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Drought and salinity: a comparison of their effects on the ammonium-preferring species *Spartina alterniflora*

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Abstract

Drought and salinity are the most serious environmental factors affecting crop productivity worldwide; hence, it is important to select and develop both salt- and drought-tolerant crops. The perennial smooth cordgrass *Spartina alterniflora* Loisel is unusual in that it is highly salt-tolerant and seems to prefer ammonium (NH_4^+) over nitrate (NO_3^-) as an inorganic N source. In this study, we determined whether *Spartina's* unique preference for NH_4^+ enhances performance under salt and drought stress. Greenhouse experiments were conducted to compare the interactive effects of N source, salinity, and low water availability on plant performance (growth and antioxidant metabolism). Drought significantly reduced growth and photosynthetic activity in *S. alterniflora*, more so with NH_4^+ than NO_3^-; in contrast, NH_4^+ enhanced growth under high salinity. The increased tolerance of *S. alterniflora* to salt stress in the presence of NH_4^+ was linked to a high level of antioxidant enzyme activity, combined with low MDA content, EL, and H_2O_2 production. In contrast, drought stress negated the growth advantages for *S. alterniflora* exposed to salt stress in the presence of NH_4^+. The susceptibility of *S. alterniflora* to drought was partly due to reduced antioxidant enzyme activities, thereby reducing

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the defense against the oxidative damages induced by osmotic stress. In conclusion, in contrast to salt stress, drought stress negates the beneficial effects of ammonium as an N source in the C_4 plant *Spartina alterniflora*.

Abbreviations — APX, Ascorbate peroxidase; CAT, Catalase; DW, Dry weight; EC, Electric conductivity; EL, Electrolyte leakage: FW, Fresh weight; GR, Glutathione reductase; GPX, Guaiacol peroxidase; MDA, Malondialdehyde; POD, Peroxidase; ROS, Reactive oxygen species; SOD, Superoxide dismutase.

1. Introduction

Drought and salinity are the most serious environmental factors affecting crop productivity worldwide (Sabagh et al. 2019). With the scarcity of precipitation and increase in evapotranspiration resulting from global warming and potential climate changes, farmers turn to irrigation practices. However, unplanned and reckless anthropogenic activity in the use of low water quality adds to existing osmotic stresses from other environmental hazards, such as salinity, soil degradation, mineral deficiency, and environmental pollution (Ben Hamed et al. 2013, Hessini et al. 2019). Studies have shown that about half of existing irrigation systems are under the influence of alkalization, salinization, or waterlogging (Munns 2002). Munns et al. (2020) defined saline soil as having an ECe above 4 dS m^{-1}, equivalent to 40 mM NaCl (mainly Na^+ and Cl^- salts), and about twice that for well-drained soil (80 mM NaCl). Such levels would diminish the productivity of most crops (Munns et al. 2020). Likewise, drought is a composite abiotic stress, occurring at any period of plant development to varying degrees (Hessini et al. 2009a, Al-Yasi et al. 2020). Drought can reduce annual crop yields by more than 50% (Boyer 1982, Farooq et al. 2009). However, in their natural biotope or in farms, plants are unavoidably exposed to multiple stresses, including salinity and drought, which can be more destructive to crop production than a single stress (Ben Hamed et al. 2013, Matthaei and Piggott 2019). One solution to this problem would be to increase crop tolerance to multiple stresses through breeding and genetic engineering, but these approaches are difficult, time-consuming, and have had restricted success (Bailey-Serres et al. 2019, Kotula et al. 2020). Screening species for tolerance to multiple stresses is an alternative approach for overcoming harsh environmental conditions. Screening can be accelerated with increased knowledge of the physiological and biochemical traits related to these stresses.

Nitrogen as a mineral nutrient is one of the crucial environmental factors regulating plant development. Plants mainly take up N as nitrate and ammonium, with most species thriving in the presence of nitrate (Hessini et al. 2009b, Marino and Moran 2019), and only a few favouring ammonium (Ashraf et al. 2018, Hessini et al. 2019).

The type of N nutrition can affect (decrease, increase, or no effect) the resistance of plants to environmental stresses (Marino and Moran 2019). The beneficial role of nitrate in plant tolerance to osmotic stress is mainly related to its function as osmoticum in osmotic adjustment (Burns et al. 2010, Hessini et al. 2019), while that of ammonium is associated with changes in plant metabolism, stimulation of antioxidant machinery, reduced Cl^- uptake, and improved K^+/Na^+ homeostasis by reducing Na^+ xylem loading (de Souza Miranda et al. 2014, Fernández-Crespo et al. 2014).

Abiotic stress can also affect soil nitrogen bio-transformations, i.e. the balance between nitrification and ammonification, with nitrification more sensitive to salinity and drought than ammonification (Hessini et al. 2009c). It is likely that the ammonium:nitrate ratio in soil solution increases under salinity and drought conditions, providing more ammonium to plants than those without stress (Hessini et al. 2019). This highlights the economic and environmental relevance of selecting for drought- and salt-resistant crop species that use NH_4^+ as the predominant N source.

