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Salicylic acid priming in *Hedysarum carnosum* and *Hedysarum coronarium* reinforces NaCl tolerance at germination and the seedling growth stage

Hanen Dallali[1], Elhem Mallek Maalej[2], Néziha Ghanem Boughanmi[1], Rabia Haouala[3]

[1]Department of Life Sciences. Faculty of Sciences of Bizerte. University of Carthage 7021 Zarzouna, Tunisia
[2]Laboratory of Biotechnology and Plant Physiology, INRAT Rue Hédi Karray 2049 Ariana, Tunisia
[3]Department of Biological Sciences and Plant protection, Higher Institute of Agronomy Chott Meriem, University of Sousse 4042, Tunisia

***Corresponding author: dallalihanen@yahoo.fr**

Abstract

In this study, the effect of 100 µM salicylic acid (SA) priming on *Hedysarum coronarium* (a cosmopolitan species) and *Hedysarum carnosum* (a species endemic to Tunisian salt flats) subjected to 200 mM of NaCl at germination and seedling growth stages was analyzed. SA priming had a beneficial effect on germination, which was related to imbibition rather than reserve mobilisation, since the dry biomass of cotyledons was unchanged by salt stress and SA priming. The results showed that SA priming at the seedling growth stage alleviated salt-induced oxidative stress by reducing malondialdehyde (MDA) and H_2O_2 content. In addition, the two species demonstrated differential H_2O_2 detoxification with the stimulation of catalase (CAT) activity in both species, but guaiacol peroxidase (GPX) stimulation was found to only occur in *H. coronarium*. Surprisingly, *H. coronarium* seedlings proved to be more tolerant to NaCl stress than *H. carnosum* seedlings. SA priming reinforced the salt tolerance of *H. coronarium* at both germination and seedling growth stages.

Keywords: germination; leguminous species; oxidative stress; priming; salicylic acid; salt stress.
Abbreviations: C - Cotyledons; CAT - Catalase; Chl - Chlorophyll; CNP - Control Non Primed seeds; CP - Control Primed seeds; GPX - Guaiacol peroxidase; H_2O_2 - Hydrogen peroxide; MDA - Malondialdehyde; R+H - Radicle + Hypocotyl; ROS - Reactive oxygen species; SA - Salicylic acid; SOD - Super oxide dismutase; TNP - Treated Non Primed seeds; TP - Treated and Primed seeds.

Introduction

Salinity is one of the major abiotic stresses, and approximately 800 million hectares of land are affected by high salt levels throughout the world (Munns, 2005), with NaCl being the most prevalent. Both osmotic and specific ionic stresses from salinity can cause stunted growth and a reduced plant yield (Munns, 2002). Ionic stress includes K^+ and Ca^{2+} deficiency and accumulation of cytotoxic ions. At the cellular level, salt tolerance is related to Na^+ and Cl^- exclusion from the cytosol and compartmentalization into vacuoles and cell walls. This mechanism is efficiently achieved in halophytes but not glycophytes, where high salt levels in the cytosol induce the death of old leaves (Munns, 2002). In addition, osmotic and ionic stress can trigger a secondary oxidative stress that leads to an increase of reactive oxygen species (ROS). At low levels, ROS act as signal molecules that induce adaptive responses (Del Rio et al., 2002); however, at high levels they are cytotoxic and can induce oxidative damage to biomolecules, and particularly lipids. The oxidation of lipids is considered to be the most damaging process that can worsen oxidative stress through the production of lipid-derived radicals (Gil and Tuteja, 2010). Lipid peroxydation, which is initiated by the hydroxyl ion (OH^-), causes damage to organelles and cell integrity and leads to an accumulation of degradation compounds such as malondialdehyde (MDA). Cells must prevent the generation of $OH^{\cdot -}$ through Fenton's reaction in order to counterbalance the absence of scavenging enzymatic mechanisms (Gil and Tuteja, 2010). Moreover, stress-induced oxidative damage have been related to the accumulation of H_2O_2 (Sairam and Srivastava, 2000), which is produced by SOD detoxification of $O_2^{\cdot -}$ ions, cellular mechanisms (photorespiration, photosynthesis) and, in stress conditions, by activities of enzymes such as NADPH oxidase (Karuppanapandian et al, 2011). H_2O_2 is detoxified by peroxidases and catalase, and the activities of these enzymes have been shown to increase in response to environmental salinity stress (Noctor and Foyer, 1998).

