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Morpho-Physiological Response to Salt Stress on Myrtle (*Myrtus communis* L.) at the plantlet stage

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- ✓ soluble sugars.

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Abstract

The objective of this study was to follow the salt stress tolerance of Myrtle (*Myrtus communis* L.) at the plantlet stage in semi-controlled greenhouse. The experiment was carried out under glass greenhouse using different saline treatments (0, 2, 4, 6, 8 and 10 g L^{-1} of NaCl). All measurements were performed on plantlet cultivated in boxes filled with peat. The data collected (28 days after the application of stress) focused on morpho-physiological parameters (the number of leave per plant, root length and cumulative plant height of the plantlet of *Myrtus communis*, chlorophyll content, soluble sugar content and proline content). The ANOVA results showed that high NaCl levels doesn't reduce plant growth, especially cumulative plant height, while the number of leaves mark their sensitivity from the dose 8 g L^{-1}. The results for chlorophyll content show that all contents studied (chlorophylls a, b and total) respond negatively to salt stress. Salinity also caused a highly significant increase in proline content and soluble sugar content in the myrtle treated, especially at the level of 10 g L^{-1}. The results show that proline content is more important at the root level followed by stems and leaves. This work has improved knowledge of the behavior and adaptation strategies of *Myrtus communis* under saline stress conditions at plantlet stage for prospective commercial exploitation of the essential oils of this species.

1. Introduction

The process of land salinization is nowadays experiencing a constantly evolving level of progression. Worldwide, nearly one billion hectares of land are affected by salinity (7% of the earth's surface) [1]. Salinity is a global problem threatening land productivity and food production in many parts of the world in general and Tunisia in particular (1.8 million hectares; i.e. more than a third of the total cultivated area of Tunisia) which negatively affects crop production [2]. The search for species adapted to salinity with economic and/or ecological potential is a fundamental issue for the

exploration of saline ecosystems. The ability to assess the performance of cultivated or spontaneous plants undergoing salt stress is very important in research programs aimed at rehabilitating and improving production in semi-arid and arid regions. The Myrtle (*Myrtus communis* L.) is an evergreen bushy sclerophyllous plant with significant ornamental interest used in arid and degraded land reforestation projects and landscaping projects [3]. It is very used in medicine by their chemical composition : its antifungal and antibacterial activity of its essential oils[4,5] , very known for the relationships between this medicinal specie and genito-urinary diseases[6,7]. It is a Mediterranean species well adapted to abiotic stresses, although it can be affected by salinity. Faced with global heating and socio-economic activity, the myrtle is considered among the species threatened by the erosion of biological and genetic diversity, which requires the creation of an integrated program of protection of its resources in addition to its development in a sustainable way[8]. In order to improve the behavior of the species in a saline environment and to ensure the yield at the site of domestication and/or plantation, the exploitation of the potentialities of the genetic variability of the origins of myrtle is an innovative procedure. Nevertheless, the evaluation of the behavior of this species at the plantlet stage with respect to salt stress is necessary, for crops in domestication and/or plantation [9,10].

In this context, an experimental study under semi-controlled greenhouse conditions (ex vitro) at the National Institute for Research in Rural Water and Forest Engineering (INGREF), Laboratory for the Management and Valorization of Forest Resources, was carried out in order to study the effect of different concentrations of salt during the plantlet stage of the Myrtle (*Myrtus communis)* on the number of leave per plant, root length and cumulative plant height of the plantlet of Myrtus communis, and also analyze the variations of chlorophyll content, soluble sugars, proline, and chlorophylls according to different concentrations of sodium chloride (NaCl) after nine months of culture.

2. Methodology

2.1 Experiments

The experiment was carried out under a semi-controlled greenhouse conditions (ex vitro) at the National Institute for Research in Rural Water and Forest Engineering (INGREF), Laboratory for the Management and Valorization of Forest Resources, in the governorate of Ariana located in the North-East of Tunisia (36° 51' 59.533" N 10° 9' 53.003" E). This region is characterized by a Mediterranean climate. It is located in the semi-arid bioclimatic stage which is characterized by a mild and short winter and a cool summer.

