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Effect of salinity on physiological, and biochemical traits of two forage species: *Albizia lebbeck* and *Faidherbia albida*

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To maximize the productivity of crops in salty areas, the search for plant species having economic importance and able of reducing the amount of salt is therefore the main selection criterion for a regeneration program. So, the determination of salinity tolerance capacity during the vegetative stage of *Faidherbia albida* and *Albizia lebbeck* was studied. The seeds of each species were germinated and transplanted individually into pots which were arranged in four randomized blocks before being irrigated with the Wacquant nutrient solution, enriched with 0, 50, 100 and 200 mM of NaCl. The results showed that architectural traits were negatively influenced by salinity through the inhibition of stem and root length, as well as the appearance of leaves. Chlorophyll and LRWC content decreased with increasing levels of NaCl, unlike total sugars content. However, these levels of NaCl had no significant effect on total protein in either species. Despite this resemblance of behavior towards NaCl at this stage of growth, the lower salt sensitivity index in *F. albida* made it the most salt resistant. It can therefore be recommended to farmers to extend the return time of salt to the soil.

Key words: Soil salinization, dynamic process, forage plants, sensitivity index.

INTRODUCTION

Salinity is one of the main problems in the efficient use of land for agriculture and it affects crop yields (Flowers et al., 2010). It affects plants by creating numerous morphological, physiological, biochemical, and molecular disturbances during their growth (Morais et al., 2012; Meguekam et al., 2014). Soil salinity is caused on the one hand by the intensive irrigation of crops with salt-rich brackish water, and on the other hand by the excessive use of fertilizers with salinity index (Yang et al., 2019). Saline soils are widespread throughout the world. In Africa, about 40 million hectares are affected (Katarzyna et al., 2022). The saline areas in Cameroon, which have low agricultural yields, are found in the arid zone and along the Atlantic coast.

NaCl in the rhizosphere causes excessive accumulation of Na^+ and Cl^- ions in organ tissues and a nutritional imbalance result mainly due to the competition between mineral elements, such as sodium and potassium;

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calcium, chloride, and nitrate; phosphate and sulfate (Ekwel et al., 2019; Meguekam et al., 2021). Note that NaCl stress generates two problems for the plant organism: the decrease in soil water potential threatens the plant's water supply, and the accumulation of toxic ions in the tissues threatens the physiological functioning of the cells (Mishra and Tanna, 2017).

The introduction of salt-stress tolerant plant species with high socio-economic value, such as forage, is an approach to rehabilitating saline soils (Kalia et al., 2015; Cherifi et al., 2017; Karoune et al., 2019). For this, the usage of local plant species would be appropriate for the following reasons: their adaptability, high biodiversity, acclimatization to local climatic and soil conditions, and their minimal requirements for soil preparation, fertilization as well as watering. They absorb soil contaminants through their roots and store them in their leaves or stems (Meguekam et al., 2014; Joseph et al., 2015). Through the technique known as phytoremediation, these plants do not only clean salt-contaminated soils, but also provide nutrients, fodder, firewood, and industrial feedstocks and, thus, help to increase the incomes of farmers in salt-affected lands (Dilla et al., 2019). It is a more effective saline-sodium soil improvement strategy than chemical amendments and can be recommended in a soil restoration program. Thus, the objective of this study is to evaluate the responses of two forage plant species under salt stress to recommend them in a saline soil restoration for the improvement of agricultural yields in a crop's association.

MATERIALS AND METHODS

The experiments were conducted under a shaded field at the Higher Teacher Training College of Yaoundé (03°51' N/11° 30'W), between July 2019 and April 2020, without climatic disturbance.

Biological material consists of the seeds of two leguminous species, namely *Albizia lebbeck* and *Faidherbia albida*, widely distributed in Africa and in Cameroon (Tchatchoua et al., 2019). They also have a good nitrogen fixation (Singh and Agrawal, 2018) and thus a promising scavenging role for the removal of metal ions from soil solutions (Mustapha et al., 2019; Ullah et al., 2020).