Spartina alterniflora Loisel is one of the most significant grass species in coastal salt marshes (Hessini et al. 2008). For its economic role as fodder and a source of green fertilizer, bioactive material, and sewage treatment (Hu and Qin 1998, Liu and Tian 2002), *S. alterniolia* has been introduced from its native biotope of the Atlantic and Gulf coastlines marshes in the USA to many countries, including China, Australia, and Morocco, and to Europe (UK, France, Denmark, Germany, and Spain) (Zheng and Zhang 1995, Lin and Li 1999). Its successful introduction into semi-arid and arid salty regions depends mostly on its ability to tolerate specific environmental restraints, such as drought, salinity, and ammonium as the dominant N source. Therefore, this study aimed to (1) understand the physiological and biochemical mechanisms of *S. alternifolia* implicated in salt and drought adaptation, and (2) determine whether *Spartina*'s unique preference for NH_4^+ improves performance under salt and drought stress.

2. Materials and methods

2.1. Growth conditions and stress imposition

In the 2010 northern spring–summer season, identical cuttings (20 cm height) of *S. alterniflora* were taken from maternal plants, washed, and separately planted into 5 L blow-molded pots filled with

sandy soil. The pots were irrigated with one-fourth diluted nutrient solution (Hewitt 1966) for one month in a greenhouse at the Biotechnology Centre of Borj-Cedria, Tunis (10°10' E, 36°48' N; 10 masl) set to 25/18°C day/night, 65–90% relative humidity, and 900–1000 μmol m^{-2} s^{-1} midday photon flux density. To avoid nitrification, we added 7.5 μl L^{-1} dicyandiamide and 4 μl L^{-1} nitrapyrin to the nutrient solution. Two alternative N sources were used: calcium nitrate [$Ca(NO_3)_2$] or ammonium sulfate [$(NH_4)_2SO_4$] at 7.5 mM of N. Plants grown with each of the N sources were subjected to: (i) salinity (0, 200 and 500 mM NaCl), or (ii) drought (100, 50, and 25% field capacity (FC)), with the 0 NaCl and 100% FC treatments acting as controls, for 60 days from 30 days after planting. The FC was assessed according to the method of Bouyoucos (1983). Hence, there were 100 pots: 2 N-sources × 5 treatments × 10 replicates. For the drought treatments, the soil water contents (SWC, %) registered by treatments 100, 50 and 25% FC were 11.5, 5.75 and 2.88% respectively. The seedlings were watered to their corresponding weights each day with nutrient solution. For the salinity treatments, sodium chloride was added in steps of 50 mmol L^{-1} every 8 hours to prevent osmotic shock. The medium having ammonium as the sole N form was buffered as described by Cantera et al. (1999). Soil evaporation was avoided by enclosing each plant pot in a plastic bag wrapped at the base of each seedling. In addition, to check water loss through evaporation from the soil surface, some pots without plants were reserved. After harvest, plants were separated into above- and below-ground portions at the end of the vegetative period (60 days after the beginning of drought and salt treatments).

2.2. Harvest and plant dry matter

At the end of the experiment, plants were harvested between 10:00 and 12:00 h. The number of leaves per plant was counted, before individually collecting leaves for leaf area determination with a portable area meter (Li-Cor 3000A, Li-Cor Inc). Shoot and root dry matter were done after oven-drying at 80°C to constant weight. Measurements were performed 60 days after the start of treatment.

2.3. Gas exchange determinations

Two days before harvest (88 DAT), net CO_2 assimilation rate (A), stomatal conductance (g_s), and transpiration rate (E) were measured using a portable gas exchange system (Li-Cor 6200, Li-Cor). Instantaneous water-use efficiency (WUE_i) was calculated as the ratios of A/E. Measurements were carried out between 10:00 and 14:00 h on fully expanded leaves acclimated to leaf chamber conditions for 10 minutes.

2.4. Lipid peroxidation assay

Oxidative damage to lipids was determined as lipid peroxidation through the expression of malondialdehyde (MDA)—a key thiobarbituric acid (TBA) reactive substance—as per Cakmak and

Horst (1991). Samples of 100 mg fresh leaf were homogenized in 5.0 mL trichloroacetic acid (0.1%, w/v) and centrifuged for 10 min at 10 000 *g*. One mL of the supernatant was mixed with 4.0 mL of 0.5% TBA in 20% TCA. The blend was heated for 30 min at 95°C, cooled over ice, and centrifuged for 10 min at 10 000 *g*. Absorbance of the supernatant was determined at 532 and 600 nm. Using a spectrometer (Sherwood Scientific Ltd., model 259), non-specific absorbance at 600 nm was subtracted from absorbance at 532 nm, and the obtained value used to calculate MDA content with an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.5. Electrolyte leakage

Electrolyte leakage (EL) was evaluated according to Dionisio-Sese and Tobita (1998). Conductivity due to the extraction of electrolytes from the extracellular spaces (EC1) was determined by incubating 200 mg of leaf fresh weight in test tubes with distilled water at 25°C for 2 h. Conductivity due to the extraction of all the electrolytes present inside the cells (EC2) was determined in test tubes containing 200 mg of leaf material in distilled water, boiled for 30 min, and then cooled to 25°C. Electrolyte leakage was done as follows:

$EL = (EC1/EC2) \times 100$

2.6. Tissue hydrogen peroxide content

Hydrogen peroxide content was assessed according to Frew et al. (1983). Fresh leaf sample weight (0.5 g) was frozen in liquid nitrogen and crushed to powder with mortar and pestle, together with 5 mL of 5 % (w/v) TCA and 0.15 g activated charcoal. After centrifuging at 10 000 *g* at 4°C for 20 min, the pH of the supernatant was adjusted to 8.4 with a 17 M ammonia solution and then filtered. The H_2O_2 content was evaluated at 505 nm (quinone imine absorption) using a spectrophotometer (Sherwood Scientific Ltd., model 259) and a standard curve created from known concentrations of H_2O_2.

