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EFFECTS OF POLYETHYLENE GLYCOL AND SODIUM CHLORIDE STRESS ON WATER ABSORPTION OF MAGNETO-PRIMED TRITICALE SEEDS

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Abstract. The effect of drought and salt stress was studied by generating the same water potential (Ψ) with polyethylene glycol (PEG) and sodium chloride (NaCl). In addition, as the magneto-priming was demonstrated to be a non-invasive method to improve germination, the study was performed by comparing water absorption of magnetically treated and non-treated seeds of triticale (*X Triticosecale Wittmack*) under stress conditions.

Seeds water uptake was inversely proportional to the PEG and NaCl concentration, although the absorption was greater for magnetically treated seeds in comparison to the untreated ones (increments up to 11%). Seeds magnetically treated absorbed more water under the influence of NaCl than PEG, especially with low Ψ levels. The adverse effect of salinity and drought on water absorption can be circumvented by exposing seeds to magnetic treatment with a stationary magnetic field induction of 3.71 mT (milliTeslas) during 10 h.

Key words: drought, salinity, magnetic treatment

1. INTRODUCTION

Germination process requires a threshold level of hydration to reactivate metabolic processes and, later on, the radicle emergence of seeds [1–3]. On the field, a limited water availability for seeds germination due to drought can be defined as decrease in the water potential [4]; as the Ψ is the free energy of water divided by the partial molar volume, its units are pressure units. When decreasing water free energy, it is more difficult for plants to take up water [5].

An important abiotic factor affecting seeds water absorption is the water stress caused by the drought and salinity, both reducing the water potential in a similar way. PEG and NaCl are often used to impose low Ψ. PEG of molecular weight higher than 6000 is the best and most used choice for imposing low Ψ since it cannot enter cells' pore [6]. NaCl is always used to study salinity effect and osmotic potential on germination studies since it facilitates the intake of ions and changes enzymatic activity of germinating seeds [7]. Considering that both compounds were reported as abiotic stressors and water absorption inhibitors, different water potentials can be obtained with different solutions of PEG6000 (as a drought stress inducer) and NaCl (as a salinity stress inducer) to study germination under water stress conditions [8].

Furthermore, seed priming was also demonstrated to improve germination parameters due to a greater water absorption, which is essential for radicle protrusion. It has been verified for many crops, especially vegetables and small seeded grasses [9]. Seeds priming had outstanding results for many seeds including the legume's ones [10].

Seed priming techniques (hydro-priming, halo-priming, osmo-priming, thermo-priming, bio-priming) were widely used to improve germination parameters [11]. Physical methods (magnetic field, ultrasounds, ionizing radiations, microwave) were also used, offering advantages over traditional treatments as fertilizers. Among these physical techniques, the advantage of using magnetic fields as pre-sowing seeds treatment is based on the low economic impact, a non-invasive and eco-friendly approach [12–13].

Changes related to seed germination and early growth of plants when exposed to magnetic fields were reported, one of the reasons being probably a greater water uptake by seeds. Other theories were proposed as well to explain these effects [14–18]. In an attempt to analyze the results obtained, many studies were conducted to find the real action mechanism of magnetic fields on germinating seeds [19–21]. The majority view is that magnetic field changes cell membrane permeability, mineral uptake and ion transportation [21].

The main objective of this study was to compare the effect on the water uptake of drought and salt stress induced on the first stage of germination. As seed germination were widely reported as influenced by magnetic treatment, a case study of water absorption of magneto-primed seeds under drought and salt stress was conducted.

2. MATERIAL AND METHODS

In two parallel experiments the effect of salinity and drought stresses caused by different potential levels of PEG6000 and NaCl on water uptake were investigated; the same water potential values were generated by PEG6000 (experiment 1) and NaCl (experiment 2), and in both cases water absorption was studied for control and magnetically primed seeds. In this paper the word control refers always to seeds not magnetically treated.

2.1. SEEDS

Spanish Office of Vegetable Varieties provided the certified seeds and guaranteed their homogeneity.