The vast majority of cultivated plants are glycophytes, among them legumes, which have high nitrogen content and constitute an important source of nutrients for both animals and humans. Salt stress affects nitrogen symbiotic fixation and plant development, in particular the germination stage, which is known for limiting plant establishment under these adverse conditions. In this primary developmental stage, salt stress affects both hydration and storage compound mobilization (Copeland and McDonald, 2001), which leads to a reduction in seedling growth (Maghsoudi and Maghsoudi, 2008). In seed technology, priming exploits the ability of mature orthodox seeds to survive cycle(s) of imbibition and drying in order to improve stress tolerance (Schwember and Bradford, 2010). This reversible germination arrest, which enables seedlings to survive sudden water stress while maintaining their desiccation tolerance (Gallardo et al., 2001), is triggered by abscisic acid (ABA) (Belin and Lopez-

Molina, 2008). Priming-induced stress tolerance correlates with the occurrence of late embryogenesis abundant (LEAs) proteins and heat shock proteins (HSPs) that accumulate during seed late maturation under abscisic acid (ABA) control (Gallardo et al., 2001). These proteins, which are involved in drying tolerance and seed longevity in orthodox seeds, are also induced by salinity and can act as osmoprotectants and antioxidants (Kalemba and Pukacka, 2007). LEAs and HSPs are synthesized *de novo* in the presence of exogenous salicylic acid (SA) and have been shown to markedly improve germination under salt stress in *Arabidopsis* seeds by inducing ROS (Rajjou et al., 2006). In *Vicia faba*, the use of SA during seed priming improved salt tolerance by reducing oxidative damage (Azooz, 2009). However, SA action was dose-dependent, whereby low levels induced an antioxidant defence, but high levels induced a programmed cell death pathway (Borsani et al., 2001). The aim of this study was to determine the effects of seed priming with SA (100 µM) on the response to NaCl (200 mM) stress of two leguminous plants: *Hedysarum coronarium*, a cosmopolitan perennial herb, that is used as fodder and hay for animals (Bassendowski et al., 1989), and *Hedysarum carnosum*, which is an endemic species to Tunisian Sebkhas (Arabic word for salt flats) and known for its tolerance to salinity. This herb spontaneously grows in central and south Tunisia (Boussaid et al., 1989). We compared the effect of seed priming with SA on these two species at two developmental stages: germination and seedling growth. For seedling growth, we focused on growth (elongation and dry biomass) and ionic status (Na^+ and K^+ concentration). Salt-induced oxidative stress was evaluated by measuring the concentration of H_2O_2, and oxidative damage was determined by measuring the MDA content and electrolyte leakage rate. H_2O_2 detoxification was measured by guaiacol peroxidase (GPX) and catalase (CAT) activities. In addition, we investigated whether SA seed priming could mitigate the adverse effects of salt.

Results

Comparative effects of SA priming on germination and seedling growth in controls and NaCl treated seeds

NaCl delayed germination for five days in *H. coronarium* and six days in *H. carnosum*. NaCl also reduced the germination rate by 46% in *H. coronarium* and 54 % in *H. carnosum*. SA priming improved germination of seeds exposed to NaCl by reducing the germination latency time by approximately three days in *H. carnosum* and two days in *H. coronarium*. In addition, SA priming caused a greater increase in the germination rate in *H. coronarium* than *H. carnosum* (40% vs. 20%, respectively) (Fig. 1).During the growth stage of control seedlings, SA priming raised the hypocotyl length in *H. carnosum* and *H. coronarium* by 25% and 16%, respectively. Exposure to salt stress resulted in a marked decrease of this parameter, with a 67% and 50% reduction observed in *H. coronarium* and *H. carnosum*, respectively. In addition, SA seed priming improved the salt-reduced length in *H. coronarium* and *H. carnosum* by 50% and 30%, respectively (Fig. 2A). Exposure to salt stress inhibited the growth of *H. carnosum* and *H. coronarium* seedlings as a result of a decrease in their radical + hypocotyl (R + H) dry biomass by 60% and 55%, respectively. Under salt stress, the cotyledon dry biomass remained unchanged in both species, and SA seed priming had no effect on biomass production (Fig. 2B).

Exposure to NaCl stress led to an increase in Na^+ content, which was more pronounced in R + H than in cotyledons of both species. In *H. carnosum*, SA potentiated salt stress which led to a three-fold increase in Na^+ content in cotyledons, whereas no such effect was observed in *H. coronarium* cotyledons or in R+H of the two species (Fig. 3A). In *H. carnosum*, salinity induced a 50% decrease in K^+ content in R + H. In contrast, this content remained unchanged in *H. coronarium*. SA priming reduced the K^+ content in R + H but a slight increase was observed in seedling cotyledons subjected to NaCl stress of both species (Fig. 3B).

SA priming reduced the induced oxidative stress and cell damage

Exposure to salt stress resulted in a 37% reduction in the total chlorophyll (chl) content in *H. carnosum*, whereas it led to an 85% increase in chl content in *H.coronarium* (Fig. 4A). In both species, this was mainly a result of a dramatic effect on chl. a content, which decreased by 72% in *H. carnosum* and increased by 77% in *H. coronarium*. SA seed priming improved chl. a content in seedlings from both species that were exposed to salt stress. This improvement was more efficient in *H. coronarium* than in *H. carnosum* compared to the control. In addition there was a decrease in chl. b content in seedlings of both species compared to the control value. Importantly, SA priming led to recovery of both chl. a and chl. b content in *H. coronarium* to the original concentration; however, it led only to the recovery of ch. b content in *H. carnosum*. SA priming raised lipid peroxidation in control seedlings, which led to an elevation in MDA content in both species that was higher in *H. coronarium* than *H. carnosum*. Exposure to salt stress increased the MDA content of cotyledons two-fold in *H. carnosum*, and 0.7 fold in *H.coronarium* (Fig. 4B). In salt stressed *H. carnosum* seedlings, SA priming induced a decrease in MDA content only in cotyledons. In salt-stressed *H. coronarium*, SA priming reduced MDA content in R + H to a similar level as in the control seedlings. The increase in lipid peroxydation in cotyledons of primed control seeds led to a 50% increase in electrolyte leakage (EL) content in *H. coronarium*, while EL content remained unchanged in *H. carnosum*. Similarly, salt stress induced a greater increase of EL in *H. carnosum* (Fig. 4C). In salt-stressed *H. carnosum* seedlings, SA priming slightly reduced EL, while it had no effect on salt-stressed *H. coronarium* seedlings.

