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Interactive effects of salinity and nitrogen forms on plant growth, photosynthesis and osmotic adjustment in maize

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ABSTRACT

To enhance crop productivity and minimize the harmful effects of various environmental stresses, such as salinity and drought, farmers often use mineral fertilizers. However, inadequate or excessive fertilization can reduce plant growth and nutritive quality and contribute to soil degradation and environmental pollution. This study investigated the effects of salinity (0, 100 or 150 mM NaCl) and nitrogen form (sole NO_3^- or NH_4^+, or combined NO_3^-:NH_4^+ at 25:75 or 50:50) on growth, photosynthesis, and water and ion status of a commercial variety of maize (*Zea mays* SY Sincero). In the absence of NaCl, the media containing ammonium only or both nitrogen forms had higher aboveground growth rates than that containing nitrate only. Indeed, the maize growth, expressed as leaf dry matter, seen on NH_4^+ in the absence of salinity, was nearly double the biomass compared to that with NO_3^- treatment. Irrespective of N form, the presence of NaCl severely reduced leaf and roots growth; the presence of ammonium in the nutrient solution diminished these negative effects. Compared to the NH_4^+ only and combined treatments, the leaves of plants in the NO_3^--only medium showed signs of nitrogen deficiency (general chlorosis), which was more pronounced in the lower than upper leaves, indicating that nitrate is partly replaced by chloride during root uptake. NH_4^+ favored maize growth more than NO_3^-, especially when exposed to saline conditions, and may improve the plant's capacity to osmotically adjust to salinity by accumulating inorganic solutes.

1. Introduction

In intensive production systems, farmers make use of mineral fertilizers to enhance crop productivity and minimize damage from various abiotic stresses (salinity, drought and nutrient deficiency). However, unreasonable fertilization can reduce plant growth and nutritive quality and contribute to soil degradation and environmental pollution (Li et al., 2017). The most widely used fertilizers are nitrogen-based and responsible for the high agricultural nitrogen footprint. The soil is a dynamic biological system that can be adversely affected by numerous environmental constraints. According to climate prediction models, crop plants will face increased environmental stresses, including salinity, drought and mineral deficiencies, often simultaneously, in the future. Despite this, studies tend to investigate plant responses to individual rather than combined stresses.

Soil microorganisms regulate bio-transformations in the nitrogen cycle, including the balance between fixation and mineralization. Plants take up nitrogen mainly in inorganic forms, as nitrate (NO_3^-) and ammonium (NH_4^+). Vast differences exist between plant species in their N-source preference; most grow best in the presence of nitrate or mixed N sources (Hessini et al., 2009a, 2013), with only a few preferring ammonium (Hessini et al., 2017; Ashraf et al., 2018). While the response of plants to nitrogen fertilization is species-dependent, several environmental factors such as drought, salinity, flooding and increased atmospheric CO_2 levels can influence their preference (Hessini et al., 2017; Coleto et al., 2019). NO_3^- may not always be beneficial under water-deficit conditions, as it can accumulate in plant leaves without contributing to biomass formation or yield increase (Berenguer et al., 2009). High nitrate concentrations in tissue can reduce the nutritional value of the product and harm human and livestock health (Britto and

Kronzucker, 2002; Hessini et al., 2009a). Many studies have shown that salinity/sodicity can affect soil microorganism activity and, consequently, N mineralization, with the effect more marked on nitrification than ammonification (Marton et al., 2012). Likewise, recent evidence from field and laboratory studies suggests that NO_3^- uptake and reduction will be suppressed at higher levels of atmospheric CO_2 (Hachiya and Sakakibara, 2017). It is also likely that more ammonium (NH_4^+) is available to plants in salt-affected soils (Cantera et al., 1999) but many crop species are sensitive to NH_4^+ (Britto and Kronzucker, 2002). The causes of NH_4^+ toxicity are not clear, and no reasonable hypothesis for its effects exists (Esteban et al., 2016). A few species are well-adapted to this N source, such as *Spartina alterniflora* (Hessini et al., 2013, 2017) and citrus (Fernandez-Crespo et al., 2012).

The problem in salt-affected soil is not the availability of nitrogen but the competition at the root surface for nitrate (NO_3^-) and chloride (Cl^-), on the one hand, and ammonium (NH_4^+) and sodium (Na^+), on the other hand. NO_3^- accumulates in the soil solution after uncontrolled N-fertilizer usage, which can result in high concentrations in plant leaves with no increase in biomass or grain yield (Berenguer et al., 2009). Products with high NO_3^- levels lose commercial value (Britto and Kronzucker, 2002). Several studies have been undertaken to produce healthy food outputs (low nitrate concentrations) with minimal disturbance to the agro-ecosystem (Hessini et al., 2015; Bitew and Alemayehu, 2017).

The selection of nutrient-efficient, salt-tolerant plant varieties that can use ammonium as the predominant N source is an emerging strategy (Iqbal et al., 2015). The intensification of food production with minimal negative environmental impacts and zero increase in land degradation is the objective of many researchers for the sustainable intensification of agriculture. We explored variability in plant NH_4^+ tolerance, in relation to factors such as the NO_3^-/NH_4^+ ratio and the presence/absence of NaCl, to identify a convenient N fertilization program for maize plants on salt-affected soils.

2. Material and methods

2.1. Plant material and growth conditions

This experiment used the seeds of an introduced variety of maize (SY Sincero), provided by the Centre for Ecology, Evolution and Environmental Changes, University of Lisboa, Portugal. The seeds were disinfected with 1% sodium hypochlorite solution for 5 min and then directly sown in 5 l plastic pots filled with sandy soil and cultivated in a greenhouse under sunlight (photon flux density $\sim$1500 μmol m^{-2} s^{-1}), with average day/night temperatures of 25/18 °C and a relative humidity of 65/90%. After emergence, the seedlings were thinned to two plants per pot, according to their stand and vigor. To prevent nitrification, 4 μl L^{-1} nitrapyrin (Nserve; Dow Chemical Co., Kings Lynn, England) and 7.5 μl L^{-1} dicyandiamide (DCD; Sigma Chemicals, St. Louis, MO) was added to the nutrient solution. Nitrogen was added as either calcium nitrate [Ca(NO$_3$)$_2$], ammonium sulfate [(NH$_4$)$_2$SO$_4$] or a mixture of the two in ratios of 25:75 or 50:50 [NO$_3^-$:NH$_4^+$] (total 6 mM N), and there were three salt concentrations (0, 100 and 150 mM NaCl). To avoid osmotic shock, NaCl was added progressively in weekly doses of 50 mmol L^{-1} d^{-1}. The medium containing NH_4^+ as the N source was buffered with 0.33 g CaCO$_3$ per kg soil dry weight (DW) (Cantera et al., 1999). Ten replicate pots for each treatment were used, which were arranged in a completely randomized design. Plants were harvested and separated into leaves and roots at the end of the vegetative stage (60 days after the beginning of the salt treatment).

2.2. Gas exchange measurements

Gas exchange parameters [net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g$_s$)] were determined using a portable gas exchange system (Li-Cor 6200, Li-Cor Nebraska, USA).