Morphologically, similar, and apparently healthy seeds of each species were selected and washed with tap water. They were then disinfected with a 70% (v/v) ethanol solution for 15 min and rinsed thoroughly with distilled water. Then, they were scarified before being placed between two layers of cotton spread in Petri dishes and supplied daily with distilled water until complete germination. Ten days after sowing (DAS), the sprouted seeds of each species were transplanted individually into 0.9 to 1.2 mm diameter culture pots containing 500 g of sand, previously treated with a 50% v/v hydrogen peroxide solution for 1 h and rinsed with distilled water. These pots were irrigated every day with the nutrient Wacquant solution (Wacquant, 1974). Ten days after transplantation (DAT), the pots were labeled and arranged in four randomized blocks representing four levels of salt treatment with five replicates each (El-Iklil et al., 2000). The used nutrient solution in the pots of the first group was void of NaCl and noted T0, while those of the other experimental groups, called (T1, T2, and T3) were respectively enriched with 50, 100, and 200 mM of NaCl (Meguekam et al., 2014).

Measurements

Morphological traits of A. lebbeck and F. albida under salt stress

The morphological traits were evaluated at the end of every five days by manual counting of the number of leaves and stem length using a cm-scale tape measure (Meguekam et al., 2021). After twenty-five days of growth, the plants of each NaCl level were carefully removed, then its shoots and roots were separated (Cherifi et al., 2017). The root lengths and the water relations were determined; the first using a tape measure and the latter using the weighing of fresh and dry leaf samples. Fresh masses of the leaf samples were determined immediately, while their dry masses were determined after 24 h of oven drying at 105°C using a precision electronic balance (0.001 g) (Meguekam et al., 2014). The physiological and biochemical parameters were evaluated on the fresh leaf sample.

Water relation

Leaf relative water content (LRWC): The water content of the leaves was calculated by the following formula based on the fresh weight matter (FW) of the leaf sample, the turgor weight (TW) after its incubation of the samples in a dark condition in distillated water and constant dry weight (DW) after it was removed from the oven (Hniličková et al., 2017).

$$RWC (\%) = [(FW - DW) / (TW - FW)] \times 100 \quad (1)$$

Sensitivity index (SI): Is the relative production of dry mass between treated plants (MSt) compared to one of the control plants (MSc) as defined by formula (2) published by Karoune et al. (2016).

$$SI = 100 (MSt - MSc) / MSc \quad (2)$$

A negative value for this sensitivity parameter reflects the inhibition of its growth by salt stress, while its positive value reflects stimulation.

Physiological parameters of A. lebbeck and F. albida under salt stress

Chlorophyll content (Chl): The determination of chlorophyll pigments was carried out, according to the modified method of Holm-Hansen et al. (1965), by their solubility in organic solvents. Approximately 100 mg of fresh leaf sample was ground with a pinch of sterile fine sand, then 5 ml of acetone was added and gently homogenized to a dark green solution. The resulting mixture was left for 10 min and then filtered through Whatman filter No. 4. The optical density of the obtained filtrate (O.D.) was read using a Jenway 6305 spectrophotometer at 663 and 645 nm, respectively, against 80% acetone. The concentrations were determined using formula 3 as published by Arnon (1949).

$$[\text{Total Chl}] = (0.0202 \times \text{O.D. } 645) + (0.00802 \times \text{O.D. } 663) \quad (3)$$

Total sugars: The total sugars (TS) were determined according to the modified method of Dubois et al. (1956) whose principle is the following: concentrated sulfuric acid causes the departure of several water molecules from the sugar monomers. The leaf sample was dried at 70°C, homogenized in 80% ethanol, and placed in a water bath at 80°C for 30 min after centrifugation at 3000 g for 5 min. Samples were washed twice with H_2O at room temperature. Each sample was resuspended in 3 ml of H_2O and boiled for 2 h with phenolic sulfuric acid. The intensity of the coloring is proportionate to the concentration of the sugar monomers. The optical density was measured at 488 nm using a UV spectrophotometer (Jenway 6305). The total sugar content was determined by the Nelson-Somogyi method as described by Oser (1979).

Protein content: Protein content (PR) was determined by modifying Bradford's (1976) method using the bovine serum albumin (BSA) as the standard protein. An extracted 0.2 ml of protein was homogenized with 0.3 ml of an already prepared sodium-phosphate buffer, pH 7. The mixture was centrifuged at 13000 rpm for 5 min at 4°C. About 1 ml of the supernatant was poured into a tube containing 1.5 ml of the Bradford reagent and, this new mixture was shaken and incubated in the dark for 15 min. The absorbance of the resulting blue complex was read at 595 nm using the spectrophotometer UV (Jenway 6305). The standard curve was obtained using BSA 1 mg/ml.

