Biochemical response of three natural populations of *Zizyphus lotus* L. at the seedling stage under a saline constraint

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Abstract— This work was carried out in order to evaluate the influence of saline stress on the content of the leaves and the roots in total soluble sugars, total soluble proteins and free amino acids. Three natural populations of *Zizyphus lotus* coming from three areas located in different pedoclimatic zones were the subjects of this study. The zones targeted during these investigations are: Ain Chifae, Fès and Guercif. The populations of *Zizyphus lotus* of these three zones are designated hereafter respectively by A, B and C. Seedlings of *Z. lotus* are subjected to a saline stress during 4 weeks with concentrations of: 0 mM, 50 mM, 100 mM and 200 mM. The results obtained showed that the increasing NaCl concentrations lead to an accumulation of these components in the foliar and root part. Indeed the results obtained showed that the population C is the one which had the advantage of accumulating total soluble sugars on the level of the leaves and the roots compared to populations A and B, whereas it synthesized less total soluble proteins on the levels of the leaves. With regard to the amino free acid content, the lowest values were recorded at the population B as well at the leaves and the roots. The analysis of the results would be associated with the environmental variation of the cultivation of *Zizyphus lotus* in the three studied zones. This would have certainly an impact on the biological and nutritive characteristics of *Zizyphus lotus*.

Keywords—Amino free acids, Saline stress, Total soluble proteins, Total soluble sugars, *Zizyphus lotus*.

1 Introduction

The jujube tree (*Zizyphus lotus*), belongs to the family of Rhamnaceae commonly called SEDRA, and is a species present in several biotopes of the arid and semi-arid areas. It easily adapts to its natural environment thanks to its root system, able to seek water over more than 50 meters deep. This plant has a paramount importance in the economic, cosmetic and therapeutic sectors since it is used in the cure of several diseases. In Morocco, the jujube tree is regarded as a medicinal plant frequently used in traditional medicine. Its analgesic anti-inflammatory and antispasmodic effects were largely highlighted [1].

These characteristics make of *Zizyphus lotus* a shrub of universal value on the arid and semi-arid ecological areas. However, this plant with such a patrimonial value is often ignored even forgotten, more especially as the farmers in spite of its industrial, economic and therapeutic virtues often grub it up.

On the other hand, the salinization of the agricultural ground starts to become extensive with the extension of the irrigated surface; nearly 500000 of hectares of the arable lands are prone to an increasing salinization, which has a harmful effect on the growth and the development of the plants [2] . It consequently causes a hydrous deficit at the plants due to the osmotic stress possibly linked to biochemical disturbances induced by the flow of sodium ions [3], [4].

Because of lack of investigation into the ground of the salinity effects on the development of the *Zizyphus lotus* seedlings in Morocco, this work aims to evaluate the impact of the saline stress on some biochemical parameters of the *Zizyphus lotus* seedlings to various concentrations in NaCl, and in three Moroccan ecotypes distributed in various pedoclimatic arid, semi-arid and wet zones.

2 Materiel and Methods

2.1 Vegetable material

The vegetable material is made of three ecotypes of *Zizyphus lotus* coming from three areas located in different pedoclimatic zones. These ecotypes come from the areas of Ain Chifae, Fès and Guercif. They are respectively indicated: "A", "B" and "C". Fruits of *Z. lotus* were harvested in August 2015. The extraction of seeds of *Z. lotus* was carried out using a hammer under the ambient conditions of the laboratory.

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

2.2.1 The cultivation of the seedlings

Before launching germination, the batches of seeds are soaked in the distilled water for 5 hours. After that, the