Instantaneous water-use efficiency (WUE$_i$) was calculated as the ratio of A/E. Measurements were initially taken under increasing light intensities (PAR from 0 to 2500 μmol m^{-2} s^{-1}) to determine the maximal net CO_2 assimilation rate (A) of 2000 μmol m^{-2} s^{-1} (data not shown). Subsequent measurements were taken at 2000 μmol m^{-2} s^{-1} PAR (saturating light intensity), 350 μmol mol^{-1} ambient CO_2 concentration and 29 ± 2 °C leaf temperature. Measurements were carried out between 10:00 a.m. and 2:00 p.m. on leaves acclimated to the leaf chamber conditions for 10 min (10 replicates per NaCl treatment). The gas exchange measurements were made 59 days after the start of the salt treatment.

2.3. Harvest and plant dry weight

At the end of the experiment, 90-day-old plants were harvested between 10:00 and 12:00 a.m. Leaf and root DWs of each plant were determined after measuring their surface area with a portable area meter (LI-3000A, Li-Cor Nebraska, USA). Plant DW was determined after oven drying samples to constant weight at 60 °C.

2.4. Measurements of water relations and osmolality

Relative water content (RWC) was calculated using the equation of Schonfeld et al. (1988):

$$RWC\,(\%) = [(FW - DW)/(TW - DW)]/100$$

where FW is leaf fresh weight, DW is leaf dry weight (7 d at 80 °C), and TW is leaf turgid weight (24 h at 4 °C in distilled water).

Leaf water potential (Ψ_w) was measured on five fully expanded leaves exposed to direct light radiation per treatment using the Scholander pressure-chamber technique 6–8 h after the onset of the light period (Scholander et al., 1965).

Solute potential (Ψ_S) was measured on freshly harvested leaves that were cut into small pieces, placed in Eppendorf tubes, and crushed with a pestle before being centrifuged at 15,000 g for 15 min at 4 °C. The supernatant was collected to measure the osmolality of leaf sap using a vapor pressure osmometer (Wescov 5500) (Puniran-Hartley et al., 2014). Osmolality is indicated by a value 'c', which is converted to Ψ_s according to the Vant'Haff equation:

$$\psi_S\,(MPa) = -c\,(mosmoles\,kg^{-1})\times 2.58 \times 10^{-3}$$

Osmotic adjustment (OA) was calculated, for each treatment, from the difference between the solute potential of the control (Ψ_{Sc}) and stressed plants (Ψ_{Ss}).

$$OA = \psi_{Ss} - \psi_{Sc}$$

Leaf water and osmotic potential were measured one day before the end of the treatments.

2.5. Proline and sugar concentrations

Free proline was extracted from 50 mL of sap cell with sulfosalicylic acid (3%) and quantified according to the protocol of Bates et al. (1973). Soluble sugars were extracted from dried plant material and determined spectrophotometrically at 640 nm using the method of Staub (1963).

2.6. Inorganic ion assay

Tissues were briefly rinsed with deionized water, oven-dried at 60 °C for at least 48 h, then weighed and ground to a fine powder. Inorganic solute accumulation was determined with an inductively coupled plasma atomic emission spectrometer after treating cell sap extracts with 0.5% nitric acid. Na^+ and K^+ were assayed in flame emission using an atomic absorption spectrophotometer (IL 151). Tissue nitrate concentrations were determined in aqueous extracts

using 0.5 g fresh weight in 5 mL distilled water (Cruz and Martins-Loução, 2002). The ammonium concentration was determined according to the reaction of Berthelot modified by Rhine et al. (1998) using 2-phenylphenol-tetra-hydrate as the chromogenic compound. Tissue nitrate concentrations were determined in aqueous extracts according to Jackson (1958).

2.7. Statistical analysis

The data were subjected to one-way ANOVA with means compared using Duncan's Multiple Range test at the 5% significance level using SPSS 16.0 software.

3. Results

3.1. Plant growth and development

In the absence of NaCl, the medium containing both nitrogen forms had the highest plant growth and the medium containing nitrate only had the lowest (Photo 1). Except for the NO_3^--only treatment, plants grown in the absence of NaCl had fully expanded, dark green leaves without any visual toxicity symptoms (Photo 1). The presence of ammonium in the medium significantly stimulated leaf growth and somewhat reduced root growth (Fig. 1).

Under the control conditions, plants in the NH_4^+-only medium had two-fold higher leaf DW than those in the NO_3^--only medium. In contrast, plants in the NO_3^--only medium had slightly higher root DW than those in the NH_4^+-only medium (Fig. 1A).

The introduction of NaCl to the irrigation solution severely reduced leaf and root growth, more so at the high salt concentration (150 mm NaCl) and with NO_3^- as the sole nitrogen source (Fig. 1) where the leaves became yellow.

The addition of 100 mM NaCl significantly reduced leaf growth, in terms of dry weight, by 11% and 7% in the sole NO_3^--fed and NH_4^+-fed plants, respectively (Fig. 1B). The impact of salinity was more pronounced for roots, with reductions of 30% and 15% in the sole NO_3^--fed and NH_4^+-fed plants, respectively (Fig. 1B). Irrespective of N source, the addition of 150 mM NaCl to the irrigation solution reduced leaf DW in maize; the effect was less marked in the mixed and sole NH_4^+ media, being 23% and 5% for the sole NO_3^-- and NH_4^+-fed plants, respectively (Fig. 1C). In the same NaCl treatment, root growth was more affected than leaf growth, with declines of 50 and 35% for the sole NO_3^-- and NH_4^+-fed plants, respectively (Fig. 1C). Leaf area responded similarly to leaf DW. Irrespective of salt concentration, NH_4^+ supplementation increased leaf expansion (Fig. 2).

In the absence of NaCl, the PA/R ratio (expressed as dry weight) increased significantly with increasing NH_4^+ concentration in the irrigation solution. Regardless of N source, the presence of NaCl increased this ratio, more so at 150 mM than 100 mM NaCl (Fig. 2).

