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EFFECT OF PRIMING ON GERMINATION AND INITIAL GROWTH OF MELON PLANTS UNDER SALT STRESS

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ABSTRACT

The objective of this work was to evaluate the effect of different osmotic agents and priming durations on the germination and initial growth of melon in salinity. The osmotic agents were NaCl and $CaCl_2$ and durations 2 and 4 days. First a response curve of germination to salinity was made. After priming the seeds were placed to germinate in a saline medium (14 dS m^{-1}). The germination percentage was determined. The two treatments with the best results (NaCl and $CaCl_2$ 2 days in both salts) were selected to evaluate the initial growth of the plants in salinity. It was sown in pots, irrigated with nutrient solution and 60 mM NaCl. The germination was reduced to 50% with 14 dS m^{-1} and inhibited with 22 dS m^{-1}. The best germination was achieved with NaCl and $CaCl_2$ 2 days with 99 and 94% respectively. Both treatments showed an increase in growth parameters and chlorophyll content.

Key words: priming, germination, salinity, stress.

RESUMEN

Efecto del priming en la germinación y crecimiento inicial de plantas de melón en estrés salino.

El objetivo del trabajo fue evaluar el efecto de diferentes agentes osmóticos y tiempos de *priming* en la germinación y crecimiento inicial de melón en salinidad. Los agentes osmóticos fueron NaCl y $CaCl_2$ y los tiempos del tratamiento 2 y 4 días. Primeramente se confeccionó una curva de respuesta de la germinación a la salinidad. Luego del *priming* las semillas se colocaron a germinar en

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un medio salino (14 dS m^{-1}). Se determinó el porcentaje de germinación. Se seleccionaron los dos tratamientos con los mejores resultados (NaCl y $CaCl_2$ 2 días en ambas sales) para evaluar el crecimiento inicial de las plantas en salinidad. Se sembró en macetas, regándose con solución nutritiva y 60 mM de NaCl. La germinación se redujo al 50% con 14 dS m^{-1} y se inhibió a 22 dS m^{-1}. La mejor germinación se logró con NaCl y $CaCl_2$ a los 2 días con 99 y 94% respectivamente. Ambos tratamientos mostraron un incremento de los parámetros de crecimiento y clorofila.

Palabras clave: priming, germinación, salinidad, estrés.

INTRODUCTION

Soil salinity is one of the most important conditions for growing melon. Salinity can conduce to a reduction of percentage and germination rate (6; 46). In addition, in severe salt stress conditions, the seedlings can show a reduction of aerial biomass (28), inhibition of stem, leaf and root growth (5), reduction of leaf water potential and stomatal conductance (29), metabolic alterations with photosynthesis decrease (14) and reduced fruit yield and quality (32; 14). However, if development of plants is considered, germination and initial seedling growth phases have the highest sensitivity to salinity, increasing tolerance between fruit development and harvest (37; 17; 9; 45). The technique of pre-conditioning seeds (priming) can become a technological alternative to overcome limitations imposed by salinity.

Priming is a controlled seed hydration technique that allows activating the metabolic and repair processes (activation of DNA repair pathways and antioxidant mechanisms) that occur in early stages of imbibition (pre-germinative metabolism), without radicle emission (germination) (38). Priming treatments have proven their advantages and are investigated for the following purposes: a) seed invigoration during storage; b) increase, acceleration and synchronization of germination and establishment of seedlings; c) overcoming or alleviation of different types of dormancy and; d) improvement of germination and establishment of seedlings under adverse environmental conditions (25; 41).

Controlled hydration can be achieved with an osmotic agent that reduces the osmotic potential (Ψo) of water (osmopriming), a substance that retains surface water (matric priming) or the addition of a limited amount of water (hydropriming) (49). Osmopriming is the main technique used because of simplicity and satisfactory results (22).

The beneficial effects of priming are not exclusive of seed germination and can be can be extended to later stages by increasing tolerance to different biotic and abiotic stresses (11; 13; 35).

