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Research Article
Morphological and Physiological Responses of Three Populations of *Ziziphus lotus* L. on Seedling Stage Under Saline Stress

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Abstract
Background and Objective: This study aims to evaluate the behavior of three natural populations of *Ziziphus lotus* namely A, B and C, respectively from three different regions under the effect of saline stress on the seedling stage. **Materials and Methods:** The areas targeted during these investigations are AinChifa (sub-humid stage), Fez (semi-arid) and Guercif (arid stage). During 4 weeks, *Z. lotus* seedlings were a subject for salt stress with different concentrations: 0, 50, 100 and 200 mM. The biomass of the leaves, stems and roots by dry matter of the plant, relative water content of leaves, total chlorophyll content and proline content were determined. **Results:** The results obtained has showed that saline stress has a significant effect on the various parameters such as leaf, stem and root biomass, relative water content, chlorophyll and proline. **Conclusion:** Salt stress affect morphological and physiological behavior of *Ziziphus lotus* because it exerts a depressive effect on all the parameters studied.

Key words: Salt stress, *Ziziphus lotus*, population, morphological, physiological

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Commonly called sedra, the Jujube (*Ziziphus lotus*) belonging to the family Rhamnaceae, is a species present in several biotopes with arid and semi-arid regions. Its fruits, leaves and roots have many nutritional, cosmetic and medicinal interests. In Morocco, the jujube is considered as a medicinal plant frequently used in traditional medicine. Anti-inflammatory, analgesic and anti-spasmodic activities have been widely emphasized[1]. In addition to its pharmacological properties, *Ziziphus lotus* L. also, a pastoral species highly appreciated by many animals (sheep, cattle, camelids and goats)[2]. *Ziziphus lotus* fruits are very rich in flavonoids, sterols, tannins and triterpenoid saponins[3,4].

These characteristics make *Ziziphus lotus* a shrub of universal value with arid and semi-arid ecological areas. However, this plant with a patrimonial value is often ignored or even forgotten, especially since it is torn away by farmers despite its industrial, economic and therapeutic virtues.

On the other hand, soil salinization was considered to be one of the main factors limiting plant development and is becoming increasingly disturbing as it reduces the area of arable land and threatens food security in areas arid and semi-arid areas. In Morocco, the salinity of agricultural soils is beginning to increase with the extension of irrigated areas. Nearly 500,000 ha of arable land are subject to increasing salinization[5]. Salinity leads to plants' water deficiency due to osmotic stress possibly coupled with biochemical perturbations induced by sodium ion inflow[6,7]. The crossing of the germination stage is decisive and crucial in any development and growth of the seedling.

Due to lack of field investigation of salinity's effects on the development of *Ziziphus lotus* in Morocco, the aim of this work is to evaluate the impact of salt stress on some morphological and physiological parameters of *Ziziphus lotus* seedlings at different NaCl concentrations for three Moroccan ecotypes distributed in different arid, semi-arid and wet zones.

MATERIALS AND METHODS

This research project was conducted from 07/2015-06/2016.

Plant materials: Plant material consists of three ecotypes of *Ziziphus lotus* from 3 regions in different pedoclimatic zones. These ecotypes originate from the regions of AinChifa, Fez and Guercif. They are, respectively, designated as "A", "B" and "C". The fruits of *Z. lotus* were harvested in August, 2015.

Effect of salinity on the development of *Z. lotus* seedlings

Production of seedlings: Before starting germination, the seeds were soaked in distilled water for 5 h. Afterwards, they were treated with 10% bleach for 15 min, then soaked in 70% ethanol for 5 min and rinsing with distilled water. The seeds were germinated in Petri dishes containing filter paper impregnated with 10 mL of sterile distilled water. Petri dishes closed tightly to prevent evaporation and thus maintain a constant relative humidity (~80%). The seeds were incubated in dark at 35±1°C and followed every 24 h for 7 days. The germination was identified by the emergence of the radicle. Young 7 day old *Z. lotus* seedlings were transplanted into plastic pots (9 cm deep and 1 kg soil capacity) containing a 1:1 sand soil mixture.

The average temperatures recorded under green house during the cultivation period (August-December, 2015) were between 18 and 40°C. The watering of the young seedlings was carried out in running water taking into consideration the field capacity. Once the seedlings were acclimatized (3 months after planting), the application of salt treatments began and irrigation was carried out using different NaCl concentrations (T1: 0 mM, T2: 50 mM, T3: 100 mM and T4: 200 mM). The number of repeats was 20 per treatment. Irrigation with saline solutions began on November 10th, 2015 and ended on December 10th, 2015.