3.2. Effect of NaCl and N form on gas exchange parameters

In the absence of NaCl, the N form did not affect net assimilation or internal leaf temperatures. However, stomatal conductance and transpiration increased with increasing NH_4^+ concentration in the irrigation solution (Table 1). The addition of 100 mM NaCl significantly reduced net assimilation, more so in the medium containing NO_3^- only. In contrast, stomatal conductance and transpiration remained the same or improved in the sole NO_3^--fed plants. The combined effect of salt and nitrogen form did not affect internal leaf temperature. Increasing the NaCl concentration from 100 to 150 mM significantly reduced all photosynthetic parameters (A, E and gs) and increased internal leaf temperature (Table 1). Leaf temperature increased the most when the irrigation solution contained ammonium as the sole nitrogen form (Table 1). In the absence of salt, the sole NO_3^--fed plants had the highest instantaneous water-use efficiency. The presence of NaCl
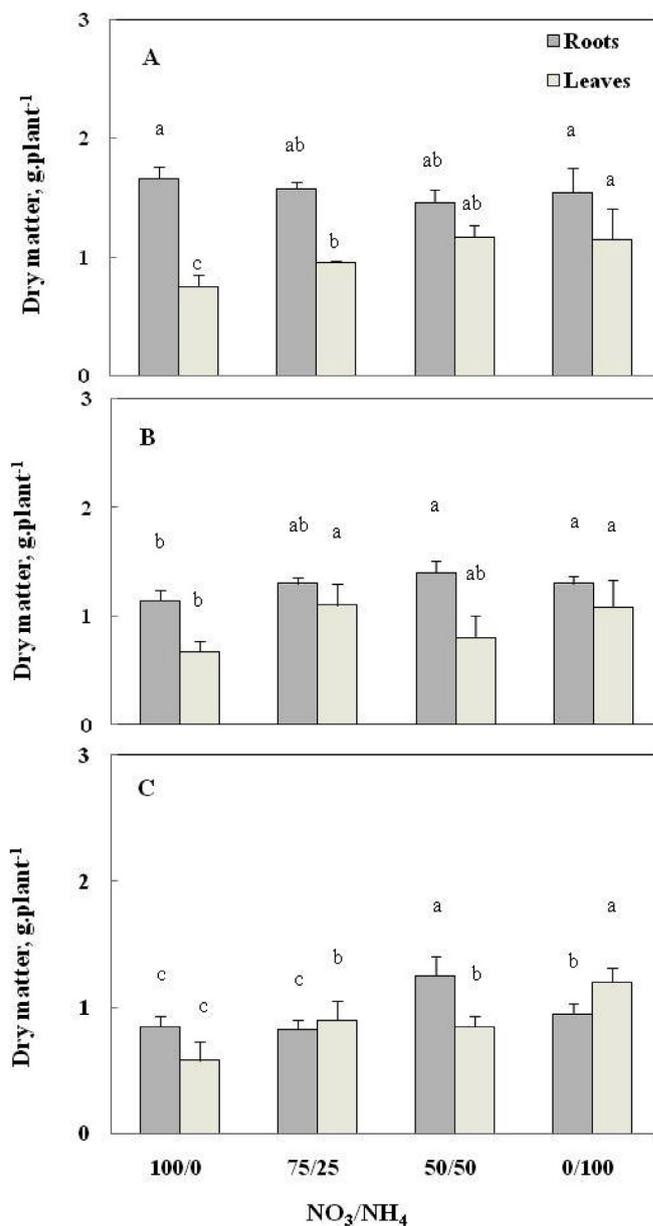

Fig. 1. Effect of nitrogen form and salinity level (A, 0; B, 100 mM; C, 150 mM NaCl) on leaf and root dry matter (DM, g plant^{-1}) in maize. Data presented as mean ± SE (n = 10). Different letters within the same column indicate significant differences between treatments at P ≤ 0.05.

severely reduced this value, more so at 150 mM NaCl and with NH_4^+ as the sole nitrogen source (Table 1).

3.3. Water relations parameters

In the absence of NaCl, the N form did not affect leaf water potential or turgor potential, but the presence of ammonium in the irrigation solution significantly reduced leaf osmotic potential (Table 2). Regardless of N form, the presence of NaCl significantly reduced the water and osmotic potentials, more so at 150 mM NaCl. The degree of OA increased with increasing NaCl concentration in the irrigation solution, more so in the presence of ammonium (Table 2).

3.4. Organic and inorganic solute concentrations

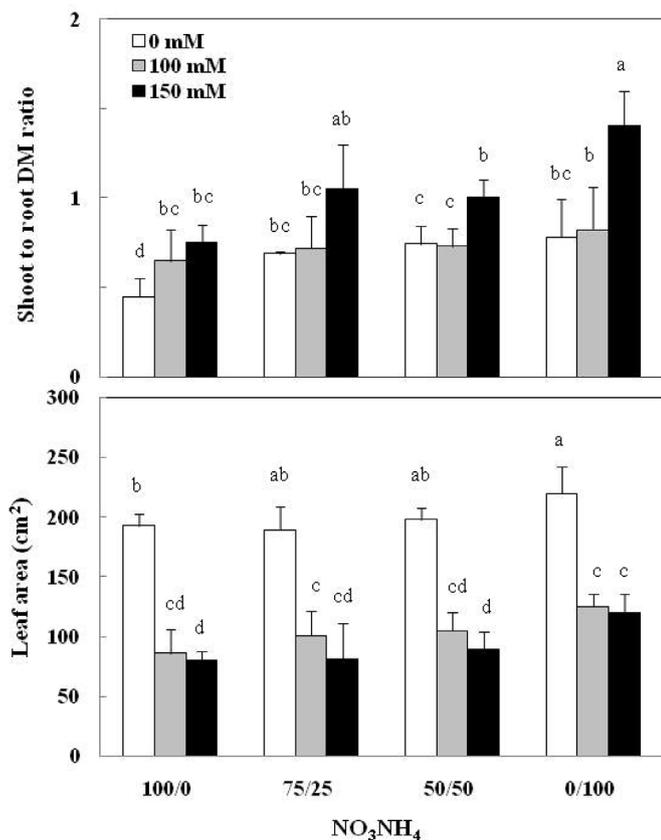Table 3 shows the different amounts of inorganic ions (Na^+, K^+,

Fig. 2. Effect of nitrogen form and salinity level on shoot to root dry matter ratio (top) and leaf area (cm^2; bottom) in maize. Data presented as mean ± SE (n = 10). Different letters within the same column indicate significant differences between treatments at P ≤ 0.05.
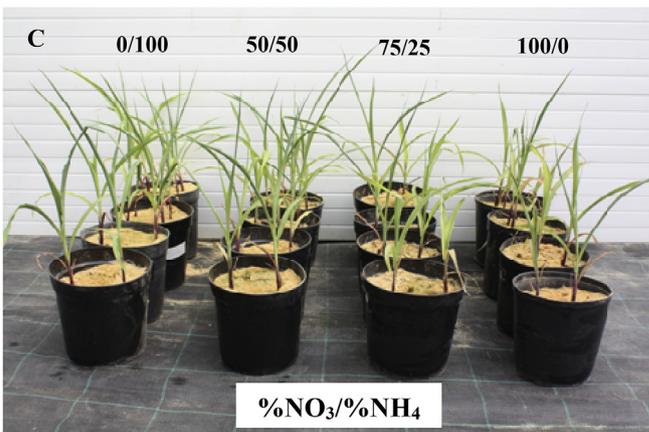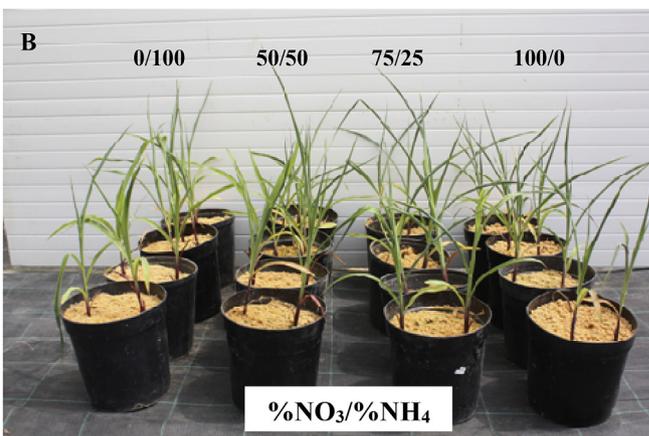

Photo 1. Effect of nitrogen form and salinity level (A, 0; B, 100 mM; C, 150 mM NaCl) on plant growth and development in maize. Each treatment consisted of eight pots, each containing two plants.