Statistical analysis

The collected data are the averages of five replicates for morphological and physiological parameters and of those of the three replicates for biochemical parameters. These averages were compared by a one-way ANOVA test for biochemical parameters and a two-way for the others. They were performed with the GraphPad Prism 8 software and the differences were reported as significant in at least one of the three levels $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$. The results were represented using plotted curves or histograms.

RESULTS AND DISCUSSION

Architectural traits of *A. lebbeck* and *F. albida* under salt stress during the growing state

Salt stress modified architectural traits of *A. lebbeck* and *F albida* plants compared to the control. The effect of NaCl levels on these traits according to the treatment's duration is as shown in Figures 1 to 3.

The analysis of Figure 1 shows that increasing doses of NaCl significantly ($P < 0.05$) inhibited stem elongation in *A. lebbeck* from 50 mM as early as the second week (Figure 1A), whereas in *F. albida*, the inhibition of stem elongation is observed at the same period but from 100 mM of NaCl (Figure 1B).

The effect of salt stress on the variation of number of leaves according to the duration of treatment is represented in Figure 2. The analysis of this figure shows that, increasing doses of NaCl in the culture medium significantly inhibited ($P< 0.05$) leaf production in the *A. lebbeck* species from 100 mM NaCl as from the 4th week of treatment (Figure 2A), whereas in *F. albida*, these treatments more significantly inhibited ($P < 0.001$) the appearance of the leaves from 50 mM of NaCl from the same period (Figure 2B).

Regarding the length of the root, increasing doses of NaCl in the culture medium more significantly inhibited ($P < 0.05$) its elongation at 50 mM of NaCl in *A. lebbecck*, and much more significantly ($P < 0.01$) from 100 mM of NaCl as well as in *F. albida* from 50 mM of NaCl compared to the control.

Leaf inhibition as well as length inhibition are thought to be an adaptive morphological response to water stress that results from high Na^+ and Cl^- concentrations, resulting in physiological and biochemical changes that inhibit architectural traits on other peanut-tolerant varieties (Meguekam et al., 2014, 2021) and, for potato (Chourasia et al., 2021). In addition, the reduction in seedling growth and the development in the two studied plant species could be due to the osmotic, and ionic effects, of salinity that are responsible for the reductions in plant growth (Nawaz et al., 2010). The osmotic effect becomes effective from the onset of salinity stress and leads to an early reduction in growth due to a lack of adequate turgor pressure in plant cells. However, osmotic stress is a transient effect, and it is followed by an ionic effect if plants are exposed to higher salinity levels for a relatively longer period (Abbas et al., 2017). The observed decrease in the growth of the vegetative apparatus could also be explained by an increase in osmotic pressure caused by NaCl in the medium of culture, which blocks the absorption of water by the roots. The plants would then adapt to this condition by reducing their growth to avoid salt damage. This reduction is also more significant at higher concentrations of 100 and 200 mM of NaCl after the first month (Theerawitaya et al., 2014).

The number of leaves would be a good indicator to identify the mortality of plants grown under saline stress.

The important decrease of the root length observed earlier in the effects of NaCl concentration compared to the control in *F. albida* plants could be attributed to ion toxicity due to the accumulation of Na^+ and Cl^- ions in tissues, resulting in osmotic stress as also observed in *Acacia stenophylla* species and other *F. albida* varieties (Abbas et al., 2017); It could also be attributed to a decrease in nutrient uptake by plants or an increase in sodium redistribution from roots (Karoune et al., 2019).

Physiological responses of *A. lebbeck* and *F. albida* after twenty-five days of salt treatment

Leaf relative water content (LRWC)

The effect of salt stress on the leaf relative water content of both species is as shown in Figure 4. The analysis shows that, leaf water content of both species is not significantly influenced ($P < 0.05$) over twenty-five days at 50 and 100 mM of NaCl as compared to the control. In contrast, in the medium enriched to 200 mM of NaCl, this parameter is significantly inhibited ($P < 0.05$) compared to the control. This maintenance of relative leaf water content is the evidence of a salinity control mechanism, and the plant species involved could grow better in a saline environment (Huang et al., 2015). Plant responses to soil salinity based on water relation have previously been observed (Meguekam et al., 2014; Helena et al.,

