Domestication of (*Rosmarinus officinalis L.*): contribution to the effect study of soil type on salinity tolerance

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Abstract- In Morocco, a large number of aromatic and medicinal species are endemic. It is therefore essential to work towards preserving and protecting them from massive and irrational exploitation, especially with the emergence of the "Bio" label. Domestication of these plants is therefore the best option to alleviate the pressure they are under. In addition a large part of Moroccan agricultural regions are affected by the soil salinisation process. Currently, almost 5% of lands are already affected by salinisation at different levels. In response to the magnitude of this problem, it is necessary to select species capable of developing in saline areas. The present work aims to evaluate the substrate effect on Rosemary morphophysiological behaviour (*Rosmarinus officinalis L.*) under a saline constraint. This was done with the purpose of elaborating a tolerance thresholds classification to saline stress, an important criterion in the choice of species to be retained in an enhancement program of the salinity affected areas. The trial is conducted using an experimental design in block. Rosmary is planted in plastic pots of 9 cm diameter and 11 cm height. The used substrates are: arenosols, sea sand and vertisols. The pots were irrigated with 40 ml of sodium chloride each 72 hours, with a concentration of 6 $g.l^{-1}$, 9 $g.l^{-1}$, 12 $g.l^{-1}$, in addition to the control. The measured parameters are: length of aerial and root parts, root volume, water content (WC), soluble sugar content (SS). Each treatment is repeated 10 times.

Another test was added, which corresponds to pots filled with sea sand. A plastic film is placed under the pots to limit drainage and placed in a vegetable garden, in order to study the interest of the drainage percentage on the plant growth maintenance. The obtained results show that saline treatments cause significant changes on root morphological parameters than on aerial morphological parameters. Plants cultivated in vertisols grow poorly, in contrast to those planted in sandy substrates, which shows the drainage importance to minimise the salt stress effect. The salt effect on the plants physiological characteristics is reflected in a significant reduction in the plants water content (P= 0.041), which leads to an increase in the soluble sugar content. The depressive effect of saline stress above 6 g/l and in the long term in vertisols induces a significant reduction of water content (P=0.032) and a significant increase of soluble sugars (P=0.018). Therefore, under salt stress, an arenosols has optimal characteristics for rosemary germination.

Index Terms- Domestication, Morphophysiological characteristics, *Rosmarinus officinalis L.*, Saline stress, Sea sand.

I. INTRODUCTION

In the last year, medicinal and aromatic plants have been increasingly in demand in several fields such as food, perfumes, pharmaceutical industry and natural cosmetics. However, as with most cultivated plants, the growth and yield of medicinal and aromatic plants can be affected by environmental constraints such as salinity and drought [1].

Salinisation poses a real threat to global food security, particularly in arid and semi-arid regions. It is estimated that more than 800 million hectares of land in the world are affected by salinity [2]. In Morocco, 5% of agricultural areas are affected by the soil salinisation process [3].

In Morocco the species *Rosmarinus officinalis L.* is found in forests, scrubland and matorrals, on well-drained calcareous substrates. It is widespread in the Eastern Rif, the Eastern Middle Atlas, the Eastern High Atlas and the Oriental highlands. It grows in semi-arid and sub-humid bioclimates with cool warm variants in the thermo-Mediterranean and meso-Mediterranean vegetation levels. It is a drought resistant plant with apparent xerophytic traits (small leaves etc.) [4].

Morocco has great potential in the field of aromatic and medicinal plants. Rosemary grows naturally in Moroccan mountains. They do not require any irrigation and their production does not impact the groundwater table [5]. In addition to their use for plant raw materials harvesting, rosemary fields are necessary for the development of local beekeeping and constitute a privileged habitat for bees.

However, it must be emphasised that overexploitation of these aromatic and medicinal plants, including Rosemary, can lead to desertification and thus contribute to global warming [5]. Therefore, efforts related to the domestication and cultivation of rosemary have been undertaken to enable the sustainable exploitation of this resource, but there is still a long way to go.

Rosemary is relatively tolerant of salt stress at moderate doses, but the salts addition at high concentrations can affect the delicate balance between growth and salt exclusion. This species responds to increasing NaCl concentrations by reducing height growth relative to the control and a decrease in relative water content is observed. Increasing the salt stress intensity in rosemary induces an increase in soluble sugar and proline content [6].

The work objective is to study the possibility of domesticating Rosemary (*Rosmarinus officinalis L.*) in Morocco under different types of substrate (vertisols, arenosols and sea sand), taking in consideration the salinity of most Moroccan soils.

