Seed priming induces biochemical changes in melon plants and increase salt tolerance

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ABSTRACT

An experiment was conducted to evaluate the effect of priming on germination and initial growth of melon plants under salinity conditions. Two osmotic agents (NaCl and $CaCl_2$) and two treatment durations (2 and 4 days) were compared. Germination percentage and growth of seedlings were evaluated at electrical conductivity 8.0 dS m^{-1}. Total chlorophyll content, relative water content, root viability, proline content, relative electrolyte leakage, malondialdehyde concentration, peroxidase and catalase activity, Na^+/K^+ ratio in green parts, and K^+ leakage from roots were measured. The best germination was obtained when seeds were soaked either with NaCl or $CaCl_2$ for two days. Priming improved growth parameters and plants presented a better response in all the evaluated biochemical parameters. Results suggest that priming could be used to improve the performance of seeds and seedlings in situations of high salinity.

Keywords: *Cucumis melo,* osmo priming, germination, salt stress, oxidative stress.

RESUMEN

Se realizó un experimento para evaluar el efecto del ´priming´ de semillas sobre la germinación y crecimiento inicial de plantas de melón en salinidad. Se compararon dos agentes osmóticos (NaCl y $CaCl_2$) y dos duraciones de tratamiento (2 y 4 días). Se evaluó el porcentaje de germinación y crecimiento de las plántulas a una conductividad eléctrica de 8.0 dS m^{-1}. Se midió el contenido total de clorofila, contenido relativo de agua, viabilidad de raíces, contenido de prolina, pérdida relativa de electrolitos, concentración de malondialdehído, actividad peroxidasa y la catalasa, proporción de Na^+/K^+ en partes verdes y pérdida de K^+ por raíces. La mejor germinación se obtuvo cuando las semillas se trataron con NaCl o $CaCl_2$ durante dos días. El ´priming´ mejoró los parámetros de crecimiento y las plantas mostraron una mejor respuesta en todos los parámetros bioquímicos evaluados. Los resultados sugieren que el ´priming´ podría usarse para mejorar el rendimiento de las semillas y las plántulas en situaciones de alta salinidad.

Palabras clave: *Cucumis melo, osmo priming, germinación, estrés salino, estrés oxidativo.*

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INTRODUCTION

Soil salinity is one of the most important stresses limiting crop production. This phenomenon affects 20% of cultivated lands of the world and causes important yield losses in crops (Qadir *et al*., 2014). Irrigation with poor quality water is the main cause of increased soil salinity in cultivated land (Yadav *et al*., 2011). Although irrigated crops represent only 15% of total cultivated land, this represents one third of the world's food production given that yields double what is produced in non-irrigated crops (Munns, 2005). For this reason, it is unlikely that crop irrigation will be abandoned as a normal practice and, instead, an increase in its application could be expected (Shabala and Munns, 2012).

Melon (*Cucumis melo* L.) is an important horticultural crop with a worldwide production of 27.3 million tons. China, Iran, Turkey, Egypt, and the United States represent 68% of the world´s production (Iqbal *et al*., 2016). It is cultivated in all regions of the world with warm and slightly rainy weather, where salinity is becoming a problem (Yasar *et al*., 2006). This crop is considered as moderately sensitive to salt (salinity threshold in the range of 0.75 - 6.8 dS m^{-1}) (Tedeschi *et al*., 2011). It has been reported that the highest sensibility occurs in early vegetative stages, while its tolerance increases between fruit development and harvest (Franco *et al*., 1993; Nukaya *et al*., 1984). High salinity can delay or inhibit germination *(Carvajal et al., 1998; Tanveer et al*., 2012), inhibit growth (Bokochoy-Nbok and Bokochoy, 1991; Botía *et al*., 1998) and reduce yield (Huang *et al*., 2012; Sivritepe *et al*., 2005).

Seed priming is a technique of controlled hydration that allows activation of numerous metabolic processes (pre-germinative metabolism) which occur in early stages of imbibition, before radicle protrusion (Paparella *et al*., 2015). There are different priming methods, which vary based on the way the entry of water is controlled: osmo-priming (seeds are soaked in an osmotic solution such as polyethylene glycol, mannitol or salts), hydropriming (seeds are soaked in a determined amount of water, or limited imbibition periods), or matrix priming (organic or inorganic solids are mixed with water to reduce osmotic potential) (Wojtyla *et al*., 2016). Priming can improve seed germination under favorable and unfavorable conditions, although its effects are more evident under adverse conditions *(Jisha et al*., 2013). The advantages of priming are not exclusive to the seed stage but it can also improve growth and yield of crops under different abiotic stressors (Castañares and Bouzo, 2017; Zheng *et al*., 2016; Farhoudi and Sharifzadeh, 2006).