2.7. Antioxidant enzyme activities

Frozen leaves samples were powdered with liquid nitrogen before homogenization with the extraction buffer for the relevant enzyme. After filtration through two layers of Miracloth (Calbiochem), homogenates were centrifuged at 20 000 *g* for 20 min at 4°C. Extract aliquots were used for the distinct enzyme assays.

Catalase (CAT) and guaiacol peroxidase (GPX)

Samples of 200 mg frozen leaves were homogenized in a mortar with 2 mL of extraction medium containing 66 mM potassium phosphate buffer (pH 7) and 0.1 mM EDTA. Total catalase (CAT, EC 1.11.1.6) activity was estimated according to the method of Beers and Sizer (1952). Losses in H_2O_2

were evaluated at 240 nm. Total guaiacol peroxidase (GPX, EC 1.11.1.7) activity was evaluated as described by the method of Castillo et al. (1984).

Ascorbate peroxidase (APX)

Total ascorbate peroxidase (APX, EC 1.11.1.1) activity was evaluated according to Hossain and Asada (1984) after the reduction in absorbance at 290 nm of a reaction medium containing 50 mM HEPES–NaOH (pH 7.6), 0.25 mM ascorbate, 0.1 mM H_2O_2, and 10–40 μL of leaf extract. Controls with 50 μm p-chloromercuribenzoate, an APX inhibitor, were included to test for non-specific peroxidases or spontaneous reactions (Ranieri et al. 1996).

Glutathione reductase (GR) and superoxide dismutase (SOD)

Fresh leaf samples (200 mg) were homogenized with an extraction medium containing 5 mM Tris-HCl (pH 7.5), 0.2% (v/v) Triton X-100, 0.1 mM EDTA, 2 mM DTT, 1 mM PMSF, and 0.1 % (w/w) polyvinylpolypyrrolidone (PVPP). After desalting with Bio Gel P6-DG (Bio-Rad laboratories), the supernatant was used to assess GR (glutathione reductase, EC 1.8.1.7) and superoxide dismutase (SOD; EC 1.15.1.1) activities. The decrease in absorbance at 340 nm was used to evaluate GR activity. The reaction medium contained 0.1 M HEPES (pH 7.8), 2.5 mM GSSG, 3 mM $MgCl_2$, 0.2 mM NADPH, and 200 μL of extract sample. SOD activity was determined based on the inhibition of its product, superoxide radical, on cytochrome c determined at 550 nm (McCord and Fridovich 1969)

2.8. Determination of GDH activity

Glutamate dehydrogenase (GDH; EC 1.4.1.2) was extracted using equal proportions of plant material to volume of extractant (1/1; w/v) containing 50 mM Tris-HCl buffer (pH 8.0), 10 mM 2-mercaptoethanol, 1 mM EDTA, 1 mM cysteine, 10 mM MgSO4, 5 mM dithiothreitol, and 0.6% polyvinylpyrrolidone. Extracts were clean and centrifuged at 35 000 *g* for 15 min. GDH activity was determined through coupled NADH oxidation determined at 340 nm (Groat and Vance 1981).

2. 9. Statistical analysis

A two-way ANOVA was performed for all the parameters measured with salinity-drought and N source as the main effects (n=10 biological replicates). As the interaction effect was statistically significant ($P \leq 0.05$) for all parameters, post-hoc mean-separation testing (Tukey HSD) was conducted on the interaction effect. All statistical analyses were performed using SAS software.

3. Results

3.1. Plant biomass accumulation

In the absence of salinity, the NH_4^+-fed plants had 64% more plant biomass than the NO_3^--fed plants (Table 1, $P \leq 0.05$). Adding 200 mM NaCl to the nutrient solution enhanced plant biomass, relative to the controls and regardless of the N form present. Adding 500 mM NaCl had no effect on plant biomass, relative to the controls and regardless of N form (Table 1).

Under water-limited conditions (50 or 25% FC), plant biomass declined, irrespective of N source, more so at 25 than 50% FC (Table 1, $P \leq 0.05$). Leaf number and area per plant responded in a similar manner to that of biomass (Table 1, $P \leq 0.05$).

3.2. Photosynthetic capacity, transpiration, stomatal conductance, and instantaneous water-use efficiency

In the control (0 mM NaCl, 100% FC), the NH_4^+-fed plants had significantly higher net photosynthesis (A) and stomatal conductance (g_s) than the NO_3^--fed plants, while transpiration rates (E) did not differ (Table 2, $P \leq 0.05$). The 200 mM NaCl treatment had similar A, g_s and E values as the control plants. At 500 mM NaCl, A and E declined in the NO_3^--fed plants, but not in the NH_4^+-fed plants. However, g_s declined by an average of 50% in the 500 mM NaCl treatment, regardless of N form (Table 2, $P \leq 0.05$). In the drought treatments (50 and 25% FC), A, E, and g_s declined significantly, irrespective of N source. Increasing salinity severity from 200 to 500 mM NaCl had no significantly effect on plant instantaneous water-use efficiency (WUEi) but increasing drought severity from 50 to 25% FC increased plant WUEi, more so in NH_4^+-fed plants than NO_3^--fed plants (Table 2, $P \leq 0.05$).

