Physio-biochemical characterization of two acacia species (*A. karroo* Hayn and *A. saligna* Labill.) under saline conditions

Abdenour Kheloufi[1]✉, Lahouaria Mounia Mansouri[1], Anas Mami[2], Mohammed Djelilate[3]

[1]Department of Ecology and Environment, University of Batna2, Batna, Algeria
[2]Department of Biology, Faculty of Science, University of Oran, Oran, Algeria
[3]Department of Biology, University of Relizane, Relizane, Algeria

✉ abdenour.kheloufi@yahoo.fr

ARTICLE INFO

Citation:
Kheloufi A, Mansouri LM, Mami A, Djelilate M (2019) Physio-biochemical characterization of two acacia species (*A. karroo* Hayn and *A. saligna* Labill.) under saline conditions. Reforesta 7: 33-49. DOI: http://dx.doi.org/10.21750/REFOR.7.04.66

Editor: Vladan Ivetić, Serbia
Received: 2019-06-06
Accepted: 2019-06-22
Published: 2019-06-28

Abstract

Drought and salinity act simultaneously in tolerance and acclimatization under saline conditions. Therefore, plants subjected to these types of stress should have developed specific structural adaptations at the early stages of development. The solution to these environmental problems is to look for species that are relatively water-efficient and resistant to recurrent episodes of various abiotic stresses such as salt stress. In this study, the salinity tolerance index, ionic homeostasis and osmoprotection were evaluated in *A. karroo* and *A. saligna* plants of 90 days old and cultured at various concentrations of NaCl for 21 days. Results showed that salt caused remarkable changes in some growth-related parameters (dry biomass) represented by the salinity tolerance index (STI). Na^+, Ca^{2+}, and $Ratio_{Na+/K+}$ content in the leaves increased with salinity levels, while K^+ contents were significantly reduced compared to the control in both acacia species. Levels of proline, total free amino acids and reducing sugars have been accumulated considerably in the leaves. *A. karroo* was more salt-tolerant than *A. saligna*. Our results showed that the adaptability of a species to salinity is closely related to ion selectivity and biomass production. The seedlings also accumulated significantly a set of important osmolytes in leaves under salt stress, showing a marked increase in secondary metabolite accumulation. This adaptation proved very specific to each species for better survival in saline environments.

Keywords

Acacia; Aridity; Germination; NaCl; Proline; Homeostasis; Osmoprotection

Contents

1 Introduction 34
2 Material and methods 35
  2.1 Growth conditions and experimental design 35
  2.2 Studied parameters 36
3 Results and discussion 37
  3.1 Salinity tolerance index 37
  3.2 Effect of salinity on ionic homeostasis 38

3.3 Effect of salinity on proline, soluble proteins, total free amino acids, starch and reducing sugars in leaves 41
4 Conclusion 44
5 Acknowledgment 45
6 References 45

1 Introduction

Vegetation is the key to restoration of degraded landscapes and its management will be the single most important consideration in any restoration project (Bâ et al. 2010). Typical desertification processes include soil erosion, deforestation and overgrazing, or salinization and other forms of environmental degradation (Gibbs and Salmon 2015). The salinity effect on plants can be described in two main stages: Shoot independent reaction occurs first in minutes to days and is thought to be related to Na^+ detection and signaling (Roy et al. 2014). In this first stage, the effects of salinity can be significant on water relations, causing stomatal closure and inhibition of leaf expansion (Miller et al. 2010). The second phase, the salt-dependent response to salinity, develops over a longer period (days to weeks) and leads to the accumulation of toxic ions in the stem and particularly in old leaves, leading to premature senescence leaves (Munns and Tester 2008). Salinity can affect the growth and yield of most plants, inducing a reduction in cell division in roots and leaves, auxesis and cell differentiation, with their complex interactions followed by significant tissue damage, resulting in plant death from prolonged exposure to salinity (Negrão et al. 2017; Kheloufi et al. 2018b). The generic result of the effect of this abiotic factor is also a loss of productivity (Zika and Erb 2009).

One of the most urgent problems to be solved today is the reconstruction of degraded ecosystems because of soil salinization. In general, woody vegetation plays a fundamental role in the structure and functioning of arid and semi-arid ecosystems (Gautier et al. 2015). It is in this context that the introduction of fast-growing exotic species has been commonly used as a solution to facilitate the ecological restoration of degraded landscapes (Schneider et al. 2014). Like most forest trees, acacias are frequently used in reforestation programs and agro-sylvo-pastoral adjustment systems for arid and semi-arid regions (Mansouri 2011). Autochthonous acacia species are often adapted to drought and thermal constraints (Kheloufi et al. 2018a), contribute to the restoration of poor soils and allow fertility gains by fixing variable amounts of atmospheric nitrogen through the association of their root systems with microorganisms (Rhizobium and Mycorrhizae) (Boukhatem et al. 2012). They are widely known and used for livestock as a source of forage (Mokoboki et al. 2011; Kheloufi and Mansouri 2017).

A. saligna is the only Acacia tree with phyllodes present in Algeria. This species colonizes all the northern Algeria and can tolerates a wide variety of soil (even alkaline soils) and behaves normally close to very saline soils (Sebkha, chott) and coasts (Kheloufi et al. 2018a). Indeed, *A. saligna* can tolerate salt spray, hot coastal sun, extreme winds and sandy soil (Mansouri 2011). It is used for the creation of good windbreak, and it is one of the fast-growing species. The best example is the project of sand quarry reforestation in association with selected rhizobia by Mansouri (2011) in the region of Aïn Temouchent (Northwest of Algeria). The zone is located at 2 meters of altitude and 400 meters from the coast. The 1840 planted trees of *A. saligna* show a great development and participate in biodiversity conservation and soil erosion prevention. This species is used as a pioneer species, promoting the improvement of soil fertility in short periods of time, and promoting a better development of other, more productive species. On the other hand, *A. karroo* is also a fast-growing tree which takes place at the second position after *A. saligna*. Leaves are composed by many leaflets. Branches are armed with very long sharp thorns. This tree is a reliable source of forage due to foliage, flowers and pods. It is a considerable source of shade in the dry regions and a site of nesting for several species of birds. The wood is hard, durable and its gum is edible (De Vynck et al. 2016). This species is listed in most of the Algerian territory and its behavior varies from an ecotype to another (Kheloufi et al. 2018a). In Algeria, it has been reported that *A. karroo* could germinate under 400 mM NaCl with 66% of final germination (Kheloufi et al. 2017a). However, the seeds of *A. saligna* could only germinate at 150 mM with only 18% of final germination (Kheloufi et al. 2016). In this study, we describe techniques that measure the impact of salinity in these two acacia species (*A. karroo* and *A. saligna*) on physio-biochemical traits associated with growth, ionic homeostasis and some biochemical components.

2 Material and methods

2.1 Growth conditions and experimental design

The seeds of *A. karroo* Hayne were collected from Aïn El Baïda salt farm area (Oran, Algeria) (latitude: 35°39'34.96"N; longitude: 0°40'4.68"W; elevation: 136 m) and those of *A. saligna* from the region of Terga (Aïn Temouchent, Algeria) (latitude: 35°26'32.26"N; longitude: 1°13'42.80"W; elevation: 2 m). Pods were collected from 10 trees and the seeds were then mixed. The thousand-seed-weight of *A. karroo* and *A. saligna* were 39 g and 15 g, respectively. Sieving and flotation were used to sort out seeds. The clean seeds were then spread on filter paper to dry. Once dried, the seeds undergo a chemical treatment which consisted of immersion in 96% sulphuric acid for 30 minutes for *A. karroo* (Kheloufi 2017) and 90 minutes for *A. saligna* (Kheloufi et al. 2017b), followed by washing in distilled water. *A. karroo* and *A. saligna* seeds need this pre-treatment to break the seed coat dormancy.