The objective of this work was to evaluate the effect of priming on germination, initial growth, and biochemical responses of melon plants grown in saline conditions.

MATERIALS AND METHODS

Seeds of F1 hybrid ´Charentais´ cantaloupe ´Planter´s Jumbo´ (Bonanza Seeds, California, USA) were used. The different reagents and salts (analytical grade) used were obtained from Sigma-Aldrich (Buenos Aires, Argentina).

Germination tests

Seeds were surface-disinfected using a 10% sodium hypochlorite solution (NaOCl in water, v/v) for 10 min. Then, the seeds were rinsed with deionized water, placed between two filter papers (Whatman N° 1) in 8.5 cm diameter Petri dishes, and soaked with either one of two different osmotic solutions: NaCl or CaCl$_2$, according to a previous study (Castañares and Bouzo, 2018), in a ratio of 1:5 (wt/v) (Farooq *et al*., 2013). The concentration of these salts was calculated to achieve an osmotic potential (Ψo) of -1.5 MPa according to the van't Hoff equation (Shabala and Munns, 2012). Two priming durations were studied: two and four days. Ten seeds per dish were used, equidistant from each other, with five replicates per treatment. The assay was performed in a germination chamber (Semedic, model I-500 PF, Argentina) at 25 °C (± 0.5 °C) in darkness (Nascimento, 2003). After priming, seeds were removed, rinsed three times in distilled water, and air-dried at 25 °C for 24 h to reduce the moisture content to < 10%. Later, the seeds were germinated between two filter papers and imbibed with an 80 mM NaCl solution (EC = 8.0 dS m^{-1}). Control treatment (Control S1) consisted of seeds without priming, treated with distilled water. Germination was recorded daily during 8 days (ISTA, 2013) and seeds were labeled as germinated when the seed coat was broken and the radicle was visible (>2 mm). Germination percentage (GP) was calculated as the relation between germinated seeds and seeds put to germinate.

Growth of seedlings

The two treatments with the highest GP from the previous experiment were chosen to evaluate the growth response of seedlings. After priming, seeds were rinsed three times in distilled water and sown in 1000 cm^3 pots previously filled with perlite, and watered with Hoagland´s nutrient solution (Hoagland and Arnon, 1950), with 2.0 dS m^{-1} electrical conductivity (EC). After first leaf emergence, plants started to be watered with the nutrient solution with the addition of approximately 60 mM NaCl, to achieve 8.0 dS m^{-1} EC. EC was monitored daily with a portable conductivity meter (Milwaukee model WP MC66 0-10MS/C, USA). Two control groups were established: Control S0, plants derived from non-primed seeds and watered with a 2.0 dS m^{-1} EC solution; and Control S1, plants derived from non-primed seeds and watered with an 8.0 dS m^{-1} EC solution. The experiment was performed in a phytotron, with a 16 h light/8 h darkness cycle and temperatures of 25/18 °C (± 1.0 °C), optimal for melon vegetative growth (Reche Mármol, 2008).

The experiment had a completely randomized design. The position of the pots was randomly changed daily to minimize positional effects in the phytotron. All values reported are the means of five replicates. Data obtained were

analyzed using Tukey's test at 0.05 confidence level using Infostat statistical software (Di Rienzo et al., 2011).

Growth evaluation

Five plants were extracted from each treatment after 40 days, and the following parameters were determined: leaf number, main stem height, leaf area, and total dry weight. Leaf area was determined using digitized images of leaves with Image J software (Schneider et al., 2012). For stem height, the distance from the substrate surface up to the insertion of the petiole of the uppermost leaf was considered.

Xylematic potential measurement

Xylematic potential (Ψx) of plants was measured using a Scholander pressure chamber (Biocontrol model 0-6 MPA, Argentina). Measurements were made on developed leaves, located in the middle third of the stem (Scholander et al., 1964), after 20 days of growth.

Determination of chlorophyll content, relative water content, root viability, proline and malondialdehyde concentration, and relative electrolyte leakage

After 20 days of growth in salinity, five plants per treatment were harvested and used for biochemical determinations.

Chlorophyll content (Ch) was determined according to Lichtenthaler and Wellburn (1983). A sample of 100 mg of fresh leaves (PF) was homogenized with 20 mL (V) of 80% (v/v) acetone in a mortar. Then the mixture was centrifuged at 8,000 × g for 10 min. The absorbance (A) of supernatant was measured using a spectrophotometer (Shimadzu model UV-1800, Japan) at 646.8 and 663.2 nm, and chlorophyll content was determined according to the following formula: Ch (mg g^{-1} FW) = (7.15 A $_{663.2\,nm}$ + 18.71 A $_{646.8\,nm}$) x (V/PF).

