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Full Length Article

Improving Salt Tolerance in Pepper by Bio-Priming with *Padina pavonica* and *Jania rubens* Aqueous Extracts

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Abstract

This study was conducted to evaluate the effect of seed bio-priming on the salinity tolerance of pepper (*Capsicum annuum* L. var Chargui). The pretreatment was carried out by the aqueous extracts of *Padina pavonica* (at 60 g/L) and *Jania rubens* (at 80 g/L). The tests were carried out at 0, 3 and 12 g/L of salt for germination and 0 and 3 g/L for the growth which was conducted in hydroponics. Seeds were soaked in these extracts, and two batches were considered (treated and un-treated (control)). The results showed that the effect of salt was harmful on the integrity of the membrane, respiration, and mineral (Potasium and Posphorus) compositions of pepper. Nevertheless, it increased proline, total soluble sugars, sodium and secondary metabolites accumulation. the pretreatment showed that the best tolerance to salt stress in pepper was due to a better oxidative status demonstrated by the reduction of oxidative damage (reduction of leakage of electrolytes and MDA content), accumulation of secondary metabolites, at better hydration resulting from more efficient osmotic adjustment due to the accumulation of osmotica (proline, total soluble sugar, potassium and phosphorus) and an increase in metabolic activity by the improving breathing. This technique of biological pretreatment may submit with agriculture an interesting tool to increase plant tolerance to salt stress and to find an alternative to any chemical use. © 2018 Friends Science Publishers

Keywords: *Capsicum annuum*; NaCl; Priming; Aqueous extracts; *Padina pavonica*; *Jania rubens*

Introduction

The major environmental factor, which participates in the limitation plant growth and productivity is the salinity (Allakhverdiev *et al.,* 2000). It causes adverse biochemical and physiological changes in germinating seeds (Ibrahim, 2016) and it is a serious threat to agricultural productivity (Parvaiz and Satyawati, 2008). Most observable effect of salt stress is germination delay and decrease in germination percentage. In addition, salinity causes the disturbance of the enzyme systems needed to establish the physiological functions of the seed during germination (Hajlaoui *et al.,* 2007). Concentrations of 100 and 150 mM NaCl resulted in a reduction in germination rate and an extension of the latency phase in pepper seeds (Chartzoulakis and Klapki, 2000).

Salinity is capable to disturb the mineral nutrition of plants by interfering with the removal of certain essential elements such as potassium and calcium, either by substitution or by competition at the level membrane absorption sites (Zid and Grignon, 1991). In addition, increases of NaCl decreases the absorption of potassium and calcium and interferes with their physiological functions (Zhu, 2000). The most sensitive stages to salinity are seed germination and seedling growth. That is why, improvement in seed germination is an active area of research and efforts are being made to improve it, for various economically important plants (Suleman *et al.,* 2011). Among the used techniques to improve germination, the seed priming, a prior encounter with a particular type of stress, attributes to plants a greater stress tolerance (Patanea *et al.,* 2009). The pretreatment of seed is a pre-sowing trick for driving the seedling development at a later stage (Taylor and Harman, 1990). Indeed, a rapid and uniform germination due to the pretreatment of the seeds (Mc Donald, 2000). It is a technique that has been used to, under unfavorable environmental situation induce an increase in the rate and uniformity of their germination and to stimulate the emergence of seedlings (Rozbeh *et al.,* 2011). To it was reported that the plantlets developed from treated seed, germinate faster, grow strongly and perform better even at less optimal conditions (Sivritepe *et al.,* 2003). Earlier

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researches on seed pretreatment in field crops like wheat (Ghiyasi *et al.,* 2008), lentil (Ghassemi-Golezani *et al.,* 2008), maize (Foti *et al.,* 2008), sugarcane also reported improved stress tolerance and growth of seedling from treated seeds. Although effect of seed priming in other crops is documented, rare reports are available on potential of various seed priming treatments and responses of the capsicum plants on subsequent exposure to salt stress (Patade *et al.,* 2011). The pepper (*Capsicum annuum* L.) is a vegetable plant belonging to the Solanaceae family, originated in Central and South America. *Capsicum* can be grown year round in frost-free conditions and grows better in warm climate. Cold condition (below 15°C) lowers fruit setting due to poor pollination and may leads to fruit splitting (Eshbaugh, 1993). The low yields of pepper due to many constraints that which are linked differences in the plant environment, including salinity.

In most studies, the used products in the seed priming are particularly industrials and chemicals products, which are often daunting, and the search of alternative, as natural products, in various fields is widely sought. Thus, among these products, seaweed thalli aqueous extracts were reported very beneficial for germination and plant growth (Ommezzine *et al.,* 2009).

This laboratory study was conducted to investigate than priming by aqueous extracts of *Padina pavonica* and *Jania rubens* can help improve resistance to salt stress in pepper by modulating sugar metabolism, membrane stability, and ionic homeostasis.

Materials and Methods

Experimental Details and Seed Materials

This laboratory trial was carried out in Laboratory, Department of Biological Sciences and Environment Protection, Higher Institute of Agronomy of Chott Meriem, University of Sousse, Tunisia. Seeds pepper (*Capsicum annuum* L., var. Chargui) used in this study were selected from a crop cultivated in Tunisia. Bioassays were carried out at germination stage on the un-treated (control) and treated seeds with *P. pavonica* and *J. rubens.* And at the growth stage, on roots and leaves of seedlings (Harvested after hydroponic cultivation) developed from treated and un-treated seeds. These bioassays were affected in saline conditions at different concentrations.

Plant Material

Two species of algae were chosen: brown algae (*Padina pavonica*) and red algae (*Jania rubens*). These algae have been chosen because their biological activities are not yet studied in the seeds priming. The identification was made based on color and morphological characters of the thallus (Taylor, 1960). Thalli algae were collected in August 2012 from Monastir sea of Tunisia at 1 m depth. After harvest, thalli algae were washed with tap water, dried in the open air for 15 days at room temperature and then ground to obtain fine powder stored at 4°C till further use. Algae powder (100 g) was macerated in 1 L of sterile distilled water for 24 h at room temperature and in the dark (Khanh *et al.,* 2005). The mixture was filtered through filter paper (Whatman No. 1) and the extracts were recovered and stored at 4°C until use in the seed pretreatment.

Seed Priming

For pretreatment the seeds were soaked for 24 h at 28 ± 2°C in algae extracts, after disinfection with sodium hypochlorite (10%) and washing with distilled water. The ratio of seed mass to volume of solution was 1: 5 (g mL^{-1}) (Farooq *et al.,* 2006). After each treatment, the seeds were washed thoroughly with distilled water and were placed in petri dishes to germinate directly after pretreatment and washing. Un-treated seeds were used as control.

Pepper Germination and Growth and NaCl Application

In germination tests, treated and un-treated seeds were placed in Petri dishes and irrigated with 5 mL of NaCl solution. The NaCl concentrations used were, 3 g/L and 12 g/L, inducing, respectively 50% and 0% of germination rate in addition of germination in absence of the salt. After germination, the germinated seeds were frozen (-80°C) for biochemical analysis.

For seedling growth, treated and un-treated seeds were germinated in Petri dishes at room temperature in the dark, with distilled water. Fifteen-day old seedlings uniform were subsequently cultured individually in a complete Hoagland's medium (Hoagland and Arnon, 1950) diluted by half in a greenhouse (16 h light/8 h dark at 20/17°C). After 15 days of acclimation, plants were cultivated in the presence of 3 g/L NaCl (concentration inducing 50% inhibition of seedling length). Salt treatment was initiated by adding increments of 1 g/L NaCl daily until the desired concentration, in order to avoid osmotic shock. Plants were grown in the final salinity solutions for 15 days. The culture media were aerated continuously and the renewal was done every 2 days. At the end of the treatment period, the plants were harvested and separated into roots and shoots, fresh or dry materials was used for the determination of different parameters.