2.2. STATIC MAGNETIC FIELD GENERATION AND MAGNETIC PRIMING

A pair of Helmholtz coils separated 15 cm, switched in parallel and connected to a power supply provided a homogeneous magnetic field. Total number of turns per coils was 124 and their radius 15 cm. According to manufacturers (Phywe), the magnetic field generated by superposition of these two fields can be considered largely uniform between the two Helmholtz coils, and depends on the circulating current I as follows

$$B = \left(\frac{4}{5}\right)^{\frac{3}{2}} \mu_0 I \frac{n}{R}, \quad (1)$$

where $\mu_0 = 4\pi\cdot 10^{-7}$ N/A^2 is the magnetic field constant or also called the permeability constant. By selecting the maximum value of current provided by the power supplier, $I = 5$A, the magnetic field obtained is 3.71 mT. Seeds were placed in a cardboard box on the axes of the pair of coils during 10 h, while control seeds were kept in between another pair of Helmholtz coils for the same time period but with a current $I = 0$.

2.3. WATER STRESS GENERATION

For the water potential assays, five solutions with of 0.0, –0.33, –0.66, –0.99 and –1.32 MPa (megapascal) were arranged according toMichel and Kaufmann [22] with PEG and NaCl concentrations. It is important to note that when we speak about water potential, more negative values mean stronger potentials than that of the pure water, or in other words, the lower the Ψ the higher the concentration of solute.

Seeds exposed to drought (water) stress were moistened with each solution of an osmotic potential [23]. Concentrations of the well-known osmotic agent PEG6000, simulating drought stress, do not affect plant metabolism whilst it reduces the seed's water absorption [24].

Table 1 shows labels of Petri dishes according to magnetic/ water potential treatments applied. Petri dishes were labeled H for seeds soaked with PEG solutions-without magnetic treatment-and BH for magneto-primed seeds with PEG solutions. Petri dishes were labeled h for seeds soaked with NaCl solutions-without magnetic treatment-and Bh for magneto-primed seeds soaked with NaCl solutions.

Table 1

Petri dish labels according to treatments in each experiment

Experiment	Ψ (Mpa)	0	–0.33	–0.66	–0.99	–1.32
Experiment 1 PEG6000	$B = 0$	H0	H1	H2	H3	H4
	$B = 3.71$mT	BH0	BH1	BH2	BH3	BH4
Experiment 2 NaCl	$B = 0$	h0	h1	h2	h3	h4
	$B = 3.71$mT	Bh0	Bh1	Bh2	Bh3	Bh4

2.4. WATER ABSORPTION TESTS

The initial weight W_i of 25 seeds for each treatment was determined before soaking, they were immediately placed on Petri dishes containing 25 ml of the corresponding PEG and NaCl solution to each Ψ. Afterwards, they were put in the MMM FRIOCELL chamber to ensure the temperature uniformity during the soaking process. The optimum temperature for Triticale seeds germination is 21°C [25] and the temperature chosen for the study 15°C. The authors wanted to carry out the experiment far from the optimal temperature in order to check if water absorption was enhanced by the magneto-priming in drought stress conditions.

At intervals of 30, 60, 120 and 300 minutes after immersion, Petri dishes were removed from incubator, seeds were removed from Petri dishes, blotted dry and forth with weighted on a precision electronic scale and returned to their Petri dishes to continue imbibition.

The water absorption percentage due to imbibition, W_a, was calculated according to McWatters formula [26], as weight change expressed as the amount of water absorbed per unit seed dry weight

$$W_a = \frac{W_f - W_i}{W_i} \cdot 100, \qquad (2)$$

being W_i the initial weight of seeds before imbibition and W_f – the weight after having removed from Petri dishes and dried.

Water absorption charts for all treatments *vs* imbibition time were plotted.

3. RESULTS AND DISCUSSION

Water absorption of seeds under different Ψ conditions was measured during 5 h, the time before seeds germination process started.

3.1. EXPERIMENT 1. WATER ABSORPTION OF SEEDS WITH PEG SOLUTION

Water absorption course of seeds in a PEG solution, expressed as %, *vs* imbibition time are shown in Figs. 1 and 2, observing high values of R^2 by using a logarithmic adjustment.