NH$_4^+$, Ca^{2+}, Mg^{2+}, Cl$^-$, NO$_3^-$) in leaf cell sap at full turgor at the end of the experiment. The concentration of Na$^+$ ions had a strong dependence on the interactive effect of N form and NaCl concentration. In general, the presence of NaCl gradually increased Na$^+$ levels in leaf cell sap, but the level of accumulation was N-dependent.

The NH$_4^+$-only treatment decreased Na$^+$ accumulation in leaf tissue by moderate (20%) and severe (82%) amounts in the presence of 100 and 150 mM NaCl, respectively. The NO$_3^-$-only medium produced higher K$^+$ concentrations in leaves than the NH$_4^+$-only medium. The presence of NaCl decreased K$^+$ accumulation, more so in the NO$_3^-$-only medium.

The ratio of K$^+$ to Na$^+$ content, which indicates the potential for plants to discriminate the two ions (Gorham et al., 1990), declined by 46% when nitrate was replaced by ammonium in the nutrient solution. In contrast, the presence of NaCl in the nutrient solution increased the K$^+$/Na$^+$ ratio by 38% and 180% at 100 and 150 mM NaCl, respectively.

Irrespective of N source, Ca^{2+} concentration was higher than that of Mg^{2+}, with the presence of NaCl increasing Ca^{2+} but decreasing Mg^{2+}. Leaf NH$_4^+$ concentrations increased and NO$_3^-$ concentrations decreased as the proportion of N supplied as NH$_4^+$ increased (Table 3). In the sole NO$_3^-$-fed plants, the addition of 150 mM NaCl in the irrigation solution decreased leaf NO$_3^-$ concentrations by about 85% but no significant differences were found at 100 mM. The opposite was observed for NH$_4^+$ concentrations that increased three-fold at 150 mM NaCl, relative to the treatment without NaCl.

The Cl$^-$ content in leaves responded similarly to that of Na$^+$. However, the presence of NH$_4^+$ in the irrigation solution significantly reduced Cl$^-$ accumulation in leaves (Table 3).

In the absence of NaCl and irrespective of N source, leaf proline concentration was low. In the presence of NaCl, plants grown in the sole NH$_4^+$-medium had higher proline contents than the other treatments, more so at the low NaCl concentration (100 mM). At high salinity (150 mM NaCl) plants grown in the sole NH$_4^+$-medium had five-fold higher leaf proline concentrations than those grown in the sole NO$_3^-$-medium.
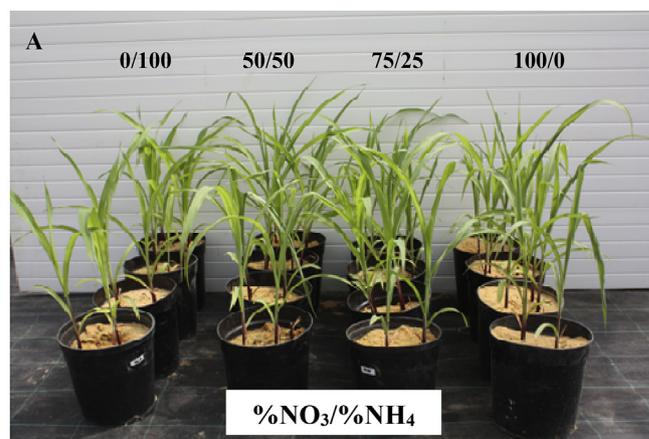
Plants grown in the sole NO$_3^-$-medium had the lowest concentrations of soluble sugars in leaves. The presence of NaCl increased the concentration of soluble sugars, more so at 150 mM than 100 mM NaCl (Table 3).

Table 1
Effect of nitrogen form and salinity level on photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs), instantaneous water-use efficiency (WUEi, µmol CO_2 m^{-2} s^{-1})/(mmol H_2O m^{-2} s^{-1})) and leaf temperature (°C) in maize leaves.

Treatments (%NO$_3$/%NH$_4$)	NaCl (mM)	A	E	g_s	WUE$_i$ (A/E)	Tc (°C)
100/0	0	15.4 ± 2.4a	1.8 ± 0.2d	80 ± 10c	8.6 ± 0.6	31.1 ± 0.7d
	100	7.6 ± 3c	2.3 ± 0.1c	80 ± 10c	3.3 ± 0.4	32.9 ± 0.2c
	150	4.1 ± 1.6cd	1.8 ± 0.1d	70 ± 10c	2.3 ± 0.4	34.7 ± 0.7b
75/25	0	18.9 ± 1.1a	4.3 ± 0.4a	200 ± 20a	4.4 ± 5.4	32.6 ± 0.1d
	100	15.4 ± 4a	2.3 ± 0.3c	100 ± 20b	6.7 ± 5.4	33.1 ± 0.2b
	150	3.1 ± 0.3d	1.6 ± 0.4d	60 ± 10b	1.9 ± 5.4	36.6 ± 0.1a
50/50	0	17.4 ± 1.1a	3.6 ± 0.1b	150 ± 40a	4.8 ± 5.4	32.7 ± 0.1c
	100	12.7 ± 3b	2.8 ± 0.4c	120 ± 20b	4.5 ± 5.4	33.2 ± 0.1bc
	150	2.8 ± 0.5d	2.7 ± 0.1c	80 ± 10c	1.1 ± 5.4	36.7 ± 0.1a
0/100	0	16.8 ± 3a	3.7 ± 0.1b	170 ± 30a	4.5 ± 5.4	32.7 ± 0.5b
	100	15.5 ± 2a	3.5 ± 0.3b	150 ± 20a	4.4 ± 5.4	33.7 ± 0.3bc
	150	3.7 ± 0.5cd	4.6 ± 0.1a	170 ± 30a	0.8 ± 5.4	35.4 ± 0.1b

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
Effect of nitrogen form and salinity level on leaf water potential (Ψw), osmotic potential (Ψs), turgid potential (Ψp), and osmotic adjustment (OA).