3.3. Oxidative stress

In the control (0 mM NaCl, 100% FC), N form had no effect on leaf H_2O_2 concentration. The H_2O_2 content of *S. alterniflora* leaves increased with increasing salinity and drought stress, regardless of N form, by 500 and 600% at 500 mM NaCl and 550 and 950% at 25% FC in the NO_3^--fed and NH_4^+-fed plants, respectively, relative to the control (Fig. 1, $P \leq 0.05$).

In the control (0 mM NaCl, 100% FC), N form had no effect on leaf electrolyte leakage. The impact of salinity on EL was only significant at 500 mM NaCl, more so in NO_3^--fed plants than NH_4^+-fed plants (73 and 10%, respectively, relative to control plants). At 50% FC, EL did not change in NO_3^--fed plants but increased by 60% in NH_4^+-fed plants (Fig. 1, $P \leq 0.05$). Irrespective of N form, the 25% FC treatment significantly increased cell membrane damage, as assessed by leaf EL, more so in NH_4^+-fed plants than NO_3^--fed plants (Fig. 1, $P \leq 0.05$).

Regardless of N form, salinity and drought stress significantly increased MDA accumulation, relative to the control (Fig. 1, $P \leq 0.05$). In contrast to salinity stress, where the highest level of lipid

peroxidation occurred in NO_3^--fed plants treated with 500 mM NaCl, drought-treated plants had similar MDA levels, independent of N source (Fig. 1, $P \leq 0.05$).

3.4. Antioxidant enzyme activities

Irrespective of stress treatment, NH_4^+-fed plants had higher enzyme activities (SOD, CAT, GPX, APX, and GR) than NO_3^--fed plants. NH_4^+-fed plants at 500 mM NaCl had higher enzyme activities than those at 200 mM NaCl, whereas, except APX, no differences were noted for SOD, CAT, GPX, and GR activities in the two water-stressed treatments (Fig. 2, $P \leq 0.05$). Generally, the salt treatments triggered higher SOD, CAT, and GPX activities than the water-stressed treatments, while the reverse was true for APX and GR activities (Fig. 2, $P \leq 0.05$).

3.5. General stress

For GDH activity, no detectible effects of salinity were observed for either N form. However, water stress significantly increased GDH activity, more so in NH_4^+-fed plants than NO_3^--fed plants (Fig. 3, $P \leq 0.05$).

4. Discussion

While *S. alterniflora* is adapted to moderate saline conditions (200 mM) caused by NaCl, the N form available for growth may disturb this performance. We investigated whether *Spartina*'s improved performance under control conditions with NH_4^+ as the N source persists under saline or drought conditions. We compared the responses of NO_3^--fed and NH_4^+-fed plants to salinity and drought in a greenhouse experiment, and confirmed the improved performance of NH_4^+-fed plants under saline but not drought conditions, indicating that *S. alterniflora* perceives each stress differently.

Although not a hard-and-fast rule, NH_4^+-fed plants have performed better than NO_3^--fed plants in some terrestrial, wetland, and marine species (Kudo and Fujiyama 2010, Fernández-Crespo et al. 2014, Munzarova et al. 2006). Plant biomass is a good indicator of plant performance for which photosynthetic activity is the main contributor (Ashraf and Harris 2013, Hessini et al. 2019). As a result, the general pattern of biomass response to N source, salinity, and drought was also found for photosynthetic and gas exchange parameters. The impact (positive or negative) of N form on these physiological processes is dose and/or species-dependent (Bassi et al. 2018, Hessini et al. 2019). In most species, photosynthesis is affected by NH_4^+ nutrition, but the underlying mechanisms appear to be complex and not understood, particularly in C4 plants such as S. *alterniflora* (Bendixen et al. 2001). It is expected that in the leaves of C4 plants, the N metabolism is adjusted to the low photorespiratory rates found in the mesophyll cells. In fact, a bundle sheath-specific GS1 isoenzyme,

with similarly high expression levels to those observed for the mesophyll cells, is characteristic of C4 leaves. This bundle sheath-specific GS1 located in close vicinity to the xylem vessels (Schlüter and Weber 2020) works as an ammonium scavenger and may prevent leaf ammonium concentrations from reaching toxic levels in the mesophyll cells; avoiding the excess NH_4^+ syndromes: inhibition of the oxygen-evolving complex, disturbance of the electron transport chain and reduction of the quantum yield of photosystem II (Markou et al. 2016, Wang et al. 2019). Our study showed that under control conditions the performance of NH_4^+-fed is better that of NO_3^--fed plants.

Regardless of the N source, salinity did not negatively affect A and E, but at high levels, it significantly reduced stomatal conductance. The integration of these observations with the better performance of ammonium- than nitrate-fed plants under salinity conditions leads to the conclusion that ammonium-fed plants are better able to fine-tune their metabolism than nitrate-fed plants, as has been detected for other plant species (Kant et al. 2007, Horchani et al. 2011). These fine-tuning adjustments may include morphological (leaf area, number of leaves or tillers per plant) and/or physiological (increased expression and/or activities of RUBISCO, phosphoenolpyruvate carboxylase PEPC and other enzymes) as observed by Hessini et al. (2009) and Ben Mansour et al. (2019).