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seeds were treated with 10% bleach for 15 minutes. Then, they were soaked in ethanol 70% for 5 minutes, followed by a rinsing with distilled water. The seeds were put to germinate in boxes of Petri containing of the filter paper soaked with 10 ml sterile distilled water. The hermetically closed boxes of Petri were to avoid evaporation and thus keep relative constant moisture (80%). The seeds were incubated in the darkness of the drying oven in 35±1°C monitored throughout 24h during 7 days. A seed was considered germinated when there was emergence of the radicle. Young seedlings of *Z. lotus* 7 days old lotuses are planted in plastic pots (9 cm in depth and a capacity of 1 kg of soil) containing a mixture of sand/soil of 1:1. Sand was washed 4 times by tap water with the aim of eliminating micro-organisms and organic waste. After that, sand was dried and mixed with soil which had gone through the drying oven at 105°C for 24 hours, then filtered to eliminate any seed likely to interfere with our seeds. The average temperatures recorded under the greenhouse during the cultivation period (August 2015-December 2015) are located between 18 and 40°C. The watering of the young seedlings was carried out with running water so as to maintain the substrate of the cultivation to field capacity. Once the seedlings acclimatized (three months after the plantation), the application of the saline treatments started and the irrigation is carried out by using various NaCl concentrations (T1: 0 mM; T2: 50 mM, T3: 100 mM and T4: 200 mM of NaCl). The pilot enviornment (T1) is watered only with running water. The number of repetition is four. Each repetition comprises five seedlings per pot and treatment. The irrigations with the saline solutions began on November 10, 2015 and ended on December 10, 2015.

2.3 Measured parameters

2.3.1 Proportioning of total soluble proteins

To determine the rate of total soluble proteins, we used the Lowry method [5] which consists of mixing 200 mg of the vegetable matter freshly crushed in 5 ml of sodium phosphates buffer (100 mm, pH 7,5), followed by a centrifugation of 4000 rpmt at 4°C for a duration of 10 minutes. The float is thus recovered for proportioning. This technique enables us to carry out a particularly sensitive colorimetric proportioning based on 2 colorimetric reactions:

*Reaction of biuret, in which $Cu^{2+,}$ in the presence of a base, reacts with the peptide connection by giving a blue-deep color.

*The chemistry of folin - ciocalteu, in which a complex mixture of inorganic salts reacts with the tyrosin residues and tryptophan of proteins, by giving a blue-green color.

The absorption was determined by spectrophotometry at 750 nm (CHROM DECH V1200). The calibration range was carried out by concentrations known as BSA (bovine serum albumin).

2.3.2 Proportioning of Total Soluble Sugars (TSS)

To carry out the proportioning of total sugars, 100 mg of the fresh matter was crushed in 4 ml of ethanol at 80%. After incubation in water bath through agitation during 30 min at 80°C, the extract was centrifuged at 4500 rpmt during (10 min). Total soluble sugars are then proportioned in means of the anthrone (150 mg of the anthrone /100 ml of sulphuric acid) [6]. The absorption was determined by spectrophotometry at 625 nm (CHROM DECH V1200). The range of calibration was prepared by glucose.

2.3.3 Proportioning of the free amino acids

The amino free acid content was extracted by crushing from 100 mg of fresh vegetable material in 5 ml Sodium Phosphates buffer (100 mm, pH 7,5). The extract was incubated at 80°C during 30 min after preliminary addition of 2 ml of ethanol at 80%. 2 ml of ethanol at 50% are then added in the tubes and the whole is mixed on vortex. After centrifugation during 10 min (5000 rpm), the float was recovered then placed at the drying oven at 80°C. The dry residues comprising the amino acids are returned into 500 µl distilled water. The proportioning of the amino acids is carried out by adding 1 ml of Ninhydrine reagent prepared extemporaneously at 100 µl 5 times diluted sample [7]. The whole is incubated during 20 min at boiling water bath. The reaction is stopped by brutal cooling in ice; 5 ml of ethanol 50% are added. Spectrometric measurements were also carried out at 625 mn (CHROM DECH V1200). The range of calibration was carried out by concentrations of Glycine (150,14mg/l).

2.4 Method of statistical analysis

The data obtained was the subject of a statistical analysis (calculation of the averages, variance analyses ANOVA, standard deviation) to seek existing variability between the various treatments used, between the two bodies and between the three populations (A, B and C). The data was treated using the software "SYS-TAT 12". A test of comparison of average was made each time there was a significant effect of factor studied by the ANOVA.

