Global Research in Environment and Sustainability – ISSN 3033-3644
March 2024, Vol. 2, No. 3, pp. 23-37.

NaCl Acclimation Enhances Salt Stress Tolerance in Huckleberry (*Solanum scabrum* Mill.) Plants

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Abstract:
This study was carried out to assess the growth and physiological response of huckleberry (Solanum Scabrum Mill) to salt stress following acclimation with NaCl in Kumba, Cameroon. To achieve this, 1-month old seedlings were transplanted in 10-L pots and subjected to control (non-acclimated, no NaCl), acclimated (5 and 10 mM NaCl) and acclimation/salinity (5 + 75 mM and 10 + 75 mM NaCl). The following parameters were measured, growth (biomass, relative growth rate (RGR), number of leaves, and leaf area), physiological parameters (leaf thickness, and water use efficiency, WUE), and yield (shoot fresh mass). Salt stress significantly reduced the total fresh weight of non-acclimatized plants (T1) by 24 % relative to controls. However, acclimation significantly improved the growth of the plants under salt stress with T4 (acclimation with 5 mM NaCl), having more enhanced growth (52% increase, from control plants). Similarly, the dry mass was also more significantly increased in acclimatized than in non-acclimatized plants, with T4 having the highest increase (72% increase, when compared with control plants). The total leaf area under salt stress was more significantly enhanced by acclimation with 5 mM NaCl (51% increase from control), while the RGR was significantly increased by acclimation under saline and non-saline conditions. The WUE was significantly elevated in acclimated plants under salt stress in T4 (46% from controls) than in the other treatments. Leaf thickness was significantly enhanced in both acclimated and non-acclimatized plants, being highest under the T4 treatment. WUE and Leaf thickness were strongly correlated with all growth parameters except number of leaves. Taken together, these results clearly indicate that acclimation with low NaCl, especially 5 mM NaCl imparts salt stress tolerance in huckleberry. Hence acclimation with 5 mM NaCl is recommended for use in the cultivation of huckleberry on saline soils.

Keyword: Water use efficiency, biomass partitioning, salinity tolerance, leaf thickness

INTRODUCTION

Salt stress, which is the accumulation of salt (especially NaCl) on the uppermost soil layer, has posed a major environmental threat to agriculture by limiting plant growth and reducing crop yield. In total there are more than 833 million hectares of land or about 8.7 % of the planet is affected by salt (Munns et al., 2008; Rahman et al., 2022). Over time, plants have acquired salt-tolerant mechanisms to survive and reproduce in saline environments. These include, osmotically adjusting the internal environment to match that of the external environment in order to maintain water uptake (Munns et al., 2020). This is achieved through the synthesis and accumulation of organic solutes such as proline, glycine betaine and sugars, or the uptake and accumulation of inorganic solutes such as Na^+ and K^+ (Munns et al., 2020). Also, antioxidants are often generated to counter the secondary oxidative stress damages. Oxidative stress arises from leakage of electrons in the electron transport chain of mostly photosynthesis, which leads to the oxidation of oxygen molecules to produce reactive oxygen species (ROS) including the superoxide anion (O_2^-), hydroxyl radical (OH•) and hydrogen peroxide (H_2O_2), which cause damages to membranes, and nucleic acids (Gill and Tuteja 2010). Finally, limiting Na^+ accumulation in photosynthetic tissues (Shoot Na^+ exclusion) constitutes an important line of defense against salt stress, especially in glycophytes (Assaha et al., 2017). With the desire to increase food production, and faced with increasing adverse environmental conditions that are limiting productivity, a lot of research has been conducted first to understand plants' responses, to salt stress, and then exploit these responses to improve plant resistance to the stress. Such efforts have included exogenous application of metabolites such as proline, minerals such as Ca^{2+} and K^+, and growth regulators such as ABA, and even microbial agents such as Mycorrhiza and Rhizobium, all of which have proven effective in increasing salt stress tolerance in the tested plants. In addition, acclimating plants to low NaCl, prior to exposing them to high salinity has equally proven effective in imparting salt stress tolerance in many plants including rice (Sriskantharajah et al., 2020), and tomato (Kamanga et al. 2020). Acclimation to external environmental changes can occur in plants thanks to internal adjustments within tissues and cells, enabling plant metabolism to proceed under these altered conditions (Demmig-Adams et al., 2008). In the context of salinity, it has been widely reported that many plant species increased the ability to tolerate salt stress after being acclimatized to low levels of the stress for a certain period of time (Pandolfi et al., 2016, 2017; Djanaguiraman et al., 2006). The beneficial effects of acclimation include both agronomical (e.g., improved survival rate; higher growth rate; less biomass reduction), and physiological (lower Na^+ accumulation in the shoot; better osmotic adjustment; increased K^+ uptake and retention in root; and enhanced vacuolar Na^+ sequestration) (Djanaguiraman et al., 2006; Pandolfi et al., 2017). But these adaptations following acclimation have not been established in huckleberry. Huckleberry (*Solanum scabrum* Mill.) or the African Nightshade belongs to the Genus: *Solanum* and Family Solanaceae. Huckleberry can be identified by its large size leaves, its flowers are either white or light purple with brown anthers (Schipper, 1998). It can be found in Europe, Asia, Australia, New Zealand, North America and the Caribbean. It is used medicinally to overcome stomach aches, duodenal ulcers, swollen glands and tooth problems (Mwai et al., 2007). Salt stress research in huckleberry began a decade ago (Assaha et al., 2013) and its growth is highly limited by high salinity (Tabot et al., 2014, Assaha et al., 2015). However, the response of the plant to salt stress is yet to be fully established. Besides none of the salt stress alleviation strategies named earlier have been investigated in the plant. Thus, the present study sought to examine the response of NaCl-acclimated and non-acclimated huckleberry plants to high salinity in pots in a screen house.