Relative water content (RWC) was calculated as described by Cao et al. (2015). The second leaves were extracted and fresh weight (FW) was determined. Then the leaves were placed in a beaker with distilled water for 5 h to determine saturated weight (SW). Finally, the leaves were dried at 80 °C until constant weight and dry weight (DW) was determined. RWC was calculated according the equation: RWC (%) = (FW–DW)/(SW–DW) × 100.

Root viability was estimated by measuring the activity of the dehydrogenase enzyme by using the 2,3,5- triphenyl tetrazolium chloride (TTC) reduction technique (Clemensson-Lindell, 1994). A sample of 500 mg of fresh roots was cut into small pieces, put into test tubes with 5 mL of TTC 0.4 % and 5 mL of phosphate buffer (pH 7.0), and incubated for 3 h at 37 °C. The samples were then extracted in ethyl acetate for 15 min. The absorbance of samples was measured at 485 nm. Results were expressed as absorbance in relation to root dry weight, determined after drying in an 80 °C oven until constant weight (A$_{485\,nm}$ g^{-1} DW).

Proline content was measured using the method developed by Bates et al. (1973). Approximately 300 mg of fresh leaf material was homogenized in 10 mL of 3% aqueous 5-sulfosalicylic acid solution, and the homogenate was filtered using Whatman No 1 paper. An aliquot of 2 mL of filtrate was reacted with 2 mL of acid ninhydrin (1,2,3-triketohydrindene hydrate) and 2 mL of glacial acetic acid ($C_2H_4O_2$) for 1 h at 100 °C. The reaction was terminated on ice for 15 min. The reaction mixture was then extracted with 4 mL of toluene (C_7H_8) and vortexed for 20 s. The absorption of the upper phase was measured at 520 nm using a spectrophotometer and proline concentration was calculated using a standard curve and expressed as µmol g^{-1} FW.

Relative electrolyte leakage (EL) was estimated according to Dionisio-Sese and Tobita (1998). A total of 100 mg of fresh leaves were cut into 5 mm segments and placed in test tubes with 10 mL of deionized water. The tubes were incubated 2 h at 30 °C and the initial EC of the medium was measured (EC1) using a conductivity meter (Altronix model CTX-II, USA). Then the test tubes were incubated at 100 °C for 15 min and the EC was measured again (EC2). EL was estimated according to the formula: EL (%) = EC1/EC2 × 100.

To evaluate the level of malondialdehyde (MDA), a sample of 300 mg of fresh leaves was macerated with 3 mL of 0.1% trichloroacetic acid ($Cl_3C_2O_2H$) (TCA). The homogenate was centrifuged at 3,000 $x\ g$ for 10 min. Then, 1 mL of the supernatant was placed in a test tube, and 1 mL of the reagent TCA-BHT-TBA (TCA 20%, thiobarbituric acid (TBA) 0.37% and butyl hydroxyl toluene (BHT) 0.01 g) was added. The sample was incubated in a water bath for 25 min at 95 °C. The reaction was terminated on ice. Then the sample was centrifuged at 10,000 $x\ g$ for 10 min. The supernatant was used to measure the absorbance at 532 and 600 nm. The content of MDA was expressed as µM g^{-1} FW (Heath and Packer, 1968).

Peroxidase and catalase activity

After 20 days of growth, fresh leaf samples (60 mg) were ground in a mortar, re-suspended with 1.5 mL of 10 mM phosphate buffer (pH 6), and centrifuged at 12,000 $x\ g$ for 20 min. The supernatant was used for the following assays.

To determine the peroxidase activity (POX), 100 µL of 10 mM sodium phosphate buffer (pH 6), 20 µL of guaiacol ((CH_3O) C_6H_4OH) 0.25% (v/v) and 50 µL of 0.88 M H_2O_2 were added to 100 µL of the extract. A reduction in absorbance at 470 nm was measured at 0.5, 1.0, 1.5, and 2.0 min. POX activity was expressed as Abs min^{-1} mg^{-1} FW (George, 1953).

Catalase activity (CAT) was measured according to Aebi (1984). An aliquot of 50 µL was extracted, and 2000 µL of 50 mM sodium potassium phosphate buffer (pH 7) and 20 µL of 0.88 M H_2O_2 were added. The activity was determined by measuring the decrease in absorbance due to H_2O_2 decomposition for 1.0 min at 240 nm and expressed as µmol of H_2O_2 reduced g^{-1} FW.

Determination Na$^+$/K$^+$ Ratio and K$^+$ leakage from roots

The following determinations were also made in seedlings after having grown for 20 days in salinity.

A sample of 2 g plant material (leaves and stems) was ground (2 mm) and placed in a muffle at 600 °C for 4 h. The ashes were boiled with 40 mL of HCl (30%) and 5 mL of HNO_3. The mixture was filtered and cooled. Deionized water was added to complete 250 mL (Campbell and Plank, 1998). K^+ and Na^+ contents were determined using a flame photometer (Metrolab, model 315, Argentina).