SA priming differentially affects detoxification in H. carnosum and H. coronarium

Salt stress induced oxidative stress in both species, as observed by the increase in H_2O_2 content, with the H_2O_2 content in the cotyledons being higher than that of R + H and more pronounced in *H. carnosum* than in *H. coronarium* (Fig. 5A). SA priming reduced the H_2O_2 content of R + H and cotyledons in seedlings exposed to NaCl in both species, particularly in the non-photosynthetic organs of *H. carnosum* seedlings. Soaking seeds with 100 µM of SA slightly decreased the CAT activity in non-photosynthetic organs of control seedlings of both species (Fig. 5B), while GPX activity was stimulated only in *H. coronarium* seedlings (Fig. 5C). Salt stress induced an 11-fold increase of CAT activity in *H.carnosum* cotyledons, whereas it only induced an 8-fold increase in such activity in *H. coronarium*. The increase in GPX activity was more pronounced in *H. coronarium* (2.5-

and 2.25-fold increases in cotyledons and R + H, respectively) than in *H. carnosum* (2- and 1,5-fold increases in cotyledons and R + H, respectively). In salt-stressed seedlings, SA priming reduce CAT activity in cotyledons but increased activity in R + H of both species, and the increased was more pronounced in *H. coronarium* than in *H. carnosum*. SA priming, led to a slight decrease in GPX activity in *H. carnosum*, whereas it induced an increase in this activity in *H. coronarium* (60% and 20% in cotyledons and R + H, respectively).

Discussion

NaCl affects germination and seedling growth

In this study, we found that the presence of NaCl reduced the germination rate of the two *Hedysarum* species compared to the control as a consequence of salt osmotic effects, which reduced water availability. During imbibitions, water entry occurs through aquaporins, which have reduced expression in the presence of salt (Boursiac et al., 2005). More specifically, this reduction in the germination rate, which was more pronounced in *H. carnosum* than *H. coronarium*, is related to the specific ionic salt stress during the second phase of germination that corresponds to the activation of embryo growth using reserve metabolites (Nonogaki et al., 2010). Salt stress affected the growth of seedlings from the two studied species by reducing the dry weight of R + H and hypocotyl length as a result of altered translocation of nutriments to the growing axis (Prisco and Vieira, 1976) from the cotyledons, which are organs of reserve in legumes. The dry weight of cotyledons was not affected by NaCl in *Hedysarum* species. This may be partially due to a reduced reserve mobilization. In cotyledons, proteases are inhibited by Na^+/H^+ antiporter activity, which induces the alkalinization of protein storage vacuoles (Baranova et al., 2011). Salt stress also affects starch mobilization by reducing amylase activity (Voigt et al., 2009**)** and lipid storage breakdown through a reduction in the activity of glyoxysomal cycle enzymes (Ben Miled-Daoud and Cherif, 1992).

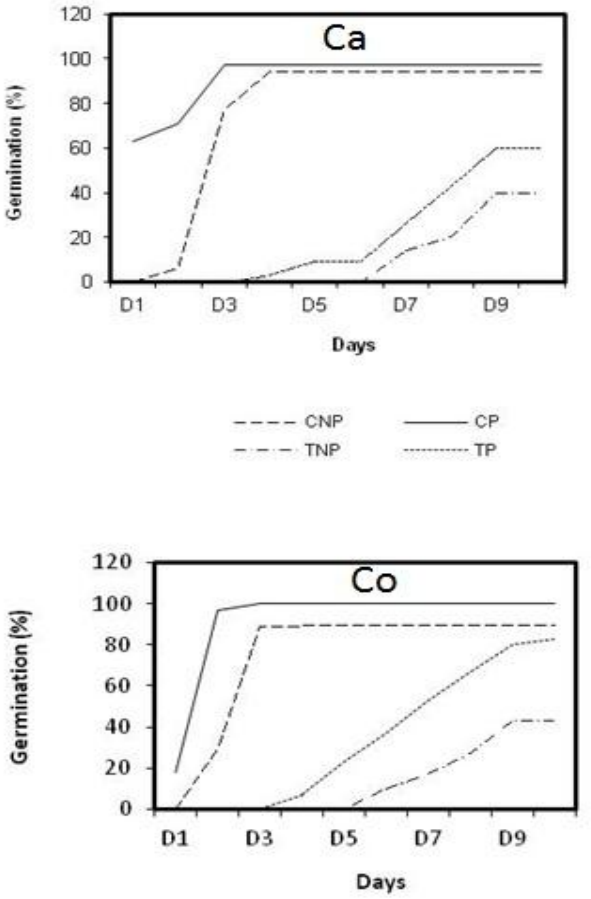

Fig 1. The effect of seed priming with 100 µM salicylic acid (SA) on the germination of *H.carnosum* (Ca) and *H. coronarium* (Co) in the presence of 0 and 200 mM of NaCl in CNP (0.0 mM NaCl-SA), CP (0.0 mM NaCl + SA), TNP (200 mM NaCl-SA), and TP (200 mM NaCl + SA).