3 Resultats and Discussion

3.1 Total soluble sugars content (TSS)

The results represented on Figure 1 and Figure 2 highlight the variation of foliar and root total soluble sugars content at the three populations (A, B and C) vis-a-vis the saline stress. The values show that the content of TSS moves in the

same direction as the concentration of the saline solution. The most significant increases in foliar and root total soluble sugars were recorded at the ecotype C. These increases on the level of the leaves are about 76% and 90% for the pilot and of 58% and 71% to 200 mM for the two populations A and B respectively, whereas they are 34% and 68% (0 mM) and 10% and 52% (200 mM) on the level of the roots. Conversely at population A and B soluble total sugars content is accumulated in the roots as well as in the leaves. The results obtained showed that the population, the concentration and the body factors have a very significant effect on the total soluble sugars content (P< 0,001).

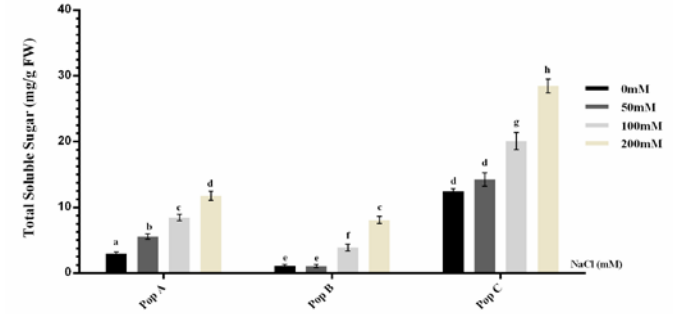

Fig. 1. Variation of total soluble sugars content of the leaves according to various NaCl concentrations at 3 populations of *Zizyphus lotus*: A (Ain chifae), B (Fès) and C (Guercif). The vertical bars correspond to the SE for N = 4.

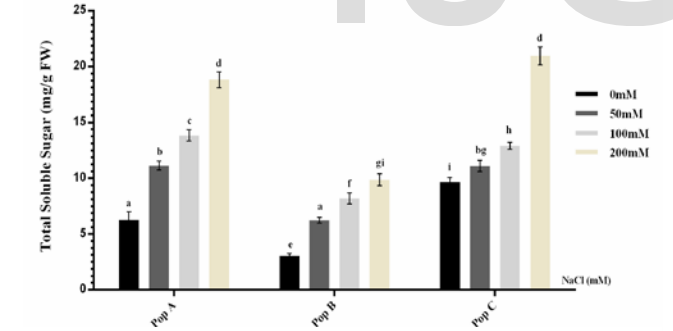

Fig. 2. Variation of total soluble sugars content of the roots according to various NaCl concentrations at 3 populations of *Zizyphus lotus*: A (Ain chifae), B (Fès) and C (Guercif). The vertical bars correspond to the SE for N = 4.

3.2 Total soluble proteins content

The variation of the total soluble proteins content extracted is presented in Figure 3 for leaves and in Figure 4 for roots. According to the results, we noticed that the ecotype B accumulates higher concentrations of total proteins compared to populations A and C in the foliar part as well in the pilot plants than stressed ones. However, by its potential of root accumulation of total soluble proteins, the population B largely exceeds the two ecotypes A and C by percentages of 48,6% and 48,3% for the pilot and of 50% and 44% per 200 mM respectively. We also noticed, according to the results obtained, that the leaves of population A accumulate more of total soluble proteins than the roots for all the studied treatments. The analysis of the variance relating to the content of total soluble proteins revealed a highly significant effect (P< 0,001) as well as for the population factor (F=140,947; ddl=2) and that of the two other concentration factors (F=35,293; ddl=3) and body (F= 31,889; ddl = 1).

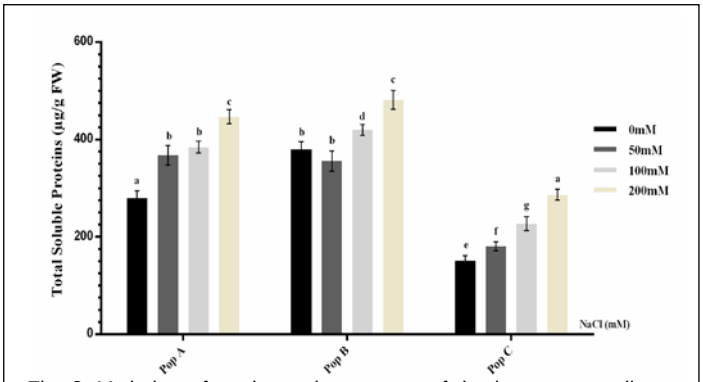

Fig. 3. Variation of total proteins content of the leaves according to various NaCl concentrations at 3 populations of *Zizyphus lotus*: A (Ain chifae), B (Fès) and C (Guercif). The vertical bars correspond to the SE for N= 4.