Measured parameters: Harvested plants were separated into leaves, stems and roots. The Dry Matter (DM) was determined after drying in an oven at 80°C during 72 h. The morphological and physiological parameters measured during this study were:

- Leaves, stems and roots biomass
- Relative water content of leaves (RWC):

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Where:

RWC = Relative water content
FW = Fresh weight of the sample
DW = Dray weight of the sample
TW = Trigger weight of the sample

Total chlorophyll content: The determination of the chlorophyll pigments were carried out in order to quantify the total chlorophyll content of the plant material according to the technique of Hiscox and Israelstam[8]. About 4 mL of Dimethyl sulfoxide (DMSO) were added to 40 mg of fresh plant material. After incubation in dark at 65°C for 15 min, the absorbance was measured at 663 nm and then at 645 nm (CHROM TECH V1200 spectrophotometer). Chlorophyll concentrations are deduced by the following equation described by Arnon[9]:

$$\text{Chl a } (\text{g L}^{-1}) = 0{,}0127\times A663 - 0{,}00269\times A645$$

$$\text{Chl b } (\text{g L}^{-1}) = 0{,}0229\times A645 - 0{,}00468\times A663$$

$$\text{Chl Tot } (\text{g L}^{-1}) = \text{Chl a} + \text{chl b}$$

Where:

Chl = Chlorophyll
A = Absorbance

Proline content: The method used for the proline dosage was determined by Rascio *et al.*[10]. About 100 mg of the fresh material were mixed with 2 mL of 80% methanol. The whole was placed at 85°C in a water bath for 1 h. Then, 2 mL of the extract, 2 mL of glacial acetic acid and 2 mL of ninhydrin reagent were placed in a boiling water for 60 min. Spectrophotometric measurements were carried out at 546 nm (CHROM TECH V1200 spectrophotometer).

Statistical analysis methods: Data were subjected to two-way analysis of variance (ANOVA) in order to determine significant differences among the treatments. The data were processed using the "SYS-TAT 12" software. A mean comparison test was performed whenever there was a significant factor effect studied by ANOVA.

RESULTS

Effect of salt stress on biomass: The dry matter of leaves, stems and roots for three *Z. lotus* populations (A, B and C) decreased in a linear way with increasing of salinity.

Leaf biomass: The results showed that 200 mM caused a significant reduction compared to other concentrations applied. Falls were recorded, respectively, for the three populations A, B and C. It can be seen that the mathematical models for all population were linear with a weighting coefficient 0.83, 0.971 and 0.9855, respectively for population A, B and C (Fig. 1). The guidelines coefficients showed the negative effect of salinity on leaves growth.

Root biomass: The effect of salt stress on root dry matter biomass for the three *Z. lotus* populations was presented in Fig. 2. Indeed, the largest reductions were recorded at 200 mM.

The linear mathematical models and the guidelines coefficients showed a negative impact of salinity on the biomass of *Z. lotus* root.

Stems biomass: Decreases in three populations A, B and C were observed (Fig. 3). The weighting coefficient were -0.0414, -0.0416 and -0.055 confirmed the negative effect of salinity on the growth of stems.

Results showed a very highly significant effect on stem, leaf and root biomass between population and salt stress ($p<0.001$). The population-salt stress interaction was not significant ($p>0.05$).

Relative Water Content (RWC): Figure 4 showed the relative water content of 3 populations stressed to the response of different NaCl concentrations. In fact, at 200 mM, the levels fell considerably for the three populations. However, the mathematical models, the weighting coefficient and guideline showed the negative effect of high salt concentrations.

The ANOVA revealed a highly significant effect between the different concentrations ($F = 25.221$, dd1 = 3, $p\leq 0.001$). On the other hand, no difference was observed between the 3 populations studied ($F = 2.172$, dd1 = 2, $p>0.05$).

Total chlorophyll content: Figure 5 showed the impact of salt stress on the chlorophyll content for the populations studied. It can be seen a negative correlation between the chlorophyll content and the populations, except for population A, which experienced a slight increase in chlorophyll content, then, dropped at 100 mM that negatively affects in the photosynthesis of stressed plants. The mathematical models revealed the negative effect of salt on the total chlorophyll content. The statistical analysis showed a highly significant differences for both population factors ($F = 10.579$, ddl = 2, $p = 0.001$) and concentrations ($F = 12.504$, ddl = 3, $p\leq 0.001$).