In contrast to salinity, a significant drop in A and E rates was observed under drought conditions. This highlighted the existence of distinct plant signalling pathways, responses to salinity and drought, and cross-talk of the N metabolism.

Our results agree with those of Kefu et al. (2003), who showed that some halophytes such as *S. alterniflora* tolerate salt stress, but not drought, and certain xerophytes tolerate drought, but not salt stress. The different responses of *S. alterniflora* to salinity and drought may be related to the effect of both stresses on stomatal conductance that, independently of the N source used for plant growth, dramatically declined under drought but not salinity. Plants modulate stomatal opening to adjust the rate of CO_2 diffusion and reduce water loss, both of which have a key role in photosynthetic machinery and water transport.

Several kinds of plant signals (hydraulic, electrical, and chemical) are identified to control the stomatal behaviour in response to varying stimuli, such as variations in atmospheric relative humidity and soil wetness. In addition to a diversity of chemical agents (Schachtman and Goodger 2008), the plant hormone abscisic acid (ABA) plays a prominent role in stomatal control (Munemasa et al. 2015). Leaf-level ABA has been correlated with the initiation of stomatal closure in response to water scarcity (Geiger et al. 2009). The rapid upregulation of ABA biosynthesis to variations in leaf-to-air vapor pressure deficit and drought stress makes ABA an appropriate candidate for vigorously

initiating stomatal closure (Munemasa et al. 2015, Zhang et al. 2018). None of these signal kinds or hydraulic parameters has been identified solely as the driving factor for stomatal closure. However, they may be at the origin of the distinct plant responses to salinity and drought, especially in halophytes.

Our study confirmed the positive effect of ammonium nutrition on salinity (Hessini et al. 2019, 2020, Dorta-Santos et al., 2020) but not on drought tolerance. In *S. alterniflora*, ammonium nutrition appears to have a priming effect against salt stress, similar to that described for maize (Hessini et al. 2019) and other plants (Chi et al. 2019, Marino and Moran 2019).

Osmotic stress, salinity and drought both involve acclimation to low water potential. However, plants growing in presence of high salinity need also manage with potentially toxic levels of specific ions. While ion uptake can provide a means for osmotic adjustment, high cytosolic amounts of certain ions such as Na^+ may disturb metabolism. Thus, regulating the distribution of ions within cells may be a crucial feature of osmotic stress resistance. However, and in accordance with previous studies (Hessini et al. 2008, 2009), *S. alterniflora* seems better adapted to salinity than drought, which may reflect its halophytic characteristics (Fuchen and Fang 2007, Hessini et al. 2009b, Carol et al. 2019). Although detrimental to most plants, moderate salinity levels (millimolar-range) can be beneficial for some halophytic C_4-type plants and non-halophytic species, such as maize (*Zea mays*; Pilon-Smits et al. 2009, Maathuis 2014, Colmenero-Flores et al. 2019). Indeed, at low water potential, several minerals, including Na^+ and Cl^-, can accumulate in cell vacuoles for osmotic adjustment, diminishing cell osmotic potential, and favoring water entry into cells, and consequently cell turgor (Al-Yasi et al. 2019). However, if cell Cl^- and/or Na^+ concentration exceeds specific thresholds, plants undergo disturbed ion homeostasis and experience photosynthetic disorders, limited growth, and even cell death (Eskandari et al. 2020, Zeeshan et al. 2020). In *S. alterniflora*, the accumulation of harmful levels of leaf Na^+ could be avoided by the high capacity for leaf sodium exudation and the secretion and accumulation of high levels of glycine betaine that increase with stress (Hessini et al. 2011, Gallego-Tévar et al. 2019).

The better performance of NH_4^+-fed plants, relative to NO_3^--fed plants, under high salinity may, at least in part, be due to the distinct interactions of NH_4^+ and NO_3^- with Na^+. On the one hand, in their hydrated forms, NH_4^+ and Na^+ have similar ionic radii, so plants may not distinguish them under saline conditions, resulting in lower Na^+ uptake in the presence of NH_4^+ than NO_3^- (Benito et al. 2014). On the other hand, Na^+-dependent nitrate transport (Junfeng et al. 2010, Nie et al. 2015) and the coupled movement of Na^+ and NO_3^- observed in many plants (Alvarez-Aragon and Rodriguez-Navarro 2017)

may fulfil osmotic adjustment (Raddatz et al. 2020), but also promote Na^+ uptake, which may be detrimental. Similar positive effects of ammonium (NH_4^+) on salinity tolerance, relative to nitrate (NO_3^-) have been observed in some species, such as *Glyceria maxima* (Munzarova et al. 2006), *Carrizo citrange* (Fernández-Crespo et al. 2014), and *Salicornia bigelovii* (Kudo and Fujiyama 2010).

It is generally accepted among plant physiologists that plant tolerance to salinity and drought occurs through a common pathway (Ding et al. 2015). However, the favourable effect of NH_4^+ nutrition on *S. alterniflora* growth observed under saline situations did not occur when plants faced drought stress, which may be due to the increased activity of GDH, an enzyme implicated in NH_4^+ assimilation in plants exposed to harsh conditions (Cañas et al. 2020, Terce-Laforgue et al. 2004).