Nine-month-old plantlets from the germination of *Myrtus communis* L. seeds harvested from shrubs in the 'Souniette' Arboretum of 'Tabarka' (in the North of Tunisia) are individually transplanted in a glass greenhouse at a temperature varying from 22 to 30°C in 15 cell trays filled with peat (7 cm in diameter and 11 cm in depth). The plants were watered with 200 ml per day.

In order to set up the trial, a completely random device with three blocks was performed. Indeed, five saline concentrations were adopted; 0, 2, 4, 6, 8 and 10 g L^{-1} of NaCl and each treatment were repeated three times with 10 plants per repetition (or block) (**Figure 1)**.

2.2 Parameters measured and plant analysis

The data collected (28 days after the application of the stress) related to physiological parameters (content of chlorophyll, number of leaves per plant and length of the roots), biochemical (content of

soluble sugars, content of proline). Regarding the length of the root, it was measured using a graduated ruler and the number of leaves was obtained by counting. Also, the chlorophyll content is determined by starting by weighing 0.1g of leaves, cut into small pieces and ground in a mortar with 10ml of 80% acetone (CH3COCH3) and a pinch of sand to facilitate grinding.

(a) The three

(b) The flowers

(c) The fruits

Figure 1. General appearance of the plant of *Myrtus communis* L.

After grinding, the solution is filtered and stored in the dark in black boxes to prevent oxidation of the chlorophyll by light. The dosage is done by taking a sample of 3ml of the solution which is put in a tank in a spectrophotometer of the UV-visible spectrophotometer type. The reading is done at two wavelengths 645 and 663 nm, the calibration of the device is done by the control solution of 80% acetone. The calculation is as follows [11]:

$$Chla = 12.7 \times (DO663) - 2.69 \times (DO\ 645)$$

$$Chlb = 22.9 \times (DO645) - 4.86 \times (DO\ 663)$$

$$Chla + Chlb = 8.02 \times (DO663) - 20.20 \times (DO\ 645)$$

The biochemical analyzes are carried out on the fresh plant material resulting from the salt stress treatments in the peat. Total soluble sugars (sucrose, glucose, fructose, their methyl derivatives and polysaccharides) are assayed by the method of Dubois et al. (1956) [11]and that of prolines according to Dreir and Goring (1974) mehtod [12]. One ml of ninhydrin reagent (1 g of ninhydrin in 24 ml of acetic acid and 16 ml of orthophosphoric acid) and 1 ml of the plant extract are put in a test tube. The whole is homogenized then 1ml of glacial acetic acid is added and vortexed. The tubes are then placed in the boiling water bath at 100°C for 60 min until the color turns pink. The tubes are cooled by soaking in melting ice for 5 min, then 5 ml of toluene are added. The mixture is then vortexed and left to stand for 30 minutes before reading the absorbance at 528 nm.

2.3 Statistical analyzes

To identify the effects of saline treatments on the various parameters measured, we carried out the analysis of variance with the "SAS" software and the comparison of the mean was carried out by the Fisher test at the 5% threshold.

3. Results and Discussion

3.1 Effect of salinity on cumulative plant height

We noted from **Figure 2** that during the four weeks of stress, salinity has no effect on the cumulative plant height for each treatment since it increased continuously in the same interval of one treatment to another as for the control for the four weeks of stress carried out. Indeed, it goes from 7.3 cm to 9.22 cm for the witness T0; for treatment (2 g L^{-1}) from 8.8cm to 10.8cm; treatment (4g L^{-1}) from 10.21 cm to 12.19 cm; treatment (8 g L^{-1}) from 10.04 cm to 12.14 cm and for (10 g L^{-1}) from 13.5 cm to 15.41 cm. The analysis of variance shows a significant difference in the effect of NaCl on the cumulative height of plants for all treatment. This significant difference is valid especially for high concentrations 10 g L^{-1} of NaCl. ($P<0.0001$ and $F= 41.04$).