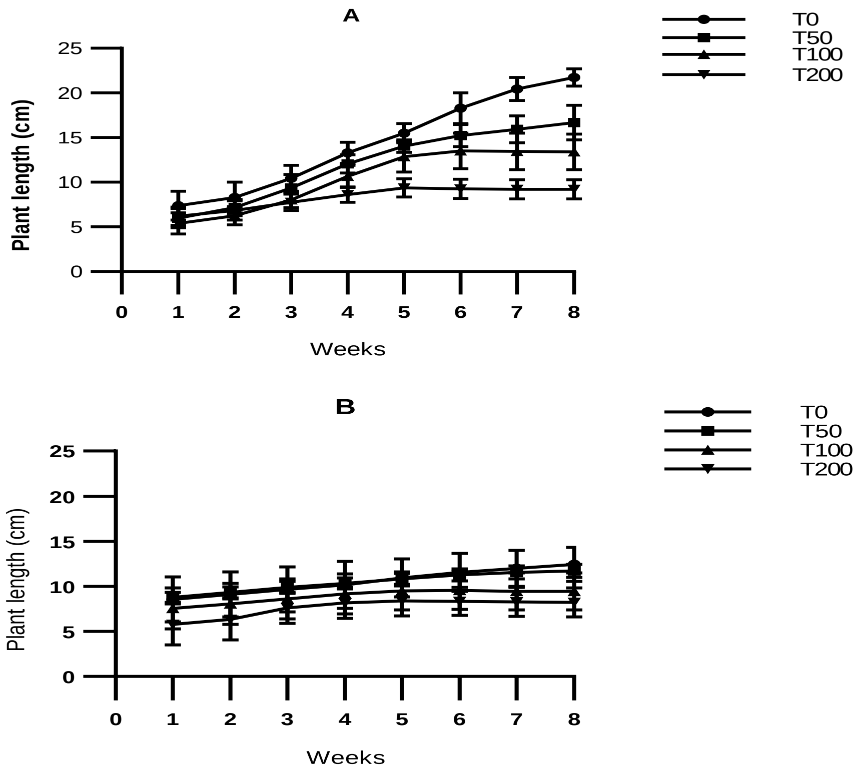

Figure 1. Plant stem length (cm) of both species as affected by different concentrations of salinity (0, 50, 100, and 200 mM NaCl), labelled T0 to T200, during twenty-five days of salt treatment. A: *A. lebbeck*, B: *F. albida*. Values are the means of 5 plant measures with P < 0.05.
Source: Author

2017; Ndouma et al., 2020; Chourasia et al., 2022).

Sensitive index (SI)

The effect of twenty-five days of salt stress on the sensitivity index is as shown in Figure 5. According to this, the salt index increases (P < 0.05) with NaCl concentration following treatment whit 50 mM in both species. The increasingly negative (signs-minus) quantities of the observed SI values, from this threshold, show that the supply of increasing doses of NaCl significantly affects, from this threshold, the morphological traits of the plants of both studied species compared to the control. The values show a significant variation on the one hand between the treatments and on the other hand between the two species. Thus, in the same level of NaCl concentration, the value of SI is more negative effect in *A. lebbeck* than in *F. albida*; the relative decrease of biomass expressed as a percentage of the control is an indicator of the sensitivity of the species to a salt stress condition. Other results, showing a negative effect of salt stress on leaf mass production, have been highlighted for a few for the genus *Acacia* (Cherifi et al., 2017; Karoune et al., 2016; Ekwel et al., 2019). The increasing values of SI are correlated with the reduction of biomass due to the highest accumulation of Na^+ concentration in leaves (Gandonou et al., 2018).

Chlorophyll content

The leaf's chlorophyll (a+b) content in both studied varieties significantly decreases (P < 0.05) in *A. lebbeck* from 100 mM of NaCl and more significantly (P< 0.01) in *F. albida* from 50 mM of NaCl, when each is compared with the control (Figure 6).

The reduction in chlorophyll content could be due to damage to chloroplasts by a high production of reactive oxygen species (Abbas et al., 2017). In addition, photosynthetic activity, due to chlorophyll degradation, is reduced by high Cl^- concentration while Na^+ restricts K^+ and Ca^{2+} nutrition. This restriction disrupts stomatal regulation that is related to chlorophyll formation in leaves (Tavakkoli et al., 2010). The chlorophyll content is the most widely used criterion to assess the general state of the plant in its environment, and it is an excellent bioindicator of stress (Karoune et al., 2016). Thus, salinity