MATERIALS AND METHODS

Location of Study Site

The study site was conducted in the screen house of the Department of Agriculture, Higher Technical Teachers' Training College, Kumba Cameroon located within 4°38'N 9°27'E and 4.63°N 9.45°E, with an elevation of 240 meters (790 ft) above sea level. This location is within Cameroon's Agroecological Zone IV with an annual rainfall of 2200 mm and an average annual temperature of 31°C (Tabot et al., 2020).

Soil Collection

The soil used in this study was collected from the Community Development Specialization Training School (CDSTS), Kumba. The soil was thoroughly mixed and samples taken and sent to the soil science laboratory of the Faculty of Agronomy and Agricultural Science of the University of Dschang, Dschang, West region, Cameroon. The characteristics of the soil are presented in

Table 1. Then the soil was used to fill thirty 10-L pots of 530.9 cm^2 surface area to the same level across all pots. The soil-filled pots were then irrigated to field capacity with fresh water prior to transplanting.

Table 1: Characteristics of the soil used in the present study

Soil Property	**Value**	**Soil Property**	**Value**
Texture	Sand/silt/clay	**Exchangeable cations (meq/100g)**	
pH (water)	6.60	Ca^{2+}	6.8
pH (KCl)	5.30	Mg^{2+}	5.9
Organic Carbon (%)	1.80	K^+	0.85
Organic matter (%)	2.25	Na^+	0.33
Total Nitrogen (g/kg)	15.6	**Total**	13.88
C/N ratio	15	**Cation Exchange Capacity (CEC)**	
		Effective CEC	1.2
		CEC pH 7	16
		S/CECS (%)	100
		Exchangeable acidity (meq/100g)	
		H^+Al (EA)	0
		Base saturation (%)	75
		Available Phosphorus (mg/kg)	29.56

Plant Material and Treatments

The huckleberry used in the present study was the Foumbot 1 variety (Agwa et al., 2019). Its seeds were purchased from a farmers' shop in Kumba central market. The plants were first raised in nursery for 1 month and then transplanted into the experimental pots prepared earlier. The plants were allowed to establish for 10 days I the pots after transplanting. Thereafter the pots were separated in to 3 sets of 6 pots. To each set, 0 (control), 5, and 10 mM NaCl (acclimation treatments) were applied respectively. The acclimation treatments were prepared by dissolving the required amount of NaCl salt in a specific volume of water to give 5 and 10 mM NaCl using the relationship in equation 1 below, while control plants received only fresh water without added NaCl.

1 Molar solution of NaCl = 58.5 g NaCl/L of water……………………………………1

The acclimation treatments lasted for 10 days. Thereafter, the 3 sets of plants were further split into 2 sets each (1 set having 3 pots), with 1 set to receive 75 mM NaCl (prepared as shown above) to give a total of six set of plants to receive the 6 treatments as shown in Table 2.

Table 2: The treatments used in the study and the replicates

SN	**Treatment**	**Replicates**
1	C (Control, non-acclimated)	3
2	T1 (75 mM NaCl, non-acclimated)	3
3	T2 (5 mM NaCl Acclimated)	3
4	T3 (10 mM NaCl Acclimated)	3
5	T4 (5 mM NaCl Acclimated + 75 mM NaCl)	3
6	T5 (10 mM NaCl Acclimated + 75 mM NaCl)	3

The pots were arranged in a Completely Randomized Design in the screen house (Figure 1). Before application of the salt stress (75 mM NaCl), pretreatment samples were collected for growth measurements. The plants were exposed to the stress for 2 weeks as shown in the experimental time line in Figure 2.

CR1	T3R3	T4R1	T1R3	T2R2	T5R3
T1R1	T5R1	T4R3	T3R2	T5R2	T3R1
CR3	T2R1	T1R2	T2R3	CR2	T4R2

Figure 1: Layout of the experimental set up. C = control, T = treatment and R = replicate

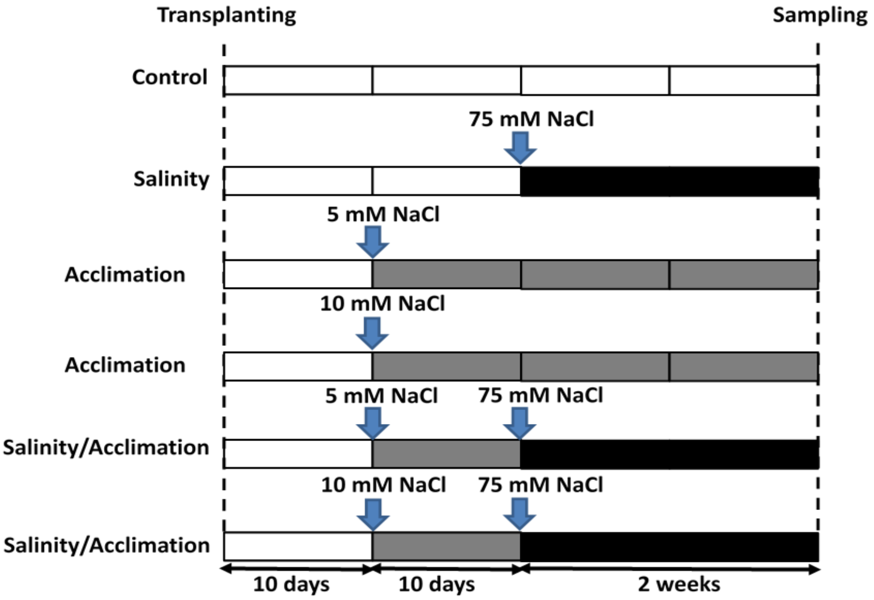

Figure 2: Duration of acclimation and/or salinity treatments during the study.