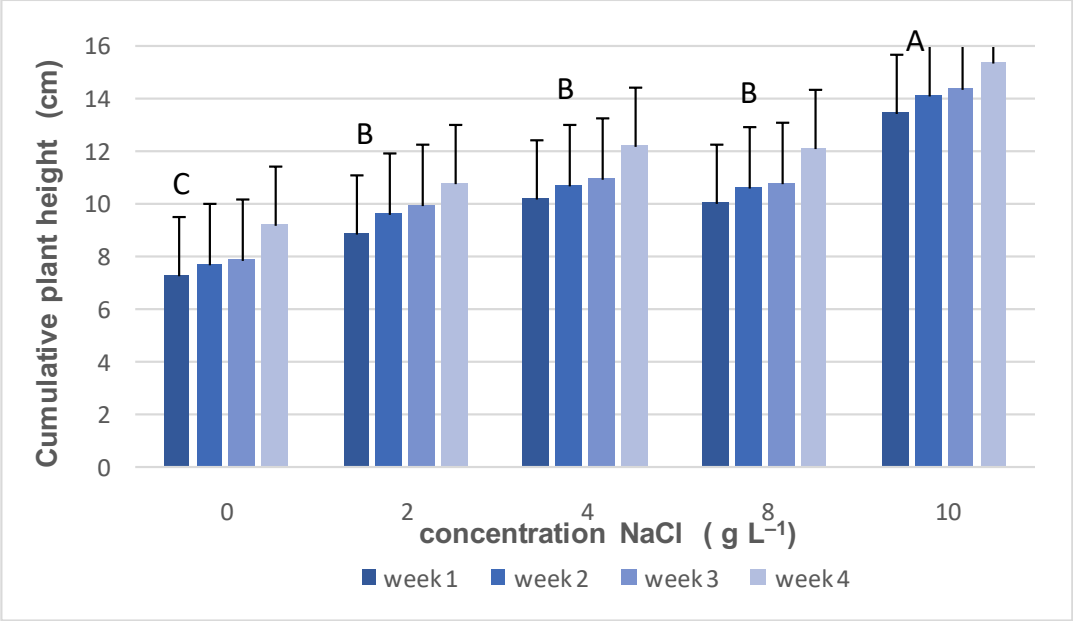

Figure 2. Effect of salinity on cumulative plant height (The values followed by the same letter are not significantly different at the 5% threshold according to the Fisher test)

3.2 Effect of salinity on the Number of Leaves

Regarding the number of leaves (Figure 3), salt stress in Myrtle is accompanied by a decrease in the number of leaves in the different treatments; this reduction is well accentuated after the first week of stress, especially at the level the treatment of 8 g L^{-1} and 10 g L^{-1} of NaCl. However, the statistical analysis shows that there is no statistical difference for the number of leaves whatever the concentrations of NaCl applied. Many studies suggest that during vegetative growth, salt stress significantly reduces growth parameters such as number of leaves per plant and total plant yield. These parameters have decreased and this decrease is all the more significant as the salt stress is high.(Ferrara et al. [13], Sam-Amoah et al[14], Hamrouni et al. [15]).