NaCl affects the ionic and oxidative status

Salinity tolerance in plants is specifically related to the efficiency of limiting Na^+ translocation from roots to sites where high Na^+ concentrations disturb metabolic activities (Akram et al., 2007), such in as cotyledons, which are sites of reserve mobilization. In response to salt treatment, the cotyledons of the two studied species maintained a lower Na^+ content compared to the R + H. High Na^+ concentrations have been shown to negatively affect K^+ acquisition (Munns, 2002). We found that NaCl induced a decrease in K^+ content in organs of the two *Hedysarum* species, with the exception of the R + H of *H. coronarium*, where the K^+ content was unchanged. Adequate K^+ nutrition is considered to be an important condition in the tolerance of glycophytes to salinity (Zhu, 2000). Based on these observations, we found that *H. coronarium* was more tolerant to salinity than *H. carnosum* due to the lower Na^+ concentration and higher K^+ concentration. Like all abiotic stresses, NaCl induced oxidative stress in *Hedysarum* seedlings, which resulted in an increase in the H_2O_2 concentration. This increase was particularly marked in the photosynthetic organs, which are a major source of ROS in plants (Edreva, 2005) and are sites known for being very sensitive to NaCl stress. At these sites, salt-induced ROS accumulation induces plastid structural alterations, which cause damage to photosynthetic pigments, and in particular chlorophyll (Munns and James, 2003). The decrease in total chlorophyll content observed in *H. carnosum* cotyledons is related to the H_2O_2 accumulation, which is known to promote the degradation of these pigments as well as reduce their biosynthesis (Zhao et al., 2007). In contrast, the salt-induced increase in chlorophyll content in *H. coronarium* may be the result of an increase in the number of chloroplasts, as observed in NaCl-stressed rice, or an increase in chlorophyll biosynthesis, which is induced by H_2O_2 signaling to reduce salt damage (Misra et al., 1997). Since variations in chlorophyll content under salt stress conditions are related to differences in ROS production and the antioxidant capacity (Thipyapong et al., 2004), they could be used as criteria for the selection of tolerant crops (Eryilmaz, 2006), particularly on the basis of chl. a content, which is involved in the conversion of light energy. Salt stress induced an increase in chl. a content in *H. coronarium* whereas it led to a decrease in chl. a in *H. carnosum*. In addition, NaCl stress increased lipid peroxidation, which led to an MDA accumulation that was more pronounced in *H. carnosum* cotyledon. This observation was similar to that observed for the H_2O_2 concentration. Since low MDA content

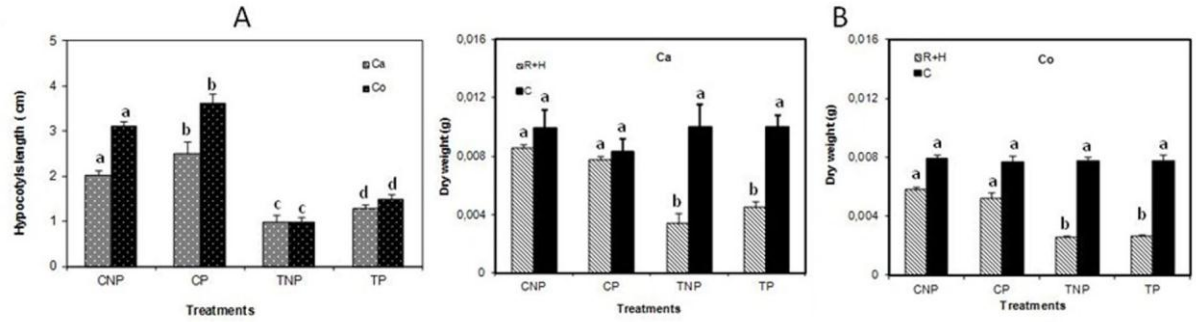

Fig 2. The effect of seed priming by 100 µM salicylic acid (SA) in *H. carnosum* (Ca) and *H. coronarium* (Co) on hypocotyl length (A) and dry weight (B) in cotyledons "C" and radicles plus hypocotyls "R + H" in 10-day-old seedlings treated with 0 and 200 mM NaCl.