Seeds were germinated in plastic pot (Top diameter: 10 cm; Bottom diameter: 7 cm; Height: 14 cm) (Figure 1) containing 1 kg of mixed substrate (two volumes of sand mixed with one volume of compost) (EC=49 $mS.m^{-1}$; pH=6.2; N=89 $g.m^{-3}$; P_2O_5=42 $g.m^{-3}$; K_2O=27 $g.m^{-3}$) and arranged according to the method of complete randomized blocks with four replicates under greenhouse conditions. Sand was sieved at 2 mm to eliminate wastes and coarser material then washed repeatedly with tap water to eliminate all carbonates and chlorides. The experiment was conducted in the green house of Ecology and Environment Department, University of Batna 2, Algeria.

Three months (90 days) old healthy seedlings of uniform size were selected as initial material and further grown in KNOP's nutrient medium. Plants were subjected to salt treatment by supplementing the nutrient medium with varied sodium chloride (NaCl) concentrations (200, 400 and 600 mM) (Table 1). The control plants were grown in the nutrient medium devoid of NaCl. The nutrient solutions were replaced with freshly prepared solutions at every 7 days intervals.

After 21 days of salt treatment, stem and root samples were harvested from control and NaCl-treated plants for estimation of various parameters. It should be noted that for each measurement/treatment or assay, a number of 4 replications were used. In addition, and to ensure the study of the effect salinity on all parameters with the same conditions of growth and development, a considerable number of plants were used (30 plants × 4 treatments × 2 species).

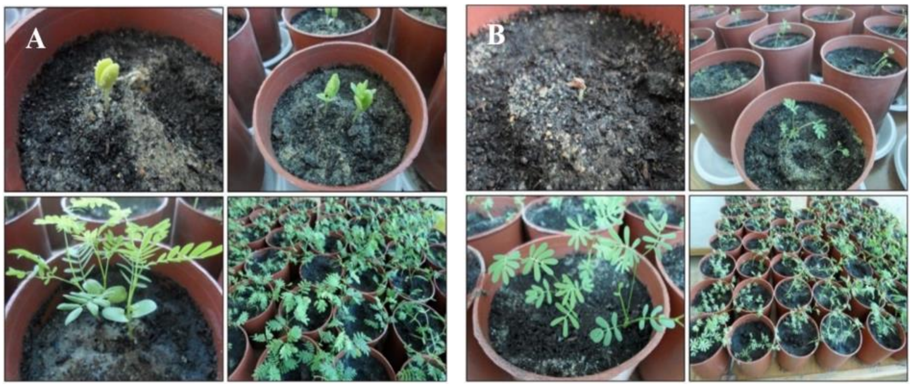

Figure 1. Experimental design and different stages of plant development of two acacia species: (A) *A. karroo* and (B) *A. saligna*.

Table 2. Preparation of saline solution and corresponding water potential.

NaCl (mM)	**NaCl (g.L^{-1})**	**Ψos Level (MPa)** (Braccini et al. 1996)
0	0	0
200	11,68	-0,83
400	23,37	-1,67
600	35,06	-2,50

2.2 Studied parameters

The estimation of biochemical parameters took place at the Biotechnology Research Center (CRBt) (Constantine, Algeria).

Salinity tolerance index

$$\mathrm{STI} = \frac{\mathrm{TDW\ at\ Sx}}{\mathrm{TDW\ at\ SI}} \times 100$$

TDW : Total dry weight of the plant (Oven at 80 °C for 48 hours)
SI: Control treatment
Sx: Treatment at a salt concentration 'x'

Estimating ion contents in leaves

Twenty-one days of NaCl treatments, four plants were used from each treatment for numerous measurements. Leaves dry matter (LDM) was measured at 60 °C for 48 h. The oven-dried leaves samples were ground into powder and kept in a desiccator for determining the contents of Na^+, K^+ and Ca^{2+}. After desiccation, the powdered samples were used to determine the ion contents by Inductively coupled plasma-mass spectrometry (Kleve, Germany) following the method of John et al. (2003).

Contents of proline, total free amino acids and soluble proteins

The levels of proline (Pr), total free amino acids (TFAA) and soluble proteins (SPt) in leaves were determined following the methods described by Ringel et al. (2003), Yemm et al. (1955) and Bradford (1976), respectively.

Contents of reducing sugars and starch

The contents of reducing sugars (RS) and starch (St) in leaves samples were estimated according to the method of Green and Schwarz (1989) and Hansen and Møller (1975), respectively.

All experiments were conducted with four replicates (n = 4) and the mean (± SD), one-way and two-way ANOVA were calculated using SAS Version 9.0 (Statistical Analysis System) (2002) software. The mean separations were carried out using Duncan's multiple range tests and significance was determined at $p \leq 0.05$. The graphics were made with Excel 2016.

3 Result and discussion

The effect of NaCl, the species and their correlation (TRT × Sp) was highly significant on all the parameters studied in this work ($p < 0.0001$) (Table 2).

3.1 Salinity tolerance index

The STI is a reliable parameter for the determination of salt tolerance (Krishnamurthy et al. 2016). According to (Table 2), it was not affected by salt stress at low and high levels ($p<0.0001$) except that there is a highly significant difference between the two species studied ($p<0.0001$). Indeed, in *A. karroo*, the increase in salinity had no effect on total dry biomass (Figure 2). In *A. saligna*, the STI recorded a 50% reduction at 200 and 400 mM NaCl, and this reduction reached 70% at 600 mM NaCl compared to control (Figure 2). Dry biomass is used to estimate the salinity tolerance index as suggested by many authors (Feng et al. 2018) in order to evaluate the threshold of salinity tolerance over a given period. Our results indicate that plants exposed to salt stress at different concentrations had delaying effects on whole plant performance, including growth and development inhibition.

Our conclusions corroborate those of Rahman et al. (2017) who worked on *Acacia auriculiformis* and concluded that the high salt tolerance index (STI) at the seedling stage indicates that the key mechanisms of salt tolerance in plants may be associated with (1) accumulation of compatible solutes such as Proline, total sugars, reducing sugars and total free amino acids; (2) increase the amount of K^+ and Ca^{2+} ions in the leaves; (3) to increase the retention of K^+ ions in photosynthetic tissues by preventing the absorption of Na^+; (4) anatomic adjustment by increasing the endodermal thickness of the stems and roots; (5) effective compartmentalization of Na^+ in vacuoles and (6) the increased exclusion of Na^+.

Table 2. Variance analysis for the traits investigated of five acacia species in response to different durations of sulphuric acid pretreatment and after 18 days of sowing.