Electrolyte Leakage

Electrolyte leakage (EL) gives an estimate of the change in membrane permeability. EL is based on the measurement of the electrical conductivity of an aqueous medium (distilled water) where samples of seeds, leaves or roots of peppers treated and untreated (control) have been immersed. According to Lutts *et al.* (1996), Electrolyte leakage (EL) was determined either, after 24 h or 48 h of incubation.

The samples were placed in test tubes containing 15 mL of distilled water. After incubation at room temperature (24 h or 48 h), the electrical conductivity (EC_1) of the medium was measured using a digital conductivity meter (type BCT-4308). Subsequently, samples were autoclaved at 121°C for 20 min to burst the walls and release all electrolytes, a second incubation was made as indicated above, and the final electrical conductivity (EC_2) Was measured.

The electrical conductivity was expressed as a percentage of the control according to the ensuing equation (Lutts *et al.*, 1996):

$$EC = (EC \text{ of treatment} / EC \text{ of control}) * 100$$

According to the equation described by EL was calculated:

$$EL = (EC_1/EC_2) \times 100$$

Lipid Peroxidation

Lipid peroxidation was measured according by Doblinski *et al.* (2003). The homogenization of the frozen vegetable tissues (200 mg) in 2.5 mL of 67 mM phosphate buffer (pH=7) and 0.05g of polyvinylpyrrolidone (PVP) was followed by centrifugation at 2000 g 15 min at 4°C. 3 mL of 0.5% TBA prepared in trichloroacetic acid (TCA) 20% was added to the supernatant (750 µL). The mixture was incubated at 90°C for 10 min. The reaction was stopped by cooling the mixture in ice. The mixture was centrifuged, and the absorbance of the supernatant was measured at 532 and 600 nm, after cooling. The MDA content was determined using the molar extinction coefficient of 155 mM^{-1} cm^{-1} (Doblinski *et al.*, 2003).

Determination of Dehydrogenases

Fresh vegetable tissues (0.1 g) were washed and dried quickly between two blotting papers, then incubated in 5 mL of a TTC solution (0.2%, pH=7) at a temperature equal to 37°C, in the course of four hours at dark. Then, adding 0.5 mL of sulfuric acid (1 M) to block the reaction. Thereafter, the tissues were removed, washed with distilled water, dried rapidly between two filter papers and crushed in a mortar containing 3.5 mL of ethyl acetate and placed on ice. The solution obtained was filtered through Whatman No. 1 paper and the volume was adjusted by adding 7 mL of ethyl acetate to each tube. Finally, the optical density was read at 485 nm. According to Sampietro *et al.* (2006), the formazan was calculated as ensuing:

$$\text{Formazan content (\%)} = DO_{485} \text{ treatment}/DO_{485} \text{ control}$$

Total Soluble Sugars Content

Soluble sugar contents were determined according to the method of Dubois *et al.* (1956). The fresh plant material (0.1 g) was placed in test tubes. Then 2 mL of 80% ethanol was added to extract the sugars. The tubes were left in the dark and at room temperature for 48 h at the time of assay. The tubes were placed in a 70°C to evaporate the alcohol. After cooling, 20 mL of distilled water was added. Subsequently, 1 mL of 5% phenol was added to 1 mL the solution to be analyzed. Finally, was rapidly added to the mixture 2 mL of H_2SO_4 (96%). The reaction was stopped by placing the tubes in an ice bath for 25 min. The absorbance was measured at 490 nm. The sugar concentration was calculated from the equation deduced from the calibration range and the quantities are expressed in µg/g MF.

Proline Content

Proline, a marker of resistance to abiotic stresses, has been studied in seeds, leaves and roots of pepper. The method used for the proline assay is that of Bates *et al.* (1973). It consists of weighing 10 mg of, previously dried, vegetable tissues in 1.5 mL Eppendorf tubes. The tubes were filled with 3% (w/v) sulfosalicylic acid (1.5 mL) to precipitate the proteins. The samples was mixed and centrifuged at 13 000 g for 10 min. The supernatant (1 mL) was added to 1 mL of glacial acetic acid and 1 mL of ninhydrin reagent (1.25 g ninhydrin in 30 mL of glacial acetic and 20 mL 6M H3PO4) and incubated at 100°C for 1 h. After cooling in an ice bath (to stop the reaction), 2 mL of toluene was removed and the absorbance was read at 520 nm. The proline concentration was calculated from a calibration curve performed by a series of proline solutions (0–100 mg mL^{-1}).

Determination of the Potassium, Sodium and Phosphorus Content

According to the method described by Martin-Prével *et al.* (1984), the Na^+ and K^+ content were measured in seeds and leaves of pepper. The calcination followed by a hydrochloric recovery of the ash was carried out as follows: we put in a muffle furnace (220°C) one gram of dry matter. During two hours and then at 550°C. For 6 h in order to be calcined. The ash obtained was dissolved with 2 mL of concentrated hydrochloric acid (HCl) and then heated on a hot plate until evaporation. Thereafter, 5 mL of HCl (N/10) was added and the mixture was kept for 10 min and then filtered and adjusted to 100 mL with distilled water. The K^+ and Na^+ concentrations were determined from calibration curves established from solutions with known concentrations previously prepared and passed on to the flame spectrophotometer. The ionic contents are expressed in percent dry matter (DM).

The phosphor (P) assay is performed by spectrophotometry at 430 nm. 5 mL of nitrovanadomolybdic reagent were added to 10 mL of mineralized (previously prepared) and then diluted to 25 mL with distilled water. The P concentration was determined from a calibration curve established from a standard phosphorus range. The P content is expressed as percent DM.

Secondary Metabolites Production in Pepper

Roots and aerial parts of pepper grown at 0 and 3 g/L of NaCl were harvested and then homogenized for 24 h at methanol (80%). We are used supernatant to complete the levels of total phenols, flavonoids and total precipitable alkaloids, after centrifugation of the homogenate at 10,000 g at 4°C/20 min (García-Sánchez *et al*., 2012).

Determination of Total Phenols

The quantification of total phenols (TP) was made the Folin-Ciocalteau method (Velioglu *et al.*, 1998). To do this, 500 µL of the Folin-Ciocalteau reagent diluted 10-fold in distilled water was added at 100 µL to the sample extract and was allowed to react for 5 min in the dark and at ambient temperature. Then, 400 µL of a sodium carbonate solution (7.5%) was added. After 90 min of incubation under the same conditions, the absorbance was measured with a UV spectrophotometer at the length of 765 nm. Total phenolics content were expressed as mg gallic acid equivalent per g of dry weight using gallic acid calibration curve ((R^2= 0.996).

Determination of Total Flavonoids

The total flavonoids (TFd) content was measured by the spectrophotometry method described by Quettier *et al.* (2000). An amount of 500 µL of methanolic solution of $AlCl_3$ (2%) was mixed with the same amount of aqueous extract. Absorbance at 430 nm was recorded after 30 min of incubation in the obscurity and flavonoid content was expressed as mg of quercetin equivalent per g of dry weight, using quercetin calibration curve (R^2 = 0.998).

Determination of Total Percipitable Alkaloids

The technique used is that described by Stumpf (1984). The aqueous extracts (300 µL) were mixed with the Dragendorff reagent (100 µL). After centrifugation at 7000g for 1 min, the supernatant was recovered and dissolved in 1 mL of NAI (2.45 M). An aliquot of 10 µL of each tube was added to 1 mL of NAI (0.49 M) by following the absorbance is measured at 467 nm. The content of total alkaloids was expressed as mg of papaverine hydrochloride equivalent per g dry weight, using papaverine hydrochloride calibration curve (R^2 = 0.951).