K^+ leakage from roots was estimated according to Chen *et al*. (2005), in plants growing. Seedlings of each treatment were randomly chosen, their roots were washed and immersed in a beaker with 10 mL of 80 mM NaCl solution for 2 h. Then seedlings were removed, roots were surface dried with paper towels and root fresh weight was measured. The amount of K^+ released into solution was determined using a flame photometer and expressed in mmol K^+ g^{-1} FW.

RESULTS

Germination in salinity

Priming with NaCl or $CaCl_2$ for two days reversed the inhibitory effect of salinity on melon seeds germination, compared to seeds without treatment (Control S1). Increasing treatment duration from 2 to 4 days reduced the favorable effect of the treatments of priming with NaCl and $CaCl_2$ (table 1).

Effect of priming on the growth of seedlings

Since the highest GP was registered for seeds exposed for two days with either salt, NaCl or $CaCl_2$, the following determinations were made on plants using these treatments.

Priming allowed improving plant performance in salinity when compared to plants without treatment (fig. 1 and 2). In this way, priming allowed to increase the vegetative growth of plants measured through leaf number, main stem height, leaf area, and total dry weight.

Effect of priming on xylematic potential

Saline stress led to a reduction in xylematic potential (Ψx). Priming mitigated this reduction, although the highest value of the xylem potential was measured in the control treatment without saline stress (table 2).
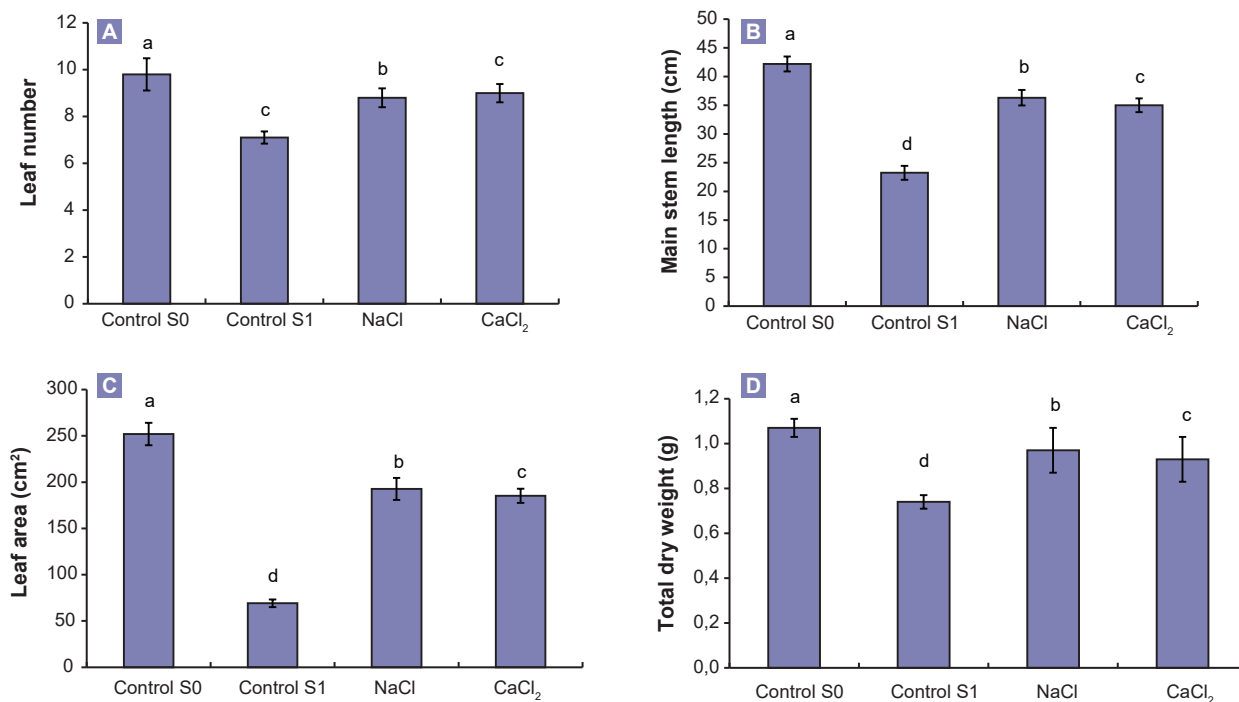
Treatment	GP (%)
Control S1	72.00 ± 7.07 b
NaCl/2-d	98.00 ± 2.00 a
NaCl/4-d	68.00 ± 7.66 b
CaCl2/2-d	94.00 ± 6.30 a
CaCl2/4-d	74.00 ± 7.00 b

Table 1. Effect of priming with NaCl and $CaCl_2$ for 2 or 4 days on melon germination percentage (GP) at 8.0 dS m^{-1}. Different letters indicate a significant difference ($P \leq 0.05$) according to Tukey's test.