Agronomic Practices

The pots were irrigated with treatments when necessary. And all the pots received the same volume of treatment solution during each irrigation to reduce bias. These irrigation volumes were recorded each time. Five grams of NPK fertilizer was applied before start of acclimation treatments, as well as at the start of salt stress treatments. Weeding was done when they appeared in the pots. The fungicide/insecticide, Chem Copp 50 (American Corporation), was used to control fungi and pests and when applied, all the plants received the same treatment.

Data Collection

At the end of the treatment, various growth and physiological parameters were measured.

Number of Leaves Per Plant:

The number of leaves were counted on all plants under each treatment.

Leaf Area:

The Length and width were collected and multiplied to get the leaf are of a leaf of each pot. This was done following the procedure in Agwa et al., (2019).

Leaf area (cm^2) = Length x width.. (**2**)

Then the total leaf area was calculated from the equivalent ratios of total leaf dry mass to total leaf area and specific leaf area to specific dry mass of a selected leaf.

***Biomass (Fresh and Dry Weights) Partitioning**:*
At the end of the experiment all the plants where were harvested and separated into root, stem and leaf. Then the fresh weights of the 3 parts were measured using a 0.01 g precision balance and recorded. Then the samples were oven-dried for 72 h at 70 ^{0}C and weighed again to determine their dry weights.

Relative Growth Rate (RGR):
This was determined by collecting dry weight of pre-treatment shoot samples (w1) and that of shoots collected at the end of the experiment (w2). The RGR was then calculated using the formula below.

$$\text{RGR} = \frac{lnW2-lnW1}{t} \quad \text{(3)}$$

Where, W1 = pretreatment dry weight, W2 = final dry weight, and t = duration of treatment in days

Leaf Thickness (cm²/g)):
This was determined by measuring the area (A) of a specific leaf, and the dry weight (m) of the leaf. Then the leaf area ratio was calculated using the formula below:

$$LAR = \frac{A}{M} \quad \text{(4)}$$

Water Use Efficiency (WUE):
This was determined as the ratio of total dry biomass (TDW) to total volume of water irrigated (V) during the experiment as shown in the following equation.

$$WUE\ (\frac{g}{L})\ \frac{TDW}{V} \quad \text{(5)}$$

Yield
Yield data was obtained by weighing the shoot (stem ad leaves) fresh mass at the end of the experiment and expressing it per pot, and then extrapolating per hectare.

Data Analyses
All data collected were subjected to one-way analysis of variance using SPSS statistical package version 21 (IBM Corp. Amonk NY, USA) after normality test. Means (n = 3) were compared using the Tukey HSD test at α = 0.05. Correlation analysis was performed using 2-tailed, Pearson correlation test.

RESULTS

Effect of Acclimation on Growth of Huckleberry Plants Subjected to Salt Stress

The overall growth of huckleberry plants grown under control, salinity stress (75 mM NaCl), NaCl acclimation (5 and 10 mM NaCl) and salinity/acclimation (5 + 75 mM NaCl and 10 + 75 mM NaCl) is shown in Figure 3. As can be observed, salinity markedly decreased the growth of huckleberry plants, whereas acclimation rescued the plants from the salt stress effects. Also, plants acclimated with 5 mM NaCl showed best growth of the plants under non-stress conditions.

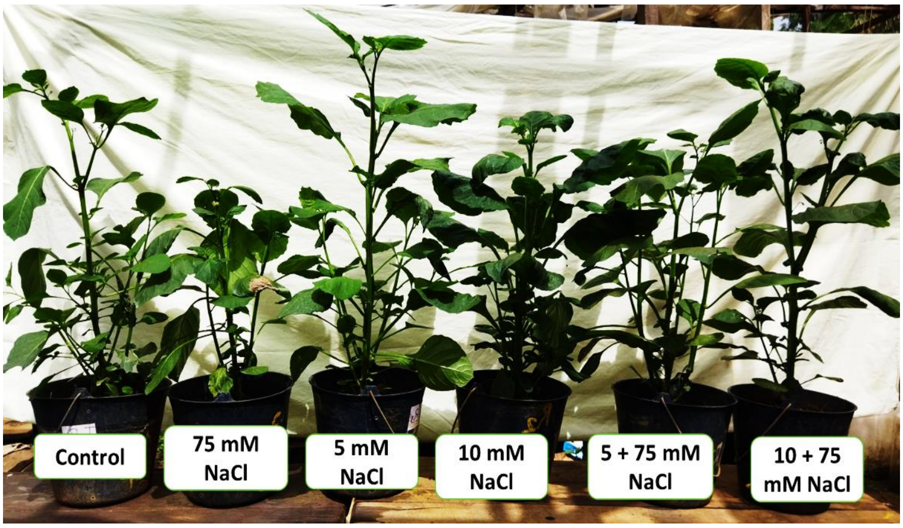

Figure 3: Effect of NaCl acclimation on the growth of huckleberry plants subjected salt stress.

Effect of Acclimation on The Fresh Weight of Huckleberry Plants Subjected to Salt Stress

The effect of salt stress on fresh plant biomass is shown in Figure 4. Salt stress (75 mM NaCl) significantly decreased ($p<0.05$) the fresh weight of total biomass of the plant (24% reduction), inhibiting the growth leaves and stems (21 and 30% reduction, respectively). However, the root biomass remained unaffected by the salinity treatment. Nevertheless, acclimation with 5 and 10 mM NaCl significantly improve the growth of the plant, both under control and salt conditions. Acclimation with 5 mM NaCl produced the best results under salt stress (34% increase relative to control), compared to the 10 mM level, implying that acclimating huckleberry with 5 mM NaCl is more suitable in impacting stress tolerance than with 10 mM NaCl.