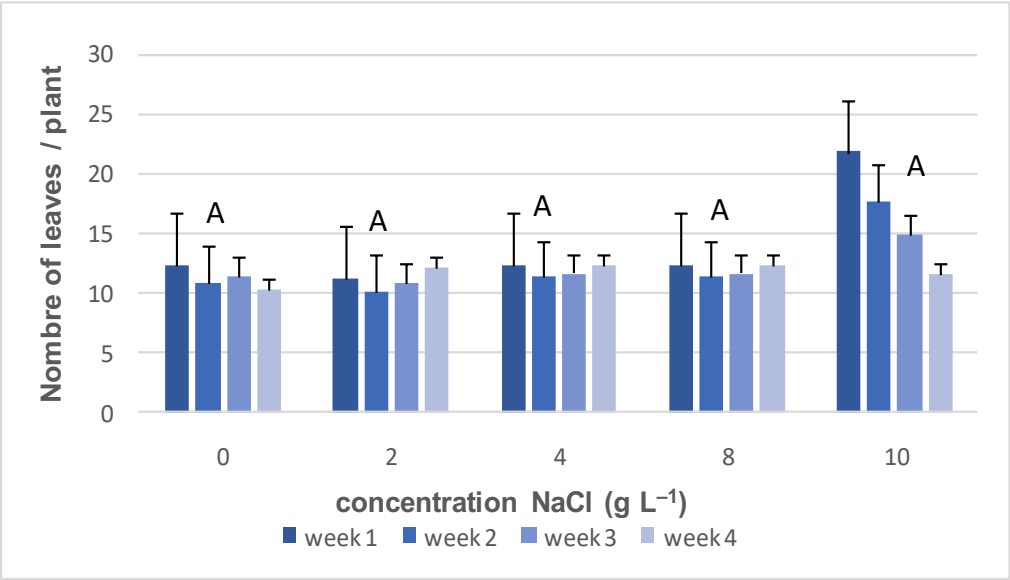

Figure 3. Effect of salinity on the Number of Leaves (The values followed by the same letter are not significantly different at the 5% threshold according to the Fisher test)

3.3 Effect of salinity on the Root length

According to the results of the length of the roots (Figure 4), it is noted that the values obtained from the root growth remain more or less invariable for the different concentrations of NaCl studied with a slight decrease for the treatments T3 (8 g L^{-1})and T4 (10g L^{-1}). Indeed, it varied from 6.7 cm for the control and to 6.2 cm for the treatment at 10 g $L^{-1}$of NaCl. This result is confirmed by the statistical analysis which shows that the salinity applied does not affect root growth with P<0.5185 and F=0.81. These results agree with those of Mülling and Läuchli cited by Ben Ahmed [16] who find that the increase in salinity leads to a decrease in the yield of the aerial part of corn sprouts, however it has no significant effect on root growth.

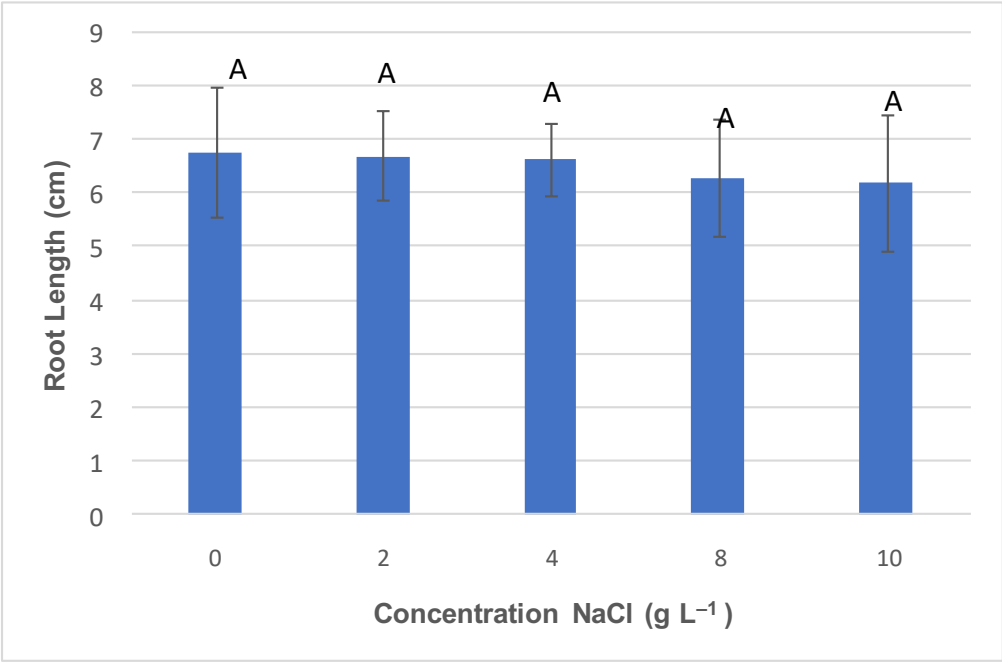

Figure 4. Effect of salinity on roots length (The values followed by the same letter are not significantly different at the 5% threshold according to the Fisher test)