Parameters	Variables	Degree of freedom	F of Fisher	*P*
STI	TRT	3	12,10	< 0,0001
	Sp	1	49,38	< 0,0001
	TRT×Sp	3	6,06	0,0032
Na^+	TRT	3	679,03	< 0,0001
	Sp	1	85,37	< 0,0001
	TRT×Sp	3	31,74	< 0,0001
K^+	TRT	3	1403,03	< 0,0001
	Sp	1	82,91	< 0,0001
	TRT×Sp	3	25,84	< 0,0001
Ca^{2+}	TRT	3	1535,10	< 0,0001
	Sp	1	339,77	< 0,0001
	TRT×Sp	3	1333,70	< 0,0001
$Ratio_{Na/K}$	TRT	3	83,66	< 0,0001
	Sp	1	35,13	< 0,0001
	TRT×Sp	3	21,23	< 0,0001
Pr	TRT	3	25042,9	< 0,0001
	Sp	1	8457,79	< 0,0001
	TRT×Sp	3	1471,76	< 0,0001
SPt	TRT	3	124,27	< 0,0001
	Sp	1	1568,16	< 0,0001
	TRT×Sp	3	7,30	< 0,0001
TFAA	TRT	3	1277,00	< 0,0001
	Sp	1	3509,24	< 0,0001
	TRT×Sp	3	75,05	< 0,0001
St	TRT	3	1758,21	< 0,0001
	Sp	1	6258,59	< 0,0001
	TRT×Sp	3	103,33	< 0,0001
RS	TRT	3	1039,46	< 0,0001
	Sp	1	897,90	< 0,0001
	TRT×Sp	3	61,09	< 0,0001

Es (Sp), Saline treatment (TRT), Salinity tolerance index (STI), Sodium ion content (Na^+), Potassium ion content (K^+), Calcium ion content (Ca^{2+}), Ratio Na^+/K^+ ($Ratio_{Na/K}$), Proline content (Pr), soluble protein (SPt), Total free amino acids (TFAA), Starch (St), Reducing sugars (RS)

3.2 Effect of salinity on ionic homeostasis

In this study, the contents of various mineral ions were analyzed to better understand the salinity effect on the mineral absorption and their accumulation in the leaves. In the leaves of both acacia species, the Na^+ and Ca^{2+} content increased gradually with increasing salt concentration except for the 600 mM concentration where the Ca^{2+} content in *A. saligna* leaves decreased by 60% compared to 400 mM NaCl (Figure 3A, Figure 3C).

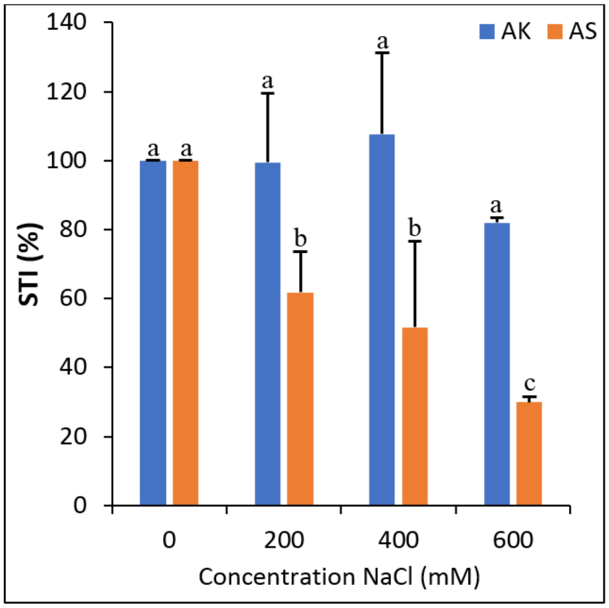

Figure 2. Effect of salt stress on the salinity tolerance index in two acacia species (*A. karroo* and *A. saligna*) after 21 days of treatment at different levels. For each species, means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test.

Na^+ and Ca^{2+} levels in leaf tissues in acacia plants stressed with sodium chloride are in continuous growth, while the K^+ content has decreased very significantly (Figure 3B). In both species studied and for all the salt treatments used, the curve of the $Ratio_{Na+/K+}$ in the leaves was similar to that of the Na^+ ion increase (Figure 3D, Figure 3A). Under the extreme stress level (600 mM NaCl), the $Ratio_{Na+/K+}$ in foliar tissues of *A. saligna* reached a maximum of 22.4 mg.g-1 LDM. This increase is three times higher than that recorded in *A. karroo* leaves (Figure 3D). Maintaining osmotic pressure and turgor in leaf tissues under saline conditions is mainly realized in halophytes using inorganic ions (Na^+, Cl^- and K^+) in order to maintain the leaves osmotic pressure and turgidity in saline conditions (Shabala and Munns 2017). These three main ions represent 80% to 95% of the osmotic pressure of the cells (Moir-Barnetson et al. 2016). However, glycophytes do this homeostasis by increasing the synthesis of new compatible solutes (Shabala and Munns 2017).

Based on several research results, salt tolerance in plants can be associated with three behaviors: (i) the exclusion of Na^+, (ii) the inclusion of Na^+ and (iii) Na^+ sequestration in vacuoles. In addition, the impact of salt stress on the absorption and transport of other minerals is also considered to be a determining factor of salt tolerance mechanism in plants (Farooq et al. 2015). In this study, the content of Na^+ and Ca^{2+} in the leaves of *A. karroo* increased continuously as a function of salt concentration, while the K^+ content was continuously reduced, leading to an increase in the Na^+/K^+ ratio (Figure 3D). In halophyte plant species, the Na^+ ions increase in the vacuole, realizing a relation with the activities of antiport localization Na^+/H^+ (NHX) and H^+-ATPase (VH^+-ATPase) vacuolar on the vacuolar membrane (Zhang et al. 2012).

In vacuoles, K^+ ions play a key role in turgor. However, under salt stress, there is an efflux of K^+ and an influx of Na^+ (Dreyer and Uozumi 2011; Bose et al. 2014). Na^+ ions also played a role in the conservation of cells turgor under salt stress (Hasegawa 2013). These data indicate that the competitive inhibition between Na^+ and K^+ uptake causes a change in the intracellular Na^+/K^+ ratio in the acacia plants studied. The significant increase in Ca^{2+} levels in the leaves of the two acacia species subjected to salt stress (Figure 3C, Figure 4B) also indicates that ion exclusion mechanisms reduce the antagonistic effects of Na^+, thus accelerating the absorption of other beneficial nutrients such as potassium, which actively participates in the activation of enzymes, the stabilization of protein synthesis, the maintenance of membrane potential and cytosolic pH, while calcium plays a key role in K^+/Na^+ selectivity and signal transduction to salt stress (Chakraborty et al. 2018).