Statistical Analysis

Four replications were performed for all biological assays. The results were analyzed by SPSS Statistics 20 for Windows. The Duncan test, the t-test and the ANOVA test were performed to analyze differences between treatments. The means were separated on the basis of the least significant differences at $p < 0.05$.

Results

Electrolyte Leakage

The priming was beneficial only with *J. rubens* extract, mainly in the presence of 12 g/L of salt, wherein the electrolyte leakage was reduced by 235% compared to untreated material, after 48 h of seeds immersion. In other cases, the electrolyte leakage was similar to the control or increased (Fig. 1A).

In leaves, the leakage of electrolyte was reduced in all cases after 24 h incubation. The reduction was an average of 159.79% in the absence of salt for the priming by the two types of extract. Similarly at 3 g/L of salt, an average reduction of 141%, compared to the control, was registered. After 48 h of immersion, the leakage of electrolyte was reduced in all cases, except for the priming with *P. pavonica* extract, where an increase of 51% was noted in the non saline conditions (Fig. 1B).

Concerning roots, the priming with *P. pavonica* extract increased electrolyte leakage, after immersion for 24, by 227 and 191%, respectively in 0 g/L and 3 g/L NaCl. With the second extract, an increase (163%) was noted at 0 g/L and a reduction of 279% compared to control, was registered at 3 g/L. After 48 h, the leak was increased in all cases (Fig. 1B).

Lipid Peroxidation

Concerning germinated seeds, the malondialdehyde content was reduced in seeds treated compared to untreated seeds under saline conditions. This average reduction was, 0.82 and 0.30 $U.g^{-1}$ FW at 3 and 12 g/L NaCl, respectively. However, improvement in salt concentration allows an increase in MDA content in untreated seeds, this content was reduced by 1.06 $U.g^{-1}$ FW (*J. rubens*) and 0.58 $U.g^{-1}$ FW (*P. pavonica*) in the presence of 3 g/L NaCl. At 12 g/L NaCl, the priming had no significant effect on the accumulation of MDA (Fig. 2A).

For leaves, the results showed that, in the absence of salt, the priming has no way to prevent the increase of the MDA content, which was increased by 5.41 and 6.61 $U.g^{-1}$ FW relative to the control with *P. pavonica* and *J. rubens*, respectively. A comparable result was noted in saline conditions. Similarly for roots, where the MDA content was increased by 2.16 and 5.64 $U.g^{-1}$ FW versus control, respectively, with *P. pavonica* and *J. rubens*, in absence of salt. At 3 g/L NaCl, this content was increased by 5.41 and 0.35 $U.g^{-1}$ FW, respectively in the roots developed from treated seeds with *P. pavonica* and *J. rubens*. For untreated ones, this increase was 3.90 $U.g^{-1}$ FW (Fig. 2B).

Dehydrogenases Activity

During germination, the formazan content was increased in the absence of NaCl, by 14% and 17%, in seeds

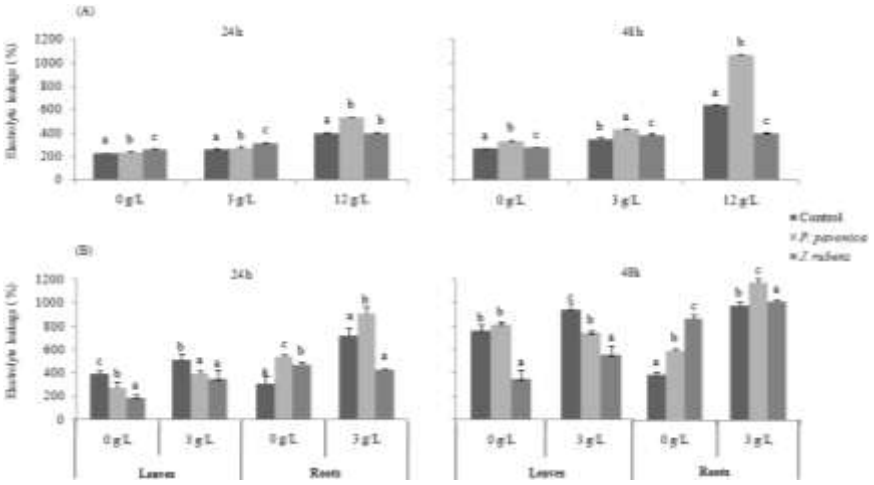

Fig. 1: Electrolyte leakage (%) from pepper germinated un-treated (control) and treated seeds with *P. pavonica* and *J. rubens* (A), and from roots and leaves of seedlings developed from primed and un-primed seeds (B) in the presence of NaCl at different concentrations, after an immersion of 24 and 48 h in distilled water. The bars on each column show standard error. Value = average ± S.E., n = 4. Different letters on columns indicate significant differences among treatments at $p < 0.05$

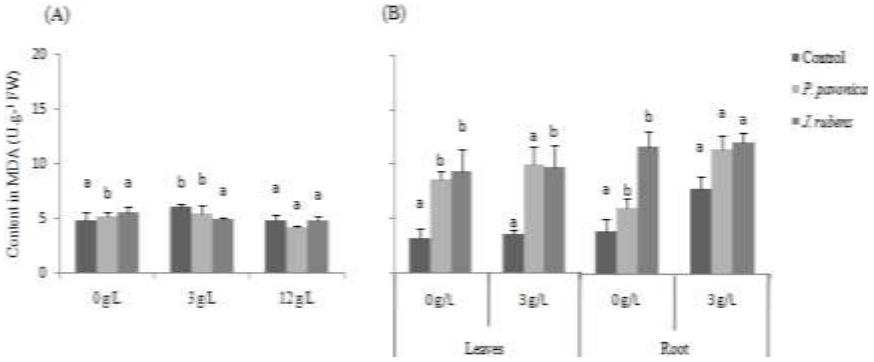

Fig. 2: Content of malondialdehyde (MDA) (U.g^{-1} FW) in pepper germinated un-treated (control) and treated seeds with *P. pavonica* and *J. rubens* (A), and in roots and leaves of seedlings developed from primed and un-primed seeds (B), in the presence of NaCl at different concentrations. The bars on each column show standard error. Value = average ± S.E., n=4. Different letters on columns indicate significant differences among treatments at $p < 0.05$

primed with, respectively *P. pavonica* and *J. rubens*. At 3 g/L of NaCl, the formazan content in primed seeds by extracts of the two respective algae showed a stimulation of 236% and 13%. These values were 32% and 14% at 12 g/L (Fig. 3A).

For leaves, the content in formazan has increased in all cases, compared to control. In non saline conditions, the percentage increase was 22% and 26%, after pretreatment with *P. pavonica* and *J. rubens* extracts. At 3 g/L NaCl, theses percentages were 24% and 41%. The formazan level was increased in saline conditions, for the roots, and there is no significant difference between the two extracts which induced an average augmentation of 216%. In non saline conditions, the best increase (25%) was noted in roots developed from seeds treated with *J. rubens* extract. The second extract induced only 8% of increase, relative to control (Fig. 3B).

Proline Content

In saline conditions, the proline content has been more or less improved in all cases (Fig. 4). In untreated germinated seeds, proline amounts were 13×10^{-3}, 11×10^{-3} and 16×10^{-3} µmol/g DM, respectively at 0, 3 and 12 g/L (Fig. 4A). The priming enhanced the proline content (1.25 times) in the absence of salt, and did not affect it in germinated seeds at 3 g/L, compared to the control (11×10^{-3} µmol/g DM). Nevertheless, the pretreatment decreased this content by an average of 0.67 times at 12 g/L. There is no significant difference between the two extracts effect (Fig. 4A).