II. MATERIALS AND METHODS

A. *Experimental design*

The study is being conducted in a greenhouse at the Ibn Tofail Faculty of Science in Kenitra from early March 2017 to January 2018, using an experimental design in block (Figure 1). Rosemary is planted in three types of substrates: arenosols, sea sand and vertisols, in plastic pots of 9 cm diameter and 11 cm height.

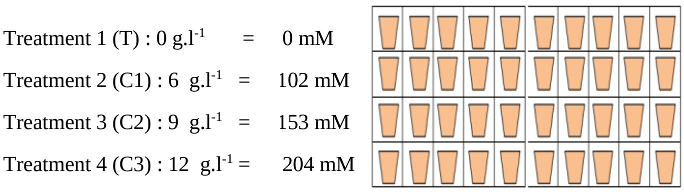

Figure 1: Germination test illustration for the three substrates

The experiment is composed of three blocks (3 substrates), each block consists of 4 lines: one line corresponding to the control and the other three lines corresponding to the different salt treatments. Each treatment is repeated 10 times.

Another trial corresponding to 20 plastic pots (5 cm diameter and 7 cm height) filled with sea sand and placed in a vegetable garden pond is added (Figure 2). A plastic film is placed under the pots to limit drainage, in order to study the drainage percentage interest on the plant growth maintenance. The four treatments are repeated five times (including the control).

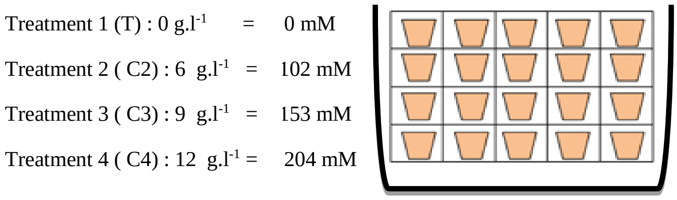

Figure 2: Germination test illustration for the Pond garden

The pots were irrigated at the beginning of the experiment with 40 ml of tap water for each pot. 15 days after, the plants were irrigated every 72 hours with 40 ml of sodium chloride (which was chosen as the stress agent). Table 1 shows the concentrations of sodium chloride used.

Table 1: Sodium chloride (NaCl) concentrations used in the experiment

Traitment	Concentration of NaCl (mg.l-1)	Concentration of NaCl (mM)
T	0	0
C1	6000	102
C2	9000	153
C3	12000	204

1. *Soil*

The soils used in this trial come from Kénitra city located in the North-West of Morocco (Kénitra, Morocco, latitude 34° 15' 39 North, longitude -6° 34' 48 West, altitude above sea level: 13 m). This region has an appreciable natural richness, notably arable land and important water resources, as well as varied ecosystems. In the present study, three soil types (Substrate) were used:

Substrate 1 (Sub 1): Arenosols from Maamora region
Substrate 2 (Sub 2): Sampled sea sand from Mehdia region
Substrate 3 (Sub 3): Vertisols from Mnasra region.
The table 2 shows the granulometric characteristics of Sub 3 and Sub 1.

Table 2: Granulometric characteristics of Sub 3 and Sub 1

Substrate type	Granulometry (%)		
	Clay	Silt	Sand
Sub (1) [7]	3.15	6.3	90.59
Sub (3) [8]	75.4	19.2	4

2. *Plant material*

The plant material used in this study is Rosemary (*Rosmarinus officinalis L.*), a species of the botanical family Lamiaceae, which prefers deep, light and permeable clay-limestone soils with an alkaline pH (7-8). It adapts to all types of topography and also tolerates a great variability in altitude [9].

A semi-arid Mediterranean climate is well suited to rosemary: high sunshine, warmth and low rainfall [9].

B. Measured parameters

The physiological and biochemical modifications analyses in rosemary are carried out in Plant Biology and Physiology Department Laboratories, Sciences Faculty Ibn Tofail-Kenitra. The first parameter evaluation on 5 pots of each concentration was performed after one month of treatment with NaCl, while the second evaluation was carried out once the plants started to wilt.

1. Morphological parameters

1.1. Overhead and underground lengths

The plants were carefully dug up, their roots washed under running water and then quickly wrung out with filter paper. The aerial parts (stems + leaves) and root parts were separated.

The lengths of the aerial parts (LAP) and root parts (LRP) were measured with a ruler graduated in mm.

1.2. Root volume

It is measured by immersion in a graduated cylinder. [10]; according to the principles of Archimedes' thrust, the volume of an immersed body is equal to the volume of the displaced liquid.

