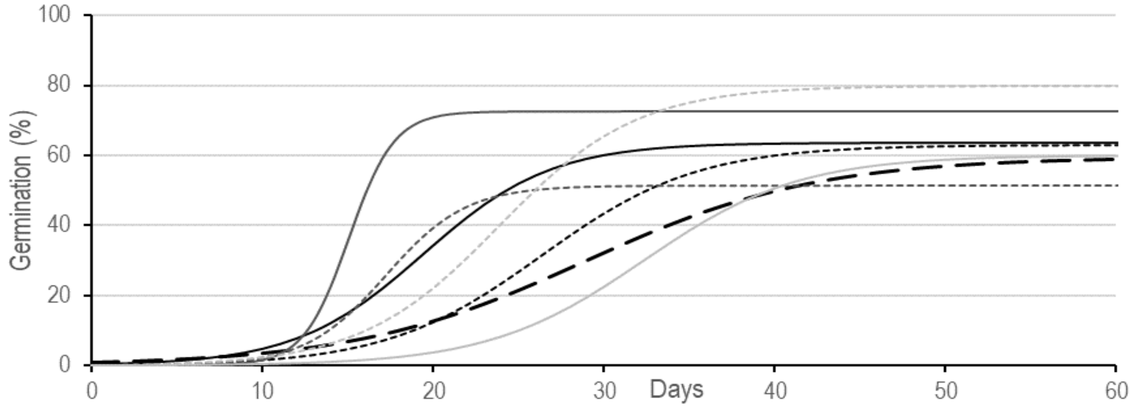

Figure 4. Logistic model adjusted to the cumulative germination obtained with caper seeds subjected to different combinations of magnetic field inductions and exposure times. (— — control; ------- 1h 150 mT; ——— 1h 250 mT; ------- 24 h 150 mT; ——— 24 h 250 mT; ------- ∞ 150 mT; ——— ∞ 250 mT).

Germination values obtained for control seeds (57%) can be considered as acceptable, and it is in line with seeds that are six months old with GA application (Juan, 2017). Therefore, the germination percentages obtained with the magnetic field application should be considered as a considerable improvement.

As mentioned in the water uptake experiment, García Reina et al. (2001) stated that the magnetic field alters the water relations in seeds, and this effect might be the reason for improvement in germination of magnetically exposed seeds. Similar response has been observed in seeds of various species, such as asparagus, calendula, corn, sage, tomato, and French marigold (Soltani et al., 2006 a, b; Flórez et al., 2007, 2012; Martínez et al., 2009; Afzal et al.,2012).

Seedling growth

Significant differences (P≤0.05) were observed between seedlings subjected to a magnetic field and control seeds (Table 3). The magnetic field induction and exposure time interaction resulted in significant (P≤0.01; Figure 5) differences. The maximum radicle growth was obtained with the chronic exposure of the seedling to B1, although it did not differ significantly (P≤0.05) from the chronic or 24 hours exposure to B2.

The greater length obtained with chronic exposure coincides with the results of seeds exposed to magnetic field inductions in corn (Flórez et al., 2007; 125 and 250 mT), wheat (Martínez et al., 2002; 6217 and 24868 J m^{-3}), and French marigold (Afzal et al., 2012; from 25 to 125 mT). Magnetic field induction at lower values also increased the radicles of lettuce (Soltani and Kashi, 2004; 2.5 T), *Ocimum basilicum* (Soltani et al., 2006 b; 3 mT) and *Asparagus officinalis* seedlings (Soltani et al., 2006 a; 7mT). The effect of high-gradient magnetic fields in cells can be related to an interference with the cytoplasmic ion currents or ion distribution and with the induction of intracellular magnetophoretic displacement of starch-containing plastids (Kuznetsov et al., 1999; Soltaniet al., 2006 a).

On the other hand, Belyavskaya (2004) pointed out that the growth of the primary roots of seedlings of different species exposed to weak magnetic fields (intensities from 100 nT to 0.5 mT), is inhibited during early germination stages in comparison with the control, due to the proliferative activity and cell reproduction in meristem of plant roots. Even tough a strong inhibition of initial root growth in wheat, pea and sugar beet seedlings occured during the first four days of exposure to a weak magnetic field, this inhibition was later partially compensated with a greater root elongation (Sitnik et al., 1984; Belyavskaya, 2004).

Table 3. Effect of magnetic field induction and exposure time in seedlings radicle length

		Radicle length (cm)
Magnetic induction (MI)		
	150 mT	1,25 b
	250 mT	1,43 a
Exposure time (ET)		
	Control	0,52 c
	1 h	1,22 b
	24 h	1,17 b
	∞	1,63 a

Analysis of Variance

Factors (degrees of freedom)	% Sum of the squares
MI(1)	2.17**
ET(3)	72.83**
MI x ET (3)	21.80**
Error (24)	3.18
Standard deviation	0.095

Mean values followed by different lower-case letters in each column indicate significant differences at P ≤ 0.05 using the LSD test. ** Indicates significant differences at P ≤ 0.01.

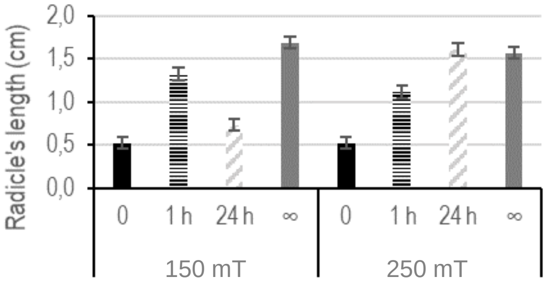

Figure 5. Magnetic induction and exposure time interaction for radicle length. Average values of 4 replications. Vertical bars represent the standard error.

CONCLUSION

Exposing caper seeds to stationary magnetic fields, improves their germination, increases germinaticon percentage and average germination rate, and reduces the time to reach 50% germination. These advantages can improve the establishment of the crop. Exposing the seeds to 250 mT for 24 h or chronic exposition to 150 mT led to maximum improvement, but 24 hours exposure to 250 mT also reduced the germination period.

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