In seedlings developed from untreated seeds, the proline amount passed from an average of 32×10^{-3}

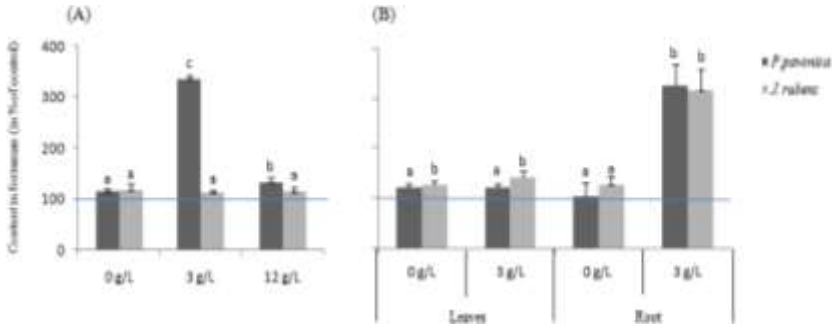

Fig. 3: Formazan content (in % of control) in pepper germinated un-treated (control) and treated seeds with *P. pavonica* and *J. rubens* (A), and in roots and leaves of seedlings developed from primed and un-primed seeds (B), in the presence of NaCl at different concentrations. The bars on each column show standard error. Value = average ± S.E., n=4. Different letters on columns indicate significant differences among treatments at $p < 0.05$

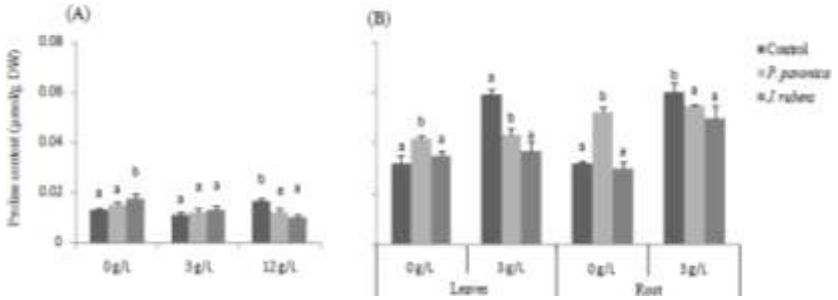

Fig. 4: Proline content (µmol / g DW) in pepper germinated un-treated (control) and treated seeds with *P. pavonica* and *J. rubens* (A) and in roots and leaves of seedlings developed from primed and un-primed seeds (B), in the presence of NaCl at different concentrations. The bars on each column show standard error. Value = average ± S.E., n = 4. Different letters on columns indicate significant differences among treatments at $p < 0.05$

µmol/g DM to 59, 5×10^{-3} µmol/g DM, for the two organs, when they were in the presence of 0 and 3 g/L of NaCl, respectively. For seedlings from primed seeds, the pretreatment with *P. pavonica* has enhanced the proline content in the absence of salt the increase was 31.14% in leaves and 62.24% in roots. In the presence of 3 g/L of NaCl, the proline content decreased compared to the control, by percentages of 26% and 10%, for the two respective organs. With *J. rubens* extract, the priming gave levels compared to the control or somewhat lower (Fig. 4B).

Total Soluble Sugars Content

During germination, the salt decreased the content of soluble sugars in germinated untreated seeds. This reduction was 20% and 65% at 3 and 12 g/L of NaCl, respectively. The priming increased this content in all cases. Indeed, in the absence of NaCl, primed seeds with aqueous extracts of *P. pavonica* and *J. rubens* had, respectively, 6.90 and 7.42 mg/g FW, which presented an average increase of 1.06 times, relative to untreated seeds (Fig. 5A). In saline condition, sugars content significantly increased in seeds treated with *J. rubens* extract, which induced a percentage increase of 61% and 227% at 3 and 12 g/L. The second extract induced an average increase of 23%, at the two NaCl concentrations (Fig. 5A).

In leaves, the priming enhanced the sugars content in all cases and the effect of the two extracts was comparable at 3 g/L. At this concentration, the sugars content increase was an average of 57.48%, compared to untreated material. In the absence of salt, *J. rubens* extract was more beneficial, it induced an increase of 39.22%, compared to the second one which gave a percentage increase of 12%, relative to the control (Fig. 5B).

Potassium, Sodium and Phosphor Contents of Pepper

For pepper germinated seeds, that priming decreased K^+ content, only in the absence of salt (Fig. 6Aa). Whereas in the presence of NaCl, this content was increased 1.07 and 1.06 times, respectively at 3 and 12 g/L, compared to the control. For untreated seeds, the K^+ content was 0.76 and 0.68 µg/mL in absence and presence of salt, respectively. In untreated seeds, the sodium content was increased by 1.2 and 2.5 times respectively at 3 and 12 g/L. The pretreatment with *J. rubens* extract has enhanced the Na^+ content 2.1 times in the absence of salt, compared to the control. Under salt, this content

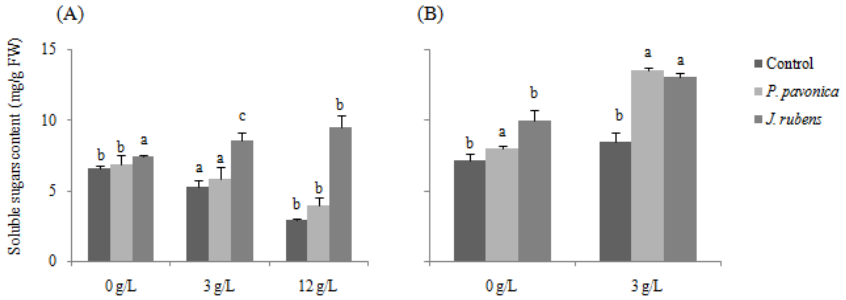

Fig. 5: Total soluble sugars content (mg/ g FW) in pepper germinated un-treated (control) and treated seeds with *P. pavonica* and *J. rubens* (A) and in leaves seedlings developed primed and un-primed seeds (B), in the presence of NaCl at different concentrations. The bars on each column show standard error. Value = average ± S.E., n = 4. Different letters on columns indicate significant differences among treatments at $p < 0.05$

was decreased by 0.87 and 0.2 times, respectively at 3 and 12 g/L of NaCl. The priming with *P. pavonica* extract improved the P content only at 12 g/L, where it was 1.64 times higher compared to untreated material. At 0 and 3 g/L NaCl, this content was, respectively 1.04% and 1.02% *J. rubens* extract, improvement P content by 1.03, 1.17 and 1.22 times, at the three salt concentrations. As for untreated seeds accumulation of P was decreased in the presence of salt of an average of 0.71 times (Fig. 6Ac).

Sodium content was increased by 3.70 times at 3 g/L of salt, in leaves developed from untreated seeds. The priming with *J. rubens* extract reduced by an average of 0.5 times, the content of Na^+, at 0 and 3 g/L NaCl. The second extract, increased this content by 1.37 times at 0 g/L, and decreased it by 0.8 times at 3 g/L (Fig. 6Bb). The priming decreased accumulation of K^+ of 0.94 and 0.40 (with *P. pavonica* extract) and 0.56 and 0.64 (with *J. rubens* extract), respectively at 0 and 3 g/L NaCl, compared to the control. For seedlings from untreated seeds, the presence of salt reduced the accumulation of K^+ 0.80 times (Fig. 6Ba). The same, the accumulation of P was decreased under the priming effect. However, this decrease is an average of 0.73 and 0.55 times at 0 and 3 g/L of salt (Fig. 6Bc).

Accumulation of Secondary Metabolites in Pepper Seedlings

The contents of total phenols, total flavonoids and total precipitable alkaloids were determined at the level in roots and leaves of developed from treated and untreated pepper seeds in saline (3 g/L) and non-saline conditions (0 g/L) (Table 1).