2. Physiological parameters

2.1. The plants Water content (WC)

The plants water content is measured by calculating the difference between fresh and dry weight [11]. This difference is expressed as a percentage of the fresh weight by the following formula:

$$WC = \left(\frac{(FW - DW)}{FW}\right) \times 100$$

WC: the plants relative water content (in %)
FW: fresh weight just after harvest (g)
DW: dry weight after oven drying at 85 °C for 36 h (g)

2.2. Total soluble sugars

Total soluble sugars (sucrose, glucose, fructose, their methylated derivatives and polysaccharides) are determined by the phenol method [12].

C. Statistical analysis

The measured data were subjected to a one-way analysis of variance (ANOVA) using SPSS (2018) statistical software. Then, a comparison of the means between the control and treated samples was performed using the post-hoc test. This test can be used to determine significant differences between group means in a variance analysis. Results are considered significant when P≤ 0.05.

III. RESULTS

A. Morphological parameters

1. Overhead and underground length

The obtained results show that the LAP and the LRP vary according to the substrate and the salt treatment (Figure 3). Indeed the LAP is more important for sub 3 (Vertisols) than for the other substrates, while the LRP is more significant (P= 0,014) for sub 1 compared to the other substrates. The substrate 2 controls LPR is higher than the garden pond controls. A decrease in plant growth in the sandy substrate was noticed after 30 days of the salt treatment, this decrease is more significant in the garden pond than in the blocks of substrate 2 (marine sand). The salt affects the growth of the plants tested in a degressive way, the lengths decrease as the substrate becomes more and more concentrated.

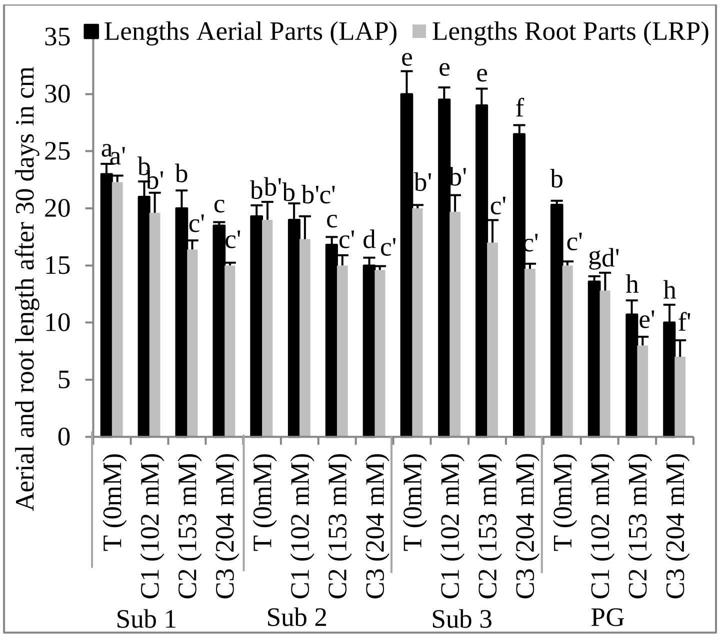

Figure 3: Aerial and root length variation in the 4 blocks under different NaCl concentration effect (Values with different letters are significantly different: $p < 0.05$)

Statistical analysis shows a highly significant difference in the garden pond between the control and the C3 stressed treatments (P: 0.017 in rosemary). The salinity effect leads to a reduction in plant size (stem and roots), which is more significant the higher the NaCl concentration. It can be observed that salinity reduced the growth of LRP more than that of LAP. The reduction is more important at higher salt concentrations. Salt stress has a negative effect on root growth.

2. Root volume

From the results we observed that the control plants root volume and root length evolved much more than those irrigated with the saline solution, both in the block test and in the pond garden (Figure 4).

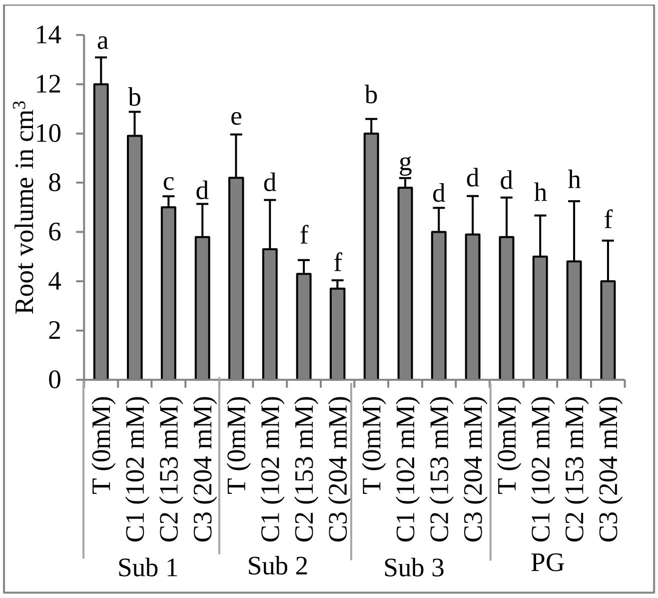

Figure 4: Root volume variation in the 4 blocks after 30 days of salt treatment (Values with different letters are significantly different: $p < 0.05$)