3.4 Effect of salinity on Chlorophyll content

The results for the chlorophyll content demonstrated that all the contents studied (chlorophylls a, b and total) respond negatively to salt stress. For the chlorophyll content (Figure 5), the highest value was recorded at Treatment of 4 g L^{-1} with 40.88µg/g FW and the lowest value for treatment of 2 g L^{-1} with 30 .73 µg/g FW. Regarding the content of chlorophylls a and b, they have the same variation between the different concentrations of sodium chloride (NaCl). However, the analysis of variance shows that this variation is not significant for the different chlorophyll contents analyzed (Cha P<0.095 and F=1.62; Chb P<0.3011 and F=1.4 and Cht with P<0.2317 and F=1.62).

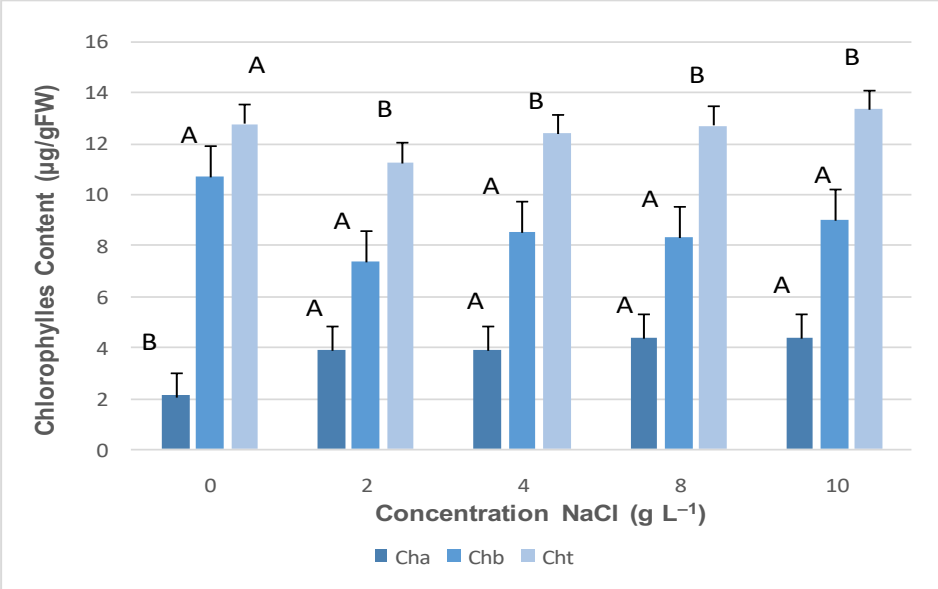

Figure 5. Effect of salinity on chlorophyll content (The values followed by the same letter are not significantly different at the 5% threshold according to the Fisher test)

We can deduce from our results that despite the NaCl concentrations following our plantlets were subjected, they were able to protect their photosynthetic apparatus while ensuring the growth and development of the aerial part, this is the case with Atriplex halimus (Benrebiha et al., 2012) [17]. These results are in agreement with those of Sharaf et al. [18] on tomato, Dali et al. [19] and Rhim et al. [20] on pepper (Capsicum annuum L.) who indicated that reduced salinity increased the amount of chlorophyll a and total chlorophyll. The effect of salt stress on the functioning of photosynthesis in stressed plantlets whose chlorophyll level has more or less decreased compared to the control. This can be explained from the fact that halophytes are successful as C4 plants. These plants can keep the stomata closed for a long time during physiological drought due to high salt concentrations, which causes a decrease in photosynthetic activity.