Treatments (% NO$_3$/%NH$_4$)	NaCl (mM)	Ψ_w (−MPa)	Ψ_s (−MPa)	Ψ_p (MPa)	OA
100/0	0	0.2 ± 0.1a	0.6 ± 0.2b	0.4 ± 0.1 ab	–
	100	0.6 ± 0.3b	0.8 ± 0.1bc	0.2 ± 0.1a	0.2
	150	0.6 ± 0.b	0.9 ± 0.1c	0.2 ± 0.1a	0.3
75/25	0	0.2 ± 0.1a	0.8 ± 0.4bc	0.5 ± 0.1b	–
	100	0.6 ± 0.2b	1.2 ± 0.3cd	0.6 ± 0.2b	0.4
	150	1.0 ± 0.3c	1.3 ± 0.4cd	0.4 ± 0.1 ab	0.5
50/50	0	0.3 ± 0.1a	0.9 ± 0.1c	0.6 ± 0.2b	–
	100	0.6 ± 0.3b	1.1 ± 0.4cd	0.5 ± 0.1b	0.2
	150	0.8 ± 0.3bc	1.4 ± 0.1d	0.6 ± 0.1b	0.5
0/100	0	0.3 ± 0.1a	0.9 ± 0.1c	0.6 ± 0.1b	–
	100	0.6 ± 0.2b	1.5 ± 0.3d	0.9 ± 0.2c	0.6
	150	0.8 ± 0.3bc	1.7 ± 0.1d	0.9 ± 0.3c	0.8

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

4. Discussion

Despite the higher energy costs of NO_3^- uptake relative to other N sources, plants are generally well-adapted to NO_3^- nutrition (Cruz et al., 2006; Glibert et al., 2016). Indeed, NO_3^- toxicity in plants is rarely observed, even when it accumulates to concentrations several times higher than those recommended for maximum plant growth (Cruz et al., 2006; Hessini et al., 2009a). In contrast, even relatively low concentrations of NH_4^+ can induce toxicity symptoms, including foliar necrosis, degradation of chloroplastic membranes, and disturbed hydraulic and osmotic status (Moschou et al., 2012; Glibert et al., 2016). Some plants, such as the maize variety used in this study, are NH_4^+ tolerant or grow better with NH_4^+ than NO_3^- (Hessini et al., 2013; Ashraf et al., 2018). Under non-buffered conditions, NH_4^+ nutrition results in acidification of the root medium—a cause of ammonium toxicity (Esteban et al., 2016)—due to disturbances in relative ion uptake and the consequent plant nutritional imbalance. Therefore, plant tolerance to NH_4^+ nutrition may depend on the buffering capacity of the root medium.

Plants can increase their biomass by increasing the size of existing leaves and roots, or by creating new ones (Hessini et al., 2017). In our study, changes in leaf biomass were accompanied by a proportional increase in leaf area, with little or no change in leaf initiation, indicating that the improved growth in the combined and sole NH_4^+ treatments was mainly due to increases in pre-existing leaf extension rather than new leaf formation. In contrast, root DW either did not change or only increased slightly in sole NO_3^--fed plants, indicating

that the beneficial effects of NH_4^+ are essentially linked to leaf growth.

Although maize is considered moderately sensitive to salinity, there are no published studies on inter- or intra-specific variability in this regard. The variety chosen for the present study (Sincero) is the most salt-tolerant of five other commercial varieties (unpublished results). Studies on the combined effect of N source and salinity on plant growth and development commenced in the 1960s. The findings of these studies were dependent on plant species, the intensity and duration of the salt stress, and the nitrogen form and concentration present in the medium. Regardless of N form, the presence of NaCl significantly inhibits maize growth, more so as the concentration increases and more on root growth than shoot growth, indicating the high sensitivity of roots to salinity.

The presence of ammonium in the nutrient solution reduced the adverse effects of NaCl (up to 150 mM) on leaf and root growth. Several studies have reported increased sensitivity to salinity in sole NH_4^+-fed plants (Lewis et al., 1989; Bybordi, 2012), but the NaCl tolerance of sole NH_4^+-fed plants in our study either did not change or increased, more so at the higher level (150 mM). In contrast, sole NO_3^--fed plants in the presence of 150 mM NaCl had pale yellow young leaves and completely yellow old leaves, as described in Zhu et al. (2014).

Although not essential for most plants, sodium (Na^+) and chloride (Cl^-) can be beneficial or even essential for some C$_4$-type species, including maize (Maathuis, 2014). Indeed, at low soil water potential, these two minerals (Na^+ and Cl^-), among others, actively accumulated in cell vacuoles (i.e., osmotic adjustment) to reduce osmotic potential and maintain turgor pressure to attract water into the cells (Hessini et al., 2009b). However, at high concentrations, Na^+ and Cl^- become toxic and cause morphological, physiological, biochemical and molecular disturbances in most plants (Ashraf and Harris, 2013; Flowers et al., 2015).

In our experiment, sole NH_4^+-fed plants grew better than sole NO_3^--fed plants and maintained lower leaf Na^+ concentrations, even when roots were exposed to 150 mM NaCl for more than 60 days. This suggests that plants fed NH_4^+ or NO_3^- interact differently to high salt concentrations. The lower leaf Na^+ content of sole NH_4^+-fed plants may have resulted from restricted Na^+ uptake by roots (Ferchichi et al., 2018), due to increased competition for transport through non-specific cation channels (NSCC; Demidchik and Maathuis, 2007). The higher leaf Na^+ content of sole NO_3^--fed plants may have resulted from the accumulation of leaf Na^+ to compensate for the negative water potential created by salts in the soil solution, as described for halophytes (Flowers et al., 2015) and some glycophytes including cereals (Genc et al., 2016). In Arabidopsis, a large proportion of the Na^+ accumulated in shoots is taken up from the medium and loaded into the xylem by systems that depend on nitrate and require the presence of the nitrate transporter NRT1.1 (Alvarez-Aragon et al., 2016). The co-transport of

Table 3
Effect of nitrogen form and salinity on maize leaf Na $^+$, K $^+$, Ca $^{2+}$, Mg $^{2+}$, NH $_4$ $^+$, Cl $^-$, NO $_3$ $^-$, proline, and total soluble carbohydrates concentrations (mM).

Solute concentration (mM) %NO3/%NH4	100/0 NaCl (mM)			75/0 NaCl (mM)			50/50 NaCl (mM)			0/100 NaCl (mM)		
	0	100	150	0	100	150	0	100	150	0	100	150
Na $^+$	12.2 ± 4.1e	343 ± 35c	1107 ± 22a	12 ± 2e	333.1 ± 22c	520 ± 50b	12.1 ± 1e	327.4 ± 30c	470 ± 60b	12.4 ± 3e	220.8 ± 25d	190 ± 23d
K $^+$	345.2 ± 10a	133.8 ± 24c	257.3 ± 13b	251 ± 34b	135.6 ± 10c	150.1 ± 9C	209.9 ± 24b	136.4 ± 8c	149.9 ± 8c	189.4 ± 15c	119.3 ± 5cd	122.7 ± 20cd
K $^+$/Na $^+$	28.3 ± 2a	0.39 ± 0.01e	0.23 ± 0.02f	20.9 ± 4b	0.4 ± 0.02de	0.29 ± 0.08e	17.3 ± 3b	0.42 ± 0.07de	0.32 ± 0.06e	15.3 ± 1c	0.54 ± 0.1de	0.65 ± 0.1d
Ca $^{2+}$	161.8 ± 11d	276.8 ± 15b	257.9 ± 18b	180.3 ± 9c	270 ± 20b	160.7 ± 15d	191.4 ± 4.6c	268.7 ± 6.3b	150.9 ± 14d	190.8 ± 11c	381.4 ± 13a	342.7 ± 11a
Mg $^{2+}$	68.5 ± 7.2a	30.6 ± 5.6b	61.9 ± 5.9a	60 ± 8a	27.6 ± 2.1 c	40.1 ± 5ab	39.8 ± 7ab	28.6 ± 3.8c	38.6 ± 6ab	36.8 ± 6ab	27.7 ± 4.5c	23.1 ± 1.1 ab
NH $_4$ $^+$	0.17 ± 0.02c	0.21 ± 0.15c	0.47 ± 0.02a	0.18 ± 0.01c	0.2 ± 0.03c	0.36 ± 0.1 ab	0.4 ± 0.15a	0.39 ± 0.1a	0.37 ± 0.11 ab	0.51 ± 0.16a	0.45 ± 0.12a	0.31 ± 0.09b
Cl $^-$	142 ± 11f	296 ± 9c	600 ± 14a	133 ± 9f	276 ± 20cd	381 ± 25b	128 ± 11f	255 ± 9d	366 ± 21b	96 ± 5j	214 ± 10e	269 ± 10d
NO $_3$ $^-$	13.9 ± 2.1a	15.5 ± 3.2a	2.1 ± 0.8c	12.2 ± 2.2 ab	13.5 ± 3.1a	1.9 ± 0.6c	9.7 ± 2.9b	9.3 ± 1.7b	0.9 ± 0.03d	trace	trace	trace
Proline	4 ± 0.6b	2.2 ± 0.2c	2.6 ± 0.3a	3.4 ± 0.2b	0.53 ± 0.2d	2.1 ± 0.3c	3.2 ± 0.6b	0.5 ± 0.1d	2.33 ± 0.5c	4.8 ± 0.2b	0.25 ± 0.1e	13.4 ± 0.8a
Soluble sugars	30.8 ± 2d	78.4 ± 8b	136 ± 13a	69.3 ± 4b	121 ± 10a	137 ± 12a	81 ± 8b	126 ± 14a	134 ± 14a	71.4 ± 3b	115 ± 9 ab	134 ± 7a