The higher concentration of NaCl caused a significant decrease in root volume (P=0.047) compared to the controls, respectively for the 4 blocks. The means Comparison by the post hoc test shows that there is a significant difference between the treatments C1, C2 and C3 and the controls at the Sub 1, Sub 2, Sub 3 and at the pond garden level. The root volume results show very highly significant differences between the 4 blocks (Table 3).

Table 3: ANOVA test for the root volume variation in the 4 blocks after 30 days of salt treatment.

ANOVA	**Root Volume**	
Plant	Variable	P
Rosemary	Blocks	0.047

B. Physiological parameters

1. Water content

The obtained results indicate that salt stress induces a reduction in water content (WC) at different NaCl concentrations (Figure 5). Indeed, the control plants show the highest water content. The water content in the substrate 2 controls is low compared to the pond garden controls. The plants water content decreases as the saline concentration in the substrate increases. The plants sown in the substrate 3 have the highest water content values, while the low values are those of the pond garden.

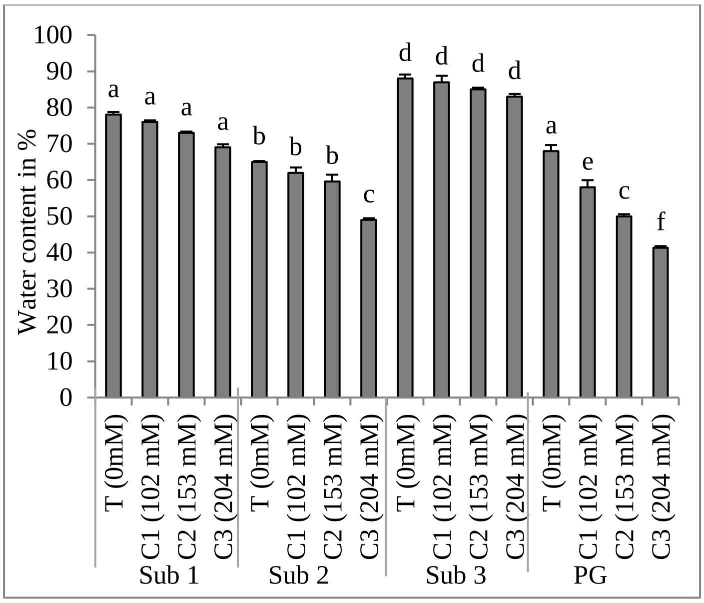

Figure 5: Water content variation in the 4 blocks after 30 days of salt treatment (Values with different letters are significantly different: $p < 0.05$)

The statistical analysis results (Figure 5) show that the NaCl concentrations added to the soil have a remarkable effect on the plant water content. Indeed, at the pond garden, the variance analysis showed a significant effect of the salt addition on the C1 (P=0.047), C2 (P=0.039) and C3 (P=0.018) treatments compared to the control plants.

The water content measured during the trials showed that there were significant differences between the blocks (P=0.041) (Table 4). Furthermore, the variance analysis showed a highly significant effect of salinity between the Rosemary blocks for water content.

Table 4: ANOVA test variation in water content in the 4 blocks after 30 days of salt treatment.

ANOVA	**Water Content**	
Plant	Variable	P
Rosemary	Blocks	0.041

3. Total soluble sugar content

The higher the substrate salt content, the higher the plants sugar content, with a variation in concentration from one substrate to another (Figure 6). The plants in the pond garden show much higher levels than those in Sub3 (Vertisols). Comparing the substrate 2 controls with the pond garden controls, we observe that the soluble sugar content is high in sub2.