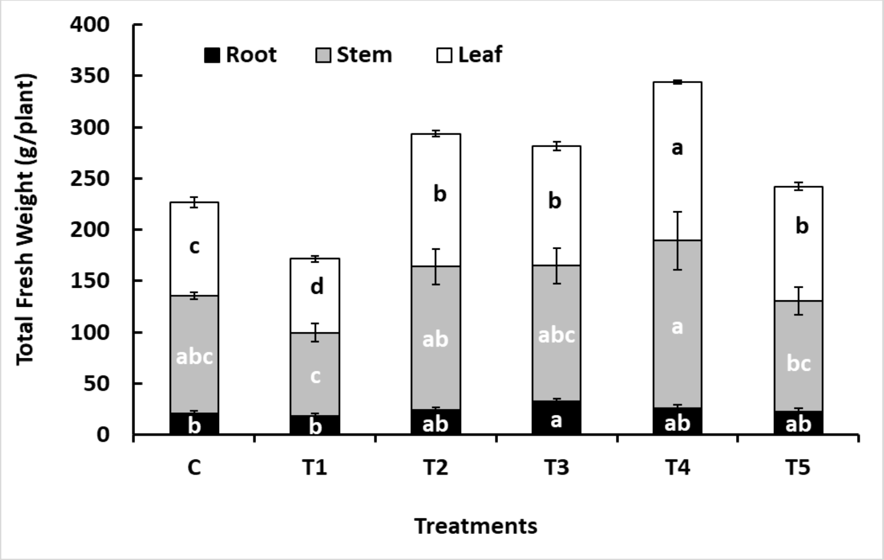

Figure 4: Effect of NaCl acclimation on the Total Fresh Weight of huckleberry plants subjected to control and salinity stress conditions. The bars represent means ± S E (n = 3), bars with the same letter for each part are not statistically different ($p \leq 0.05$). C = control,

T1 = 75 mM NaCl, T2 = 5 mM NaCl, T3 = 10 mM NaCl, T4 = 5 + 75 mM NaCl, and T5 = 10 + 75 mM NaCl.

Effect of Acclimation on Dry Weight of Huckleberry Plants Subjected to Salt Stress

The effects of salinity on the dry weight of acclimatized and non-acclimatized huckleberry plants are presented in Figure 5. Salt stress (75mM NaCl) reduced the total biomass accumulation of huckleberry plants (13% reduction), although this reduction was not statistically significant ($p>0.05$). Acclimation significantly increased the total biomass accumulated under salinity and control conditions, as well as the mass of the different organs under control and stress conditions, except T2 and T5. Overall, T4 (5 mM + 75 mM NaCl) yielded the highest dry mass (49% relative to control plants). The root dry mass wasn't significantly affected by T3 (10 mM and 75mM stress), which is significantly higher compare to the other treatments. However, the leaf dry weigh was significantly higher in T4 ($P<0.05$) compared to control. Overall T4 (5 mM-acclimated plants) produced drier biomass under all experimental conditions indicating enhanced salt stress tolerance.

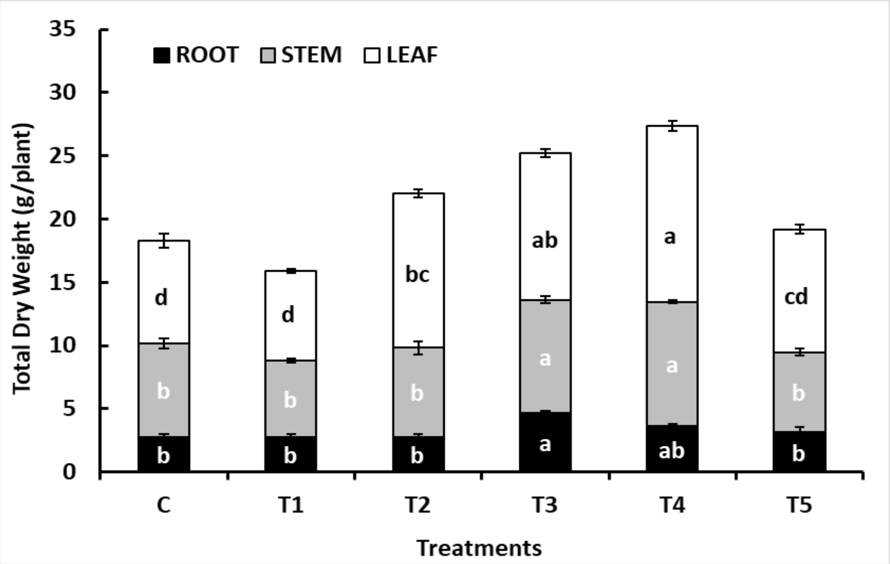

Figure 5: Effect of NaCl acclimation on the total dry weight of huckleberry plants subjected to control and salinity stress conditions. The bars represent means ± SE (n = 3), bars with the same letter for each part are not statistically different ($p \leq 0.05$). C = control, T1 = 5 mM NaCl, T2 = 10 mM NaCl, T3 = 75 mM NaCl, T4 = 5 + 75 mM NaCl, and T5 = 10 + 75 mM NaCl.

Effect of Acclimation on Total Leaf Area of Huckleberry Plants Subjected to Salt Stress

The effect of NaCl acclimation on huckleberry plants grown under salt stress and control conditions is given in Figure 6. Salt stress significantly decreased ($p<0.05$) the total leaf Area (32% reduction). However, acclimation with 5 mM NaCl significantly improved the leaf area (50% increase from salt stress levels) to values comparable to those of control, whereas acclimation with 10 mM NaCl did not alleviate the stress-induced reduction. A significant increase was seen in acclimation with 5 mM NaCl under non-stress condition, which produced the largest leaf area (almost 10000 cm^2), when compared with control values (less than ~8000 cm^2).