3.5 Effect of salinity on Proline content

The irrigation of *Myrtus Communis* plantlets by adding sodium chloride with different concentrations showed a difference in the proline content which is manifested by an accumulation of the latter in the leaves of the treatment T0 (0 g L^{-1}) 19 .75 mg/gFW and increased to 68.88 mg/g FW for T4 (10 g L^{-1}) (**Figure 6**). The results show that the proline content is higher at the root level followed by the stems and the leaves. At the level of the stems, the proline varies from 39.41 mg/g MF for T0. It reaches the value of 113.98 mg/gFW for T4 (10 g L^{-1}), the same for the roots which have the highest values between 68.65 mg/g FW for the T0 treatment (0 g/L) and reached 131.78 mg/g FW for the T4 treatment (10 g L^{-1}). Salinity caused a very significant increase in proline

content in *Myrtus communis* treated especially at the level of 10 g L^{-1} in the greenhouse. Proline accumulation is a form of osmotic adjustment and is a means of salinity tolerance. The accumulation of proline, induced by stress, can be the result of three complementary processes: stimulation of its synthesis, inhibition of its oxidation and/or alteration of protein biosynthesis. Proline is thought to be synthesized from glutamic acid via 5 carboxylic acid 1 pyrroline (P5C), but also via arginine and ornithine [16]. Our results also agree with those of Mezni [21] who showed that the proline content increases in the different organs with increasing salt concentration for alfalfa and those of Ben Ahmed [16] for the two species of Sulla.

3.6 Effect of salinity on Total sugar content

According to **Figure 7**, which represents the total sugar content, we observed a decrease in the total sugar content by increasing the dose of NaCl. Thus, the highest accumulation is recorded in the control with a value of 188 mg/g FW and the lowest sugar content is observed with the T4 treatment of 10 g $L^{-1}$with the value of 80 mg/g FW.

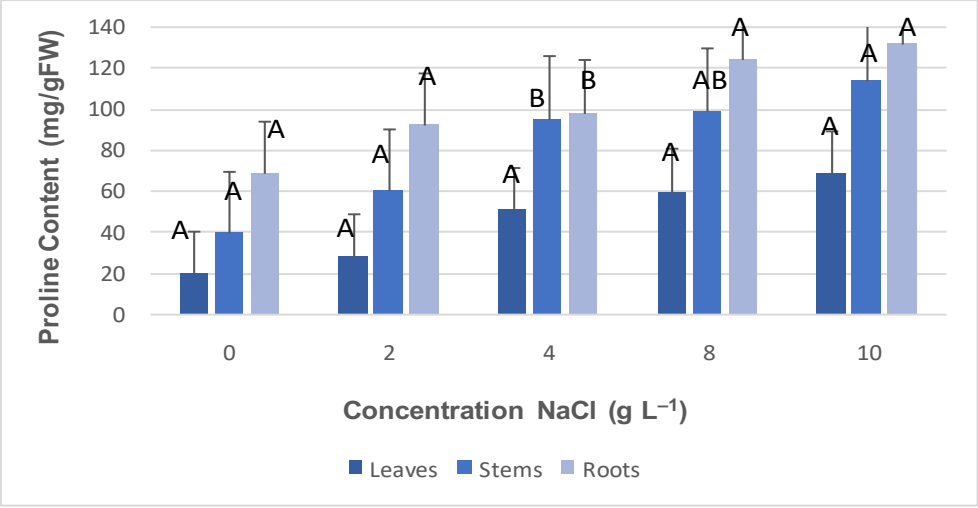

Figure 6. Effect of salinity on Proline Content (The values followed by the same letter are not significantly different at the 5% threshold according to the Fisher test)