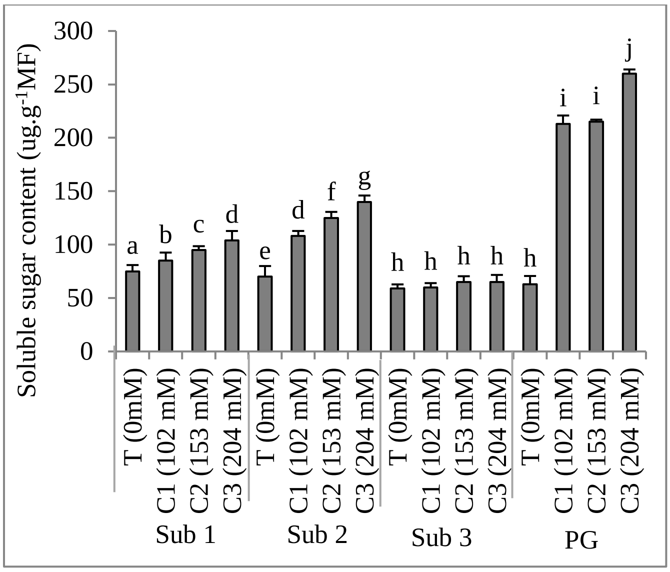

Figure 6: Total soluble sugar content evolution in leaves in the 4 blocks (Values with different letters are significantly different: $p < 0.05$)

The analysis of variance revealed a significant salinity effect on the leaves sugar content in the pond garden; indeed we notice a strongly significant difference between the controls and the treatments C1 (P=0.045), C2 (P=0,031) and C3 (P=0.000). The analysis of variance showed a highly significant difference between the blocks (P=0.003) (Table 5)

Table 5: ANOVA test for Total Soluble Sugar content evolution in leaves for the 4 blocks

ANOVA	Total Soluble Sugar	
Plant	Variable	P
Rosemary	Blocks	0.003

C. The long-term salt stress depressive effect

Plants in Sub3 (Vertisols) have a longer lifespan than those in sandy substrate (Table 6).

Table 6 Rosemary optimal survival time under salt stress

Blocks	The optimal survival time in days
Sub 1	70
Sub 2	40
Sub 3	90
PG	30

Salt has a depressive effect on plants development and growth, especially at high salt concentrations and for long exposure periods.

1. Salinity effect on plant morphology

The results show that long-term substrate salt treatment induces reductions in the aerial and root parts (Figure 7).

The ANOVA analysis for aerial parts length showed that there was no significant long-term effect between the three treatments and the control except for the plants germinated in substrate 1 (Figure 7). While for root parts length, the effect of salt stress is significant for all three substrates compared to the control.

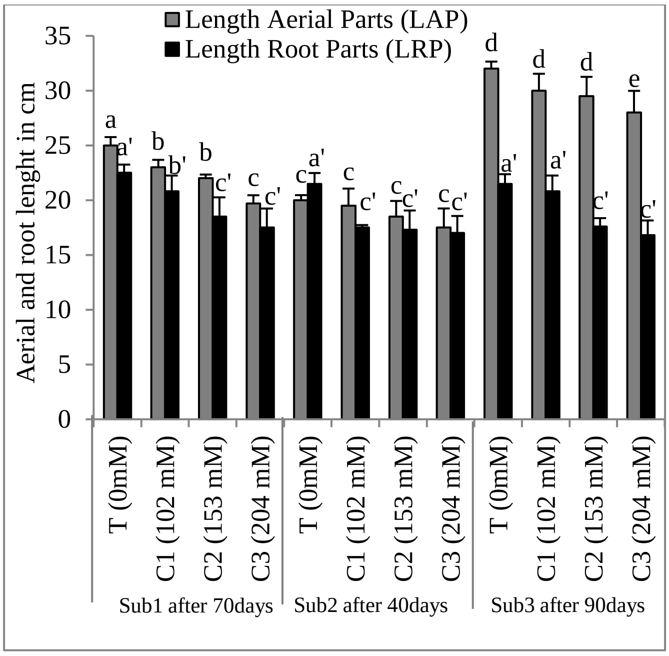

Figure 7: The long-term salt treatment effect on the aerial and root parts length (Values with different letters are significantly different: $p < 0.05$)

In terms of comparison between the three substrates it can be seen that plants germinated in substrate 3 have a significant (P=0.02) length of aerial parts compared to substrate 1 and substrate 2.

The root volume system examination showed that the salt stress had a negative effect on the roots volume (Figure 8). Indeed, statistically for germinated plants in all three substrates, the three treatments had a significantly lower effect on root volume compared to the control (Sub1 (P=0.022); Sub2 (P=0.04); Sub3 (P=0.018)).