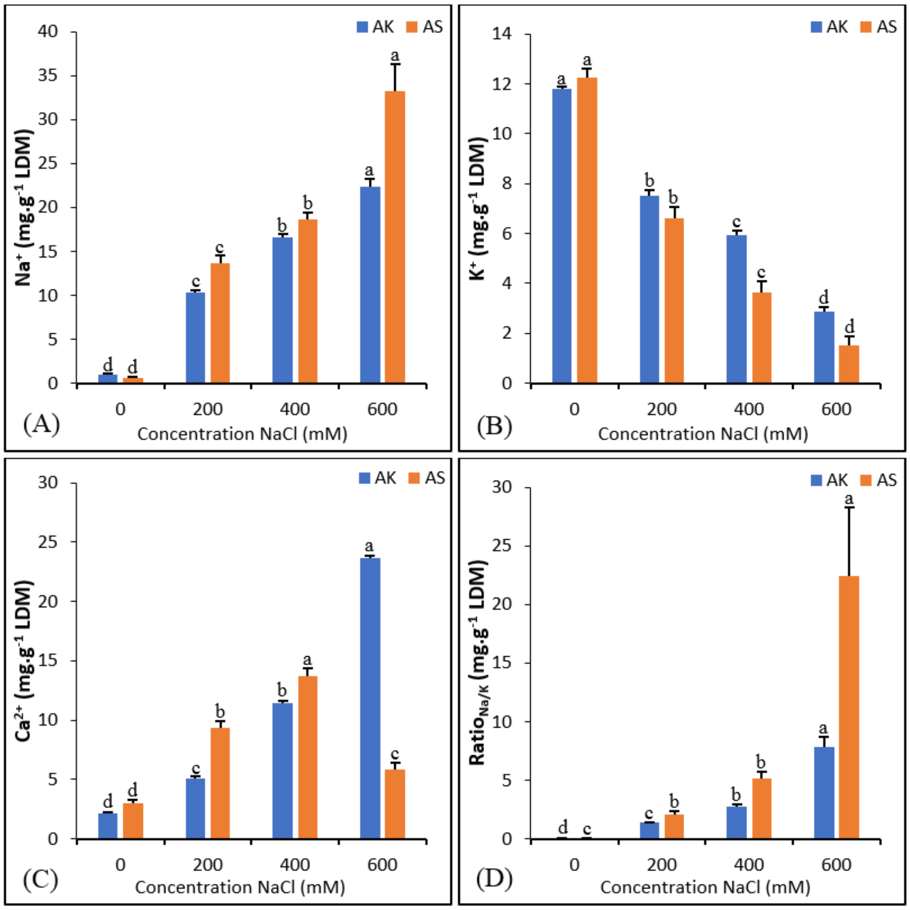

Figure 3. Effect of salt stress on the content of (A) sodium ions, (B) potassium ions, (C) calcium ions and (D) the ratio Na^+/K^+ ($Ratio_{Na/K}$) in the leaves of *A. karroo* and *A. saligna* after 21 days of saline treatment at different levels. For each species, means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test. (LDM : leaf dry matter).

In both acacia species, we observed an increase in leaf Ca^{2+} under salt stress conditions, which contrasted strongly with the reduction of Ca^{2+} in other plant species when plants were subjected to the same type of stress (Prasath and Gomathinayagam 2016; Diouf et al. 2018). Choi et al. (2017) reported that Ca^{2+} reduces salinity, protects plants and confers salt tolerance in glycophytes. It has also been reported that exogenous Ca^{2+} attenuates salinity in halophytes (Nedjimi 2017). Indeed, Ca^{2+} plays a key role in stabilizing the cell wall structure, maintaining the structural and functional integrity of the cell membrane, regulating transport and ion exchange, and acting as a messenger in saline stress signaling (Tahjib-Ul-Arif et al. 2018).

This regulation of Na$^+$ and K$^+$ homeostasis also implies the ability of *A. karroo* to maintain sufficient K$^+$ uptake to maintain a reduced cytosolic Na$^+$/K$^+$ ratio (Figure 3D, Figure 4A), which is a screening criterion for Salt Tolerance (Deinlein et al. 2014). This corroborates with the findings of previous work of Nemati et al. (2011) and Bader et al. (2015) who reported an increase in the Na$^+$/K$^+$ ratio or a reduction in the K$^+$/Na$^+$ ratio, respectively. A similar observation (accumulation of Na$^+$ and reduction of K$^+$) has been reported in other acacia species (*A. longifolia*, *A. ampliceps* and *A. auriculiformis*) grown under saline conditions (Morais et al. 2012; Theerawitaya et al. 2015; Rahman et al. 2017). Chen et al. (2010) also confirmed that high salt concentrations induced Na$^+$ uptake and transport and reduced K$^+$ content in leaf tissues.

The increase in Ca^{2+} accumulation despite the increase in Na$^+$ is interpreted by the fact that Na$^+$ interferes with the absorption of Ca^{2+} in the presence of salt (Amtmann et al. 2018). Ca^{2+} accumulation under extreme salinity conditions (600 mM NaCl) could help to protect the two acacia species from the toxic effects of Na$^+$ by activating the SOS pathway, which protect against salinity-induced cell membrane damage (Almeida et al. 2017).

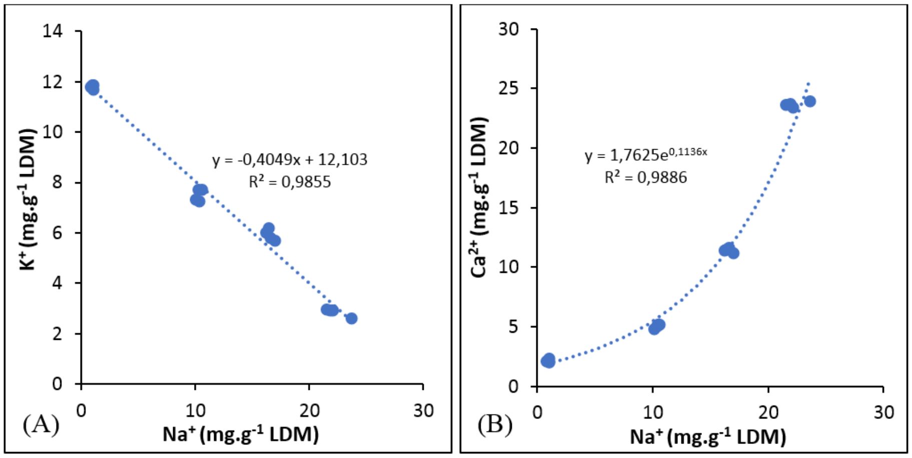

Figure 4. Example of correlation in *A. karroo* leaves: (A) Sodium and potassium ion content and (B) Sodium and calcium content. (LDM : leaf dry matter).

Our results therefore suggest that the highest levels of K$^+$ and Ca^{2+} in the leaves support the optimal functioning of metabolic processes and salt tolerance in *A. karroo* and *A. saligna*. In addition, intracellular K$^+$ homeostasis is a prerequisite for the optimal functioning of the plant's metabolic mechanism and its overall performance (Amtmann et al. 2018). This result may indicate the important role played by divalent cations such as Ca^{2+} as blockers of potassium efflux channels, allowing efficient retention of Ca^{2+} in photosynthetically active leaf tissues (Almeida et al. 2017).

3.3 Effect of salinity on proline, soluble proteins, total free amino acids, starch and reducing sugars in leaves

According to Figure 5A, the leaves proline content in *A. karroo* increased very remarkably by 320, 645 and 1454% after exposure to 200, 400 and 600 mM NaCl, respectively, compared to the control. The same increase was observed in *A. saligna* except that the values are lower because the proline content was low in the control plants (Figure 5A).

A progressive increase in the total free amino acid content was observed when the levels of exposure to salinity increased and this in both studied species (Figure 5C). This increase is much more expressed under 600 mM NaCl. Indeed, in the leaves of the two species studied, the content of free amino acids exceeds 85% of the control (Figure 5C). At the same time and according to the same nature of biomolecules, the total protein content in the leaves underwent a regressive evolution in both species following the increase of the NaCl concentration but with much more pronounced values in *A. karroo* (Figure 5B). In fact, the total protein content decreases from approximately 30% at 600mM NaCl in the leaves of both species (Figure 5B).

There is also a positive relationship between NaCl concentrations and reducing sugar contents, and maximum levels are observed at the highest salinity level (600 mM) in the leaves of the both species after 21 days of exposure to salt stress. Under this concentration, the reducing sugar content exceeds the control plants of 300% and 200% respectively in *A. karroo* and *A. saligna* (Figure 5E). On the other hand, the evolution of the starch content is inversely proportional to the increase of the salinity levels. Indeed, Figure 5D clearly shows that the starch content is reduced at 50% in both species under 600 mM NaCl. It should be noted that the values recorded in all these secondary metabolites are high in the leaves of *A. karroo* and this in both stressed or unstressed plants (Figure 5).