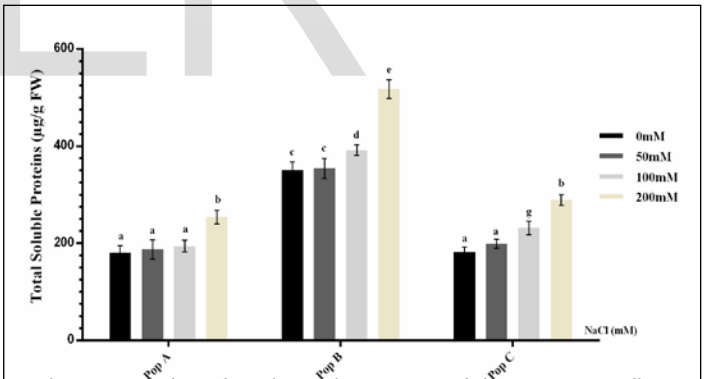

Fig. 4. Variation of total proteins content of the roots according to various NaCl concentrations at 3 populations of *Zizyphus lotus*: A (Ain chifae), B (Fès) and C (Guercif). The vertical bars correspond to the SE for N= 4.

3.3 Amino free acid content

Salinity had a marked effect on the amino free acid content of the three populations of *Z. lotus* (Fig. 5) (Fig. 6). The results obtained made it possible to highlight that the population C accumulates high amino free content concentrations in the root part compared to ecotypes A and B with percentages of 48% and 83% for the pilot and of 54% and 79% for 200 mM respectively. On the level of the foliar part, the population B accumulates less amino free acids compared to A and C as well at the pilot plants and the

stressed ones. The statistical analysis of the results obtained shows highly significant differences (P<0,001) of the three factors: population (F= 378,03; ddl=2), concentration (F= 81,209; ddl=3) and body (F=17,728; ddl=1).

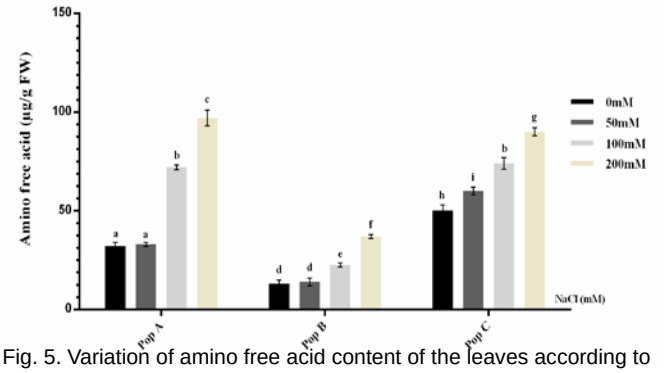

Fig. 5. Variation of amino free acid content of the leaves according to various NaCl concentrations at 3 populations of *Zizyphus lotus*: A (Ain chifae), B (Fès) and C (Guercif). The vertical bars correspond to the SE for N=4.

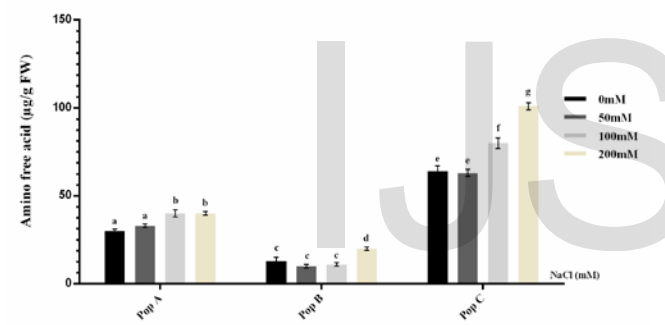

Fig. 6. Variation of amino free acid of the roots according to various NaCl concentrations at 3 populations of *Zizyphus lotus*: A (Ain chifae), B (Fès) and C (Guercif). The vertical bars correspond to the SE for N=4.