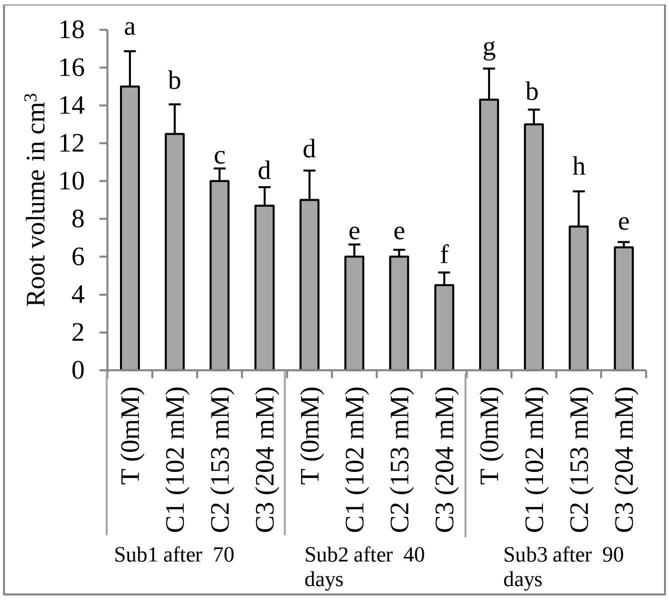

Figure 8: The long-term salt treatment effect on root volume (Values with different letters are significantly different: $p < 0.05$)

Visually (Figure 9) the salt negative effect on plants is remarkable. During cultivation, the different tested plants reacted in a similar way to an excess of salinity. A decrease in the aerial part growth and a yellowing of the leaves were noted. At high concentrations, above 6000 mg of salts per liter, a modification of the leaf morphology is noticed, becoming narrow and rigid, in addition to a wilting of certain plants. Leaf senescence accelerates with increasing concentration.

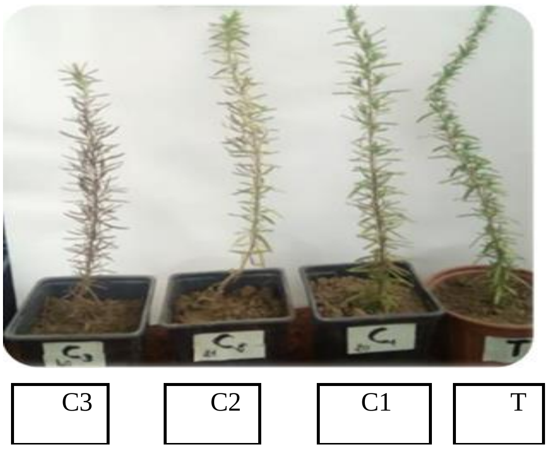

Figure 9: Comparison between *Rosmarinus officinalis L.* plants for the three treatments for sub 1

2. *Salinity effect on plant physiological characteristics*

According to the obtained results, the plants show a reduction in water content (Figure 10) and an increase in soluble sugars (Figure 11) gradually with increasing salinity. It was found that long-term salt stress causes a very significant reduction in water content for the three substrats compared to the control (Sub1 (P=0.033); Sub2 (P=0.03); Sub3 (P=0.027).

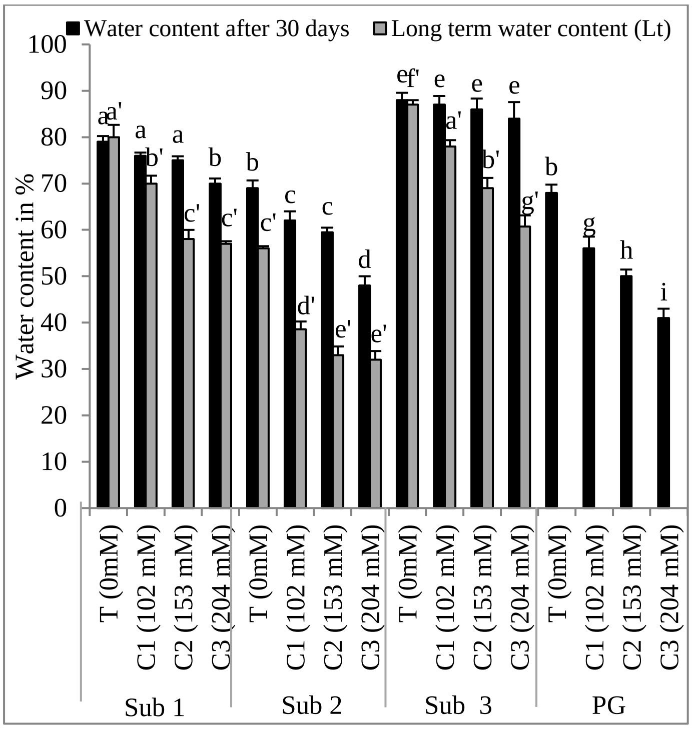

Figure 10: Long-term salt treatment effect on physiological traits (water content) (Values with different letters are significantly different: $p < 0.05$).