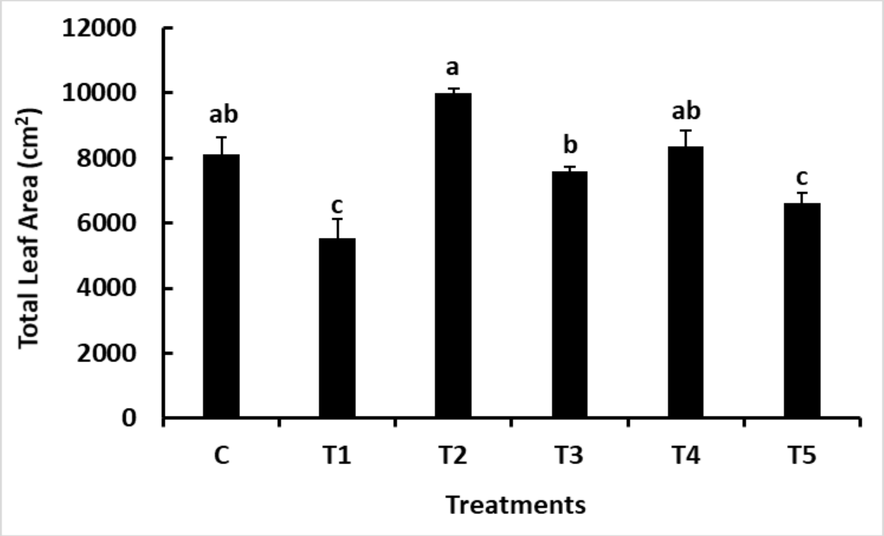

Figure 6: Effect of NaCl acclimation on the total dry weight of huckleberry plants subjected to control and salinity stress conditions. The bars represent means ± SE (n = 3), bars with the same letter are not statistically different ($p \leq 0.05$). C = control, T1 = 5 mM NaCl, T2 = 10 mM NaCl, T3 = 75 mM NaCl, T4 = 5 + 75 mM NaCl, and T5 = 10 + 75 mM NaCl.

Effect of Acclimation on Relative Growth Rate (RGR) of Huckleberry Plants Subjected to Salt Stress

The effect of NaCl acclimation on RGR on huckleberry plants grown under salt stress and control conditions is shown in Figure 7. According to the Figure, salt stress is seen to significantly reduce the RGR of the huckleberry plants. However, this reduction is rescued by acclimating the plants with 5 mm NaCl, raised the RGR well above control levels (14% increase relative to salt stress alone), while acclimation with 10 mM NaCl, restored the RGR to a level comparable to control. This implies that acclimation with 5 mM is more suitable in impacting salt stress tolerance for improved growth, by enhancing the growth rate.

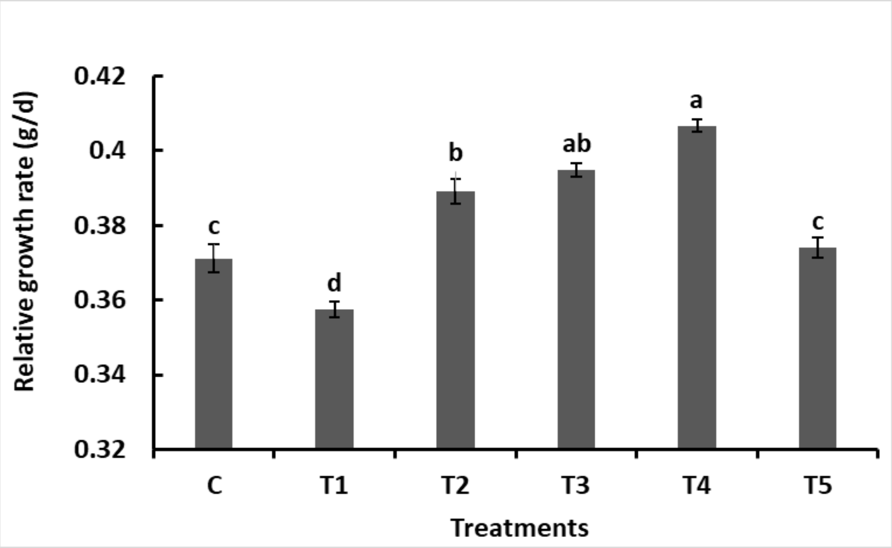

Figure 7: Effect of NaCl acclimation on the relative growth rate of huckleberry plants subjected to control and salinity stress conditions. The bars represent means ± SE (n = 3), bars with the same letter are not statistically different ($p \leq 0.05$). C = control, T1 = 5 mM NaCl, T2 = 10 mM NaCl, T3 = 75 mM NaCl, T4 = 5 + 75 mM NaCl, and T5 = 10 + 75 mM NaCl.

Effect of Acclimation on Number of Leaves of Huckleberry Plants Subjected to Salt Stress

The effect of salt stress on number of leaves is presented in Figure 8. This shows that NaCl induces leave production which is further enhanced when they are acclimatized with 5 mM NaCl.