Figure 1. Effect of priming on growth parameters of 40 days-old melon plants: leaf number (A), main stem length (B), leaf area (C), and total dry weight (D). [Control S0: non-stressed plants, irrigated with nutrient solution with 2.0 dS m^{-1}; Control S1: stressed plants, watered with 8.0 dS m^{-1} nutrient solution; NaCl and $CaCl_2$: plants from primed seed during two days with NaCl and $CaCl_2$ respectively and watered with 8.0 dS m^{-1} nutrient solution]. Different letters indicate a significant difference ($P \leq 0.05$) according to Tukey's test.
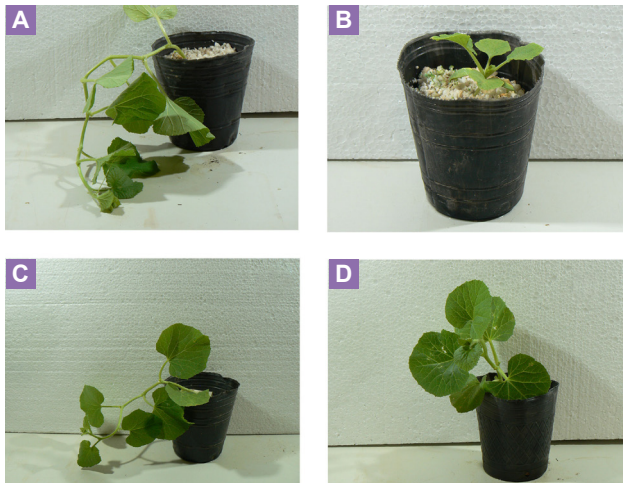
Figure 2. Melon plants of 20 days old. A: Control S0: non-stressed plants, irrigated with nutrient solution with 2.0 dS m⁻¹; B: Control S1: stressed plants, watered with 8.0 dS m⁻¹ nutrient solution; C and D: plants from primed seeds during two days with NaCl and $CaCl_2$ respectively and watered with 8.0 dS m⁻¹ nutrient solution.

Effect of priming on Na⁺/K⁺ Ratio and K⁺ leakage from roots

Na⁺/K⁺ ratio as well as K⁺ leakage by roots had a reduction in plants derived from primed seeds, compared with plants without previous treatment (Control S1). There were no significant differences between both salts (table 2). It was not possible to completely reverse the effect of salinity on these parameters.

Biochemical changes in plants

All biochemical parameters analyzed were affected by salinity (fig. 3). Priming allowed reversing partially this negative effect.

Reduction in total chlorophyll content, relative water content, and root viability were partially alleviated through priming treatments. In general, no differences were observed between the use of NaCl or $CaCl_2$ (fig. 3 A, B, and C).

An increase in relative electrolyte leakage and malondialdehyde (MDA) was measured in stressed plants. A lower increase was measured in plants derived from primed seeds (fig. 3 E and F).

Proline content, which is associated with the response to stress and with adaptation, was higher in stressed plants, and an even higher increase was registered in plants from primed seeds (fig. 3 D).

The activity of antioxidant enzymes increased under saline stress conditions and this increase was higher in priming treatments (fig. 3 G and H). The activity of peroxidase and catalase enzymes was higher in priming treatments. The NaCl treatment was even higher.

DISCUSSION

Priming conditions of the experiment were based on the research conducted by Nascimento (2003), who studied the effect of several treatments on melon germination at 17 and 25 °C. When comparing seed exposure to different osmotic potentials (Ψo, from -1.0 to -1.5 MPa) during different times (3, 6, 9, or 12 days), the best results were obtained with Ψo between -1.3 and -1.5 MPa for 3 days. On the other hand, Bradford (1986) suggested that Ψo lower than -2.0 MPa can damage seeds. Values close to 0 MPa could allow germination during treatment. In this work, an osmotic potential of -1.5 MPa and 2 days of priming allowed to reverse the negative effect of salt stress during the first vegetative stages of melon plants. With this osmotic potential, there was no damage to the seeds, and germination was prevented during the priming treatment.

The choice of the osmotic agents was based on previous work (Castañares and Bouzo, 2018) in which the effect of different agents (polyethylene glycol, $KNO_3+K_3PO_4$, NaCl and $CaCl_2$) on melon germination in saline stress was studied.