Short-term salt stress (30 days) is less compromising for plant development than long-term salt stress (Lt). Under control conditions, the obtained total soluble sugar contents are similar for all studied substrates. The total soluble sugars content increases considerably when switching to salinity conditions, especially at 6000, 9000 and 12000 mg/l, and accumulations differ from one substrate to another.

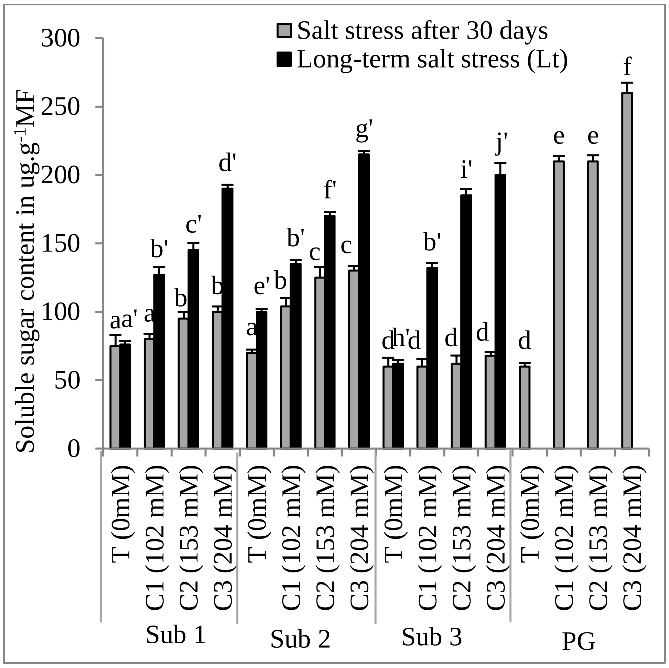

Figure 11: Long-term salt treatment effect on soluble sugar content (Values with different letters are significantly different: $p < 0.05$)

The depressive effect of NaCl is clearly apparent as soon as the plant starts to wilt, at the long term exposure to salinity. We note a very significant increase in the soluble sugar (SS) content in the long term compared to that measured after 30 days of exposure to salinity (Sub1 (P=0.014); Sub2 (P=0.028); Sub3 (P=0.008)).

IV. DISCUSSION

The salinity effects are visible in the plant physiology and morphology for the different used concentrations.

Plants absorb water by osmosis. This absorption is conditioned by the difference in osmotic pressure of their sap and soil solution. The relative water content analysis in the 4 blocks allows a global description of rosemary water status.

In the pond garden, salt stress caused a high regression of the relative water content due to excessive amount of soluble salts in arenosols after the lowering of the drainage compared to Sub 2 which contains the same substrate.

The increased soluble sugar content in the substrate 2 controls and the low water content and high root growth compared to the garden pond plants controls indicate the water stress presence in addition to salt stress in the substrate 2 plants. Indeed, arenosols (more than 55% sand) are light, easy to work, have low cohesion between particles, have low water reserves and are poor in nutrients, they cannot become salty by irrigation because they do not retain water [13].

Plants grown in Sub 3 (vertisols) after 30 days show the best water content which seems to be weakly correlated with salinity compared to plants grown in Sub 1 (Mamora soil) and Sub 2 (sea sand). Furthermore, for an adsorbed sodium ionic fraction inferior to 40%, the clay is in crystallite form, calcium is fixed on the internal surface sites and sodium on the external surface sites [14]. Whereas, for an adsorbed sodium ionic fraction superior to 40%, the crystallites disintegrate and the sodium is located on both inner and outer clay surfaces causing a clay flocculation with a decrease in the soluble sodium amount at the soil level [14], and this delays the salt stress severity in plants with time.

According to the obtained results, salinity reduced the *Rosmarinus officinalis* aerial and root parts growth, which is quite normal. Indeed according to the results reported by [15] and [16], the plant adapts to salt stress first by reducing its root system thus preserving the aerial part in order to maintain photosynthesis, then by downsizing the aerial part with the increase in salinity.

Similar results were reported for rosemary by [6], for bean (*Phaseolus vulgaris L.*) by [17] and [18], and for rice by [19].

In the garden pond, the decrease in the plants root length in treatment C3 is significant compared to the control plants. This decrease is probably due to the ionic toxicity of the soluble Na ions in the soil solution, favoured by the drainage lowering in the garden pond.

Based on the achieved results, the root system volume is inversely proportional to the applied stress intensity. The root volume reduction reflects the growth inhibition due to the low root system branching. The variance analysis at a classification criterion showed that there is a highly significant difference between the controls and the plants that underwent the C3 treatment for all three soil types.

From the obtained results, it appears that salt treatments cause significant changes on root morphological parameters more than on aerial morphological parameters. These results are supported by other studies [20; 21; 22].