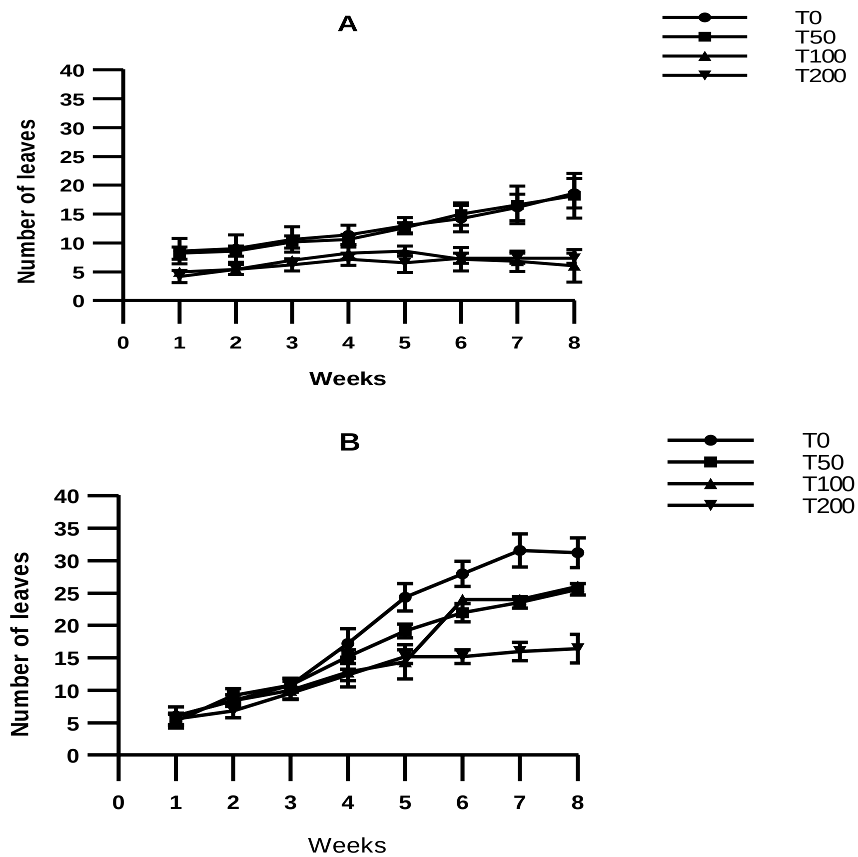

Figure 2. Number of leaves of both plant species as affected by different concentrations of salinity (0, 50, 100 and 200 mM NaCl), labelled T0, T50, T100, T200, during twenty-five days of salt treatment. A: *A. lebbeck*, B: *F. albida*. Values are the means of 5 plant measures with P < 0.05.
Source: Author

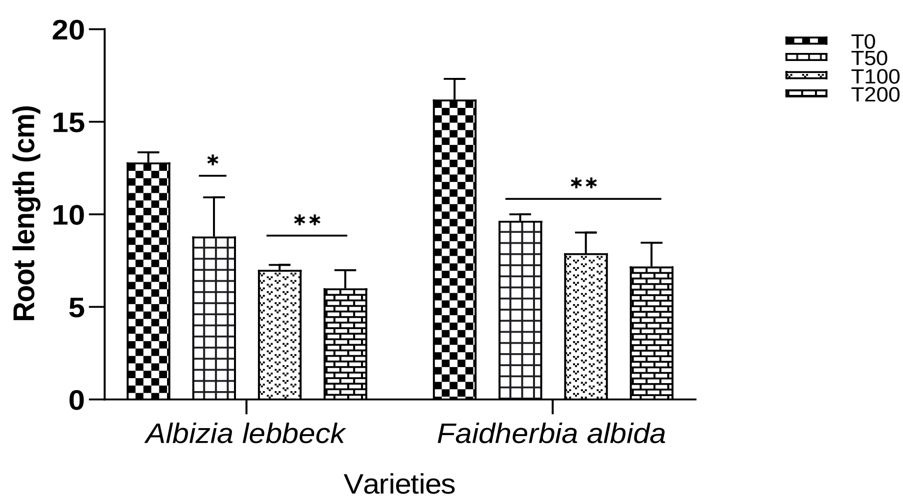

Figure 3. Root length (cm^2) of both plant species as affected by different concentrations of salinity (0, 50, 100, and 200 mM NaCl), labelled T0 to T200, after twenty-five days of salt treatment. Values are the means of 5 plant measures. (*) P< 0.05, (**) P < 0.01, (***) P < 0.001.
Source: Author