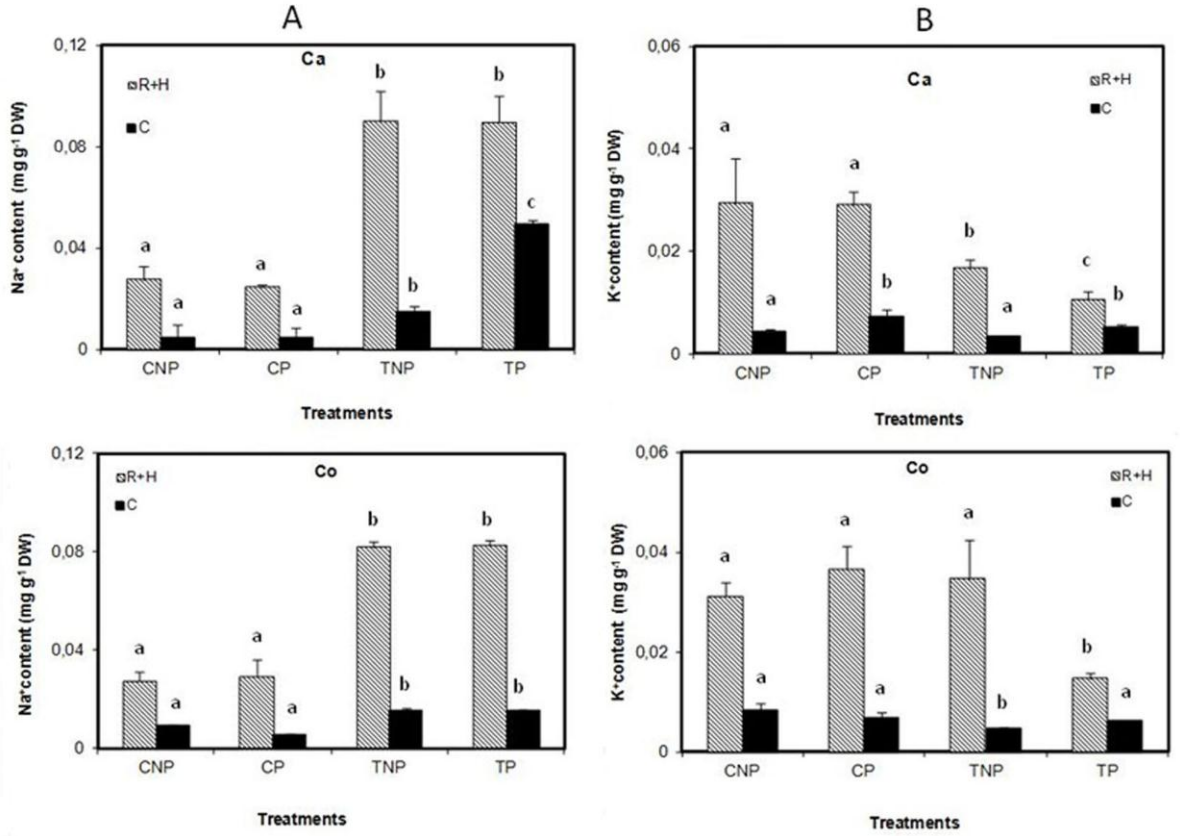

Fig 3. The influence of seed priming of *H.carnosum* (Ca) and *H. coronarium* (Co) on Na^+ (A) and K+ (B) content in cotyledons "C" and radicles plus hypocotyls "R+H" in 10-day-old seedlings treated with 0 and 200 mM NaCl.

is important in terms of salt tolerance, *H. coronarium* appeared to be more tolerant to salt stress than *H. carnosum*. The increase in MDA content was due to an accumulation of OH^- ions that oxidize membrane lipids, which leads to leaky membranes (Katsuhara et al., 2005). Salt treatment also increased in EL of cotyledons in the two studied species, but this increase was higher in *H. carnosum* than in *H. coronarium*. Based on the studied parameters, we found that *H. coronarium* is more tolerant to salt stress than *H. carnosum* at both the germination and seedling growth stages. The sensitivity of *H. carnosum* seedlings was unexpected, since this species is endemic to Tunisian Sebkhas and is known for its tolerance to salinity. Our results confirm previous studies that have shown that salt tolerance is developmentally regulated (Borsani et al., 2001). In *H. carnosum*, the mechanisms of salt tolerance are triggered later during the adult stage, which enables the plant to adapt to the high salt levels that are characteristic of Tunisian Sebkhas.

SA priming reinforces salt tolerance

SA priming improved the germination rate of the control, but had an even more substantial effect on the treated seeds of the two species evaluated, particularly *H. coronarium*. The increased germination rate by SA priming was also observed in the control as well as salt-stressed *Brassica napus* seedlings (Dolatabadian et al., 2008). SA might act in the imbibition stage by increasing osmotic adjustment (Escobar et al., 2010), but in the two studied species subjected to salinity conditions, the Na^+ concentration was unchanged in both species and the K^+ concentration was reduced in both