Salinity and drought stress tend to generate increased loads of oxidative stress. Depending on the plant species and conditions, ammonium nutrition may (Misra and Gupta 2006, Wang et al. 2010, Huang et al. 2013, Cruz et al. 2011, Liu and Von Wiren 2017, Mirfattahi and Eshghi 2020) or may not (Dominguez-Valdivia et al. 2008) be associated with increased oxidative stress. In our study, NO_3^--fed plants had higher levels of H_2O_2, but lower SOD activity than NH_4^+-fed plants, implying that the downstream activity of CAT and APX in NO_3^--fed plants was insufficient to maintain H_2O_2 concentrations at levels compatible with optimal physiological activity. In *S. alterniflora*, ammonium nutrition appears to protect plants under saline conditions, but how NH_4^+-fed plants protect themselves from oxidative stress is not known (Bendixen et al. 2001).

When NH_4^+ was combined with salinity or drought, the activities of major antioxidant enzymes responsible for ROS scavengers increased significantly. The mechanisms by which these activities increased with ammonium as the sole N source is not known but has been detected in several other organisms (Marino and Moran 2019, Wu et al. 2020). Nevertheless, the NH_4^+ ion or one of its assimilation products (e.g. glutamine or glutamate) may be a stress signal for generating the activation of many enzymes responsible for stress acclimation (Rios-Gonzalez et al. 2002, Marino and Moran 2019).

Some reports have stated that ROS production on ammonium media is higher than under other N sources (Misra and Gupta 2006, Wang et al. 2010). However NO_3^-, as an electron sink under photosynthetic C-acceptor limitation, can partly alleviate photoinhibition and the consequent production of ROS (Yi et al. 2014). This leads to a cumulative stress effect, frequently observed when cadmium, aluminium or salt stress conditions coexists with NH_4^+ nutrition (Tan et al. 1992, Speer and Kaiser 1994, de Sousa Leite and Monteiro 2019).

The combined effect of drought and ammonium nutrition affected the activity of enzymes involved in the oxidative response system. The response pattern of APX is interesting as it differed in the three treatments (N source, salinity, and drought): $NH4^+$-fed plants always had higher activities than NO_3^--fed plants, as did plants grown under drought over plants under salinity. However, the higher APX activity under drought was not enough to overcome drought stress. Despite the preference for NH_4^+ over NO_3^- under non-limited water conditions, *S. alterniflora* suffers from high oxidative stress when carbon flux is diminished by drought, as happens in non-NH_4^+ specialists. We showed that NH_4^+-fed plants subjected to severe water limitation accumulate more MDA, indicating an increase in lipid oxidation and insufficient membrane protection, consistent with high electrolyte leakage.

Conclusion

Our study demonstrates that ammonium as the sole N form favours the growth of *S. alterniflora* under high salinity more than nitrate, as ammonium is linked to enhanced antioxidant capacity of plants to limit oxidative damage. In contrast, drought-stressed *S. alternifolia* did not benefit from this form of nitrogen. The sensitivity of this species to drought may, at least in part, be related to drastic stomatal closure, which affects plant performance and severely inhibits growth. As a result, *S. alterniflora* tolerates salinity, but not drought, even at moderate levels.