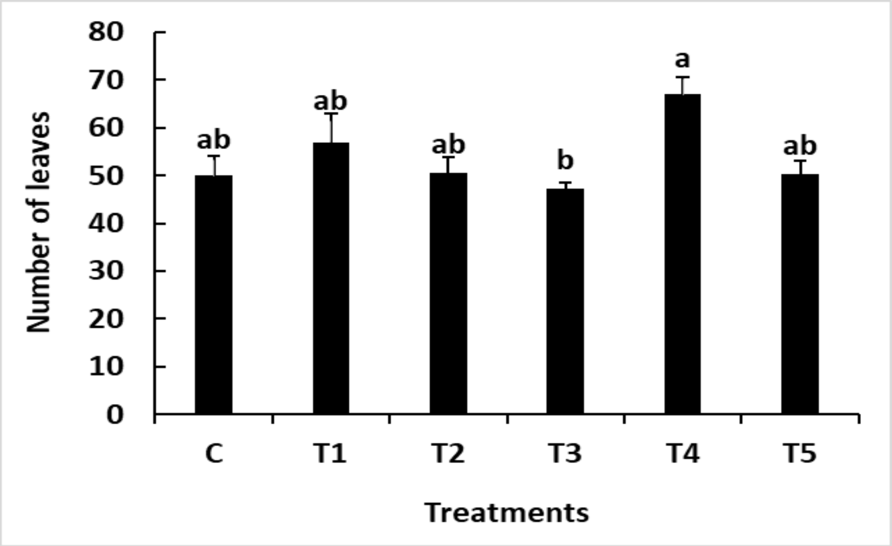

Figure 8: Effect of NaCl acclimation on the number of leaves of huckleberry plants subjected to control and salinity stress conditions. The bars represent means ± SE (n = 3), bars with the same letter are not statistically different ($p \leq 0.05$). C = control, T1 = 5 mM NaCl, T2 = 10 mM NaCl, T3 = 75 mM NaCl, T4 = 5 + 75 mM NaCl, and T5 = 10 + 75 mM NaCl.

Effect of Acclimation on Leaf Area Ratio (LAR) of Plants Subjected to Salt Stress

The LAR is often used to evaluate leaf thickness under salt stress, which is an indication of more volume to accommodate accumulated salts. The effect of salt stress on leaf mass per area is shown in Figure 9. Salt stress significantly increased the LAR ratio, which is further enhanced by 5 mM NaCl acclimation under salt stress. However, under control conditions, only the 10 mM NaCl acclimation significantly increased the thickness (33% increase) when compared to control. This indicates that salt stress had a positive influence on LAR. Nevertheless, acclimation with 5 mM NaCl (T4) had the highest increase in LAR

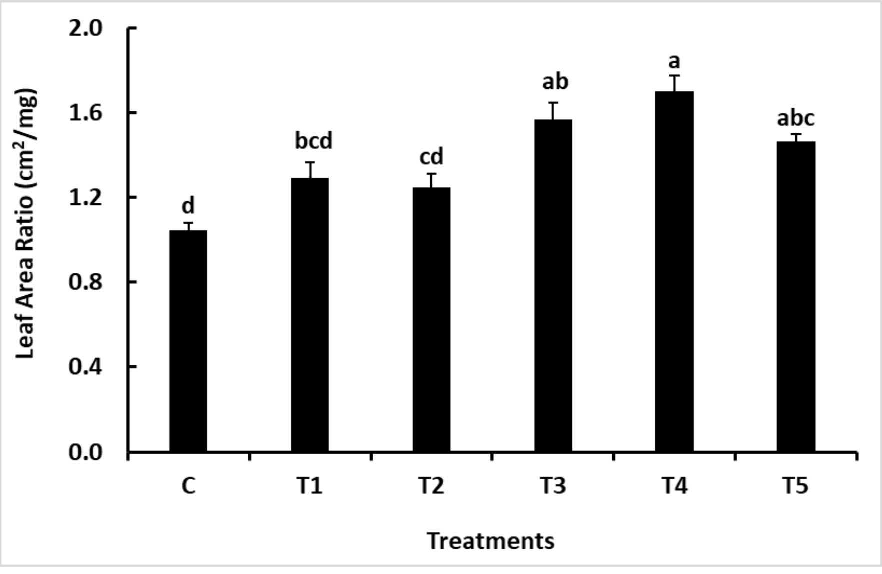

Figure 9: Effect of NaCl acclimation on the leaf area ratio of huckleberry plants subjected to salinity stress. The bars represent means ± SE (n = 3), bars with the same letter are not

statistically different (p ≤ 0.05). C = control, T1 = 5 mM NaCl, T2 = 10 mM NaCl, T3 = 75 mM NaCl, T4 = 5 + 75 mM NaCl, and T5 = 10 + 75 mM NaCl.

Effect of Acclimation on Water Use Efficiency (WUE) of Huckleberry Plants Subjected to Salt Stress

The effect of NaCl acclimation on WUE of huckleberry plants subjected to salt stress and control conditions is given in Figure 10. The WUE decreased under salt stress in non-acclimatized plants, but this reduction was not statistically significant. However, under salt stress conditions, acclimated plants (especially T4) significantly enhanced the WUE (50% increase), when compared to controls. Under control conditions, acclimatized plants had marked increases in WUE when compared with non-acclimatized controls. The T5 treatment (10 + 75 mm NaCl) did not significantly alter the WUE from non-acclimatized control and salt stressed plants. These results indicate that WUE in part is responsible for observed differences in growth of the plants.