Total Phenols (TP) Content

In leaves, the priming increased the levels of TP, in the absence of salt, by an average factor of 1.16 times, relative to the untreated seed. Similarly, at 3 g/L NaCl, these levels have been increased compared to the control. This increased was 1.30 and 1.07 times, respectively, for leaves from seeds treated *P. pavonica* and *J. rubens.*

In roots developed in the absence of salt, quantities of TP were increased, compared to the control, by an average factor of 2.07 times, after the priming with the two extracts. Similarly, in the presence of salt, the contents of TP were improved by an average of 1.60 times, for all treatments compared to untreated seeds (control) (Table 1).

Total Flavonoids (TFd) Content

In salt condition, the TFd content was increased in leaves and roots for untreated seeds, by respectively, 1.24 and 1.19 times. The priming, with *P. pavonica* and *J. rubens* extract, has improved the TFd content. This content was increased of a means 1.17 times, in the presence of the salt, relative to the control.

In roots developed in the absence of salt, quantities of TFd were increased, compared to the control, by an average factor of 1.16 times, following the priming with the two extracts. Similarly, in the presence of salt, the contents of TP were improved by an average of 1.33 times, for all treatments compared to untreated (control) seeds (Table 1).

Total Precipitable Alkaloids (TA) Content

Under saline conditions, TA content was increased of 1.63 times in pepper seedlings from untreated seeds. The pretreatment with the two extracts, increased the TA accumulation, in roots, by an average of 2.38 times at 0 g/L and 2.27 times at 3 g/L and by 2.35 times in leaves at 3 g/L (Table 1).

Discussion

The aim of this study was to evaluate the seed priming by aqueous extracts of *J. rubens* and *P. pavonica* on the germination and growth of pepper (*C. annuum* L. var Chargui) in the presence and the absence of NaCl. Salt stress caused a depressive action on germination, expressed by reduction and delay in germination rate, and pepper growth, which it's manifested by a decrease in root and shoot length and their behavior, was comparable. This effect is reported for many plant species (Jamil *et al.*, 2006).

Table 1: Total phenols content (TP) (mg GA/g FW), flavonoids content (TFd) (mg QE/g FW) and precipitable alkaloids content (TA) (mg PAHE/g FW) in roots and leaves of seedlings developed from un-treated (control) and treated seeds with *P. pavonica* and *J. rubens*, developed at 0 and 3 g/L NaCl

Leaves				
	Concentration (g/L)	TP	TFd	TA
Control	0	4.40 ± 0.01d	3.95 ± 0.06a	1.00 ± 0.05d
	3	5.72 ± 0.4f	4.92 ± 0.06b	1.96± 0.19cd
P. pavonica	0	5.21 ± 0.03 a	4.90± 0.03b	1.86 ± 0.07c
	3	7.48 ± 0.57 e	5.93 ± 0.04d	2.21 ± 0.17b
J. rubens	0	5.05 ± 0.17 b	4.88 ± 0.01b	1.78 ± 0.16a
	3	6.14± 0.3 c	5.66 ± 0.05b	2.40 ± 0.01a
Roots				
	Concentration (g/L)	TP	TFd	TA
Control	0	0.58 ± 0.06a	0.36± 0.005a	1.50± 0.01bc
	3	0.68 ± 0.01a	0.43 ± 0.01c	1.97± 0.501c
P. pavonica	0	1.25 ± 0.11c	0.40± 0.02bc	1.88 ± 0.01b
	3	1.15± 0.02bc	0.49± 0.01ab	2.42 ± 0.07a
J. rubens	0	1.16± 0.01bc	0.44 ± 0.01d	1.70 ± 0.06a
	3	1.03 ± 0.17b	0.47± 0.006c	2.07 ± 0.1ab

All values are the average of five measurement ± standard deviation. Different letters indicate significant differences between treatments at $p<0.05$

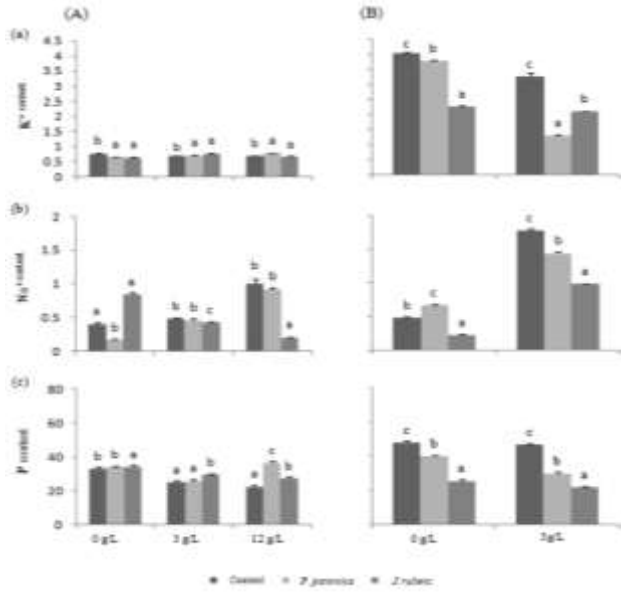

Fig. 6: Potassium (a), sodium (b), and phosphorus (c) content (in %) of pepper germinated un-treated (control) and treated seeds with *P. pavonica* and *J. rubens* (A) and of leaves seedlings developed from primed and un-primed seeds (B), in the presence of NaCl at different concentrations. The bars on each column show standard error. Value = average ± S.E., n = 4. Different letters on columns indicate significant differences among treatments at $p < 0.05$

However, roots were more sensitive in some cases, and aerial parts in other cases (Jamil *et al.,* 2006).

Also, physiological and biochemical parameters were affected by salt stress. The results obtained show an accumulation of certain measured parameters such as; sodium, proline, secondary metabolites and total soluble sugars. However, a decrease in electrolyte leakage (EL) and lipid peroxidation was recorded in the presence of salt (Fig. 1). An increase in MDA content was noted, when NaCl concentration increased, in pepper seeds and seedlings (Fig. 2). The same results were obtained by Ghezal *et al.* (2016) in pea. Similarly, Falleh *et al.* (2012) found that salinity increases lipid peroxidation.

The decrease in the activity of dehydrogenases could be a reflection of cell damage. Indeed, under salt stress, the lower production of formazan was noted in germinated seeds and pepper roots, where mitochondrial respiration has been, significantly, reduced (Fig. 3). While in the leaves the application of salt stress increased the accumulation of formazan.

Salt stress affects ionic transport processes in plants, which can modify nutritional status and ionic equilibrium (Laüchli and Epstein, 1990). At high salinity, plants have developed complex mechanisms allowing adaptation to ionic and osmotic stress. These mechanisms include lowering the concentration of toxic ions in the cytoplasm by restricting the influx of Na^+ or its sequestration in the vacuole and/or its extrusion (Hajibagheri *et al.,* 1987). In this study, an increase of Na^+ by 1.2 and 2.5 times was observed at 3 and 12 g/L in the untreated seeds and 3.70 times at 3 g/L of salt in leaves. Whereas, a decrease in potassium and phosphorus was noted in saline condition (Fig. 6).

The accumulation of proline in plants in response to environmental stress has been considered by some researchers as an adaptive trait for stress tolerance (Rhodes and Hanson, 1993). Determination of its rate is a useful test to monitor the physiological state and evaluate the tolerance of the plant to stress (Abrahám *et al.,* 2010). Application of salt stress increased the accumulation of proline in sprouted seeds, leaves and roots of pepper (Fig. 4). Our finding agrees with research done on okra (Habib *et al.,* 2012), Pistachier of the atlas (Benhassaini *et al.,* 2012), rice (Joseph *et al.,* 2015) in two varieties of bananas (Belfakih *et al.,* 2013), tomato (Hernandez *et al.,* 2000), barley (Hassani *et al.,* 2008) and finally on ornamental species (Denden *et al.,* 2005). It is established that proline is certainly one of the most widespread osmolytes. At an osmotic stress, its biosynthesis is increased in chloroplasts (Székely *et al.,* 2008) and its accumulation in stressed plants has a protective function (Verbruggen and Hermans, 2008), following the disruption of protein metabolism (Lepengue *et al.,* 2012). The process of accumulation of proline in foliar tissues is considered as a criterion of adaptation (Szabados and Savouré, 2010). Stewart (1981) suggests that the accumulation of proline to a given stress is both the result of a decrease in protein synthesis and a conversion of glutamate to proline.