Germination reduction in saline conditions can be attributed to a combination of osmotic and toxic effects. Water absorption during imbibition is diminished with the reduction of Ψo caused by solutes (Ibrahim, 2016). On the other hand, high salinity can affect germination due to the toxic effects of Na⁺ and Cl⁻ on the viability of the embryo (Daszkowska-Golec, 2011).

Treatment	Ψx (atm)	Na+/K+ ratio	K+ leakage (mmol g-1 DW)
Control S0	-2.83 ± 2.29 c	0.022 ± 0.006 c	3.07 ± 0.33 c
Control S1	-8.50 ± 0.50 a	0.098 ± 0.004 a	35.85 ± 1.84 a
NaCl	-4.40 ± 0.36 b	0.056 ± 0.006 b	21.55 ± 1.06 b
CaCl2	-5.73 ± 0.25 ab	0.060 ± 0.004 b	24.91 ± 0.29 b

Table 2. Effect of priming on xylematic potential (Ψx), Na⁺/ K⁺ ratio, and K⁺ leakage. [Control S0: non-stressed plants, irrigated with nutrient solution with 2.0 dS m⁻¹; Control S1: stressed plants, watered with 8.0 dS m⁻¹ nutrient solution; NaCl and $CaCl_2$: plants from primed seed during two days with NaCl and $CaCl_2$ respectively and watered with 8.0 dS m⁻¹ nutrient solution]. Different letters indicate a significant difference (P ≤ 0.05) according to Tukey's test.
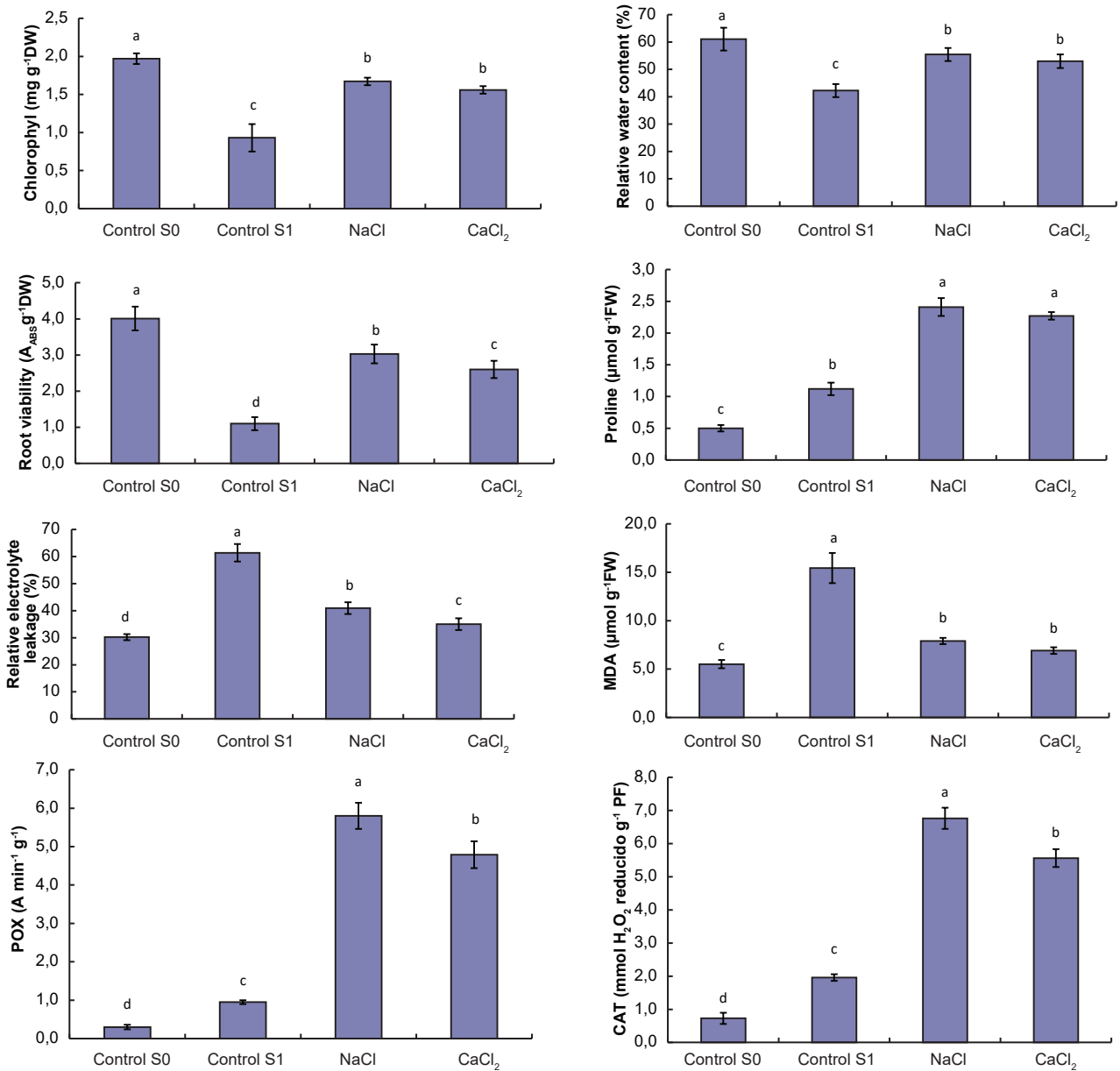
Figure 3. Effect of priming on total chlorophyll (A), relative water content (B) root viability (C), proline (D), relative electrolyte leakage (E), MDA content (F), peroxidase (G) and catalase (H) activity. [Control S0: non-stressed plants, irrigated with nutrient solution with 2.0 dS m^{-1}; Control S1: stressed plants, watered with 8.0 dS m^{-1} nutrient solution; NaCl and CaCl$_2$: plants from primed seed during two days with NaCl and CaCl$_2$ respectively and watered with 8.0 dS m^{-1} nutrient solution]. Different letters indicate a significant difference (P ≤ 0.05) according to Tukey's test.