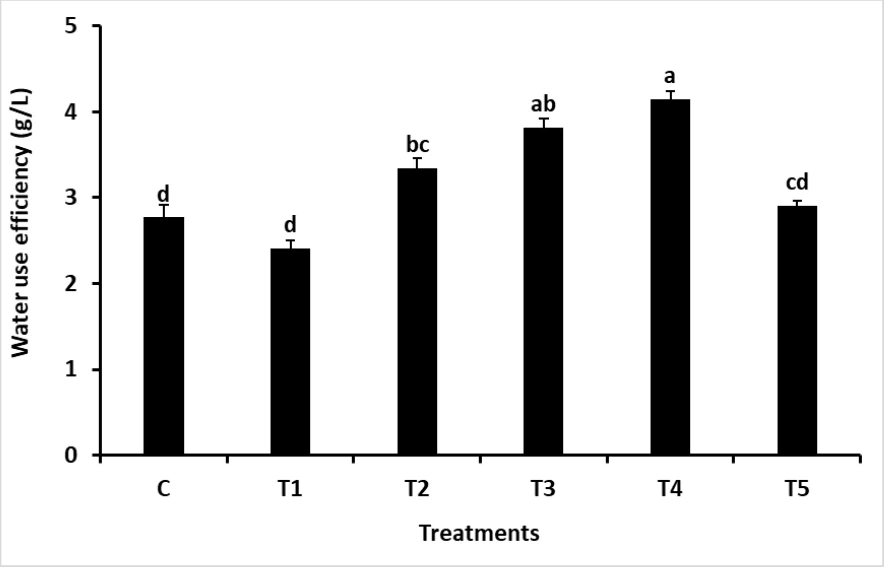

Figure 10: Effect of NaCl acclimation on the water use efficiency (WUE) of huckleberry plants subjected to salt stress. The bars represent means ± SE (n = 3), bars with the same letter are not statistically different (p ≤ 0.05). C = control, T1 = 5 mM NaCl, T2 = 10 mM NaCl, T3 = 75 mM NaCl, T4 = 5 + 75 mM NaCl, and T5 = 10 + 75 mM NaCl.

Effect of Acclimation on The Yield of Huckleberry Plants Subjected to Salt Stress

Results of yield per pot and per hectare (ha) is given in Figure 11 A and B below. While salt stress significantly reduced the yield of non-acclimatized huckleberry plants, it did not significantly alter that of plants acclimatized with 10 mm NaCl (T5), but significantly elevated that of plants acclimatized with 5 mM NaCl (T4), which produced the highest yield per pot (318 g) and per ha (45.4 tons). While salt stress reduced the yield of non-acclimatized plants by 25%, the yield is improved through acclimation with 5 mM NaCl by 54 % (T2) (Figure 11B).

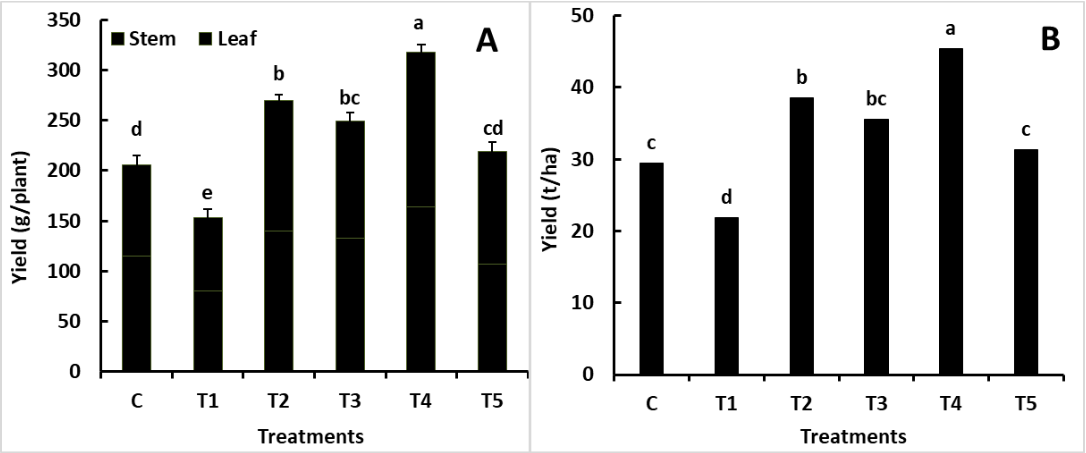

Figure 11. Effect of NaCl acclimation on the yield of huckleberry plants subjected to control and salinity stress conditions. A) Yield per pot, and B) yield per ha.

The bars represent means ± SE (n = 3), bars with the same letter are not statistically different ($p \leq 0.05$). C = control, T1 = 5 mM NaCl, T2 = 10 mM NaCl, T3 = 75 mM NaCl, T4 = 5 + 75 mM NaCl, and T5 = 10 + 75 mM NaCl.

Correlation

Correlation among the different measured variables is presented in Table 3. From the coefficients it can be noticed that WUE and RGR had a strong and positive correlation with all growth parameters measured, except with number of leaves. Leaf mass per area was also positively correlated with the dry weight of leaf stem and root, as well as with leaf and root fresh weights, but had no correlation with stem fresh weight. Furthermore, the leaf area was only positively correlated with leaf dry weight, leaf and stem fresh weight.

Table 3: Correlation among the different growth and physiological variables of acclimated and non-acclimated huckleberry plants subjected to salt stress and non-stress conditions

		1	2	3	4	5	6	7	8	9	10	11
1	LDW	1										
2	SDW	**.714****	1									
3	RDW	**.499***	**.636****	1								
4	TLA	**.619****	.395	.071	1							
5	LMA	**.653****	**.639****	**.639****	-.091	1						
6	LFW	**.953*****	**.678****	.343	**.597****	**.617****	1					
7	RFW	**.518***	**.584***	**.746*****	.272	**.537***	.365	1				
8	SFW	**.742*****	**.633****	.447	**.614****	.372	**.679****	.304	1			
9	RGR	**.952*****	**.853*****	**.623****	**.619****	**.650****	**.894*****	**.599****	**.795*****	1		
10	WUE	**.936*****	**.893*****	**.706****	**.518***	**.731****	**.866*****	**.652****	**.745*****	**.980*****	1	
11	NL	.301	.266	-.035	-.124	.318	.327	-.069	.341	.249	.265	1

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, values are correlation coefficients, LDW = leaf dry weight, SDW = stem dry weight, RDW = root dry weight, TLA = total leaf area, LMA = leaf mass per area, LFW = leaf fresh weight, RFW = root fresh weight, SFW = stem fresh weight, RGR = relative growth rate, WUE = water use efficiency, and NL = number of leaves