In a highly saline environment, the main physiological response of plants is to perform osmotic adjustment through two processes: the accumulation of ions in the vacuole and the synthesis of compatible solutes in the cytosol (Hajiboland et al. 2014; Razzaghi et al. 2015). Therefore, salinity-induced changes in levels of various organic metabolites such as reducing sugars, starch, total proteins, total free amino acids and proline were analyzed to determine the role of these organic metabolites in osmotic adjustment and salt tolerance in *A. karroo* and *A. saligna*. We observed a significant increase in the reducing sugar content under extreme salt level and a gradual decrease in starch content with an increase in salinity (Figure 5). Decreasing the starch content and increasing the reducing sugar content under higher salinity conditions could be due to conversion of starch to provide more sugar and energy for osmotic protection under increased salinity (Thalmann and Santelia 2014). Sugars play a key role in adaptive processes related to NaCl tolerance via interdependent mechanisms of growth and osmoregulation (Sharif and Khan 2016). For example, Chaum et al. (2009) found that the total soluble sugar level of a salt-resistant rice variety is higher than that of the salt-sensitive variety, and that the sugars enhance salt-induced osmotic stress resistance in rice plants.

In the leaves of the two acacia species studied in this work, the total content of free amino acids and proline increased under the three NaCl concentrations compared to the control, especially under the 600 mM treatment. The high accumulation of free amino acids may be due to the high biosynthesis of amino acids or the progressive activity of the protease for osmotic adjustment (Parida and Das 2005).

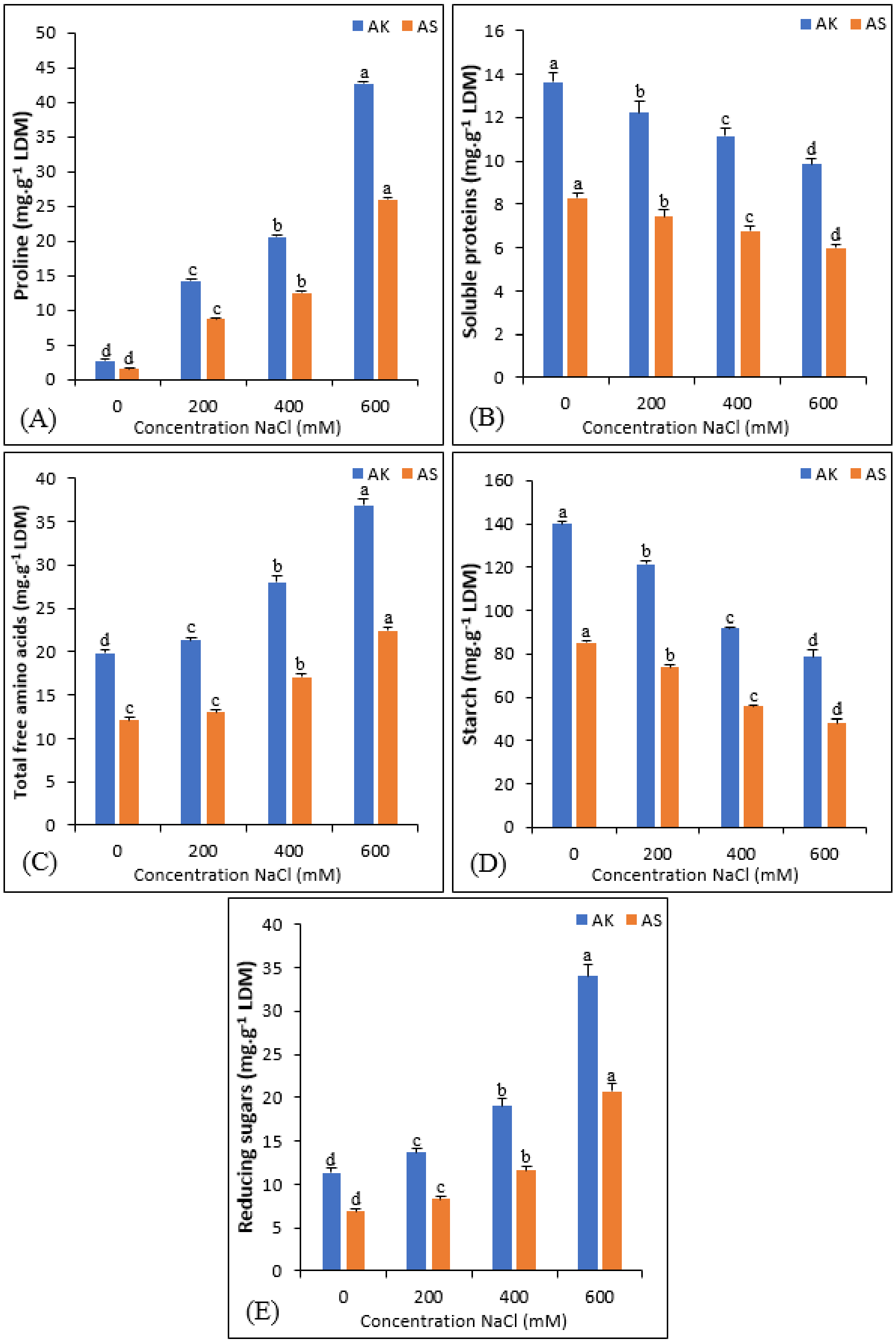

Figure 5. Effect of salt stress on the content of (A) proline, (B) soluble proteins, (C) total free amino acids, (D) starch, and (E) reducing sugars in the leaves of *A. karroo* and *A. saligna* after 21 days of saline treatment at different levels. For each species, means, in each box, with similar letters are not significantly different at the 5% probability level using Duncan's test. (LDM : leaf dry matter).

Proline is known to provide improved protection against salinity by eliminating free radicals, stabilizing membranes, proteins and enzymes, and maintaining ionic homeostasis (Dagar and Minhas 2016). The present study showed an increase in proline content at all salt concentrations. The increase in saline-induced leaf proline in these two acacia species may be due to improved proline biosynthesis and/or may be due to the inhibition of proline catabolism. The increase in leaf proline content in response to salt stress has been reported in many forest and fruit trees: Olive tree (Ahmed et al. 2012), Date palm (Yaish 2015), Lemon tree (Balal et al. 2011), Eucalyptus (Chaum et al. 2013), *Acacia auriculiformis* (Patel et al. 2010), *Acacia saligna* (Soliman et al. 2012), *Acacia arabica* (Lassouane et al. 2013), *Acacia longifolia* (Morais et al. 2012) and *Acacia senegal* (Patel et al. 2011). Proline production induced by salt stress has been demonstrated in halophyte and glycophyte species; except that halophytes accumulate more leaf proline under normal and stressed conditions (Himabindu et al. 2016). It has been shown that proline also has an osmoregulatory function interpreted by membrane protection and enzyme stabilization (Zouari et al. 2016). Therefore, the osmoregulatory function of proline in *A. karroo* and *A. saligna* shows clearly through the increase of its content, joining an increase in the content of total amino acids, playing a role in the maintenance of osmotic equilibrium of the cell under high salinity conditions. In fact, by maintaining high levels of free amino acids, halophytes are able to satisfy the rise demand for amino acids during protein metabolism (Suprasanna et al. 2016). The observed decreases in starch and protein corroborated the increase in levels of sugars and amino acids in *A. karroo* leaves under different levels of salinity (Figure 6).