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

Na $^+$ and NO $_3$ $^-$ by the NRT1.1 protein, whose synthesis is induced by the presence of NO $_3$ $^-$, may explain the distinct responses of sole NH $_4$ $^+$- and NO $_3$ $^-$-fed plants to the presence of NaCl. While root Na $^+$ uptake and tissue accumulation fulfill the primary function of osmotic adjustment, the processes may lead to long-term toxicity, as evidenced by the reduction in biomass accumulation and leaf chlorosis observed in sole NO $_3$ $^-$-fed plants.

The effect of salinity on NO $_3$ $^-$ uptake is controversial. In some C $_4$ plants, such as *Amaranthus tricolor* L., Na $^+$ specifically enhances NO $_3$ $^-$ uptake, which has a significant positive effect on plant growth (Ohta et al., 1989). However, in other species, NaCl treatments have reduced NO $_3$ $^-$ uptake (Ehlting et al., 2007). In the NaCl treatments (100 and 150 mM) of our study, the leaves of plant fed NO $_3$ $^-$ as the sole nitrogen source showed visual symptoms of toxicity characteristic of N deficiency that were accompanied by increased Cl $^-$ accumulation in leaves, suggesting that NO $_3$ $^-$ is partially replaced by Cl $^-$ during root uptake. The cellular targets for Cl $^-$ toxicity remain elusive, but high concentrations can interfere with root NO $_3$ $^-$ uptake (Bazihizina et al., 2019). Inhibition of NO $_3$ $^-$ uptake by Cl $^-$ depends on plant species and the concentrations of NO $_3$ $^-$ and Cl $^-$ in the uptake medium (Cerezo et al., 1997). In root cells, the high-affinity saturable system for NO $_3$ $^-$ uptake that operates at low NO $_3$ $^-$ concentrations is inhibited by high external Cl $^-$, whereas the low-affinity linear system that operates at high NO $_3$ $^-$ concentrations is inhibited by high internal Cl $^-$ (Cerezo et al., 1997). The competition between Cl $^-$ and NO $_3$ $^-$ is stronger in salt-sensitive plants than salt-tolerant plants (Leidi et al., 1992). Tissue NH $_4$ $^+$ levels result from the balance between the reactions that consume (GS, GDH) and produce (NR, amino acid catabolism) ammonium, which can be altered under salt exposure (Hessini et al., 2009d). The 150 mM NaCl treatment in our study increased leaf NH $_4$ $^+$ concentration in plants fed NO $_3$ $^-$ as the sole nitrogen source. According to Hessini et al. (2009d), this can result from amino acid and protein catabolism activated by reactive oxygen species (ROS) and/or a lack of carbon skeletons to incorporate ammonium into organic molecules due to a decline in photosynthetic activity in salt-stressed plants.

The antioxidant response to salinity is a possible salt-tolerance mechanism that may offer significant advantages to sole NH $_4$ $^+$-grown plants. For example, in *S. alterniflora*, SOD activity—the 'first line of defense' against oxidative stress—was about three times higher in sole NH $_4$ $^+$-grown than sole NO $_3$ $^-$-grown plants, which was associated with less electrolyte leakage and lower concentrations of MDA and H $_2$O $_2$ in sole NH $_4$ $^+$-grown plants (Hessini et al., 2013). It is well-known that salinity disturbs mineral-nutrient relations in plants through their effects on nutrient availability, transport and partitioning. Competition between NH $_4$ $^+$ and Na $^+$ for root uptake sites was also observed for K $^+$. Compared to sole NO $_3$ $^-$-fed plants, the increased K $^+$/Na $^+$ ratio in sole NH $_4$ $^+$-fed plants with NaCl indicated that competition between NH $_4$ $^+$ and Na $^+$ for root uptake sites was more pronounced than between NH $_4$ $^+$ and K $^+$. However, the apparent competition between cations for transport through the NSCC in sole NH $_4$ $^+$-fed plants did not involve Ca $^{2+}$, as its concentration increased in the leaves. Many publications have shown that when plants are exposed to stress, an essential function of Ca $^{2+}$ is that of a secondary messenger in stress signaling (Hessini et al., 2009c). Liu and Zhu (1998) demonstrated its crucial role in triggering a signaling cascade to active Na $^+$/H $^+$ antiporters. Additionally, salinity stress induces ion deficiencies or imbalances due to competition for nutrients including Cl $^-$ and NO $_3$ $^-$ (Rahneshan et al., 2018). The presence of high Cl $^-$ concentrations in the irrigation solution reduces NO $_3$ $^-$ uptake but using NO $_3$ $^-$ as the N form also reduces Cl $^-$ uptake and accumulation (Jabeen and Ahmad, 2011).

Plant growth is under the control of many physiological, biochemical and molecular processes, particularly photosynthesis (Ashraf and Harris, 2013). The impact of salinity on photosynthesis is strongly dependent on N form, plant species, and the duration and intensity of the salt stress (Hessini et al., 2013). In our study, in the absence of NaCl, variations in net assimilation and leaf temperature were independent of

N form. However, stomatal conductance and transpiration were higher in the sole NH_4^+-fed plants. The lack of coordination between increases in stomatal conductance and transpiration, on the one hand, and photosynthesis, on the other hand, is characteristic of C_4 plants (Ashraf and Harris, 2013), where the net CO_2 assimilation rate is conserved independently of stomatal conductance (Hessini et al., 2013). An increase from 100 to 150 mM NaCl significantly decreased leaf gas exchanges rates (A, E and g_s), with significant effects on leaf temperature, which may be considered an early indicator of stress (O'Neill et al., 2011).