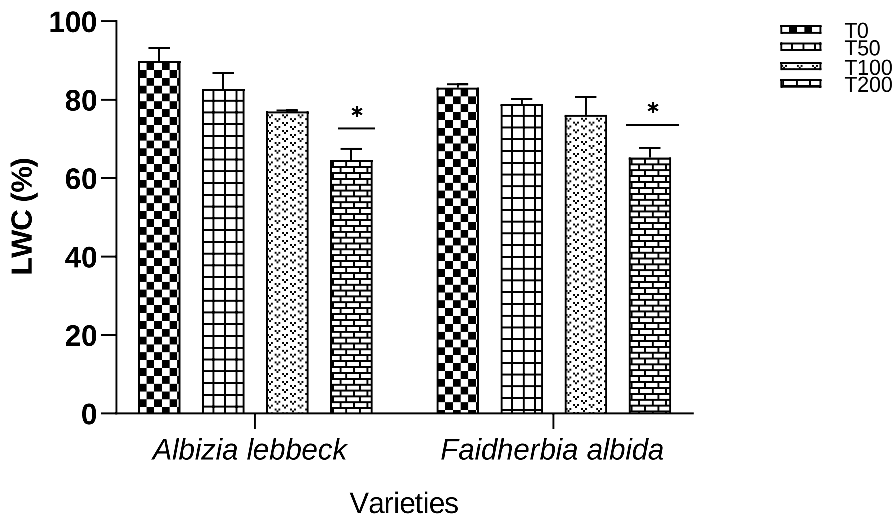

Figure 4. Leaf relative water content (LRWC) of both plant species after twenty-five days salt treatment. Values are the means of 5 plant measures. (*) P< 0.05, (**) P < 0.01, (***) P < 0.001.
Source: Author

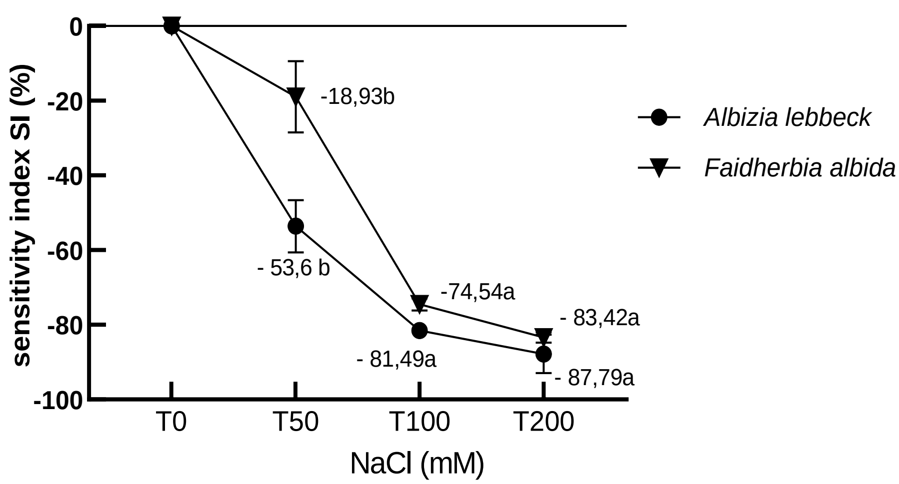

Figure 5. Sensitivity index of both plant species as affected by different concentrations of salinity (0, 50, 100 and 200 mM NaCl), labelled T0, T50, T100, T200, twenty-five days of salt treatment. Values are the means of 5 plant measures. Those with different letters are significantly different at P < 0.05.
Source: Author

is known to inhibit photosynthesis in many species following stomatal closure, thereby limiting of CO_2 into the leaf and diffusion into chloroplasts (Munns et al., 2016; Chutipaijit et al., 2011).

Similar results were obtained in leaves of jojoba (*Sismmondsia chinensis*) seedlings (Hassan and Ali, 2014). The decrease in chlorophyll in *Atriplex canescens* leaves also becomes meaningful above moderate concentrations of NaCl (Nedjimi, 2014).