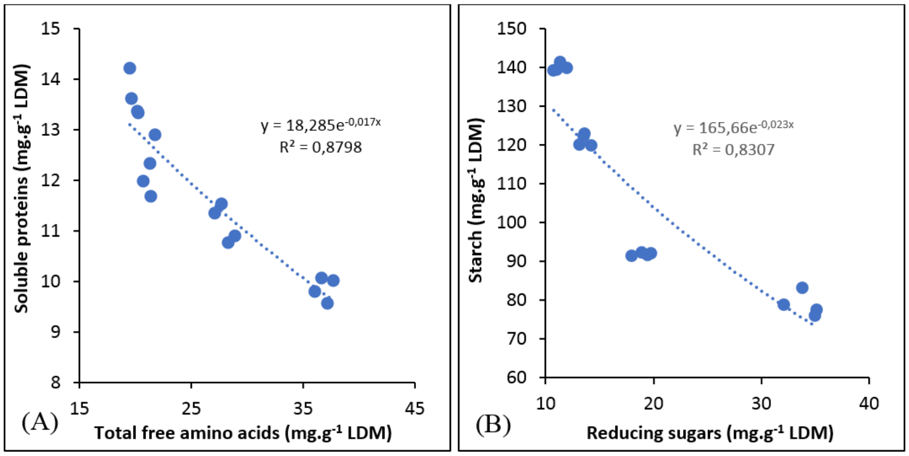

Figure 6. Example of correlation in *A. karroo* leaves: (A) Soluble proteins and (B) Starch and reducing sugars. (LDM : leaf dry matter).

4 Conclusion

Although the plants of *A. karroo* and *A. saligna* showed some changes under moderate and high saline stress, they continued their vegetative growth and maintained a considerable and fairly stable dry biomass was observed in *A. karroo* under salinity conditions. This adaptation proved very specific to each species for better survival in saline environments. Evaluation of seedling survival or leaf Na^+ content may not be significant as a predictor of salinity tolerance without further information, such as the effect of salinity on various growth parameters at the time of application in other organs such as stems and roots. In this work, we sought to describe the mechanisms used to measure some of the processes that can contribute to salinity tolerance. The parameters estimated during this study are valid only for the case of young plants in pots and under well-defined conditions. It would be necessary to validate our results with an afforestation program. On the other hand, this study does not exclude that these acacia species are considered as potential halophytic species to be cultivated in saline lands, thus making them favorable to agroforestry practices, especially since these forest trees have the capacity to revegetate nutrient-poor soils.

5 Acknowledgment

The present work was realized within the framework of the project "Contribution to the study of the effects of drought and salt stress on Acacia species in Algeria". We are grateful to Bruce Roger Maslin, Senior Principal Research Scientist (Department of Parks and Wildlife, Australia) and creator of the WorldWide-Wattle website.

6 References

Ahmed CB, Magdich S, Rouina BB, Boukhris M, Abdullah FB (2012) Saline water irrigation effects on soil salinity distribution and some physiological responses of field grown Chemlali olive. J Environ Manage 113:538-544. https://doi.org/10.1016/j.jenvman.2012.03.016

Ajmi A, Vázquez S, Morales F, Chaari A, El-Jendoubi H, Abadía A, Larbi A (2018) Prolonged artificial shade affects morphological, anatomical, biochemical and ecophysiological behavior of young olive trees (cv. Arbosana). Sci Hortic-Amsterdam 241:275-284. https://doi.org/10.1016/j.scienta.2018.06.089

Almeida DM, Oliveira MM, Saibo NJ (2017) Regulation of Na^+ and K^+ homeostasis in plants: towards improved salt stress tolerance in crop plants. Genet Mol Biol 40(1):326-345. https://doi.org/10.1590/1678-4685-gmb-2016-0106

Amtmann A, Armengaud P and Volkov V (2018) Potassium nutrition and salt stress. Annual Plant Reviews 328-379. https://doi.org/10.1002/9781119312994.apr0151

Bâ AM, Diédhiou, AG, Prin Y, Galiana A, Duponnois R (2010) Management of ectomycorrhizal symbionts associated to useful exotic tree species to improve reforestation performances in tropical Africa. Ann Forest Sci 67(3):301. https://doi.org/10.1051/forest/2009108

Bader B, Aissaoui F, Kmicha I, Salem AB, Chehab H, Gargouri K, Chaieb M (2015) Effects of salinity stress on water desalination, olive tree (*Olea europaea* L. cvs 'Picholine', 'Meski' and 'Ascolana') growth and ion accumulation. Desalination 364:46-52. https://doi.org/10.1016/j.desal.2015.01.002

Balal RM, Ashraf MY, Khan MM, Jaskani MJ, Ashfaq M (2011) Influence of salt stress on growth and biochemical parameters of citrus rootstocks. Pak J Bot 43(4):2135-2141.

Bose J, Rodrigo-Moreno A, Shabala S (2014) ROS homeostasis in halophytes in the context of salinity stress tolerance. J Exp Bot 65(5):1241-1257. https://doi.org/10.1093/jxb/ert430

Boukhatem ZF, Domergue O, Bekki A, Merabet C, Sekkour S, Bouazza F, Galiana A (2012) Symbiotic characterization and diversity of rhizobia associated with native and introduced acacias in arid and semi-arid regions in Algeria. FEMS Microbiol Ecol 80(3):534-547. https://doi.org/10.1111/j.1574-6941.2012.01315.x

Braccini ADL, Ruiz HA, Braccini MDC, Reis MS (1996) Germinação e vigor de sementes de soja sob estresse hídrico induzido por soluções de cloreto de sódio, manitol e polietileno glicol. Revista Brasileira de Sementes 18(1):10-16. https://doi.org/10.17801/0101-3122/rbs.v18n1p10-16

Bradford MM (1976) A rapid sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-Dye Binding. Anal Biochem 72:248-254. https://doi.org/10.1016/0003-2697(76)90527-3

Chakraborty K, Basak N, Bhaduri D, Ray S, Vijayan J, Chattopadhyay K, Sarkar RK (2018) Ionic Basis of Salt Tolerance in Plants: Nutrient Homeostasis and Oxidative Stress Tolerance. In Plant Nutrients and Abiotic Stress Tolerance (pp. 325-362). Springer, Singapore. https://doi.org/10.1007/978-981-10-9044-8_14

Chaum S, Somsueb S, Samphumphuang T, Kirdmanee C (2013) Salt tolerant screening in eucalypt genotypes (*Eucalyptus spp.)* using photosynthetic abilities, proline accumulation, and growth characteristics as effective indices. In Vitro Cellular and Developmental Biology-Plant 49(5):611-619. https://doi.org/10.1007/s11627-013-9537-5

Chaum S, Supaibulwattana K, Kirdmanee C (2009) Comparative effects of salt stress and extreme pH stress combined on glycinebetaine accumulation, photosynthetic abilities and growth characters of two rice genotypes. Rice Sci 16(4):274-282. https://doi.org/10.1016/S1672-6308(08)60091-8

Chen S, Polle A (2010) Salinity tolerance of *Populus*. Plant Biology 12(2):317-333. https://doi.org/10.1111/j.1438-8677.2009.00301.x