Author contributions

HK, JK, and CC carried out experimental and analysis work. HK, and JK: design and interpretation of all experiments. HK, CC and SK wrote the manuscript.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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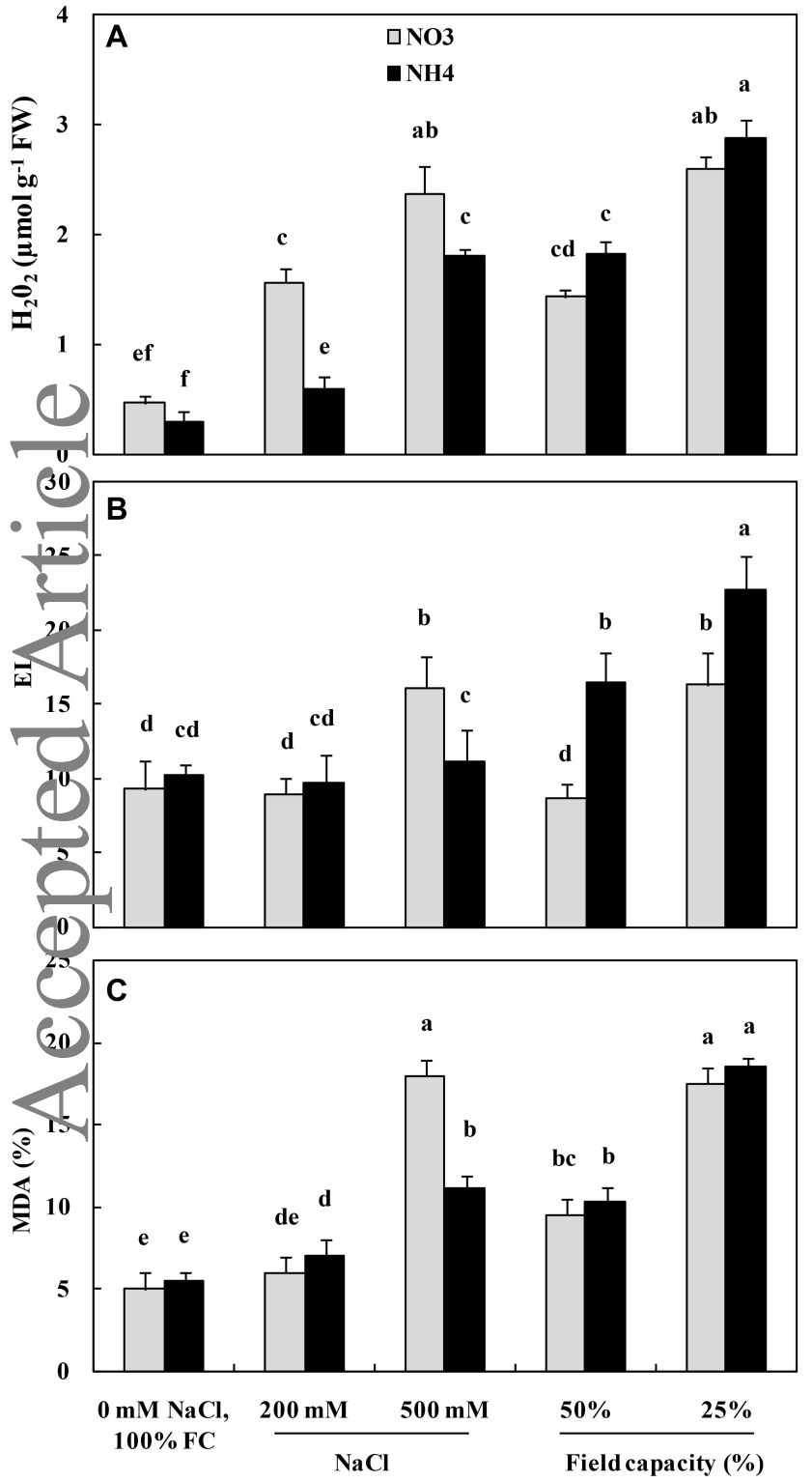
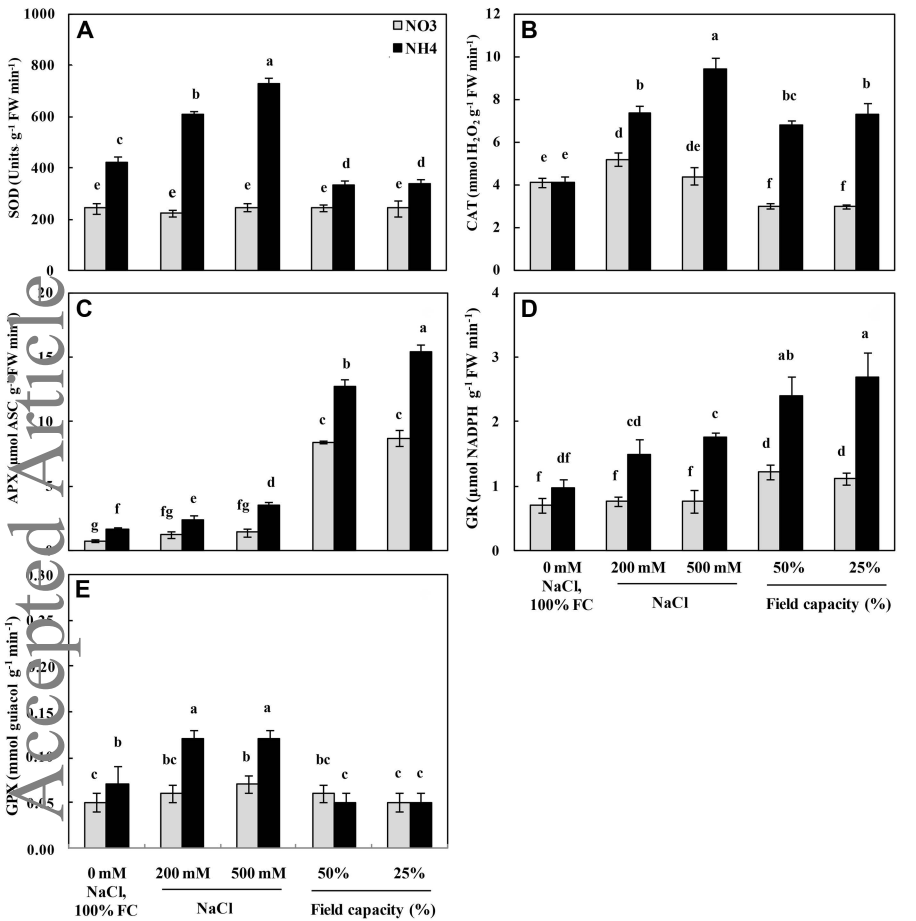
Figure captions

Fig. 1. Interactive effects of nitrogen form (NO_3^- and NH_4^+) and osmotic stress (salinity or drought) on hydrogen peroxide (H_2O_2) concentration (μmol g^{-1} FW), electrolyte leakage (EL, %) and malondialdehyde (MDA) concentration (μmol g^{-1} FW) in leaves of *S. alterniflora*. Each data point is the mean ± SD of three replicates per plants. Values with different letters are significantly different at P=0.05.