DISCUSSION

The aim of this study was to evaluate the growth and physiological responses of huckleberry (*Solanum scabrum* Mill.) to salt stress following acclimation to NaCl. Previous studies on the growth and physiological responses of huckleberry to salt stress have been carried out (Assaha et al 2013, 2015a, 2015b) and showed that the plant is sensitive to high salinity. Since there has been no research works on salt stress alleviation in huckleberry, the current study sought to use low NaCl to impart stress tolerance in huckleberry as has been observed in other plants including tomato (Kamanga et al., 2020), rice (Sriskantharajah et al., 2020), and maize (Pandolfi et al., 2016). In this light, the huckleberry plants were acclimated with 5 and 10 mM NaCl and then subjected to high salinity. The results obtained showed that the acclimation treatments enhanced the growth and therefore tolerance of the huckleberry plants to salinity. Hence the following paragraphs will focus on discussing how this enhanced tolerance might have been brought about in huckleberry.

It is known that leaf number and leaf area is directly proportional to net photosynthetic rate (Cai et al., 2007), implying that a reduction in leaf area will lead to a reduction in photosynthesis, while an increase in leaf area will result in increased photosynthesis. In the present study, while salt stress significantly reduced the total leaf area of non-acclimated plants, the total leaf area of huckleberry plants acclimated with 5 mM NaCl, significantly increased under salt stress, compared to non-acclimated salt-stressed plants. Thus, acclimation restored the leaf area to control levels (Figure 6), similar to observations on some rice cultivars acclimated with 1 mM NaCl (Sriskantharajah et al., 2020). Since increase in leaf area often corresponds to increased photosynthesis, and hence increase in biomass production, culminating in enhanced growth (Kanno et al., 2009; Negrão et al., 2017), it is possible that the enhanced leaf area under salt stress in the present study might have contributed to enhanced growth of acclimated huckleberry plants under salt stress as leaf area was strongly correlated with all growth parameters. However, there was no significantly correlation between leaf number and biomass accumulation in terms of dry and fresh weights, indicating that salt stress tolerance in huckleberry is not associated with leaf number.

In addition, WUE, a function of efficient water usage by crops in biomass production, is an important salt stress tolerance trait, as there is a positive correlation between WUE and salt stress tolerance (Negrão et al., 2017) implying that a decrease in the WUE of a plant under salts stress will negatively impact the growth while enhanced WUE will increase stress tolerance. In the present study, WUE was found to be positively correlated with all growth parameters (Table 3). However, salt stress reduced the WUE of non-acclimated plants (T2), although this reduction was not statistically significant, but the plants acclimated with 5 mM NaCl had significantly higher WUE under salt stress (T4), (Figure 10). This increase was even higher than that of control plants, implying that acclimation induced enhanced WUE in huckleberry plants that helped them adapt to high salinity without any growth penalties as observed in the strong correlation between biomass and WUE.

Furthermore, leaf thickness, which is a measure of the ratio between leaf area and leaf mass is an important salt stress tolerance trait (Negrão et al., 2017), as it indicates the volume of the leaf space that is required to accommodate excess salts accumulated plants (; Acosta-Moto et al., 2017). Lim et al. (2020), induced tissue succulence in Arabidopsis and observed an increase in WUE of the plants which enhanced tolerance to both water deficit and salt stress. This observation indicates that there is a close association between succulence or leaf thickness, with WUE, implying therefore that an increase in leaf thickness will result in increased WUE signifying tolerance, whereas a decrease will indicate susceptibility. In the present study, salt stress significantly enhanced the thickness of non-acclimated plants, while acclimation further significantly enhanced the thickness, especially of plants acclimated with 5 mM NaCl (see Figure 9). In addition, a strong and positive correlation was found between WUE and leaf thickness (Table 3), clearly showing that leaf thickness contributed in the salt stress tolerance of acclimated plants relative to non-acclimated ones, through enhanced WUE.

Moreover, RGR, the rate of increase of biomass per unit of biomass already existing (Nieves et al., 2011), is also an important indicator of salt stress tolerance (Rahnama et al., 2010). In a study conducted by Nieves et al. (2011) aimed at understanding the rate limiting factor in the growth of palms under salt stress, found that the leaf n content was responsible for RGR reduction in the palms. This suggests that leaf N availability is correlated with RGR, implying that reduction in leaf N will lead to reduced RGR and hence growth reduction and consequently salt stress susceptibility and vice versa. In the present study, salt stress significantly reduced the RGR of non-acclimated plants, but acclimation rescued the plants under salt stress, as 10 mM (T5) restored the RGR to control levels, while 5 mM (T4) significantly increased the RGR above control levels (Figure 7). This clearly indicates that acclimation induced stress tolerance in huckleberry by enhancing its RGR, possibly owing to enhanced leaf N. in addition the strong correlation between RGR, WUE and leaf thickness (LAR), clearly indicates that the latter two have a big role to play in the RGR of huckleberry plants under salt stress, as well as in the yield of the plants. This yield might have been associated more with WUE, as it is correlated with enhanced leaf and stem biomass, than with leaf thickness, which only correlated with leaf biomass.

CONCLUSIONS

The importance of vegetables in the human diet is universally recognized. They are very important food crops consumed on a daily basis because of their nutritive and health properties especially huckleberry. This study shows that salt stress negatively affects the growth and yield of huckleberry. However, acclimation with NaCl proves to be a good way of alleviating the salt stress effects in the plants and improving their yield under both normal and saline conditions. Acclimation with 5 mM NaCl produced the best results and so would constitute a suitable option for managing the production of huckleberry on saline soils. However, further research is needed to establish the physiological and molecular basis of acclimation-induced salinity tolerance in huckleberry.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Higher Educations Research Modernization Allowance to DAVM

Conflict of Interest

The authors declare that they have no conflicts of interest

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