Proline content: Salt had a significant effect on proline accumulation. The results showed that the proline evolves in

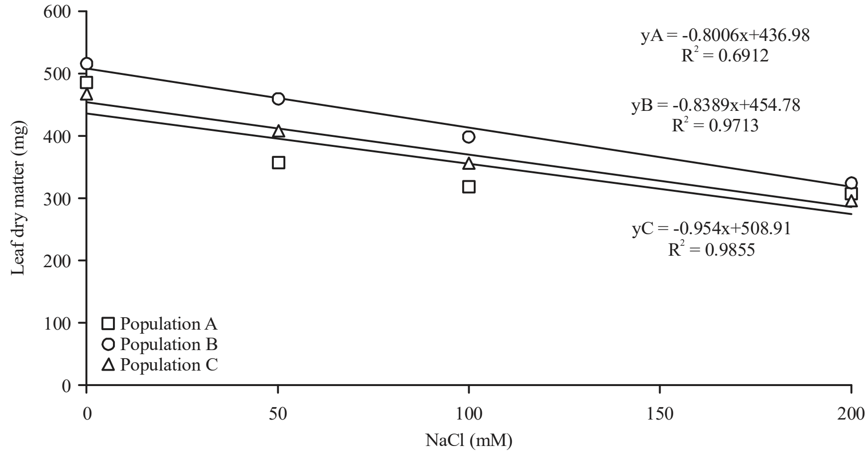

Fig. 1: Effect of salt stress on leaf dry matter biomass in three *Z. lotus* A, B and C populations
SE (n = 4)

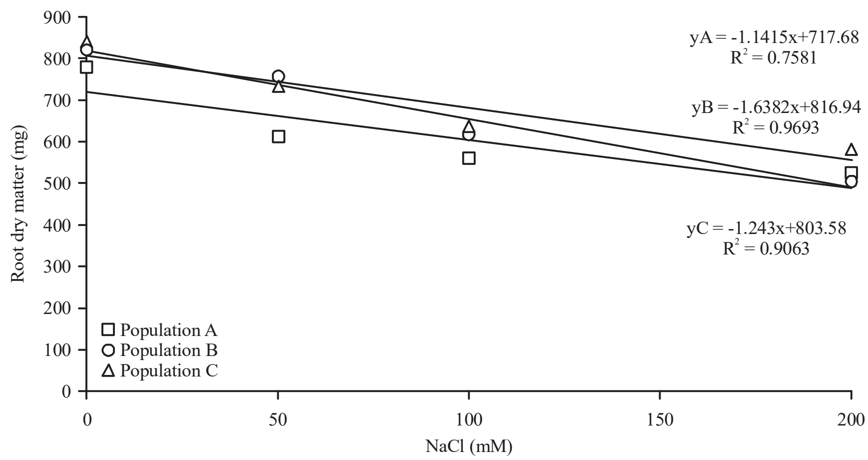

Fig. 2: Effect of salt stress on root dry matter biomass in three *Z. lotus* A, B and C populations
SE (n = 4)

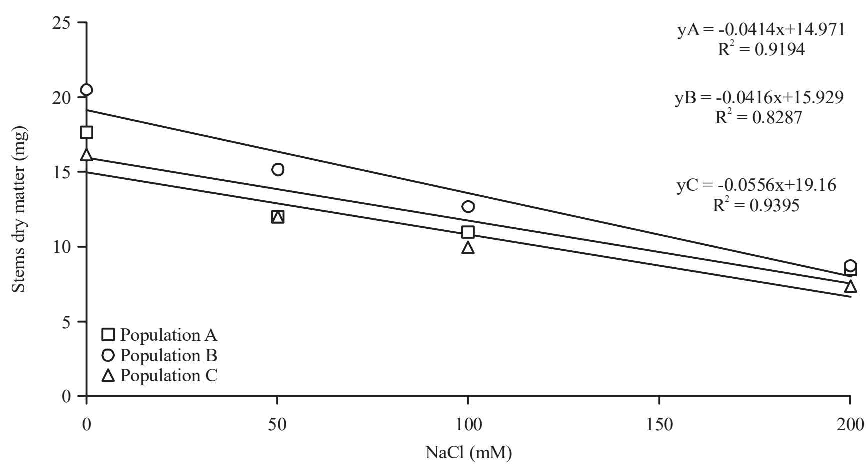

Fig. 3: Effect of salt stress on stems dry matter biomass in three *Z. lotus* A, B and C populations
SE (n = 4)

