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IMPACT OF HALOPHYTE ASSOCIATION ON THE PHYSIOLOGICAL RESPONSES OF *LAVANDULA DENTATA* UNDER SALT STRESS CONDITIONS

Issam El-Khadir [1], Yassine Mouniane [2], Ahmed Chriqui [3], Mohamed Kouighat [4], Amir Souissi [5], Mouloud Lamtai [6*] and Driss Hmouni [7]

[1,2,3,7] Natural Resources and Sustainable Development Laboratory, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco.
[4] Research Unit of Plant Breeding and Plant Genetic Resources Conservation, Regional Agricultural Research Centre of Meknes, National Institute of Agricultural Research, Avenue Ennasr, P.O. Box 415, Rabat 10090, Morocco.
[5] Swift Current Research and Development Centre, Agriculture and Agrifood Canada, Swift Current, SK, Canada.
[6] Laboratory of Biology and Health, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco.
*Corresponding Author Email: mouloud-lamtai@hotmail.fr

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Abstract

Salinity represents a major challenge to plant growth, prompting exploration of strategies to improve resistance. This study focuses on *Lavandula dentata* and its associations with the halophytes *Atriplex prostrata* and *Plantago macrorhiza* under salt stress conditions. Key morphological and physiological parameters were analyzed, including biomass, above-ground length, root length, root volume, leaf water content, chlorophyll, proline, and total sugar content. The results show that combining *Lavandula dentate* with the halophytes *Atriplex prostrata* and *Plantago macrorhiza* produced significant improvements over cultivation alone. Biomass increased by 35%, above-ground length by 25%, and root length by 40% in the presence of halophytes, while leaf water content rose by 15% and chlorophyll by 20%, while proline content fell by 10%. The correlation results show significant correlations between several physiological parameters, including a strong correlation of 0.856 between biomass and length of aerial part, as well as other notable associations between the different parameters measured. Concerning principal component analysis (PCA), the first two principal components explain 81.4% and 8.2% of the total variance, respectively. Prin1 is mainly influenced by biomass, above-ground length, and chlorophyll content, while Prin2 is influenced by biomass, above-ground length, and total sugar content. These analyses offer valuable insights into the interactions and adaptations of *Lavandula dentata* in response to salt stress, potentially applicable to optimizing plant resilience in saline environments.

Keywords: Salinity; *Lavandula Dentata*; Halophyte Associations; *Atriplex Prostrata*; *Plantago Macrorhiza*.

1. INTRODUCTION

Soil salinization is a major obstacle to agricultural particularly in arid and semi-arid regions where irrigation and high evaporation contribute to soil salinization output since it drastically restricts plant development. The use of saline soils can aid in addressing the present issues with food security in the face of the combined stresses of population increase and climate fluctuation [1, 2]. Based on 73% of the land identified to far, the FAO estimates that the worldwide area of salt-affected soils consists of 424 million hectares of topsoil (0–30 cm) and 833 million hectares of subsoil (30–100 cm) [3].

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Among the plants affected by this stress, *Lavandula dentate*, an aromatic and medicinal plant renowned for its therapeutic properties and use in cosmetics, suffers particularly from high soil salinity [4]. This sensitivity considerably reduces its productivity and quality, thus limiting its economic potential [5]. The impacts of salt stress on plants include reduced growth, decreased photosynthesis, disruption of ionic balance and increased production of stress compounds such as proline [6]. The ability of plants to tolerate salt stress depends on their ability to maintain ionic homeostasis, protect their photosynthetic apparatus and modulate their metabolism to cope with adverse conditions [7].

To overcome these challenges, innovative strategies are needed. One promising approach is to combine salt-sensitive plants with halophytes plants capable of tolerating and even thriving in saline environments [8]. Halophytes, such as *Atriplex prostrata* and *Plantago macrorhiza*, have developed unique adaptation mechanisms that enable them to survive and grow in high-salinity conditions [9]. These mechanisms include the exclusion or sequestration of toxic ions, the production of osmoprotective compounds and the enhancement of water use efficiency [10]. Interactions between *Lavandula dentata* and these halophytes could potentially mitigate the negative effects of salt stress [11].

This study aims to explore these interactions by evaluating the effects of associating *Lavandula dentata* with *Atriplex prostrata* and *Plantago macrorhiza* under different NaCl levels (0, 34, 86 and 172 mM). Parameters measured included biomass, above-ground length, root length, root volume, leaf water content, chlorophyll content, proline concentration and total sugar content. By measuring these parameters, we hope to determine whether association with halophytic plants can improve *Lavandula dentata*'s tolerance to salt stress.

2. MATERIALS AND METHODS

2.1 Plant Materials

This study uses three plant species: *Lavandula dentata*, a salt-sensitive glycophyte and two salt-tolerant halophyte plants *Atriplex prostrata* and *Plantago macrorhiza* collected near Oued Maleh (Figure 1), a Moroccan region renowned for its saline soils offering potential benefits when combined with salt-sensitive plants.