Leaf temperature is dependent on plant water status. Irrespective of N form, leaf water potential declined significantly in the presence of NaCl, more so at the high concentration (150 mM NaCl). In sole NO_3^--fed plants, leaf water content also declined, indicating that maize struggled to maintain tissue hydration (Hessini et al., 2017). In contrast, sole NH_4^+-fed plants maintained or even improved tissue hydration in the presence of NaCl. At the same time, osmotic potential declined significantly in the presence of NaCl, more so in plants in the NH_4^+-only medium than the NO_3^--only or mixed media. This indicates that sole NH_4^+-fed plants faced with saline conditions use strategies similar to those observed in halophytes—reducing cell osmotic potential and maintaining cell turgidity (Hessini et al., 2008, 2009b, 2017). A broad group of low-molecular-weight organic solutes (amino acids, tertiary sulphonium, quaternary ammonium compounds, sugars and polyhydric alcohols) accumulate in the cytoplasm of several species to balance the osmotic potential of the accumulated Na^+ and Cl^- in the vacuole. For example, glycine betaine is a quaternary ammonium compound that is an effective compatible solute and accumulates in the chloroplasts of many species when exposed to harsh conditions. It could play a role in maintaining intracellular osmotic equilibrium during stress conditions (Slama et al., 2015). In addition to their role in osmoregulation, these compounds can protect plants from osmotic stress by cellular osmotic adjustment, detoxification of ROS, protection of membrane integrity, and stabilization of enzymes/proteins (Ashraf et al., 2018; Slama et al., 2015). The nature of organic solutes accumulated in response to the combined effects of NaCl and N form depend more on the species, nature and duration of stress, and N concentration than N form (Slama et al., 2015).

In the present study, positive correlations occurred between the degree of osmotic adjustment and proportion of NH_4^+ in the NO_3^-:NH_4^+ ratio and between the degree of osmotic adjustment and amount of biomass production, which is common in sole NH_4^+-fed plants (Hessini et al., 2013). Except for sole NO_3^--fed plants, the reduction in osmotic potential (or development of active osmotic adjustment) is due to the active accumulation of organic and inorganic solutes, more so inorganic ions than organic solutes (proline and soluble sugars).

5. Conclusions

The stimulating effects of NH_4^+ nutrition on maize performance under buffered media suggest that acidification of the external medium is an important component of plant NH_4^+ toxicity. The presence of ammonium was beneficial or even necessary for maize growth and development, especially under saline conditions. Our results suggest that it is possible to exploit moderately salt-contaminated soils by growing higher salt-tolerant ecotypes and using NH_4^+-based fertilizers. Such practices will enhance the development of new saline agriculture in calcareous soils in arid and semiarid regions.

Author contributions

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

References

Alvarez-Aragon, R., Haro, R., Benito, B., Rodríguez-Navarro, A., 2016. Salt intolerance in Arabidopsis: shoot and root sodium toxicity, and inhibition by sodium-plus-potassium over accumulation. Planta 243, 97–114.

Ashraf, M., Harris, P.J.C., 2013. Photosynthesis under stressful environments: an overview. Photosynthetica 2, 163–190.

Ashraf, M., Muhammad, S., Imtiaz, M., Rizwan, M.S., Arif, M.S., Kausar, R., 2018. Nitrogen nutrition and adaptation of glycophytes to saline environment: a review. 64 (9), 1181–1206.

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

Bazihizina, N., Colmer, T.D., Cuin, T.A., Mancuso, S., Shabala, S., 2019. Friend or foe? Chloride patterning in halophytes. Trends Plant Sci. 24 (2), 142–151.

Berenguer, P., Santiveri, F., Boixadera, J., Lloveras, J., 2009. Nitrogen fertilization of irrigated maize under Mediterranean conditions. Eur. J. Agron. 30, 163–171.

Bitew, Y., Alemayehu, M., 2017. Impact of crop production inputs on soil health: a review. Asian J. Plant Sci. 16 (3), 109–131.

Britto, D.T., Kronzucker, H.J., 2002. NH_4^+ toxicity in higher plants: a critical review. J. Plant Physiol. 159, 567–584.

Bybordi, A., 2012. Effect of different ratios of nitrate and ammonium on photosynthesis, and fatty acid composition of canola under saline conditions. Intl. J. Agric. Crop Sci. 4 (10), 622–626.

Cantera, R., Urdaniz, A., Lerga, J., Garcia-Mina, J.M., 1999. Soils with Mediterranean type of climate. In: Bech, J. (Ed.), Acidifying Product as a Source of Stabilized Ammonia Nitrogen under Greenhouse Conditions. UB Publicaciones, Barcelona, pp. 233–235.

Cerezo, M., Garcia-Agustin, P., Serna, M.D., Primo-Millo, E., 1997. Kinetics of nitrate uptake by citrus seedlings and inhibitory effects of salinity. Plant Sci. 126, 105–112.

Coleto, I., Viga-Mas, I., Glauser, G., González-Moro, M.B., Marino, D., Ariz, I., 2019. New insights on *Arabidopsis thaliana* root adaption to ammonium nutrition by the use of a quantitative proteomic approach. Int. J. Mol. Sci. 20 (4), 814.

Cruz, C., Bio, A.F.M., Domíguez-Valdivia, M.D., Aparicio-Tejo, P.M., Lamsfus, C., Martins-Loução, M.A., 2006. How does glutamine synthetase activity determine plant tolerance to ammonium? Planta 223, 1068–1080.

Cruz, C., Martins Loução, M.A., 2002. Comparison of methodologiesfor nitrate determination in plants and soils. J. Plant Nutr. 25, 1185–1211.

Demidchik, V., Maathuis, F.J.M., 2007. Physiological roles of nonselective cation channels in plants: from salt stress to signalling and development. New Phytol. 175, 387–404.

Ehlting, B., Dluzniewsk, P., Dietrich, H., Selle, A., Teuber, M., Hänsch, R., Nehls, U., Polle, A., Schnitzler, J.-P., Rennenberg, H., Gessler, A., 2007. Interaction of nitrogen nutrition and salinity in grey poplar (*Populus tremula* × *alba*). Plant Cell Environ. 30, 796–811.

Esteban, R., Ariz, I., Cruz, C., Moran, J.F., 2016. Review: Mechanisms of ammonium toxicity and the quest for tolerance. Plant Sci. 248, 92–101.

Ferchichi, S., Hessini, K., Dell'Aversana, E., D'Amelia, L., Woodrow, P., Ciarmiello Lo, F., Fuggi, A., Carillo, P., 2018. *Hordeum vulgare* and *Hordeum maritimum* respond to extended salinity stress displaying different temporal accumulation pattern of metabolites. Funct. Plant Biol. https://doi.org/10.1071/FP18046.

Fernandez-Crespo, E., Camanes, G., Garcia-Agustin, P., 2012. Ammonium enhances resistance to salinity stress in citrus plants. J. Plant Physiol. 169, 1183–1191.