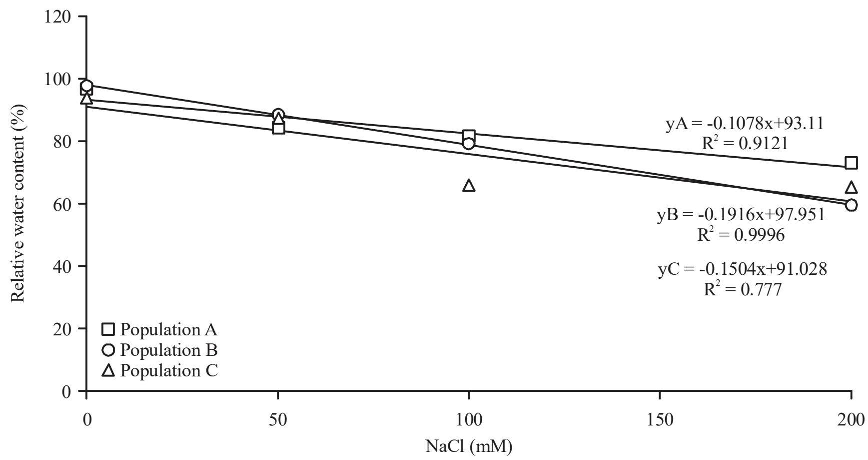

Fig. 4: Relative Water Content (RWC) of leaves of three populations of *Z. lotus* in term of different concentrations of NaCl SE (n = 4)

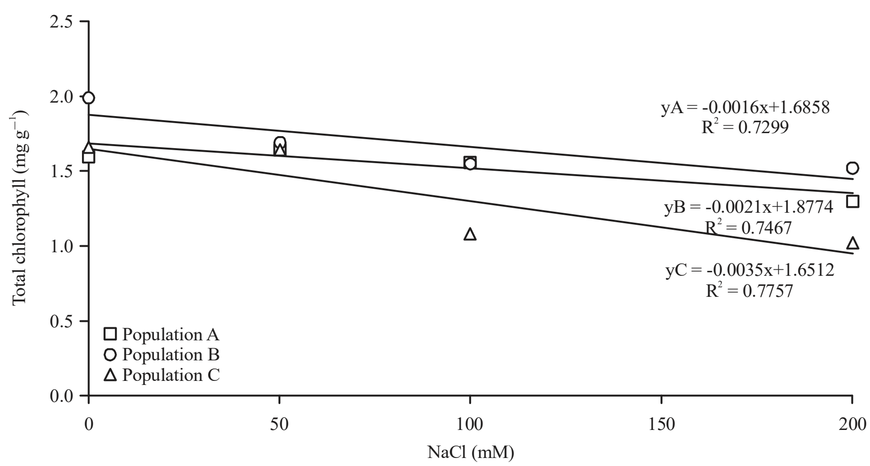

Fig. 5: Total chlorophyll content of the leaves of three populations of *Z. lotus* in terms of different concentrations of NaCl SE (n = 4)

the same direction with saline solution. This amino acid accumulates more when the culture medium was enriched with NaCl. It found that the mathematical models for the three populations A, B and C were linear (Fig. 6). The guidelines coefficients showed the positive effect of salinity on the proline accumulation on leaves of the three studied populations.

Analysis of relative variation to proline content revealed a highly significant effect ($p<0.001$) for both, population factor ($F = 31.765$, ddl = 2, $p\leq 0.001$) and concentrations factor ($F = 212.827$, ddl = 3, $p\leq 0.001$).

DISCUSSION

The increase in NaCl concentration caused a reduction in the biomass (stems, leaves and roots), relative water and chlorophyll content of the three populations studied. The decrease in growth observed in *Z. lotus* plants can be explained by the fact that NaCl acts by increasing the osmotic pressure of the medium, thus preventing the absorption of water by roots. This leads to a reduction in growth which was the result, at the cellular level, of a decrease in the number of cell divisions[11]. In our case, there was a slight reduction in growth for plants studied. This could be explained, either by the fact that the applied salt stress (50, 100 and 200 mM NaCl) was not severe enough or that the three populations would had a better resistance compared to others earlier studies. The relative water content in the leaves was a good indicator of water status, it decreases slightly in plants stressed. This was particularly noticeable when the plants were subjected to 200 mM NaCl, which appeared to be a salt stress resistance behavior. The relative water content allows the overall