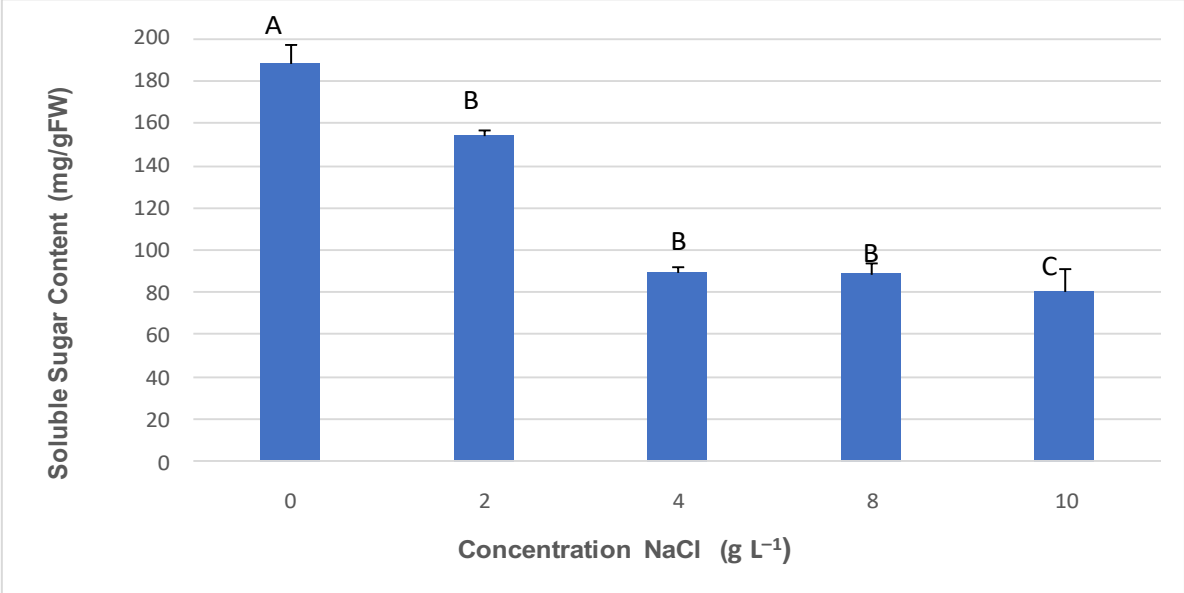

Figure 7. Effect of salinity on Total sugar Content(The values followed by the same letter are not significantly different at the 5% threshold according to the Fisher test)

The analysis of variance seems in agreement with the decrease in sugar which marks a highly significant effect in stressed plants and the highest for the 10g/L concentration ($P<0.0002$ and $F=17.68$). This is the case with *Medicago sativa* L. where a decrease in total sugars has been noted in the event of stress [22] (**Figure 7)**. On the other hand, other physiological studies have shown that under saline stress, different plant species accumulate sugars and polyols, following the hydrolysis of starch [23]. The level of tolerance to salinity is strongly correlated with the accumulation of sugars [24]. Sugars could act as an osmoticum, protect specific macromolecules (enzymes) and contribute to the stability of membrane structures[25].

Conclusion

This study allowed us to highlight some behaviors of *Myrtus communis* under saline stress conditions and to understand the mechanisms by which this species manages to tolerate salt up to certain levels of salinity. The factors assessed concerned morpho-physiological parameters at plantlet stage. The ANOVA results showed that high NaCl levels doesn't reduce plant growth, especially cumulative plant height, while the number of leaves mark their sensitivity from the dose 8g / L. Salinity caused a very significant increase in proline content in *Myrtus communis* treated especially at the level of 10 g/L in the greenhouse. This accumulation of proline constituted a form of osmotic adjustment and a means of salinity tolerance. This work has improved our knowledge of the behavior and adaptation strategies of *Myrtus communis* under saline stress conditions in greenhouse cultivation methods at the plantlet stage. The results obtained under our experimental conditions must be completed by developing the study of the physiological responses to the late stages of development of this species, namely flowering and fruiting.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest. *Compliance with Ethical Standards:* This article does not contain any studies involving human or animal subjects.

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