Total sugars content

In *A. lebbeck*, the total sugar content increased significantly (P < 0.05) from 50 mM of NaCl while in *F. albida,* it did so from 50 mM of NaCl and more significantly P < 0.05 to 200 mM of NaCl compared to the control (Figure 7). This total sugar content in the leaves is positively correlated with increasing doses of NaCl in both species grown after 25 days of saline stress

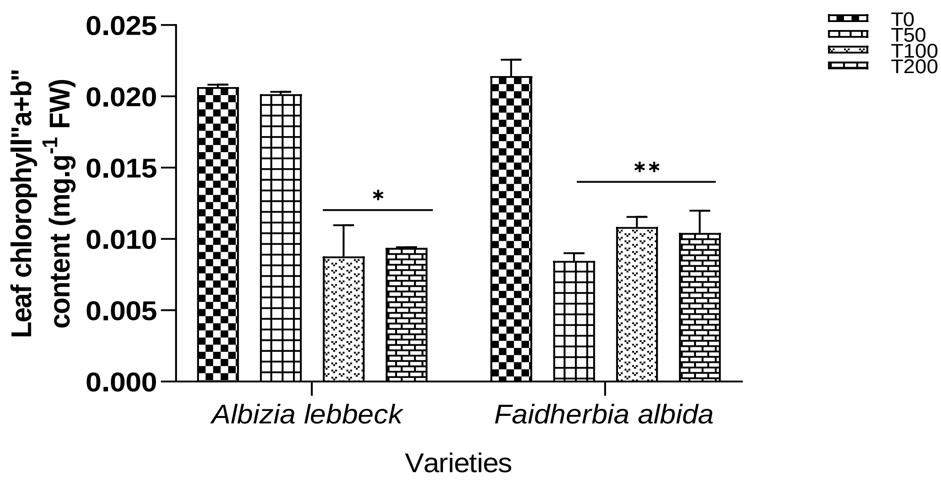

Figure 6. Leaf chlorophyll (a+b) content of both plant species after twenty-five days salt treatment. Values are the means of 5 plant measures. (*) P< 0.05, (**) P < 0.01, (***) P < 0.001.
Source: Author

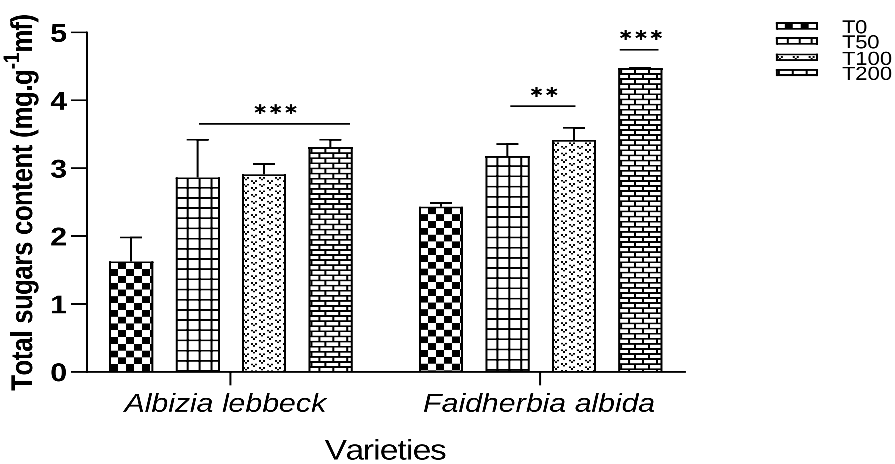

Figure 7. Leaf total sugars content of both plant species after twenty-five days of salt treatment. Values are the means of 5 plant measures. (*) P< 0.05, (**) P < 0.01, (***) P < 0.001.
Source: Author

Studies conducted on Nipa palm, grown under 16.6 dS m^{-1} EC, also showed accumulation of total sugars including sucrose, glucose, and fructose (Theerawitaya et al., 2014). Sucrose, which is considered the primary carbon source for growing seedlings, as well as glucose, would act as osmoprotectants and help maintain bio-membrane homeostasis and integrity during an abiotic stress (Redillas et al., 2012).