Fig. 2. Interactive effects of nitrogen form (NO_3^- and NH_4^+) and osmotic stress (salinity or drought) on the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX) in leaves of *S. alterniflora*. Each data point is the mean ± SD of three replicates per plants. Values with different letters are significantly different at P=0.05.

Fig. 3. Interactive effects of nitrogen form (NO_3^- and NH_4^+) and osmotic stress (salinity or drought) on the activities of glutamate dehydrogenase (GDH, nmol NADH oxidized g^{-1} protein min^{-1}) in leaves of individual *S. alterniflora* plants. Each data point is the mean ± SD of three replicates per treatment. Values with different letters are significantly different at P=0.05.





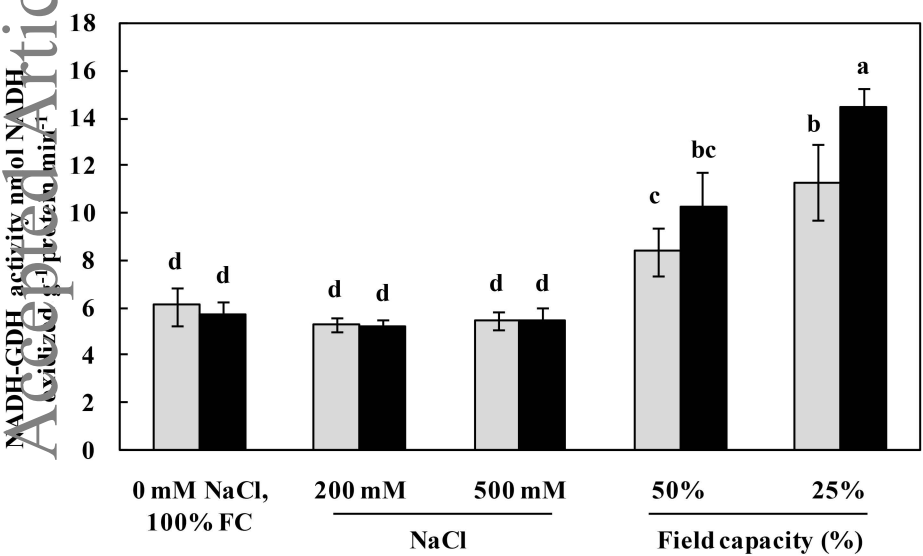


Table 1. Interactive effects of nitrogen form (NO_3^- and NH_4^+) and osmotic stress (salinity or drought) on plant performance (dry weight, leaf number and area) of *Spartina alterniflora*

	NO_3^-			NH_4^+		
	DW (g plant^{-1})	**Leaf area (cm^2 plant^{-1})**	**Leaf number**	**DW (g plant^{-1})**	**Leaf area (cm^2 plant^{-1})**	**Leaf number**
100% FC, 0 mm NaCl	2.8±0.8b	152.6±89.7b	12.6±3.2b	4.6±1.7ab	221.5±85.9ab	13.4±4.3ab
200 mM NaCl	4.8±1.5a	255.5±89.7a	16.5± 4.7a	5.4±2.1a	270.9±74.2a	15.4± 3.8a
500 mM NaCl	2.4±0.9b	78.8±17.3cd	8.3±1.2c	4.7±0.9ab	262.3±41.6a	16.8 ± 4.9a
50% FC	2.3 ± 0.9b	94 ± 5.6c	8.2 ± 1c	2.4 ± 0.2b	115 ± 27c	8.4 ± 2.4b
25% FC	1.4 ± 0.2c	91 ± 17c	6.8 ± 1.7cd	1.45 ± 0.2c	85 ± 16cd	5.4 ± 1.3c

Values represent the mean ± SE of ten replicates per treatment. Different letters within the same column indicate significant differences between treatments at $P \leq 0.05$.

Table 2. Interactive effects of nitrogen form (NO_3^- and NH_4^+) and osmotic stress (salinity or drought) on photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance (g_s), instantaneous water use efficiency (WUEi, mol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}) in *Spartina alterniflora*

	NO_3^-				NH_4^+			
	A	**E**	**g_s**	**WUEi**	**A**	**E**	**g_s**	**WUEi**
100% FC, 0 mm NaCl	12.5±0.6a	2.1±0.3a	153±9a	6.1±1.1b	17.8±0.9a	2.6±0.2a	153±9b	7±1c
200 mM NaCl	11±1b	1.6±0.3b	139± 12ab	6.9±1.3ab	17±2a	2.1±0.8b	180±15a	8±2b
500 mM NaCl	8 ± 1c	1.1±0.2c	75±5c	7.9±1.9a	16.9±0.5a	2.2±0.1ab	104±5c	7.7±1.6bc
50% FC	7.4 ± 1.1c	1.7 ± 0.2b	23 ± 3 d	4.4±0.4d	4.5 ± 0.3b	0.5 ± 0.1c	28± 3d	9±1b
25% FC	2.4 ±0.4d	0.4 ± 0.1d	7.3 ± 1.9e	6.1±0.4c	3.3 ± 0.5 c	0.2 ± 0.1d	10 ± 3.9e	16.5±1.5a

Values represent the mean ± SE of ten replicates per treatment. Different letters within the same column indicate significant differences between treatments at $P \leq 0.05$.