The objective of this work was to evaluate the effect of different osmotic agents and priming durations on germination and initial growth of melon plants under salinity stress.

MATERIALS AND METHODS

Seeds of Honeydew melon (*Cucumis melo* L.) were used in three experiments to determine the following effects: 1) salinity stress in germination; 2) priming on seed germination in salinity; and 3) priming in the initial growth and chlorophyll content of melon plants in salinity.

Experiment 1: Effect of salinity on germination

Seeds were placed between two filter papers (Whatman no 1), in 8.5 cm diameter Petri dishes, soaked with different NaCl concentrations (0 to 22 dS m^{-1}) in a ratio 1: 5 (weight: volume) (15). Ten seeds per dish were used, equidistant from each other, with 5 replicates per concentration. The assay was performed in a germination chamber (Semedic I-500 PF) at 25 °C (± 0.5 °C) and 1000 lux of light intensity. After 8 days germination was recorded (23).

Experiment 2: Effect of priming on germination in salinity conditions

Priming was performed in culture chamber in dark and at 25 °C (± 0.5 °C). The seeds were positioned equidistant from each other on a filter paper (Whatman no 1) in 8.5 cm diameter Petri dishes, soaked with two different osmotic solutions: NaCl and $CaCl_2$, in a ratio of 1: 5 (weight: volume). The concentration of these osmotic agents was calculated to achieve an osmotic potential (Ψo) of -1.5 MPa according to the Van't Hoff equation for Ψo (44). Two treatment durations were studied: 2 and 4 days. After priming the seeds were removed and rinsed three times in distilled water. Later the seeds were put to germinate in the same way as in the previous experiment, imbibed with the corresponding saline solution that caused a 50% reduction of germination. Control treatment consisted in sowing seeds without priming treatment. After 8 days the number of germinated seeds was recorded and germination percentage (GP) was calculated (23). Five replicates were used with 10 seeds per treatment. A completely randomized design was used. The data obtained from the experiment were analyzed using an analysis of variance (ANOVA).

Experiment 3: Effect of priming on initial growth of melon plants under salinity conditions

The two treatments of the previous experiment in which the best response in germination was recorded were chosen.

Sowing was done in 1000 cm^3 pots, previously filled with perlite. Irrigation was performed daily with the Hoagland´s nutrient solution (21). After first leaf emission, plants were divided into two groups: a) irrigated with nutritive solution with 2.0 dS m^{-1} electrical conductivity (EC) (S0); and b) irrigated with nutritive solution with the addition of 60 mM NaCl, to achieve an EC of 8.0 dS m^{-1} (S1). The EC was monitored daily with a Milwaukee conductivity meter, model WP MC66 0-10MS/C.

The experiment was performed in a phytotron, with a 16 h light/8 h darkness cycle (3000 lux light intensity) and temperatures of 25/18 °C (± 1.0 °C), considered as optimal for the growth of this specie (10; 30). Five plants were extracted from each treatment every 10 days and the following parameters were determined: total dry weight, main stem length, number of leaves, leaf area from digitalized images of leaves using the Image J © computer program, and total leaf chlorophyll by spectrophotometry (33). 50 plants per treatment were used in a completely randomized design. Data measured during the experiment were statistically analyzed using ANOVA.

RESULTS

Experiment 1

Reduction in germination was gradual with increasing EC. Values between 14 and 16 dS m^{-1} reduced germination to 50%, while it was completely inhibited at 22 dS m^{-1} (Figure 1).

Experiment 2

Priming partially reversed the inhibitory effect of salinity. However the best results were obtained, for both salts, with 2 days duration (Table 1).

It was observed that although priming enhanced germination percentage with both salts, increasing priming duration, from 2 to 4 days, decreased the favorable treatment effect.