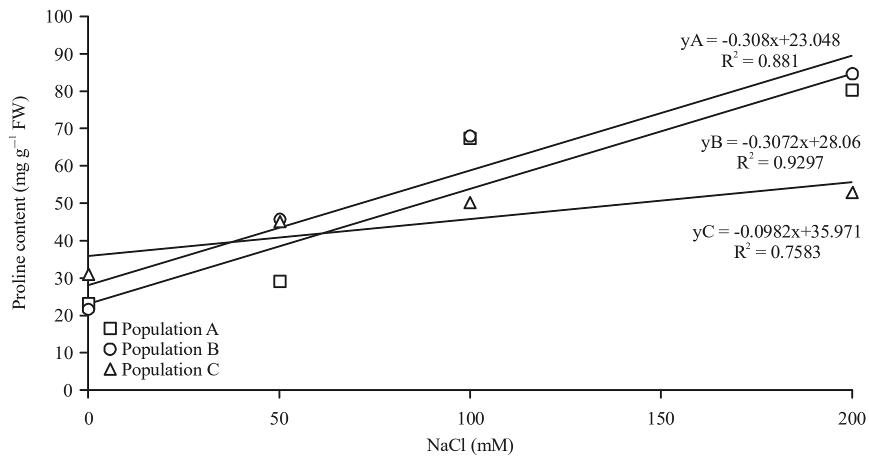

Fig. 6: Proline content of leaves of three *Z. lotus* populations in term of different concentrations of NaCl SE (n = 4)

description of the water status in the plant and the ability to perform good osmoregulation and maintain cell turgidity[12]. A sudden increase salinity results in an immediate reduction in leaf growth. This was associated with a decrease in turgor, itself related to the decrease in the gradient of water potential between the plant and the medium. According to our results, salt stress caused a delay in the growth of the plant, the plant grows but it was delayed compared to the control. It results in decreased leaf area accompanied by stress symptoms such as chlorosis and foliar necrosis, leading to leaf death. This same behavior was observed by Chartzoulakis and Klapaki[13], explained by a specific hazard of the Cl-ions accumulated at levels exceeding the compartmentalizing capacity. It was also reported that the total chlorophyll content was negatively influenced by salt regime. However, the supply of 50 and 100 mM stimulates the chlorophyll biosynthesis. Above of 100 mM, the chlorophyll content decreased. Salinity had a depressive effect by the reduction in chlorophyll content[14-16]. The decrease in the chlorophyll synthesis may be due to a decrease in 5-aminolevulinic acid[17]. The NaCl inhibited the synthesis of 5-aminolevulinic acid, which is a chlorophyll precursor[18]. In addition, its confirmed that salt stress damage photosystem II (PSII)[19] and photosynthetic enzymes[20].

The applied salt stress causes a linear increase in proline levels, similar results were found in okra[21], castor[22], pistachio of the atlas[23] and rice[24]. It was established that proline is certainly one of the most widespread osmolytes. During osmotic stress, proline is synthesized in chloroplasts[25] and accumulated[26] to protect stressed plant against the disruptions of protein metabolism[27]. The accumulation of proline in foliar tissues is considered as a criterion of adaptation[28]. It allowed the plants to support the lack of water by a decrease of the osmotic potential[29]. In the three populations of *Z. lotus*, proline accumulation is negatively correlated with total chlorophyll content. These results suggested the existence of a probable connection between the biosynthetic of chlorophyll content and proline[30]. A competition between these two compounds on their common precursor, glutamate, may be the origin of this evolution[31,32].

CONCLUSION

It can be concluding that salt stress is a limiting factor of *Ziziphus lotus* growth, because it exerts a depressive effect on all the studied parameters of the three jujube populations. The mathematical models linear with negative or positive weighting coefficient justified plant resistance vis-vis a salt stress. Thus, it was found that the population of *Fez* (B) is more tolerant of salt stress than AinChifa (A) and Guercif (C) for the most morphological and physiological parameters studied.

SIGNIFICANCE STATEMENT

The determination of the different morphological and physiological parameters in the three populations of *Ziziphus lotus* L. from different regions contributes to a better knowledge of the species and makes it possible to demonstrate the behavioral nature of the studied populations with respect to climatic variations.

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