Figure 1 shows that non-exposed to PEG solutions seeds (H0) absorbed more water than those under any water stress condition. The lower the Ψ the smaller the water absorption.

For all PEG concentrations, magneto-primed seeds (Fig. 2) absorbed more water than untreated ones (Fig. 1). Once again, by increasing the concentrations of this osmotic agent seeds water absorption was reduced. The greatest water absorption was reached by seeds labeled BH0. Through all the hydration course is clear to see that among all treatments BH0 provided the highest water absorption.

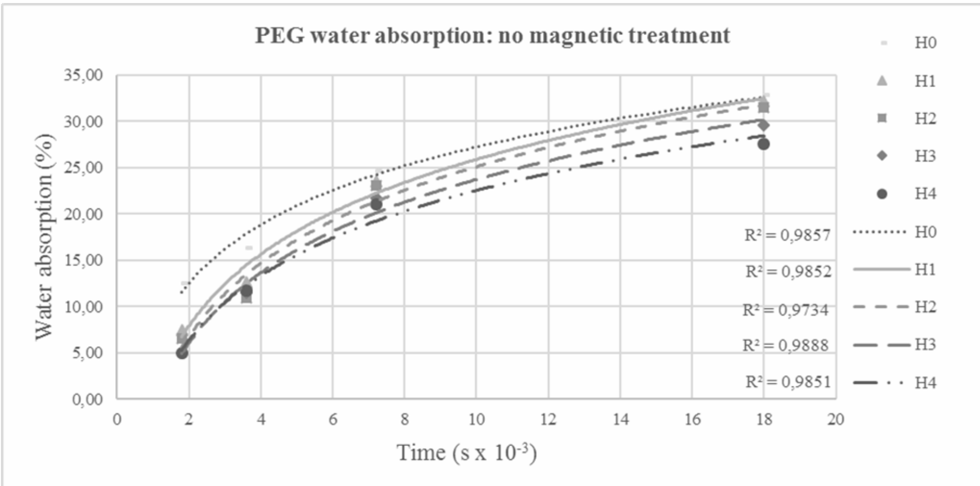

Fig. 1 – Water absorption *vs* imbibition time for control seeds, in PEG solutions.

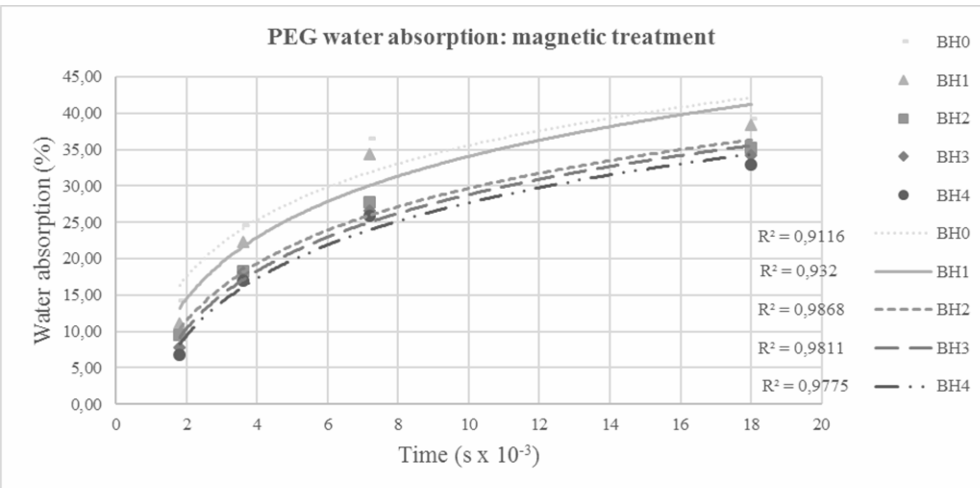

Fig. 2 – Water absorption *vs* imbibition time for magneto-primed seeds, in PEG solutions.