3.4 Discussion

The results obtained show an accumulation of the total soluble sugars content, of total proteins and amino free acids when the three ecotypes are subjected to high concentrations in NaCl. Indeed, the analysis of the results shows that the total soluble sugars content at the three populations moves in the same direction as the concentration of the saline solution. These results seem to highlight important variability between vegetable species, and the aptitude of accumulation of total soluble sugars. NaCl proportionally leads to increases in the contents of the glucidic compounds proportionally with the applied chemical amounts [8]. This accumulation of TSS is suggested as an index of resistance not only to the saline stress but also to the hydrous stress since salinity is equally dryness of a physiological type [9]. At the plants, sugars is an indicator of the stress degrees and its accumulation would be an adopted means by the plants in case of stress, in order to resist the constraints of environment [10]. In addition, it was indicated that metabolic sugars such as (glucose, galactose, the saccarose and fructose) allows resistance to different stress [11]. This TSS has a particular importance because of its direct relationship to physiological processes like photosynthesis, the translocation and breathing [12]. It is appropriately noted that the ecotype C (Station of Guercif) is characterized by an increased capacity to accumulate total soluble sugars in the leaves and the roots as well as in the two other populations A and B. Our results show also an accumulation of total proteins in the various bodies of the plants treated with NaCl. This accumulation is important at the level of the leaves. This content is positively correlated with the saline stress in the two bodies (roots and leaves). A study of the effect of the saline stress at two varieties of tomato (Campbell 33 and Mongal) showed that the evolution of the proteins content at these two varieties knew a variable response within the seedlings of the two varieties. Campbell 33 shows a significant reduction according to the intensity of the stress, while a light increase in proteins is witnessed with Mongal [13]. Another study on clover contradicting our result showed a reduction in the foliar content of total soluble proteins under a saline constraint which is due to the inhibiting effect of NaCl on the coring and the symbiotic fixing of nitrogen [14]. Several authors also report that the alteration of the intra-nodular diffusion of oxygen [15], and the inhibition of the enzymatic activity of the glutamine synthetase (GS) and the glutamate synthesis dependent on NADH (NADH-GOGAT) under the saline constraint are among the factors limiting proteinic biosynthesis [16], [17], [18]. The applied saline concentrations cause a linear increase in the amino free acid contents. It is necessary to highlight, as in the case of soluble sugars and of proteins, the important accumulation of these compounds, in the presence of the saline constraint in the two bodies (leaves and roots), in which the weakest accumulation that was recorded was at population B. The results obtained are in agreement with those of [19]. They worked on the effects of 4 levels from NaCl (pilot, 50,75 and 100) on the morpho-physiological behavior of 5 varieties of sunflower, and showed that the accumulation of the amino free acids were more important than the NaCl level in the cultivation environment where they were higher. The biosynthesis and the accumulation of the amino acids were the subject of many studies on plants exposed to the abiotic stress [20], [21]. The changes of composition cause a strong reduction in

the concomitant quantity of glutamate and an increase in the quantity of alanine, valin, serine and aspartate and glutamate as it is an amino donor [22]. Some authors proposed a function for these accumulated amino acids like donors of electrons for the respiratory chain of electrons transport [23].

4 Conclusion

The obtained results enabled us to highlight an important variation of the studied biochemical parameters. This variation seems to be related with the adaptation of three ecotypes A, B and C of *Zizyphus lotus* with its life environment, which accumulated all the different components (total soluble proteins, total sugars and amino free acids) but to differing degrees. It is worth noting that the area of Guercif, which is characterized by an arid type climate, accumulated these components with strong concentrations on the level of two bodies (leaves and roots) compared to ecotypes A and B. The various adopted strategies by *Zizyphus lotus* vis-a-vis the saline stress can explain the capacity of adaptation, development and its distribution in different pedoclimatic soils particularly arid areas. It would be perhaps interesting to use techniques based on the description of the behaviors, the genetic analysis of the characters and research of the molecular markers for an improvement of tolerance to the saline stress.

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