The accumulation of soluble sugars in leaves under saline stress in pepper has also been demonstrated in many species such as barley (Hassani *et al.,* 2008) and alfalfa

(Ibriz *et al.,* 2004). In addition, salinity reduced, curiously, the sugars in germinated seeds of the pepper (Fig. 5) Chargui variety, although these findings unusual for salinity were reported by Ottow *et al.* (2005) in the leaves of the olive tree and in Belfakih *et al.* (2013) in banana roots. These results may suggest the sensitivity of Chargui variety to salt. However, the accumulation of soluble sugars observed at the foliar level is among the most observed phenomena in the response to stress (Hajihashemi *et al.*, 2006). This increase would be due, according to some authors to a modification of enzymatic activities related to carbohydrate metabolism.

Secondary metabolites play a major role in adapting plants to environmental conditions, mainly stress conditions. In fact, these metabolites often accumulate in plants subjected to various biotic and abiotic stresses (El-Rokiek, 2007). In this study and in salt conditions, the pepper seeds untreated stimulate rather the accumulation of all secondary metabolites tested (Table 1). The results are in agreement with the findings of Ghezal *et al.* (2016).

The aqueous extracts of both algae selected for this study were used in pepper seed priming, and the results in previous studies showed improved germination and seedling growth.

However, the priming increased membrane permeability in pepper seeds germinated and seedling with increasing NaCl stress and with time of incubation. In this context, Ghezal *et al.* (2016) reported that electrolyte leakage (EL) increased in pea germinated seeds and seedlings, developed from seeds primed with aqueous extract of *Typha angustifolia* leaves, under salt stress. In some cases, the priming was beneficial in all cases where the electrolyte leakage was reduced compared to untreated seeds which seem due to better membrane repair during the re-drying process following priming, Farooq *et al.* (2011) obtained similar results.

MDA is one of the most studied parameters for evaluating a plant's tolerance to environmental stresses. Data herein demonstrated that MDA content of pepper germinated seeds was reduced in the presence of salt stress (Fig. 2). Similar results were observed in the corn seeds (Zheng *et al.,* 2008) and in seeds of *Pisum sativum* treated with aqueous extract of the leaves of *Typha angustifolia* L (Ghezal *et al.*, 2016). For pepper seedling MDA content, it was increased in saline conditions. In this context, Ghezal *et al.* (2016) reported that the content of MDA in seedling from pea seeds treated with leaves aqueous extract of *T. angustifolia*, was increased in the presence of salt. In consequent, a decrease in EL and MDA content in primed seeds and seedlings developed from primed seeds in normal or salt conditions, suggesting a membrane protection.

The decrease in the activity of dehydrogenases could be a reflection of cell damage due to exposure to NaCl. Indeed, the lower production of formazan was recorded in the presence of salt stress, where mitochondrial respiration appears to be reduced significantly. The priming was beneficial in all cases, as for, data showed that the formazan level was higher in primed pepper germinated seeds, roots and leaves indicating greater metabolic activity (Fig. 3). Similar results were reported by Ghezal *et al*. (2016) where the content of fromazan in seedlings of pea from seed treated, have been enhanced in the absence and presence of salt which indicates the improvement of the metabolic activity of cells. Orzesko and Podlaski (2003) reported that priming made the nutrients available to the plants, hence, causing extensibility in cell wall of the roots and an increase in seed respiration intensity.

Consistent with its role in enhancing growth and development under salinity, priming lead a increase in proline content in pepper germinated seeds, leaves and roots in seedling under saline conditions (Fig. 4). This same results are found in Szepesi *et al.* (2005) reported that pretreatment with salicylic acid offers protection against salinity in tomato plants, probably due to the accumulation of osmolytes such as proline. The accumulation of proline can regulate many processes necessary for the survival of plants under saline condition (Maggio *et al.,* 2002). According to Girija *et al.* (2002) the accumulation of proline could possibly be due to degradation of proline rich protein or denovo synthesis of proline.

By treated seeds, total soluble sugar synthesis was beneficial in seedlings from the treated seeds as compared to the untreated seeds (Fig. 5). This same results are found in (Ghezal *et al.,* 2016) and coriander (Ben Fredj *et al.,* 2014). The increased accumulation of soluble sugars in primed plants under stress emphasizes the positive role of priming in protecting plants from the damaging effects of salinity (Tamimi, 2016). Due to increased starch hydrolysis, assures the important role of priming in either increasing the activities of hydrolytic enzymes or inducing the de novo synthesis thereby producing germination metabolites in requisite amounts (Lee and Kim, 2000).

In this study Salinity and priming had moreover significant impact on mineral balance in pepper. An increase in Na^+ while decrease in K^+ and P were registered, but with significant ($P<0.05$) differences in the presence of NaCl (Fig. 6). Ghezal *et al.* (2016) reported the same results in pea seed treated with the aqueous extract of *T. angustifolia* under salt condition. Similarly, Demirkaya (2014) who investigated the tomato plants developed from hydroprimed seeds, and in saline conditions, recorded the same results. Seed priming obtained the inhibitory effect of salt stress on pepper germinated seeds and leaves and all of them positively responded to seed priming. Previous results obtained the same effect of saline stress in celery (Pardossi *et al.,* 1999) and pepper (Chartzoulakis and Klapaki, 2000). However, seed priming induced avoidance of coriander shoot from toxic and nutrient deficiency effects of salinity on growth because of less Na^+ but more K^+. The antagonistic relation between Na^+ and K^+ indicates that, priming could play a role in modifying K^+/Na^+ selectivity

under salt stress (Azooz and Al-Fredan, 2009).

Secondary metabolites play a major role in the adaptation of plants to environmental conditions and the circumvention of stress conditions. Indeed, it has been reported that accumulation often occurs in plants subjected to various biotic and abiotic stresses (Camacho-Cristábal *et al*., 2002). In this study the enhanced accumulation of total phenols, flavonoids and alkaloids in roots and aerial parts of pepper were recorded as a response to the presence of NaCl and also to the priming treatment. Many researchers agree that higher concentrations of secondary metabolites might result in a more resistance in plants against environmental stresses (Baldwin *et al*., 2002). Priming of pepper seeds with thalli aqueous extracts of *J. rubens* and *P. pavonica* stimulates rather the production of secondary metabolic: polyphenols, flavonoids and alkaloids with or without salt conditions.

Conclusion

Primed seeds were better able to germinate and develop into a seedling under salt stress by modulating membrane stability, sugar metabolism and ionic homeostasis. These findings suggest that the seed bio-priming with thalli extracts of *J. rubens* et *P. pavonica* could be considered as an effective alternative method to improve the resistance salt stress in pepper. In perspective, it is important to continue the study by testing priming effect on seed yield on the field and during the culture later stages.