Flowers, T.J., Munns, R., Colmer, T.D., 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Ann. Bot. 115, 419–431.

Genc, Y., Oldach, K., Taylor, J., Lyons, G.H., 2016. Uncoupling of sodium and chloride to assist breeding for salinity in crops. New Phytol. 210, 145–156.

Glibert, P.M., Wilkerson, F.P., Dugdale, R.C., Raven, J.A., Dupont, C.L., Leavitt, P.R., Parker, A.E., Burkholder, J.M., Kana, T.M., 2016. Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. Limnol. Oceanogr. 61, 165–197.

Gorham, J., Jones, R.G.W., Bristol, A., 1990. Partial characterization of the trait for enhanced $K^+ - Na^+$ discrimination in the D genome of wheat. Planta 180, 590–597.

Hachiya, T., Sakakibara, H., 2017. Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. J. Exp. Bot. 68 (10), 2501–2512.

Hessini, K., Ben Hamed, K., Gandour, M., Mejri, M., Abdelly, C., Cruz, C., 2013. Ammonium nutrition in the halophyte *Spartina alterniflora* under salt stress: evidence for a priming effect of ammonium? Plant Soil 370, 163–173.

Hessini, K., Cruz, C., Gandour, M., Soltani, A., Abdelly, A., 2009d. Do reactive oxygen species (ROS) induced by NaCl contribute to ammonium accumulation in *Spartina alterniflora*? J. Plant Nutr. Soil Sci. 172, 851–860.

Hessini, K., Ferchichi, F., Ben Youssef, S., Koyro, H.-W., Cruz, C., Gandour, M., 2015. How does salinity duration affect growth and productivity of cultivated barley? Agron. J. 107, 174–180.

Hessini, K., Gandour, M., Megdich, W., Soltani, A., Abdely, C., 2009c. How does ammonium nutrition influence salt tolerance in *Spartina alterniflora* Loisel? In: Ashraf,

M., Ozturk, M., Athar, Habib-ur-Rehman (Eds.), Salinity and Water Stress – Improving Crop Efficiency. Springer, Netherlands, pp. 91–96.

Hessini, K., Ghandour, M., Albouchi, A., Soltani, A., Koyro, H.-W., Abdelly, C., 2008. Biomass production, photosynthesis, and leaf water relations of *Spartina alterniflora* under moderate water stress. J. Plant Res. 121, 311–318.

Hessini, K., Kronzucker, H.J., Abdelly, C., Cruz, C., 2017. Drought stress obliterates the preference for ammonium as an N source in the C$_4$ plant *Spartina alterniflora*. J. Plant Physiol. 213, 98–107.

Hessini, K., Lachaal, M., Cruz, C., Soltani, A., 2009a. Role of ammonium to limit nitrate accumulation and to increase water economy in *Wild Swiss* chard. J. Plant Nutr. 32, 821–836.

Hessini, K., Martínez, J.P., Ghandour, M., Albouchi, A., Soltani, A., Abdelly, C., 2009b. Effect of water stress on growth, osmotic adjustment, cell wall elasticity and water-use efficiency in *Spartina alterniflora*. Environ. Exp. Bot. 67, 312–319.

Iqbal, N., Umar, S., Khan, N.A., 2015. Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). J. Plant Physiol. 178, 84–91.

Jabeen, N., Ahmad, R., 2011. Foliar application of potassium nitrate affects the growth and nitrate reductase activity in sunflower and safflower leaves under salinity. Not. Bot. Horti Agrobot. 39 (2), 172–178.

Jackson, M.L., 1958. Nitrogen determination for soil and plant tissue. In: Soil Chemical Analysis. Prentice Hall, Englewood Cliffs, NJ, pp. 181–204.

Leidi, E.O., Silberbush, M., Soares, M.I.M., Lips, S.H., 1992. Salinity and nitrogen nutrition studies on peanut and cotton plants. J. Plant Nutr. 15, 591–604.

Lewis, O.A.M., Leidi, E.O., Lips, S.H., 1989. Effect of nitrogen source on growth response to salinity stress in maize and wheat. New Phytol. 111, 155–160.

Li, S., Li, J., Zhang, B., Li, D., Li, G., Li, Y., 2017. Effect of different organic fertilizers application on growth and environmental risk of nitrate under a vegetable field. Sci. Rep. 7, 017–020.

Liu, J.P., Zhu, J.K., 1998. A calcium sensor homolog required for plant salt tolerance. Science 280, 1943–1945.

Maathuis, F.J.M., 2014. Sodium in plants: perception, signaling, and regulation of sodium fluxes. J. Exp. Bot. 65 (3), 849–858.

Marton, J.M., Herbert, E.R., Craft, C.B., 2012. Effects of salinity on denitrification and greenhouse gas production from laboratory-incubated tidal forest soils. Wetlands 32, 347–357.

Moschou, P.N., Wu, J., Cona, A., Tavladoraki, P., Angelini, R., Roubelakis-Angelakis, K.A., 2012. The polyamines and their catabolic products are significant players in the turnover of nitrogenous molecules in plants. J. Exp. Bot. 63 (14), 5003–5015.

O'Neill, B.F., Zangerl, A.R., DeLucia, E.H., Casteel, C., Zavala, J.A., Berenbaum, M.R., 2011. Leaf temperature of soybean grown under elevated CO$_2$ increases *Aphis glycines* (Hemiptera: Aphididae) population growth. Insect Sci. 00, 1–7.

Ohta, D., Yasuoka, S., Matoh, T., Takahashi, E., 1989. Sodium stimulates growth of Amaranthus tricolor L. plants through enhanced nitrate assimilation. Plant Physiol. 89, 1102–1105.

Puniran-Hartley, N., Hartley, J., Shabala, L., Shabala, S., 2014. Salinity-induced accumulation of organic osmolytes in barley and wheat leaves correlates with increased oxidative stress tolerance: in planta evidence for cross-tolerance. Plant Physiol. Biochem. 83, 32–39.

Rahneshan, Z., Nasibi, F., Moghadam, A.A., 2018. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. J. Plant Interact. 13 (1), 73–82.

Rhine, E.D., Sims, G.K., Mulvaney, R.L., Pratt, E.J., 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Sci. Soc. Am. J. 62, 473–480.

Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemmingsen, E.D., 1965. Sap pressure in vascular plants. Science 148, 339–346.

Schonfeld, M.A., Johnson, R.C., Carver, B.F., Mornhinweg, D.W., 1988. Water relations in winter wheat as drought resistance indicator. Crop Sci. 28, 526–531.

Slama, I., Abdelly, C., Bouchereau, A., Flowers, T., Savouré, A., 2015. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. Ann. Bot. 115 (3), 433–447.

Staub, A.M., 1963. Extraction, identification et dosages des glucides dans les extraits d'organes et les corps bactériens. In: Loiseleur, J. (Ed.), Techniques de Laboratoire. Tome 1 et 2. Masson et Compagnie, Paris, France, pp. 1307–1366.

Zhu, Y., Fan, X., Hou, X., Wu, J., Wang, T., 2014. Effect of different levels of nitrogen deficiency on switchgrass seedling growth. Crops J 2, 223–234.