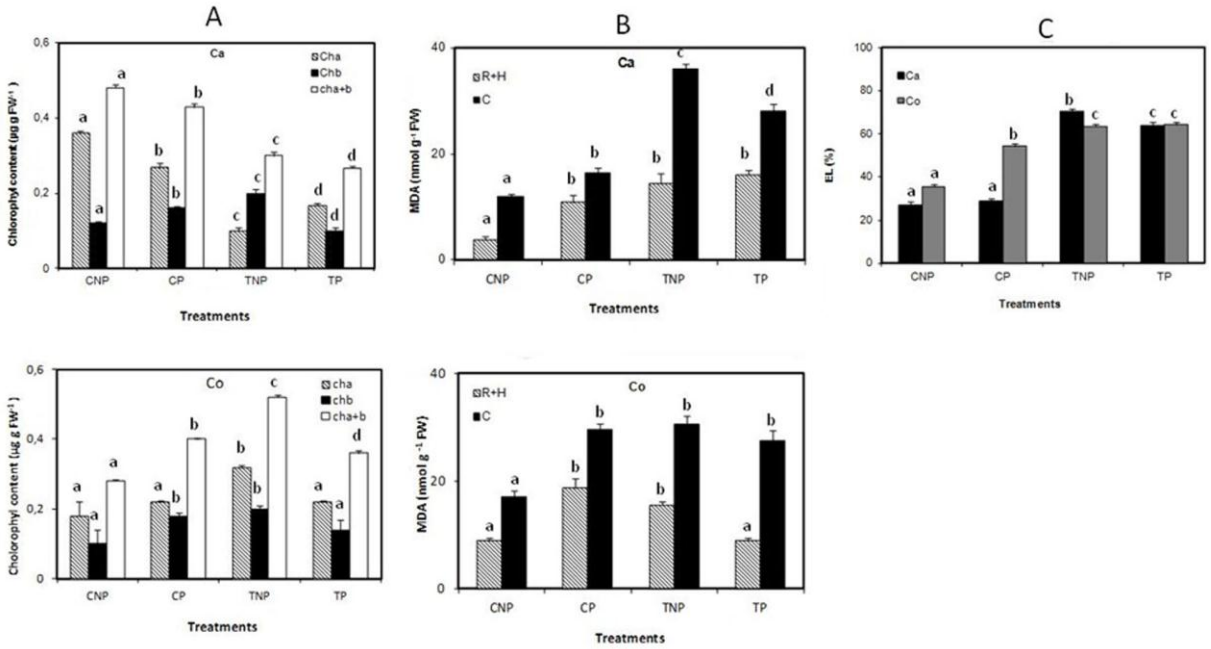

Fig 4. The influence of seed priming with 100 µM salicylic acid in *H.carnosum* (Ca) and *H. coronarium* (Co) on chlorophyll a, b, and total chlorophyll content (A), malondialdehyde (MDA) (B), and electrolyte leakage (EL) (C) in different organs of 10 day-old seedlings treated with 0 and 200 mM NaCl.

species in non-photosynthetic organs. This priming did not contribute to enhanced osmoregulation, which rather could be the result of an accumulation of compatible solutes (Szepesi et al., 2009). The shortening of the latency time observed in the two studied species as a result of SA priming, induced faster seed imbibitions in both the control and salt-stressed seedlings. This faster seed imbibition may be the result of aquaporin activation (Boursiac et al., 2005) through SA-induced acidification of the cytosol (Verdoucq et al., 2008). The SA-induced increase in the germination rate observed in the presence of NaCl may also be due to the activation of the second stage of germination, which corresponds to the embryo growth stage, through enhanced synthesis of proteins that are essential for germination (Rajjou et al., 2006**)** and the activation of the mobilization of reserve metabolites with low molecular weights (Nonogaki et al., 2010). LEAs and HSPs are synthesized in the presence of exogenous SA and have been shown to enable germinating *Arabidopsis* seeds to show a marked tolerance to salt stress (Rajjou et al., 2006). SA priming of *Hedysarum* seeds did not alleviate the adverse effects of NaCl on the seedlings' dry biomass production. In contrast, it has been shown that soaking seeds of *Vicia faba* with a high dose of SA (200 µM) improved the dry biomass production (Azooz, 2009). SA -priming induced stimulation of hypocotyl elongation in the control and, to a lesser extent, in salt-stressed seedlings, as a result of accelerated division induced by SA (Dolatabadian et al., 2008). Hypocotyl elongation may also be the result of an increase in SA-induced cell expansion, which acts as a proton donor and activates the plasma H^+-ATPase involved in wall-loosening according to the acid-growth theory of auxin action (Hager, 2003). Some studies have described the beneficial effects of SA -priming on specific ionic salt stress. For example, SA inhibits toxic Na^+ and Cl^- accumulation (Gunes et al., 2005), but also reduces potassium and calcium deficiencies (Al-Hakimi, 2006). In the *H. carnosum* and *H. coronarium* species, SA-priming at 100 µM did not provide any beneficial effect on K^+ and Na^+ nutrition. We even observed a potentiation of the NaCl-induced K^+ deficiency and Na^+ accumulation in the cotyledons of *H. carnosum* and in the H + R of both species, which was also observed by Borsani et al (2001) in a dose-dependent manner. In the two *Hedysarum* species from this study, exogenous SA treatment induced oxidative stress that increased lipid peroxidation (Gill and Tuteja, 2010) and led to an accumulation of MDA content, which was more pronounced in the cotyledons compared to the R + H. Borsani et al. (2001) also observed that SA action is potentiated at photosynthetic organs. While SA reduced the total chl content in photosynthetic organs of *H. carnosum*, it increased chl content in the organs of *H. coronarium*. In addition, SA had no effect on H_2O_2 content in control seedlings. This may be due to the activation of antioxidative defence mechanisms (Rajjou et al., 2006). In salt-stressed seedlings, SA -priming reduced MDA content in *H. carnosum* cotyledons and in the R + H of *H. coronarium*, which was most likely a result of the reduced OH^- ion production by Fenton's reaction. SA is known to more efficiently activate antioxidant defenses in *H. coronarium* seedlings than *H. carnosum*. Moreover, the former species maintains a higher total chl content than the latter. In salt-stressed seedlings, and particularly cotyledons, SA -priming reduced the salt oxidative stress, as determined by the reduction of MDA content, which may have been the result of a lower production of OH^- ions through Fenton's reaction. Exogenous SA treatment led to an increased antioxidant capacity (Azooz, 2009). The decline in H_2O_2 content was the result of CAT activity stimulation in the R + H of both species and increased GPX activity in both organs of *H. coronarium*. Detoxification of H_2O_2 was not achieved at the same level in the two species and did not justify the observed reduction in H_2O_2 content. Therefore this reduction may have been achieved by other detoxifying mechanisms that stimulate SA-induced peroxidases, particularly under stress conditions (Dixon et al., 1995), and through the involvement