The Rosemary root system resistance to salt stress may be due to a decrease in carbon allocation for leaf growth in the favor of root growth [23]. The reduction in growth may also be related to salt-induced disturbances in the levels of growth regulators (abscisic acid and cytokinins), sometimes to a reduction in photosynthetic capacity due to a decrease in stomatal CO_2 conductance under salt stress [24].

In nature, plants lose water through transpiration and tend to replace it by uptake from the soil solution [25]. The salts presence in the growing medium limits the availability of water to the plant and consequently, the plant is in a state of water deficit [26].

Our results show that salt stress reduces the plants water content. Indeed, the water and osmotic potentials of plants become feeble with increasing salinity, while the turgor pressure is increased [27; 28; 29]. Similar results were obtained by [30] on barley; by [31] on okra and by [32] on *Carum carvi L.*

The solutes accumulation allows plants to resist water shortage and toxicity. This accumulation varies in large proportions depending on the species, the development phase and the salinity level [6].

One of the main physiological characteristics of tolerance to the saline environment constraints is the osmotic adjustment. This is achieved through an accumulation of osmo-regulatory compounds leading to a reduction in osmotic potential, thus allowing the turgidity potential maintenance [33].

In terms of soluble sugar content, the obtained results show an increase in soluble sugars in stressed plants compared to control plants for the three soil types. This change in soluble sugars is reflected in particular by the phenomenon of high accumulation with increasing the medium salinity. However, Carbohydrates such as sugar (glucose, fructose, sucrose, fructans) and starch accumulate under salt stress [34], playing a role in osmoprotection, osmotic adjustment, carbon storage and radical scavenging, sugars can also act as signalling molecules under stress [35]. The same observations were noted by [31; 17; 36].

It should be mentioned that salinity affects the soil in which the plant will germinate at the first place. In fact, in vegetable nutrition, Ca and Mg deficiencies can be induced by the carbonates precipitation from pH 7-8 and due to the non-selective sodium adsorption, there is a relative excess of the sodium ion in soils with a high exchangeable sodium percentage (ESP) and therefore a higher adsorption by the roots [37]. Nevertheless, the fundamental influence of exchangeable Na is on the soils physical properties due to the respective attractive and repulsive forces of sodium and calcium ions. As the thickness of the diffuse double layer is thicker in the presence of sodium, the sodium ion creates a more favourable environment for peptisation. This clays peptisation affects the soil hydrodynamic properties [37].

In the light of the results obtained, it can be said that the plants reaction to salinity varies considerably according to the substrate, the exposure duration to this stress and to the environment salinity degree. Thus, plants react differently to these variations in the biotope, either by triggering resistance mechanisms [38], i.e. modifications in their morpho-physiological [20], biochemical [39] and mineral behaviour, as in the case of glycophytes, or by disappearing [40].

V. CONCLUSION

The medicinal and aromatic plants future in Morocco depends on the development of their cultivation, through thoughtful and harmonious work from the wild species domestication to the varietal and cultural improvement and cultivation of those already cultivated.

The salinity effects on growth depend on several factors. They vary according to the NaCl concentration applied to the treatment, the species, the substrate and the exposure to salinity duration.

Under the conditions of our experiment, the results obtained show that

- Salt stress induced significant reductions in the growth of the aerial and root parts, as well as induced a reduction in root volume in Rosemary.
- The higher the degree of salinity and the treatment duration, the greater the impact on the plant morphological and physiological parameters.

Concerning physiological traits, the depressive effect of NaCl is manifested by :

- A decrease in water content (WC) following the water potential reduction growing medium by the salt.
- The total soluble sugar content analysis showed that plants in the sandy substrate were characterised by a high soluble sugars accumulation.

In the vegetable garden pond, salt stress caused a high soluble sugar accumulation because of the soluble salts excessive amount in the arenosols due to the drainage lack, while the high soluble sugars accumulation in substrate 2 plants (marine sand) is caused by its low cation exchange capacity and the water non-retention by the substrate.

The long-term salt stress depressive effect induces a high reduction in water content and an increase in soluble sugars in Rosemary. The obtained results confirm the importance of drainage in minimising salinity impact on plant growth and development.

In terms of comparison between the three types of substrates, it can be seen that the most influenced plants by the salt treatment are those grown in substrate 3 (vertisols). Indeed, a soil with low permeability and fine texture represents a real menace to the plants grown in it, due to the high accumulation of salts in its porous matrix, as is the case for the plants grown in the vertisols. Earlier studies for cultivar selection showing long-term tolerance would be more favourable for long period harvesting with respectable plant material for export.

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