Experiment 3

This experiment, in which the effect of salinity on melon plants was studied, was performed with the two treatments in which the best germination percentage was recorded (NaCl and $CaCl_2$, and 2 days). The effect of these treatments on growth parameters is represented in figure 2. High EC had a negative effect on total dry weight, number of leaves, leaf area and main stem length. In the measured variables, priming allowed increasing salinity tolerance, with a positive differentiation respect to control. However, in low salinity condition (S0) there were no differences between the treatments and the control, therefore the treatments with priming did not present increases in the measured variables (Figure 2).

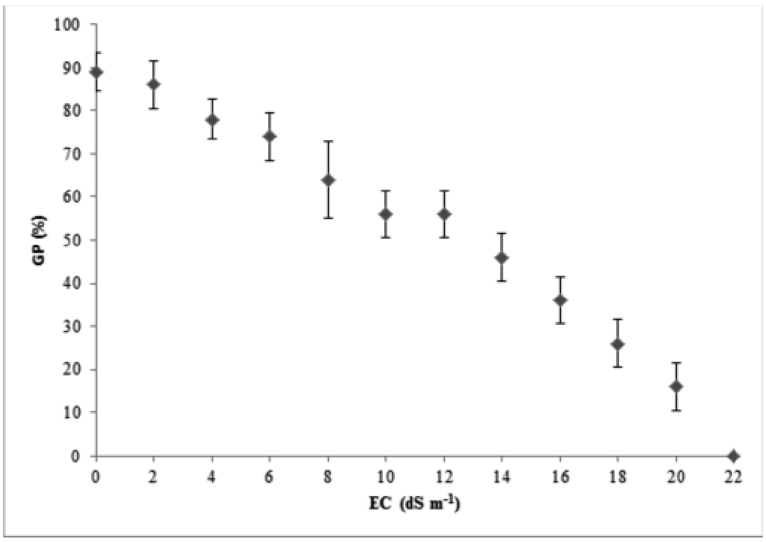

Figure 1. Variation in germination percentage (GP) with EC increase. Bars show ± SE.

Table 1. Germination percentage at 14 dS m^{-1} with different priming variations.

Priming	GP
NaCl 2.days	99.0 a
$CaCl_2$ 2.days	94.0 a
NaCl 4.days	74.4 b
$CaCl_2$ 4.days	66.2 b
Control	50.2 c

Different letters indicate a significative difference (p ≤ 0.05) according to Tukey.