It is known that sugars play a role in the osmotic adjustment at the cellular level in the plant's defense mechanism against salt. In plants, sugars function not only as substrates for energy production, but also act as osmolytic compounds and messengers in signal transduction. In addition, these sugars modulate growth, development, and gene expression (Abbas et al., 2017). The accumulation of sugars in leaves under salt stress is an adaptive mechanism for seedlings to maintain leaf turgor by decreasing water potential as well as water uptake (Zraibi et al., 2012, 2011). This would justify their relative salt tolerance by inhibiting normal plant growth as well as increasing leaf areas (Meguekam et al., 2021). Sugars are good osmoregulators because they participate in metabolic events and prevent the dehydration of plants (Redillas et al., 2012). The

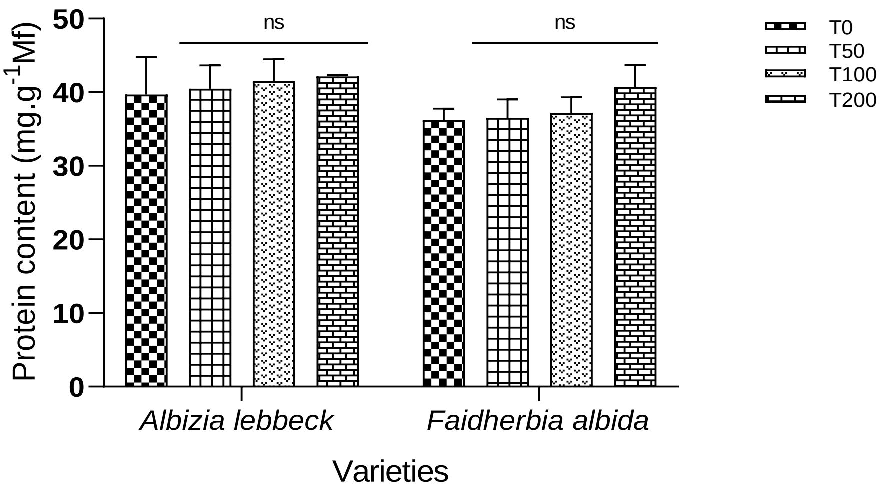

Figure 8. Leaf protein content of both plant species after twenty-five days of salt treatment. Values are the means of 5 plant measures. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$.
Source: Author

accumulation of sugars is suggested as an index of resistance to salt stress (Meguekam et al., 2014).

Biochemical response of *A. lebbeck* and *F. albida* after twenty-five days of salt treatment

Protein contents

The total protein contents of *A. lebbeck* and *F. albida* leaves after twenty-five days of salt treatment are presented in Figure 8. The analysis of this figure shows that the accumulation of total proteins in the leaves of the seedlings with increasing NaCl concentration of the medium is not significant compared to the control. Nevertheless, this accumulation is more important in the leaves of *F. albida* in the medium enriched with 200 mM of NaCl compared to its control, although the effect is not statistically significant.

Unlike some species of *Arachis hypogaea* L. that uses the biochemical pathway to respond to salt stress (Meguekam et al., 2021), the species studied in this paper tend to use physiological pathway such as transpiration limitation and leaf apoptosis. In those species that use total protein accumulation in leaves under salt stress, it serves as a biochemical adaptation mechanism that facilitates water uptake and retention to ensure lipid and peroxidase metabolism and photosynthetic activity in tolerant plants (Paul and Lade, 2014; Hand et al., 2017). This accumulation may also contribute to nitrogen storage for later reuse and plays a role in osmotic adjustment. Proteins could be re-synthesized in response to salt stress or be constitutively present at low concentrations such as in some peanut varieties (Meguekam et al., 2014).

Conclusion

At the end of this study, whose objective was to identify, between two species of leguminous *A. lebbeck* and *F. albida*, the one that could be retained for a saline soil purification program and limit the progression of soil's contamination, it was found that architectural traits were negatively influenced by salinity through the inhibition of stem and root heights, as well as the appearance of leaves. In addition, the Chl and LRWC content decreased with increasing levels of NaCl, unlike total sugars content. Moreover, these levels of NaCl had no significant effect on total protein accumulation in both species, though, the lower salt sensitivity index in *F. albida* makes it the most salt-resistant species. To sum up, both forage species tolerated 50 mM of NaCl well and 100 mM of NaCl better. However, they remained sensitive to a higher dose like 200 mM NaCl. Although a large variability between the two species' responses to salt was revealed at the stage of growth, it should be noted that *A. lebbeck*, had good architectural parameters under salt stress compared to *F. albida* which also had a lower SI. This enables *F. albida* to be proposed to farmers first, followed by *A. lebbeck* to extend the return time of salt to the soil in order to solve the problem of crop production through sustainable agriculture, and that they are likely to be used for soil by Phyto purification.

For future works, it would be important to investigate the mineral content to know more about the mechanisms that these species put in place to overcome salt stress and to have more information about their adaptation modalities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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