3.2. EXPERIMENT 2. WATER ABSORPTION OF SEEDS WITH NaCl SOLUTION

Water absorption course of seeds with NaCl solution, expressed as %, *vs* imbibition time are shown in Figs. 3 and 4, observing R^2 values higher than 0.89 by using a logarithmic adjustment. As noticed in PEG solutions, for all NaCl concentrations, magneto-primed seeds absorbed more water than untreated ones and, by increasing the concentrations of this osmotic agent seeds water absorption was reduced. The greatest water absorption was reached by seeds labeled Bh0, magneto-primed seeds with NaCl.

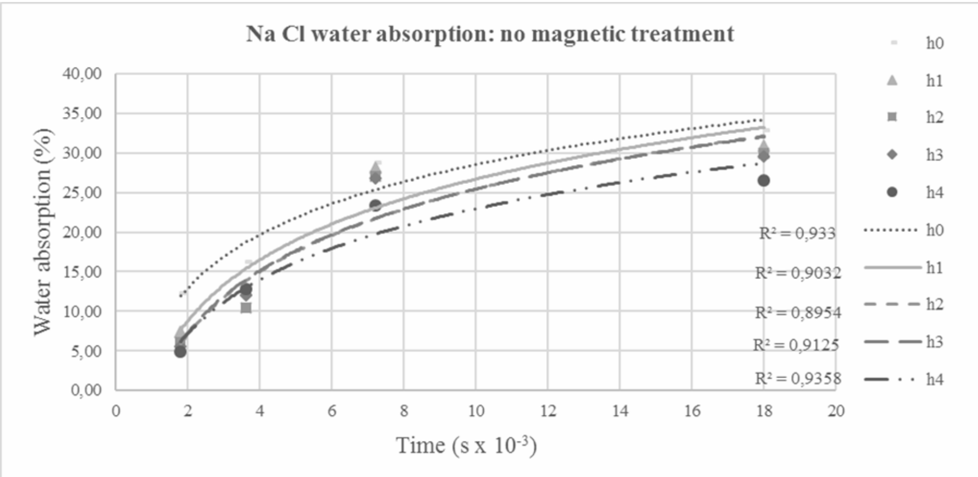

Fig. 3 – Water absorption *vs* imbibition time for control seeds, in NaCl solutions.

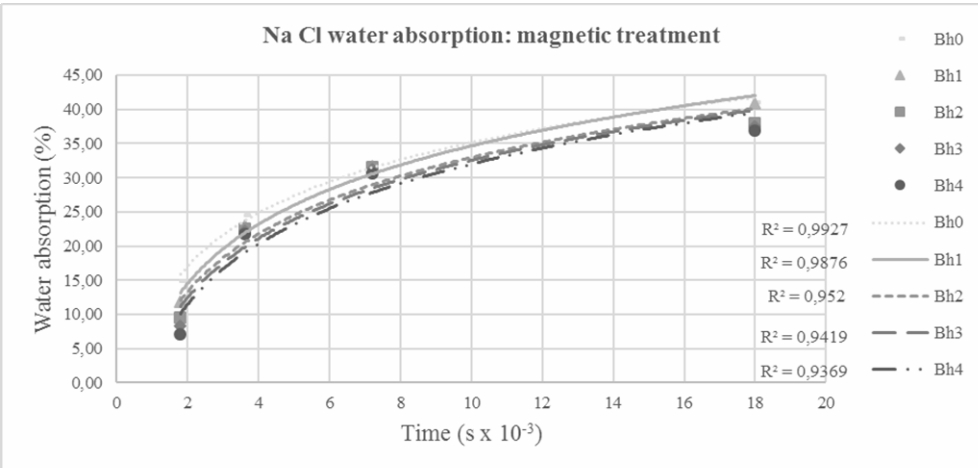

Fig. 4 – Water absorption *vs* imbibition time for magneto-primed seeds, in NaCl solutions.

By jointly analyzing both experiments, there were no large differences in water absorption during soaking process, when contrasting PEG (Fig. 1) and NaCl (Fig. 3) under no magnetic treatment. For instance, after 5 h, the water absorption of seeds labeled H0 *vs* h0 were similar and the same behavior was observed by comparing H1 *vs* h1, or H3 *vs* h3.