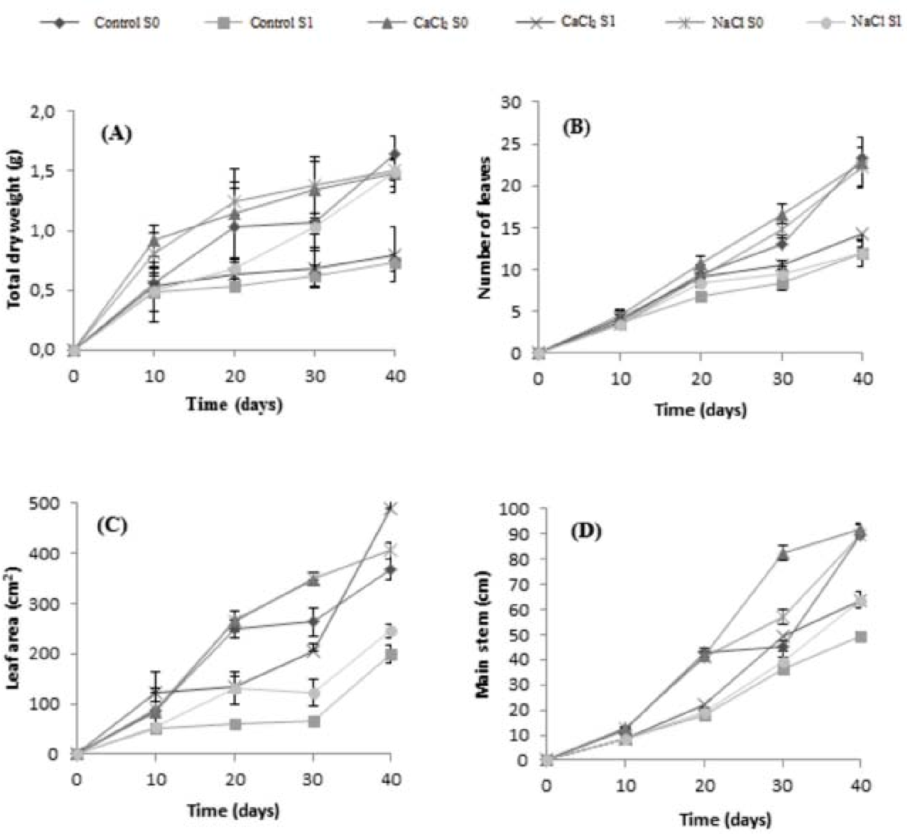

Figure 2. Variation of different growth parameters according the EC increasing for different priming variations: dry weight (A), leaves number (B), leaf area (C), and length of main stem (D). Control S0: Control treatment with EC of nutrient solution 2 dS m^{-1}; Control S1: Control treatment with EC of nutrient solution 8 dS m^{-1}; $CaCl_2$ S0: priming with $CaCl_2$ and EC of nutrient solution 2 dS m^{-1}; $CaCl_2$ S1: priming with $CaCl_2$ and EC of nutrient solution 8 dS m^{-1}; NaCl S0: priming with NaCl and EC of nutrient solution 2 dS m^{-1}; NaCl S1: priming with NaCl and EC of the nutrient solution 8 dS m^{-1}. Bars show ± SE.

The concentration of total leaf chlorophyll after 40 days of sowing showed a reduction due to salinity, although a higher concentration was measured in plants coming from seeds with priming treatment, both in low (S0) and high (S1) salinity conditions (Figure 3).

DISCUSSION

Ψo of -1.5 MPa was used because lower Ψo (< -2.0 MPa) could be harmful to seeds (7) and Ψo close to 0 MPa could allow germination during priming. The above considerations are further justified since for this species Ψo of -1.3 MPa has been determined as the most suitable for safe priming (36).

From the point of view of osmotic agents, polyethyleneglycol (PEG) has traditionally been used for priming, since it´s an inert compound and because of the relatively high molecule size it reduces the Ψo of the solution without absorption of the molecule into the seed (50). However, this compound has an extremely high viscosity which limits oxygen transfer within the solution, as well as the subsequent removal from seeds (38). For this reason, the use of inorganic salts could overcome these constraints, in addition to reducing costs (20; 31). The results obtained confirm the beneficial effect of NaCl and $CaCl_2$ as osmotic agents.

For these salts, their use has been studied for different species (38). In case of NaCl, Farhoudi *et al*. (16), working with canola (*Brassica napus* L.), observed that the po-