Germination improvement after priming (table 1) confirms the beneficial effect of this treatment. This effect can be attributed to the activation of metabolic processes that allow faster radicle emission while accelerating water absorption (Bewley and Black, 1994). Similar results were reported by Sivritepe *et al*. (1997) who studied melon germination in salinity conditions after priming with NaCl, registering an improvement in total germination and a reduction in the mean time of germination.

When comparing both priming durations (2 or 4 days) a decrease in germination was observed by extending the imbibition time (table 1). Prolonged partial imbibition above the optimum for a given species will lead to a decrease of reserves, with the consequent reduction of vigor and viability of seeds, and irregular water absorption and loss of vital electrolytes for seeds (Jett *et al*., 1996). Also, rapid seedling establishment might minimize crop risk due to environmental conditions or insect, and disease problems during field emergence.

From the analysis of the growth parameters (fig. 1) it is concluded that, although priming could not completely re-

verse the effect of salt stress, an increase in tolerance was recorded. Sivritepe *et al.* (2003) observed that with EC values of up to 9.0 dS m^{-1}, there was a difference in the dry weight of melon plants from primed seeds. At higher levels of salinity, there was no difference between treated and untreated plants.

Xylematic potential (Ψx) indicates water tension in plants' vessels which is related to the degree of stress (Selles and Ferreyra, 2005). The reduction of Ψx registered in plants growing under salinity conditions (table 2) confirms its effect on the hydric state of plants, due to the reduction of water availability (Shabala and Munns, 2012). The decrease in Ψx was lower in NaCl treatment, which suggests the activation of osmotic adjustment mechanisms, such as accumulation of compatible osmolytes, mainly proline (fig. 3 D), glycine betaine and sucrose, which reduce water potential of cells, protect against membrane damage and stabilize proteins and enzymes (Singh *et al.*, 2015). Proline accumulation in plants partially reduces the negative effects of stress given the ability of this molecule to participate in processes of osmotic adjustment and neutralization of ROS (Rejeb *et al.*, 2014). As observed in fig. 3 D, stressed plants increased proline content and an even higher content of this amino acid was measured in priming treatments. The increase in proline content in salt stressed plants was widely documented. In primed seeds, for example, was registered in pepper (*Capsicum annuum* L.) (Aloui *et al.*, 2014), rice (*Oryza sativa* L.) (Li and Zhang, 2012) and corn (*Zea mays* L.) (Bakht *et al.*, 2011).

Reduction of chlorophyll content in plants under saline stress can be related to a reduction of plant biomass and an increase in lipid peroxidation of the chloroplast's membrane due to oxidative stress, and the accumulation to toxic levels of ions that affect synthesis (Ashraf and Harris, 2013). The increase or reduction of chlorophyll content in saline stress has been proposed in melon as an indicator of tolerance or sensitivity to salinity, respectively (Romero *et al.*, 1997). There was a lower reduction of this pigment's content in primed treatments (fig. 3 A). When working with wheat (*Triticum aestivum* L.), Farooq *et al.* (2013), found a high correlation between the reduction of chlorophyll content in plants under salt stress and the degree of lipid peroxidation and membrane damage. Our results confirm this relationship (fig. 3 A, E, and F).

Reduction of relative water content (RWC) in stressed plants (fig. 3 B) can be related to lower water absorption due to damage of the root system or the low Ψ of the substrate (Kukreja *et al.*, 2005). In this way, the increase of RWC in priming treatments can be explained, on the one hand, by the lower root damage, evidenced with the highest values of reaction to TTC (fig. 3 C), as an indicator of root viability (Ruf and Brunner, 2003).