References

Abrahám, E., C. Hourton-Cabassa, L. Erdei and L. Szabados, 2010. Methods for determination of proline in plants. *Methods Mol. Biol*., 639: 317–331

Allakhverdiev, S.I., A. Sakamoto, Y. Nishiyama, M. Inaba and N. Murata, 2000. Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant Physiol*., 123: 1047–1056

Azooz, M.M. and M.A. Al-Fredan, 2009. The inductive role of vitamin C and its mode of application on growth, water status, antioxidant enzyme activities and protein patterns of *Vicia faba* L cv. Hassawi grown under seawater irrigation. *Amer. J. Plant Physiol.*, 4: 38–51

Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205–207

Baldwin, I.T., A. Keßler and R. Halitschke, 2002. Volatile signaling in plant-plant-herbivore interactions: what is real? *Curr. Opin. Plant Biol*., 5: 351–354

Belfakih, M., M. Ibriz, A. Zouahri and S. Hilali, 2013. Effet de la salinité sur la croissance des deux variétés de bananier « grande naine » et « petite naine » et leur nutrition minérale au Maroc. *J. Appl. Biosci*., 63: 4689–4702

Ben Fredj, M., K. Zhani, C. Hannachi, T. Mehwachi and A. Belanger, 2014. Effect of priming on growth, biochemical parameters and mineral composition of different cultivars of coriander (*Coriandrum sativum* L.) under salt stress. *J. Stress Physiol. Biochem.*, 10: 84–109

Benhassaini, H., A. Fetati, A.M. Hocine and M. Belkhodja, 2012. Effect of salt stress on growth and accumulation of proline and soluble sugars on plantlets of *Pistacia atlantica* Desf. subsp. atlantica used as rootstocks. *Biotechnol. Agron. Soc. Environ.*, 16: 159–165

Camacho-Cristábal, J.J., D. Anzelloti and A. González-Fontes, 2002. Changes in phenolic metabolism of tobacco plants during short-term boron deficiency. *Plant Physiol. Biochem.*, 40: 997–1002

Chartzoulakis, K. and G. Klapaki, 2000. La réponse de deux verts piments hybrides maison à la salinité de NaCl lors de différent stades de croissance. *Sci. Hortic.*, 86: 247–260

Demirkaya, M., 2014. Improvement in tolerance to salt stress during tomato cultivation. *Turk. J. Biol.*, 38: 1–7

Denden, M., T. Benttai, A. Salhi and M. Mathlouthi, 2005. Effect of salinity on chlorophyll fluorescence, proline content and floral production in three ornamental species. *Tropicultura*, 23: 220–225

Doblinski, P.M.F., M.L.L. Ferrarese, D.A. Huber, C.A. Scapim, A.L. Braccini and O. Ferrarese-Filho, 2003. Peroxidase and lipid peroxidation of soybean roots in response to p-coumaric and p-hydroxybenzoic acids. *Braz. Arch. Biol. Technol.*, 46: 193–198

Dubois, M., K.A. Gilles, P.A. Hamilton, A. Ruberg and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, 28: 350–356

El-Rokiek, K.G., 2007. Evaluation the physiological influence of benzoic and cinnamic acids, alone or in combination on wheat and some infested weeds comparing with the herbicide isoproturon. *Ann. Agric. Sci*., 52: 45–58

Eshbaugh, W.H., 1993. History and Exploitation of a Serendipitous New Crop Discovery. *In: New Crops*, pp: 132-139. Janick, J. and J.E. Simon (eds.). Wiley, New York, USA

Falleh, H., I. Jalleli, R. Ksouri, M. Boulaaba, S. Guyot, C. Magné and C. Abdelly, 2012. Effect of salt treatment on phenolic compounds and antioxidant activity of two *Mesembryanthemum edule* provenances. *Plant Physiol. Biochem*., 52: 1–8

Farooq, M., T. Aziz, H. ur Rehman, A. ur Rehman, S.A. Cheema and T. Aziz, 2011. Evaluating surface drying and re-drying for wheat seed priming with polyamines: effects on emergence, early seedling growth and starch metabolism. *Acta Physiol. Plant.*, 33: 1707–1713

Farooq, M., S.M.A. Basra and K. Hafeez, 2006. Rice seed invigoration by osmohardening. *Seed Sci. Technol*., 34: 181–187

Foti, R., K. Abureni, A. Tigere, J. Gotosa and J. Gerem, 2008. The efficacy of different seed priming osmotica on the establishment of maize (*Zea mays* L.) caryopses. *J. Arid Environ*., 72: 1127–1130

García-Sánchez, M., I. Garrido, I. De Jesús Casimiro, P.J. Casero, F. Espinosa, I. García-Romera and E. Aranda, 2012. Defence response of tomato seedlings to oxidative stress induced by phenolic compounds from dry olive mill residue. *Chemosphere*, 89: 708–716

Ghezal, N., I. Rinez, H. Sbai, I. Saad, M. Farooq, A. Rinez, I. Zribi and R. Haouala, 2016. Improvement of Pisum sativum salt stress tolerance by bio-priming their seeds using *Typha angustifolia* leaves aqueous extract. *S. Afr. J. Bot.*, 105: 240–250

Ghassemi-Golezani, K., AA. Aliloo, M. Valizadeh and M. Moghaddam, 2008. Effects of hydro and osmo-priming on seed germination and field emergence of lentil (*Lens culinaris* Medik.). *Not. Bot. Hortic. Agrobot. Cluj-Napoca*, 36: 29–33

Ghiyasi, M., A. Abbasi Seyahjani, T. Mehdi, A. Reza and S. Hojat, 2008. Effect of osmopriming with polyethylene glycol (8000) on germination and seedling growth of wheat (*Triticum aestivum* L.) seeds under salt stress. *Res. J. Biol. Sci.*, 3: 1249–1251

Girija, C., B.N. Smith and P.M. Swamy, 2002. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). *Environ. Exp. Bot.*, 47: 1–10.

Hassani, A., A. Dellal, M. Belkhodja and M. Kaid-Harche, 2008. Effet de la salinité sur l'eau et certains osmolytes chez l'orge *Hordeum vulgare*. *Eur. J. Sci. Res.*, 23: 61–69

Habib, N., M. Ashraf, Q. Ali and R. Perveen, 2012. Response of salt stressed okra (*Abelmoschus esculentus* Moench.) plants to foliar-applied glycine betaine and glycine betaine containing sugarbeet extract. *S. Afr. J. Bot.*, 83: 151–158

Hajlaoui, H, M. Denden and M. Bouslama, 2007. Étude de la variabilité intraspécifique de tolérance au stress salin du pois chiche (*Cicer arietinum* L.) au stade germination. *Tropicultura*, 25: 168–173

Hajibagheri, M.A., D.M.R. Harvey and T.J. Flowers, 1987. Quantitative distribution within the root cells of salt-sensitive and salt-tolerant maize varieties. *New Phytol.*, 105: 367–379

Hajihashemi, S., K. Kiarostami, S. Enteshari and A. Saboora, 2006. The effects of salt stress and paclobutrazol on some physiological parameters of two salt tolerant and salt sensitive cultivars of wheat. *Pak. J. Biol. Sci.,* 9: 1370–1374

Hernandez, J., A. Jimenez, P. Mullineaux and F. Sevilla, 2000. Tolerance of pea plants (*Pisum sativum*) to long-term salt stress is associated with induction of antioxidant defences. *Plant Cell Environ.,* 23: 853–862

Hoagland, D.R. and D.I. Arnon, 1950. The water-culture method for growing plants without soil. *Calif. Agric. Exp. Station*, 347: 1–32

Ibrahim, E.A., 2016. Seed priming to alleviate salinity stress in germinating seeds. *J. Plant Physiol.*, 192: 38–46

Ibriz, M., I. Thami Alami, L. Zenasni, C. Alfaiz and M. Benbella, 2004. Production des luzernes des régions pré-sahariennes du Maroc en conditions salines. *Fourrages*, 180: 527–540

Jamil, M., D.B. Lee, K.Y. Jung, M. Ashraf, S.C. Lee and E.S. Rha, 2006. Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. *J. Cent. Eur. Agric*., 7: 273–282