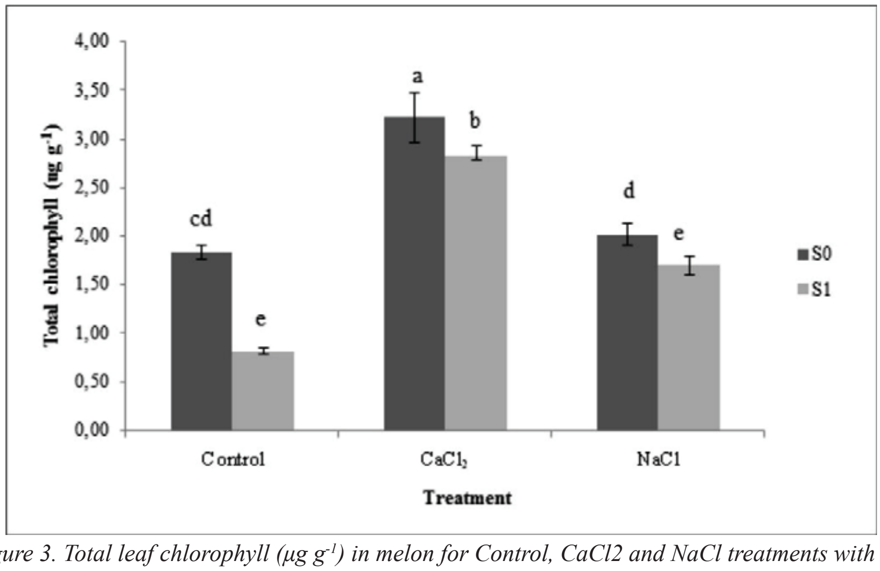

Figure 3. Total leaf chlorophyll (µg g^{-1}) in melon for Control, CaCl2 and NaCl treatments with 2 (S0) and 8 (S1) dS m^{-1}, measured 40 days after transplant. Different letters on the bars indicate a significative difference ($p \leq 0.05$) according to Tukey. Vertical bars show ± SE.

sitive response of priming to germination and initial plant growth could be associated with the activation of antioxidant systems and proline accumulation. This is consistent with that observed by Aloui *et al*. (1), who worked with pepper (*Capsicum annuum* L.). Passam and Kakourioitis (39) reported an increase in germination in such conditions, although the effects would not extend beyond this initial state. Nakaune *et al.* (34) proposed that the effect of priming with NaCl in tomato (*Solanum lycopersicum* L.) would be related to an increase of gibberellin content, a hormone linked to germination, as a consequence of the activation of genes associated with its synthesis. Also in this specie it was detected a higher Na^+ and Cl^- content in seeds and roots, which conduces to a greater capacity of osmotic adjustment (11).

In relation to $CaCl_2$, in bean (*Cajanus cajan* L.), a semi-perennial shrub legume, Jyotsna and Srivastava (26), observed that seedlings from primed seeds, when growing in saline soil, showed an increase in their protein composition and soluble sugars. In addition to the own advantages of a seed with a controlled start of its germination provided by priming, possibly the use of $CaCl_2$ in saline conditions could be associated to the influence of Ca^{2+} on the membrane integrity, as well as the antioxidant effect of several enzymes in which participates as cofactor (19; 51). In maize (*Zea mays* L.) growing in salinity it was observed that, when comparing different chlorine salts as osmotics agents, with $CaCl_2$ it was registered a higher content of Ca^{2+} in seeds, mesocotyl and radicle (4).

The lower germination measured when priming duration increased (Table 1) can be explained by the fact that an extensive partial imbibition, without the radicle emission, could cause a depletion of seminal reserves. This situation leads to a reduction in seed vigor and the consequent reduction of germination under unfavorable conditions (8; 27) as well as irregular water absorption with the consequent loss of vital electrolytes for seeds (24).

The improvement in growth parameters in plants from primed seeds (Figure 2), evidences that the advantages of priming can extend beyond the seed state, phenomenon known as "priming memory" (12), which results in a better adaptive capacity for the saline stress situation.

The measured reduction in chlorophyll concentration in plants growing in saline conditions (Figure 3) is considered as a result of oxidative stress (47) and would be associated with the presence of ions of the salts in some stages of the chlorophyll synthesis, causing the reduction of this molecule (2). However the activation of degradation mechanisms mediated by the chlorophyllase enzyme (42; 48) cannot be ruled out either. Priming allowed increasing the chlorophyll concentration (Figure 3). Some antecedents that explain this effect are related to the fact that priming allows to reverse partially the damage caused by oxidative stress (3; 40; 43), evidenced in the increase of chlorophyll concentration.

CONCLUSIONS

Priming with NaCl and $CaCl_2$ allowed increasing the germination percentage, growth´s parameters and chlorophyll contents of plants in salinity stress. The 2 days duration of treatment results more adequate for the studied conditions.

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