Stress in plants triggers numerous processes that lead to the generation of ROS. These ROS do not necessarily have a negative effect on plants, but they do have an important function in intracellular communication that allows the best acclimatization to environmental stress (Rejeb *et al.*, 2014). However, excessive accumulation causes the initiation of a significant number of auto oxidative chain reactions, mainly lipid peroxidation, DNA damage and protein degradation (Mittler, 2002), since these are molecules with a highly unstable configuration. Therefore, radicals quickly react with other molecules, generating more free radicals (Ashraf, 2009). Hydrogen peroxide (H_2O_2), which was not measured here, is the most harmful ROS and has been widely studied because of its relative stability (Mhamdi *et al.*, 2010). Antioxidant enzymes, particularly peroxidases (POX) and catalases (CAT) are very efficient at degrading H_2O_2 (Li *et al.*, 2014). Therefore, the high values measured here of these enzymes (fig. 3 G and H) indicate effective mitigation of the possible damage of H_2O_2 on melon plants. Saline stress caused an increase in the activity of POX (fig. 3 G) and CAT (fig. 3 H) enzymes. Priming led to an even greater increase, primarily for NaCl followed by CaCl$_2$. These results agree with was reported by Islam *et al.* (2015) who studied the response of priming with CaCl$_2$ and KCl in two wheat cultivars (*Triticum aestivum* L.) grown in saline soil, and reported a higher antioxidant activity. Salah *et al.* (2015) cultivated rice plants (*Oryza sativa* L.) under toxic levels of Zn, and observed a higher POX and CAT activity, and lower levels of MDA, in plants from seeds primed with PEG.

Under high salinity conditions, Na$^+$ has the ability to compete with K$^+$. Many physiological studies have demonstrated that Na$^+$ toxicity is caused by the ability of Na$^+$ to compete for K$^+$ binding sites to disrupt K$^+$ homeostasis (Hasegawa, 2013). One of the main characteristics of salt tolerant plants is the ability to maintain a low Na$^+$/ K$^+$ ratio (Tester and Davenport, 2003). In our experiments, plant growing in saline conditions increased Na$^+$/K$^+$ (table 2). This can be explained by a greater Na$^+$ accumulation in tissues and an increase in K$^+$ leakage by cells (Poustini and Siosemardeh, 2004). The reduction of Na$^+$/K$^+$ ratio in plants from primed seeds contributes to a better adaptation to salinity. In wheat, Abbasdokht and Edalatpisheh (2012) observed that plants from primed seeds had low Na$^+$/K$^+$ ratio, while the untreated plants exhibited higher ratios. Working with tomato (*Solanum lycopersicum* L.), Cayuela *et al.* (1996) reported that priming with NaCl allowed reducing Na$^+$ content in plants that best grow in salinity. Plants growing in salt stress can experience an irreversible loss of K$^+$, which is particularly important in roots. This is related to the loss of integrity of membranes, reducing the ability to retain K$^+$ (Demidchik *et al.*, 2014). Low K$^+$ leakage from roots is accepted as an indicator of salt tolerance (Chen *et al.*, 2005). The lower K$^+$ release measured in priming treatments (table 2) confirms the greater stability of membranes, also estimated from relative electrolyte leakage (fig. 3 E) and MDA (fig. 3 F). K$^+$ is recognized as an important factor limiting yield and quality of crops, and functions include maintenance of cell turgor, growth, activation of numerous enzymes, stabilization of protein synthesis, the formation of membrane potential, and maintenance of cytosolic pH (Cakmak, 2005). Relative electrolyte leakage is considered an estimator of membrane integrity (Demidchik *et al.*, 2014). A greater release of electrolytes, estimated from EC, indicates membrane damage. This was measured in stressed plants (Control S1)

while priming allowed reducing this damage (fig. 3 E). The final product of polyunsaturated fatty acids oxidation is malondialdehyde (MDA). This molecule reacts with thiobarbituric acid (TBA) and generated a pink compound detectable by spectrophotometry (Hodges *et al*., 1999). The increase of the MDA level in the conditions of the experiment (fig. 3 F) confirms the membrane damage, which corroborates the results discussed above by the electrolyte leakage measurement (fig. 3 E). Thus, priming allowed improving the integrity of membranes under the conditions of the experiment. This response is in agreement with what was observed by Randhir and Shetty (2005) in corn (*Zea mays* L.) and Amooaghaie (2011) in alfalfa (*Medicago sativa* L.).

CONCLUSIONS

The results of this study show that priming induces biochemical changes in melon seedlings increasing the adaptive response to salinity. Melon is cultivated intensively and with complementary irrigation, which promotes the accumulation of salts in the soil. Given that the studied salts have a relatively low cost and are easy to manipulate, the information obtained could be of interest to producers.

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