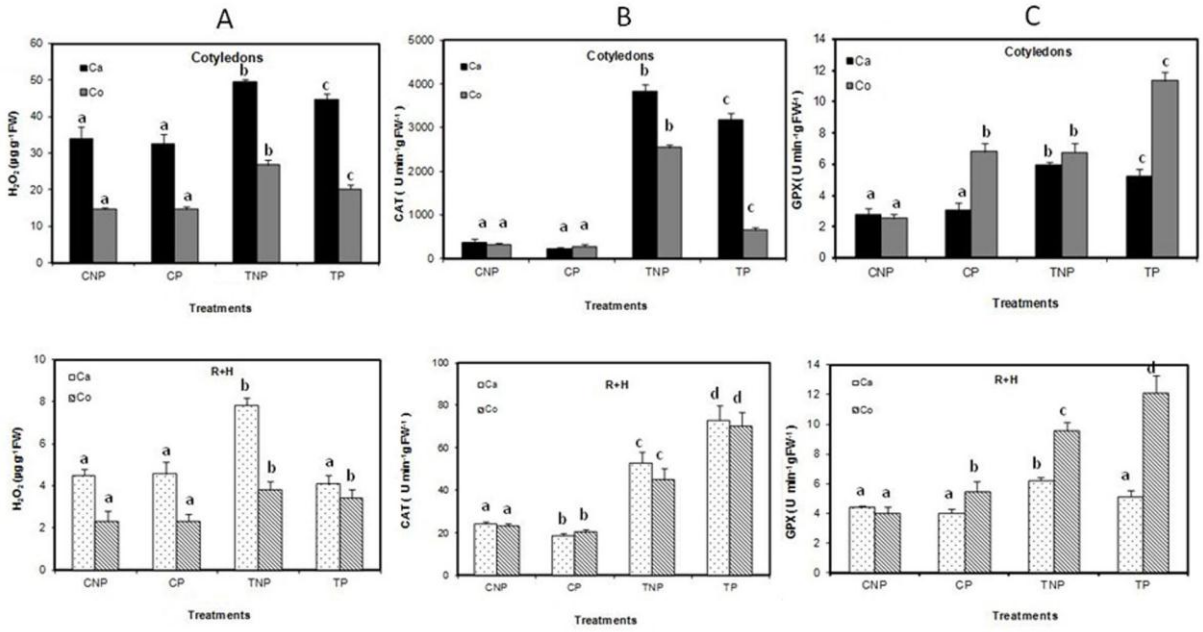

Fig 5. The effect of seed priming with 100 µM salicylic acid in *H.carnosum* (Ca) and *H. coronarium* (Co) on H_2O_2 concentration (A), catalase (CAT) (B), and guaiacol peroxydase (GPX) (C) activities in cotyledons and radicles plus hypocotyls "R+H" in 10-day-old seedlings treated with 0 and 200 mM NaCl.

of antioxidant molecules, such as glutathione (GSH) (Borsani et al., 2001). The results of this study indicate that SA seed - priming alleviates salt oxidative stress in seedlings of both *Hedysarum* species, but in a more pronounced manner in the salt -tolerant *H. coronarium* species.