Choi WG, Miller G, Wallace I, Harper J, Mittler R, Gilroy S (2017) Orchestrating rapid long-distance signaling in plants with Ca^{2+}, ROS and electrical signals. The Plant Journal 90(4):698-707. https://doi.org/10.1111/tpj.13492

Dagar JC and Minhas PS (2016) Saline irrigation for productive agroforestry systems. In Agroforestry for the Management of Waterlogged Saline Soils and Poor-Quality Waters (pp. 145-161). Springer, New Delhi. https://doi.org/10.1007/978-81-322-2659-8_9

De Vynck JC, Van Wyk BE, Cowling RM (2016) Indigenous edible plant use by contemporary Khoe-San descendants of South Africa's Cape South Coast. South African Journal of Botany 102:60-69. https://doi.org/10.1016/j.sajb.2015.09.002

Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI (2014) Plant salt-tolerance mechanisms. Trends Plant Sci 19(6):371-379. https://doi.org/10.1016/j.tplants.2014.02.001

Diouf IA, Derivot L, Bitton F, Pascual L, Causse M (2018) Water Deficit and Salinity Stress Reveal Many Specific QTL for Plant Growth and Fruit Quality Traits in Tomato. Front Plant Sci 9:279. https://doi.org/10.3389/fpls.2018.00279

Dreyer I, Uozumi N (2011) Potassium channels in plant cells. The FEBS Journal 278(22):4293-4303. https://doi.org/10.1111/j.1742-4658.2011.08371.x

Farooq M, Hussain M, Wakeel A, Siddique KH (2015) Salt stress in maize: effects, resistance mechanisms, and management. A review. Agronomy for Sustainable Development 35(2):461-481. https://doi.org/10.1007/s13593-015-0287-0

Feng K, Cui L, Lv S, Bian J, Wang M, Song W, Nie X (2018) Comprehensive evaluating of wild and cultivated emmer wheat (*Triticum turgidum* L.) genotypes response to salt stress. Plant Growth Regul 84(2):261-273. https://doi.org/10.1007/s10725-017-0337-5

Gautier D, Garcia C Negi S, Wardell DA (2015) The Limits and Failures of Existing Forest Governance Standards in Semi-Arid Contexts. Int Forest Rev 17(S2):114-126. https://doi.org/10.1505/146554815815834831

Gibbs HK, Salmon JM (2015) Mapping the world's degraded lands. Appl Geogr 57:12-21. https://doi.org/10.1016/j.apgeog.2014.11.024

Green MB, Schwarz JH (1989) Anomaly cancellations in supersymmetric D=10 gauge theory and superstring theory. In Supergravities in Diverse Dimensions: Commentary and Reprints (*In* 2 Volumes) (pp. 1146-1151). https://doi.org/10.1142/9789814542340_0071

Hajiboland R, Norouzi F, Poschenrieder C (2014) Growth, physiological, biochemical and ionic responses of pistachio seedlings to mild and high salinity. Trees 28(4):1065-1078. https://doi.org/10.1007/s00468-014-1018-x

Hamed KB, Dabbous A, El Shaer H, Abdely C (2018) Salinity responses and adaptive mechanisms in halophytes and their exploitation for producing salinity tolerant crops. In *Salinity* Responses and Tolerance in Plants, Volume 2 (pp. 1-19). Springer, Cham. https://doi.org/10.1007/978-3-319-90318-7_1

Hansen J, Møller IB (1975) Percolation of starch and soluble carbohydrates from plant tissue for quantitative determination with anthrone. Anal Biochem 68:87-94. https://doi.org/10.1016/0003-2697(75)90682-X

Hasegawa PM (2013) Sodium (Na^+) homeostasis and salt tolerance of plants. Environ Exp Bot 92:19-31. https://doi.org/10.1016/j.envexpbot.2013.03.001

Himabindu Y, Chakradhar T, Reddy MC, Kanygin A, Redding KE, Chandrasekhar T (2016) Salt-tolerant genes from halophytes are potential key players of salt tolerance in glycophytes. Environ Exp Bot 124:39-63. https://doi.org/10.1016/j.envexpbot.2015.11.010

John VS, Catalina C, Charlotte P, Juan B (2003) Efficient leaf ion partitioning, an overriding condition for abscisic acid-controlled stomatal and leaf growth responses to NaCl salinization in two legumes. J Exp Bot 54:2111-2119. https://doi.org/10.1093/jxb/erg231

Kheloufi A (2017) Germination of seeds from two leguminous trees (*Acacia karroo* and *Gleditsia triacanthos*) following different pre-treatments. Seed Sci Technol 45:1-4. https://doi.org/10.15258/sst.2017.45.1.21

Kheloufi A, Boukhatem ZF, Mansouri LM, Djelilate M (2018a) Inventory and geographical distribution of Acacia Mill. Fabaceae Mimosaceae) species in Algeria. Biodiversity Journal 9(1):51-60.

Kheloufi A, Chorfi A, Mansouri LM. 2016. Comparative effect of NaCl and CaCl2 on seed germination of Acacia saligna L. and Acacia decurrens Willd. International Journal of Biosciences 8(6):1-13. https://doi.org/10.12692/ijb/8.6.1-13

Kheloufi A, Chorfi A, Mansouri LM (2017a) Germination kinetics in two *Acacia karroo* Hayne ecotypes under salinity conditions. Open Access Library Journal 4:1-11. https://doi.org/10.4236/oalib.1103319

Kheloufi A, Chorfi A, Mansouri LM, Benyamina H (2018b) Morpho-physiological characterization and photosynthetic pigment contents of *Acacia karroo* Hayne seedlings under saline conditions. Agricult Forest 64(2):87-99. https://doi.org/10.17707/AgricultForest.64.2.06

Kheloufi A, Mansouri LM (2017) Effect of sulphuric acid on the germination of a forage tree *Acacia nilotica* (L.) subsp. *tomentosa*. Livestock Research for Rural Development 29:1-11.

Kheloufi A, Mansouri LM, Boukhatem ZF (2017b) Application and use of sulfuric acid to improve seed germination of three acacia species. Reforesta 3:1-10. https://doi.org/10.21750/REFOR.3.01.25

Krishnamurthy SL, Gautam RK, Sharma PC, Sharma DK (2016) Effect of different salt stresses on agro-morphological traits and utilization of salt stress indices for reproductive stage salt tolerance in rice. Field Crop Res 190:26-33. https://doi.org/10.1016/j.fcr.2016.02.018

Lassouane N, Aïd F, Lutts S (2013) Water stress impact on young seedling growth of Acacia arabica. Acta Physiol Plant 35(7):2157-2169. https://doi.org/10.1007/s11738-013-1252-7

Mansouri LM (2011) Production d'inoculum de *Rhizobium* associés à *Acacia saligna*pour revégétaliser la carrière de Terga (Aïn Temouchent). Mémoire de magistère, Université d'Oran Es-sénia, Oran, Algérie 116 pp.

Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell and Environment 33(4):453-467. https://doi.org/10.1111/j.1365-3040.2009.02041.x

Moir-Barnetson L, Veneklaas EJ, Colmer TD (2016) Salinity tolerances of three succulent halophytes (*Tecticornia spp*.) differentially distributed along a salinity gradient. Funct Plant Biol 43(8):739-750. https://doi.org/10.1071/FP16025

Mokoboki HK, Ndlovu LR, Malatje MM (2011) Intake and relative palatability indices of acacia species fed to sheep and goats. Agroforest Syst 81(1):31-35. https://doi.org/10.1007/s10457-010-9352-5

Morais MC, Panuccio MR, Muscolo A, Freitas H (2012) Salt tolerance traits increase the invasive success of *Acacia longifolia* in Portuguese coastal dunes. Plant Physiol Bioch 55:60-65. https://doi.org/10.1016/j.plaphy.2012.03.013

Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651-681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Nedjimi B (2017) Calcium application enhances plant salt tolerance: a review. In Essential Plant Nutrients (pp. 367-377). Springer, Cham. https://doi.org/10.1007/978-3-319-58841-4_15

Negrão S, Schmöckel SM, Tester M (2017) Evaluating physiological responses of plants to salinity stress. Ann Bot-London 119(1):1-11. https://doi.org/10.1093/aob/mcw191

Nemati I, Moradi F, Gholizadeh S, Esmaeili MA, Bihamta MR (2011) The effect of salinity stress on ions and soluble sugars distribution in leaves, leaf sheaths and roots of rice (*Oryza sativa* L.) seedlings. Plant Soil Environ 57(1):26-33. https://doi.org/10.17221/71/2010-PSE

Nie W, Gong B, Chen Y, Wang J, Wei M, Shi Q (2018) Photosynthetic capacity, ion homeostasis and reactive oxygen metabolism were involved in exogenous salicylic acid increasing cucumber seedlings tolerance to alkaline stress. Sci Hortic-Amsterdam 235:413-423. https://doi.org/10.1016/j.scienta.2018.03.011

Onodera M, Nakajima T, Nanzyo M, Takahashi T, Xu D, Homma K, Kokubun M (2019) Regulation of root-to-leaf Na and Cl transport and its association with photosynthetic activity in salt-tolerant soybean genotypes. Plant Prod Sci 1-13. https://doi.org/10.1080/1343943X.2018.1561198

Parida AK, Das AB (2005) Salt Tolerance and Salinity Effects on Plants: A Review. Ecotox Environ Safe 60:324-349. https://doi.org/10.1016/j.ecoenv.2004.06.010

Patel AD, Jadeja H, Pandey AN (2010) Effect of salinization of soil on growth, water status and nutrient accumulation in seedlings of *Acacia auriculiformis* (Fabaceae). J Plant Nutr 33(6):914-932. https://doi.org/10.1080/01904161003669939

Patel NT, Panchal NS, Pandey IB, Pandey AN (2011) Implications of calcium nutrition on the response of *Acacia senegal* (Mimosaceae) to soil salinity. Anal Biol 33, 23-34.

Prasath A, Gomathinayagam M (2016) Variation in mineral contents of *Ceriops decandra* (Griff.) Ding Hou under NaCl stress-A true mangrove species. Ann Plant Sci 5(12):1458-1462. https://doi.org/10.21746/aps.2016.12.001

Rahman MM, Rahman MA, Miah MG, Saha SR, Karim MA, Mostofa MG (2017) Mechanistic Insight into Salt Tolerance of *Acacia auriculiformis*: The Importance of Ion Selectivity, Osmoprotection, Tissue Tolerance, and Na^+ Exclusion. Frontiers in plant science 8:155. https://doi.org/10.3389/fpls.2017.00155

Razzaghi F, Jacobsen SE, Jensen CR, Andersen MN (2015) Ionic and photosynthetic homeostasis in quinoa challenged by salinity and drought–mechanisms of tolerance. Funct Plant Biol 42(2):136-148. https://doi.org/10.1071/FP14132

Ringel C, Siebert S, Wienhaus O (2003) Photometric determination of proline in quartz microplates: remarks on specificity. Anal Biochem 313:167-169. https://doi.org/10.1016/S0003-2697(02)00565-1

Roy SJ, Negrão S, Tester M (2014) Salt resistant crop plants. Curr Opin Biotech 26:115-124. https://doi.org/10.1016/j.copbio.2013.12.004

Schneider T, Ashton MS, Montagnini F, Milan PP (2014) Growth performance of sixty tree species in smallholder reforestation trials on Leyte, Philippines. New Forest 45(1), 83-96. https://doi.org/10.1007/s11056-013-9393-5

Seydi AB (2003) Determination of the salt tolerance of some barley genotypes and the characteristics affecting tolerance. Turk J Agric For 27:253-260.

Shabala S, Munns R (2017) Salinity stress: physiological constraints and adaptive mechanisms. Plant stress physiology, 2nd edn. CABI, Wallingford 24-63. https://doi.org/10.1079/9781780647296.0024

Sharif F, Khan AU (2016) Effect of salinity on tissue nutrient contents of the four dryland tree species of Indus flood plains. Arid Land Res Manag 30(1):65-78. https://doi.org/10.1080/15324982.2015.1054004

Soliman AS, Shanan NT, Massoud ON, Swelim DM (2012) Improving salinity tolerance of *Acacia saligna* (Labill.) plant by arbuscular mycorrhizal fungi and Rhizobium inoculation. Afr J Biotechnol 11(5):1259-1266. https://doi.org/10.5897/AJB11.2287

Suprasanna P, Nikalje GC, Rai AN (2016) Osmolyte accumulation and implications in plant abiotic stress tolerance. In Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies (pp. 1-12). Springer, New Delhi. https://doi.org/10.1007/978-81-322-2616-1_1

Tahjib-Ul-Arif M, Roy PR, Sohag AAM, Afrin S, Rady MM, Hossain MA (2018) Exogenous Calcium Supplementation Improves Salinity Tolerance in BRRI Dhan28; a Salt-Susceptible High-Yielding *Oryza Sativa* Cultivar. Journal of Crop Science and Biotechnology 21(4):383-394. https://doi.org/10.1007/s12892-018-0098-0

Thalmann M, Santelia D (2017) Starch as a determinant of plant fitness under abiotic stress. New Phytologist 214(3): 943-951. https://doi.org/10.1111/nph.14491

Theerawitaya C, Tisarum R, Samphumphuang T, Singh HP, Cha-Um S, Kirdmanee C, Takabe T (2015) Physio-biochemical and morphological characters of halophyte legume shrub, *Acacia ampliceps* seedlings in response to salt stress under greenhouse. Front Plant Sci 6:630. https://doi.org/10.3389/fpls.2015.00630

Yaish MW (2015) Proline accumulation is a general response to abiotic stress in the date palm tree (*Phoenix dactylifera* L.). Genet Mol Res 14(3):9943-9950. https://doi.org/10.4238/2015.August.19.30

Yemm EW, Cocking EC, Ricketts RE (1955) The determination of amino-acids with ninhydrin. Analyst 80(948):209-214. https://doi.org/10.1039/an9558000209

Zhang YM, Liu ZH, Wen ZY, Zhang HM, Yang F, Guo XL (2012) The vacuolar Na^+–H^+ antiport gene TaNHX2 confers salt tolerance on transgenic Alfalfa (*Medicago sativa*). Functional Plant Biology 39(8):708-716. https://doi.org/10.1071/FP12095

Zika M, Erb KH (2009) The global loss of net primary production resulting from human-induced soil degradation in drylands. Ecol Econ 69(2):310-318. https://doi.org/10.1016/j.ecolecon.2009.06.014

Zouari M, Elloumi N, Ahmed CB, Delmail D, Rouina BB, Abdallah FB, Labrousse P (2016) Exogenous proline enhances growth, mineral uptake, antioxidant defense, and reduces cadmium-induced oxidative damage in young date palm (*Phoenix dactylifera* L.). Ecol Eng 86:202-209. https://doi.org/10.1016/j.ecoleng.2015.11.016