However, when comparing water absorption after 5 h of magneto-primed seeds, those under NaCl influence absorbed more water specially under low Ψ, or in simpler words, the magneto-priming was more effective when seeds were imbibed with a NaCl solution than with one of PEG, as shown in Table 2.

Table 2

Average weight increase (%) after 5 hours of imbibition

Control seeds	PEG	NaCl	Magneto-primed seeds	PEG	NaCl
Ψ = 0.0 MPa	32.85	32.88	Ψ = 0.0 MPa	39.2	41.04
–0.33	32.24	30.95	–0.33	38.42	40.93
–0.66	31.51	29.93	–0.66	35.22	37.94
–0.99	29.55	29.61	–0.99	34.24	37.45
–1.32	27.54	26.6	–1.32	32.93	36.96

To go further, authors conducted a two factor ANOVA with replication analysis in order to elucidate if there were significant differences for $p < 0.05$ level between the means of the following groups of seeds: control seeds imbibed with PEG *vs* NaCl (treatments H0–H4 *vs* h0–h4), magneto-primed seeds with PEG *vs* with NaCl (BH0–BH4 *vs* Bh0–Bh4), magneto-primed seeds with PEG *vs* control PEG seeds (BH0–BH4 *vs* H0–H4) and magneto-primed seeds with NaCl *vs* control NaCl seeds (Bh0–Bh4 *vs* h0–h4). For ANOVA analysis only full data after 5 hours of imbibition were considered. As in many statistical hypothesis tests, two hypotheses were stated: H0 (null hypothesis) and Ha (alternative hypothesis). In the experiments, H0 meant that no statistically significant difference existed between the means of both treatments considered in each case. On the contrary, Ha meant that those means were different.

The results are summarized in Table 3. Excepting the pair, H0–H4 *vs* h0–h4, for all other combinations the hypothesis Ha was accepted which indicated that population means were not equal and thus there were significant differences between the mentioned groups of treatments.

Table 3

Two Factor ANOVA Analysis with Replication

	Control: PEG *vs* NaCl	Magneto-primed: PEG *vs* NaCl	PEG: treated *vs* Control	NaCl: treated *vs* Control
F value	0.71	6.67	24.1	72.6
p-value	0.41	1.38E-02	1.77E-05	2.40E-10
F critical value	4.10	4.10	4.10	4.10

As noted above, despite the isosmotic concentrations of PEG and NaCl, absorption is more negatively affected by PEG, which is consistent with the results obtained in [27]. This absorption decline appears to be the result of a greater ion uptake and osmotic adjustment in seeds under the influence of NaCl.

In low water stress case, both PEG and NaCl had an inhibitory effect on water absorption; this effect is observed for treated and control seeds. Our results are agreed with those given by Thomas *et al.* [28] who reported that magneto-priming circumvented the effect of salinity stress on germination in chickpea seeds; the percentage germination decreased with increasing salinity in magneto-primed and unprimed seeds, although magneto-primed had higher germination than control at all salinity levels.

Without considering the magnetic field effect, the water absorption decreased with salinity and drought. Whilst the magnetic treatment enhanced water uptake during the imbibition period [29], salinity and drought decreased the effect [16, 20, 28]. Therefore, magnetic priming acts as a compensatory of the negative effect for the abiotic stressors.

Some studies about the combined effect of stationary magnetic field [16, 30–31] with salt and drought stress reported similar results in maize [32], mungbean ping [33] or chickpea [28]. Kataria and Bagel [16] reported that static magnetic fields alleviated the adverse effects produced by salt stress in maize and soybean seedlings.

4. CONCLUSIONS

The adverse effect of salinity and drought on water absorption can be circumvented by seeds magnetoprimed with a stationary magnetic field induction of 3.71 mT during 10 h. Water uptake of seeds under stress conditions generated by PEG and NaCl was improved by implementing seeds magnetic treatment prior to imbibition.

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