Joseph, E.A., V.V. Radhakrishnan and K.V. Mohanan, 2015. A study on the accumulation of proline an osmo protectant amino acid under salt stress in some native rice cultivars of north kerala, India. *Univ. J. Agric. Res.*, 3: 15–22

Khanh, T.D., N.H. Hong, T.D. Xuan and I.M. Chung, 2005. Paddy weeds control by medicinal and leguminous plants from Southeast Asia. *Crop Prot.*, 24: 421–431

Lepengue, A-N., I. Mouaragadja, S. Ake and B. M'batchi, 2012. Quelques aspects biochimiques de la réaction de la roselle (*Hibiscus sabdariffa* L. var. sabdariffa) au stress salin. *J. Appl. Biosci.*, 49: 3452–3458

Laüchli, A. and E. Epstein 1990. Plant responses to saline and sodic conditions. *In*: *Agricultural Salinity Assessment and Management*, pp: 113–137. Tanji, K.K. (ed.). American Society of Civil Engineers. 1801 Alexander Bell Drive. Reston, Virginia, USA

Lutts, S., J.M. Kinet and J. Bouharmont, 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann. Bot.*, 78: 389–398

Lee, S.S. and S. Kim, 2000. Total sugars, α-amylase activity, and germination after priming of normal and aged rice seeds. *Kor. J. Crop Sci.*, 45: 108–111

Maggio, A., S. Miyazaki, P. Veronese, T. Fujita, J.I. Ibeas, B. Damsz, M.L. Narasimhan, P.M. Hasegawa, R.J. Joly and R.A. Bressan, 2002. Does proline accumulation play an active role in stress-induced growth reduction? *Plant J.*, 31: 699–712

Martin-Prével, P., J. Gonard and P. Gautier, 1984. *Méthodes Analytique de Référence, in L'analyse Végétale Dans le Contrôle de L'alimentation des Plantes Tempérées et Tropicales*. Edition Lavoisier TEC & DOC

Mc Donald, M.B., 2000. Seed priming. *In: Seed Technology and its Biological Basis*, pp: 287-325. Black, M. and J.D. Bweley (eds.). Sheffield Academic press Ltd., Sheffieid

Ommezzine, F., H. Haoula, A. El Ayeb and N. Boughanmi, 2009. Allelopathic and antifungal potentialities of *Padina pavonica* (L.) extract. *J. Plant Breed. Crop Sci.,* 1: 94–203

Ottow, E., M. Brinker, E. Fritz, T. Teichmann, W. Kaiser, M. Brosche, J. Kangasjarvi, X. Jiang and A. Polle, 2005. *Populus euphratica* Displays Apoplastic Sodium Accumulation, Osmotic Adjustment by Decreases in Calcium and Soluble Carbohydrates, and Develops leaf succulence under salt stress. *Plant Physiol.*, 139: 1762–1772

Orzesko, R.A. and S. Podlaski, 2003. The effect of sugar beet seed treatment on their vigour. *Plant Soil Environ.,* 49: 249–254

Pardossi, A., G. Bagnoli, F. Malorgio, C.A. Campiotti and F. Tognoni, 1999. NaCl effects on celery (*Apium graveolens* L.) grown in NFT. *Sci. Hortic*., 81: 229–242

Patade, V.Y., K. Maya and A. Zakwan, 2011. Seed priming mediated germination improvement and tolerance to subsequent exposure to cold and salt stress in Capsicum. *J. Seed Sci. Technol.,* 4: 125–136

Patanea, C., V. Cavallaro and S.L. Cosentino, 2009. Germination and radicle growth in unprimed and primed seeds of sweet sorghum as affected by reduced water potential in NaCl at different temperatures. *Indust. Crops Prod.,* 30: 1–8

Parvaiz, A. and S. Satyawati, 2008. Salt stress and phyto-biochemical responses of plants-a review. *Plant Soil Environ.,* 54: 89–99

Quettier, D.C., B. Gressier, J. Vasseur, T. Dine, C. Brunet, M.C. Luyckx, J.C. Cayin, F. Bailleul and F. Trotin, 2000. Phenolic compounds and antioxidant activates of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *J. Ethnopharm.*, 72: 35–42

Rhodes, D. and A.D. Hanson, 1993. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annu. Rev. Plant Physiol. Mol. Biol.*, 44: 357–384

Rozbeh, F., S. Saeed and M. Delfie, 2011. The effect of NaCl seed priming on salt tolerance, antioxidant enzyme activity, proline and carbohydrate accumulation of Muskmelon (*Cucumis melo* L.) under saline condition. *Afr. J. Biotechnol*., 6: 1363–1370

Sampietro, D.A., A.V. Marta and I.I. Maria, 2006. Plant growth inhibitors isolated from sugarcane (*Saccharum officinarum*) straw. *J. Plant Physiol.*, 163: 837–846

Sivritepe, N., H.O. Sivritepe and A. Eris, 2003. The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. *Sci. Hortic.*, 97: 229–237

Suleman, M.K., N.R. Bhatt, S. Jacob and R.R. Thomas, 2011. Germination studies in *Ochradenus baccatus* delile, *Peganum harmala* L. and *Gynandris sisrinchium* parl. *Res. J. Seed Sci.*, 4: 58–63

Stewart, C.R., 1981. Proline accumulation: Biochemical aspects. *In*: *Physiology and Biochemistry of Drought Resistance in Plants*, pp: 243-259. Paleg, L.G. and D. Aspinall (eds.). Sydney, Acad, Press

Stumpf,D.K., 1984. Quantification and purification of quaternary ammonium compounds from halophyte tissue. *Plant Physiol*., 75: 273–274

Szabados, L. and A. Savouré, 2010. Proline: a multifunctional amino acid, *Trends Plant Sci*., 15: 89–97

Székely, G., E. Abrahám, A. Cséplo, G. Rigó, L. Zsigmond, J. Csiszár, F. Ayaydin, N. Strizhov, J. Jásik, E. Schmelzer, E. Koncz and L. Szabados, 2008. Duplicated *P5CS* genes of Arabidopsis play distinct roles in stress regulation and developmental control of proline biosynthesis. *Plant J*., 53: 11–28

Szepesi, A., J. Csiszar, S.Z. Bajkan, K. Gemes, F. Horvath, L. Erdei, A. Deer, L.M. Simon and I. Tari, 2005. Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt. *Acta Biol. Szagediensis*, 49: 123–125

Tamimi, S.M., 2016. Effect of seed priming on growth and physiological traits of five Jordanian wheat (*Triticum aestivum* L.) landraces under salt stress. *J. Biosci. Agric. Res.,* 11: 906–922

Taylor, A.G. and G.E. Harman, 1990. Concepts and technologies of selected seed treatments. *Annu. Rev. Phytopathol.*, 28: 321–339

Taylor, W.R., 1960. *Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas*, pp: 234-235. Ann. Arbor: Univ. Michigan Press, Michigan, USA

Velioglu, Y.S., G. Mazza, L. Gao and B.D. Oomah, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.,* 46: 4113–4117

Verbruggen, N. and C. Hermans, 2008. Proline accumulation in plants: a review. *Amino Acids*, 35: 753–759

Zheng, Y., A. Jia, T. Ning, J. Xu, Z. Li and G. Jiang, 2008. Potassium nitrate application alleviates sodium chloride stress in winter wheat cultivars differing in salt tolerance. *J. Plant Physiol.,* 165: 1455-1465

Zhu, J.K., 2000. Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Physiol*., 124: 941–948

Zid, E. and C. Grignon, 1991. *Les tests de sélection précoce pour la résistance des plantes au stress. Cas des stress salin et hydrique*, pp : 91-108. Dans : L'Amélioration des Plantes pour l'Adaptation aux Milieux Arides. Ed. Aupelf-Uref, John Libbey Eurotext, Paris, France

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