Materials and methods

Plant material and germination conditions

The seeds of *H. carnosum* Desf. were collected from central Tunisia (Kairouan, Tunisia), whereas those of *H. coronarium* L. were obtained from Northern Tunisia (Bizerte, Tunisia). Homogenous seeds of both species were surface-sterilized using a 10% sodium hypochlorite solution for 5 min and then rinsed thoroughly with distilled water. The seeds of each species were scarified to remove tegument dormancy. The seeds were then primed using 100 µM of SA for 8 h. The optimal concentration and time was determined in preliminary experiments. After incubation, the seeds were air dried for 24 h and sown in Petri dishes with 35 seeds in each dish and four replicates for each treatment. Primed (P) or non-primed (NP) seeds were imbibed with distilled water (control: C) or with 200 mM NaCl solution (treated: T). The seeds from *H. carnosum* and *H. coronarium* were incubated at 25°C and 20°C, respectively, on filter paper. The upper sheet was removed after two days to allow light entry. All parameters were measured on 10 day-old seedlings, in photosynthetic organs (cotyledons) and non-photosynthetic organs (H + R).

Physiological and biochemical analysis

Germination and Growth parameters: Radicle protrusion was the criterion used to detect germination, and the rate was determined daily by comparing the number of germinated seeds to the total number of seeds. Hypocotyl length was measured in 10-day-old seedlings. Dry weight (DW) was determined after desiccation at 80 °C for 48 h. *Ionic status*: Na^+ and K^+ concentrations were assayed using a flame emission spectrophotometer after nitric acid extraction (HNO_3; 0.5N for 24 h) of the finely ground dry matter. *Oxidative status*: the H_2O_2 concentration was measured on fresh tissue crushed in cold trichloroacetic acid (TCA; 0.1%) and then centrifuged for 15 min at 12000 x g in a refrigerated centrifuge. The supernatant (0.5ml) was added to 10 mM phosphate buffer (pH 7) and 1 M of iodate potassium solution and the absorbance was measured at 390 nm (Sergiev et al., 1997).

Oxidative damage

The chlorophyll content (Chl. a and Chl. b) was determined by spectrophotometry as previously described (Arnon, 1949). Cotyledons of both species were immersed in 80% acetone for 24 h before being ground and then centrifuged at 3000 x g min^{-1}. The absorbance of the supernatant was measured at 645 and 663 nm. Lipid peroxidation was determined using the thiobarbituric acid (TBA) reaction followed by measurement of MDA content (Heath and Packer, 1968). Tissues (100 mg) were ground in 2 ml of 25% TBA prepared in 10% TCA solution. The mixture was incubated at 95°C for 30 min, cooled in an ice bath, and then centrifuged at 10000 x g for 15 min. The absorbance of the supernatant was measured at 532 nm and non-specific absorbance was measured at 600 nm. The MDA concentration was defined by its extinction coefficient of 155 mM^{-1} cm^{-1}. Membrane damage was assayed by measuring ion leakage rate as previously described Rizhsky et al. (2002). Cotyledons (0.1g) were placed abaxial side up in 8 ml of double distilled water for 20 h at 4°C. Following incubation, the conductivity of the bath solution was measured with a conductivity meter (value A). The cotyledon discs were then placed back into the bath solution and incubated at 95°C for an additional 30 min. After cooling the samples to room temperature, the

conductivity of the bath solution was measured again (value B). The ion leakage (%) is equal to

$$\frac{Value\ A}{Value\ B} \times 100$$

Detoxification was evaluated by measuring CAT and GPX activities. The samples were homogenized in phosphate buffer (50 mM, pH 7) and the entire procedure was conducted at 4°C. Each homogenate was centrifuged for 15 min at 15000 x g and the supernatant was used for subsequent analyses. Enzyme activity was measured using a UV/Visible light spectrophotometer (Jenway 6105). CAT activity, which corresponded to H_2O_2 destruction, was monitored by the decrease in absorbance at 240 nm for 1 min (Aebi, 1984). GPX activity was determined by the increase in absorbance (formation of tetraguaiacol) at 560 nm for 2 min (Fielding and Hall, 1978).

Statistical analyses

Parameters were recorded for 12 replications. STATISTICA 5 software was used to study statistical significance by using a one way analysis of variance (ANOVA) and DUNKAN test. The tests were performed separately for cotyledons and R + H samples. Means followed by the same letter(s) were not significantly different. P = 0.05 was considered to be statistically significant.

Conclusion

Priming of *Hedysarum* seeds with 100 µM SA improved their germination with 200 mM NaCl. SA stimulated the growth of both control and salt-stressed seedlings, but to a lesser extent for the salt-stressed condition. However, growth stimulation only stimulated elongation and not biomass production. SA priming did not alleviate the salt-induced ionic stress, but did improve the oxidative status, particularly for *H. coronarium*. In addition, detoxification was partially achieved through differential stimulation of H_2O_2 detoxifying enzyme activity. We observed that CAT activity was induced in both species but GPX activity was only induced in *H. coronarium*.

In conclusion, we found that SA priming of *Hedysarum* seeds reinforces the salt tolerance of *H. coronarium* at both germination and seedling growth stages.

Based on the studied parameters, *H. coronarium* appears to be more tolerant to NaCl stress at both stages than *H. carnosum*. In contrast, *H. carnosum*, which is specific to Tunisian Sebkhas, was able to grow better in higher salinity levels during the adult stage than *H. coronarium*.

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