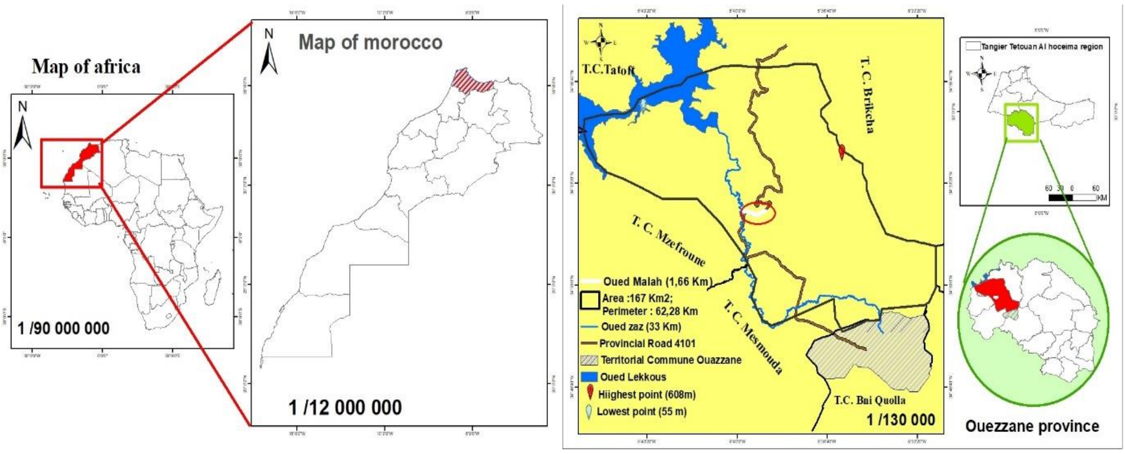

Figure 1: Geographic Origin of Halophytic Species: *Atriplex Prostrata* and *Plantago Macrorhiza* were Collected in Oued Malah (Ouezzane Region) [12]**.**

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2.2 Plant Preparation and Experiment Design

The experiment was carried out in 2.5 L pots at the Ibn Tofail University greenhouse in Kénitra. A standard substrate composed of maamoura forest soil, maritime pine leaf compost and peat was used. *Lavandula dentata* cuttings were prepared from healthy mother plants and transplanted. Plants were divided into three groups: lavender alone, lavender with *Atriplex prostrata*, and lavender with *Plantago macrorhiza*. This configuration enables us to assess the impact of salinity on lavender in different associations.

2.3 Salt Treatments

The plants received a saline treatment that involved irrigating the pots with a sodium chloride (NaCl) solution. For a month, this was carried out every 48 hours. A 100 ml standard NaCl solution was used for each irrigation. With the use of these treatments, it was hoped to evaluate how plants responded to salt stress and get a thorough understanding of how salinity affected plant physiology and morphology in both solitary cultures and in relation to halophytes.

2.4 Measured Parameters

Several parameters were measured to assess the impact of salt stress on *Lavandula dentata*, alone and in association with halophytes. Biomass was determined by measuring the fresh weight of above-ground plant parts and roots. Above-ground length was measured from the crown to the tip of the plant, while root length was measured from the crown to the tip of the longest root. Root volume was assessed by water displacement using a graduated cylinder.

Plant water status was examined by measuring leaf water content, determined by the difference between fresh and dry leaf weight. The amount of chlorophyll was measured by extracting 0.5 g of fresh leaves in 10 ml of acetone at an 80% concentration, then storing the mixture at 4 °C in darkness for a night. Following a 5-minute, 4°C centrifugation at 10,000 tr/min, the absorbance was measured at 645 and 663 nm. The chlorophyll content has been calculated using Arnon's formulas [13].

The proline concentration was assessed at 520 nm, following the protocol described by [14]. The amount of soluble sugars in the dried leaves was measured using the method described by [15].

2.5 Statistical Analysis

The results were subjected to a normality test before the two-way analysis of variance (ANOVA). Tukey's test was applied to compare the means of the variables in case of a significant F test at 5%. Additionally, these data underwent Pearson correlation analysis and Principal Component Analysis (PCA).

3. RESULTS

3.1 Biomass

The average biomass of *Lavandula dentata* alone decreases significantly with increasing NaCl concentration, from 12.13 g at 0 mM to 4.63 g at 172 mM. In contrast, the biomass of plants grown with *Atriplex prostrata* and *Plantago macrorhiza* is slightly higher at all NaCl concentrations compared to lavender alone, with *Atriplex prostrata* showing slightly better efficiency.

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At 34 mM, the length of the aerial part of *Lavandula dentata* alone is 23.6000 cm, 26.1667 cm with *Atriplex prostrata* (10.88% increase), and 24.5667 cm with *Plantago macrorhiza* (4.10% increase). At 86 mM, length is 20.9667 cm alone, 24.1667 cm with *Atriplex prostrata* (15.26% increase), and 23.4333 cm with *Plantago macrorhiza* (11.75% increase). At 172 mM, the length is 19.5000 cm alone, 21.4000 cm with *Atriplex prostrata* (9.74% increase), and 22.2000 cm with *Plantago macrorhiza* (13.85% increase) (Figure 2).

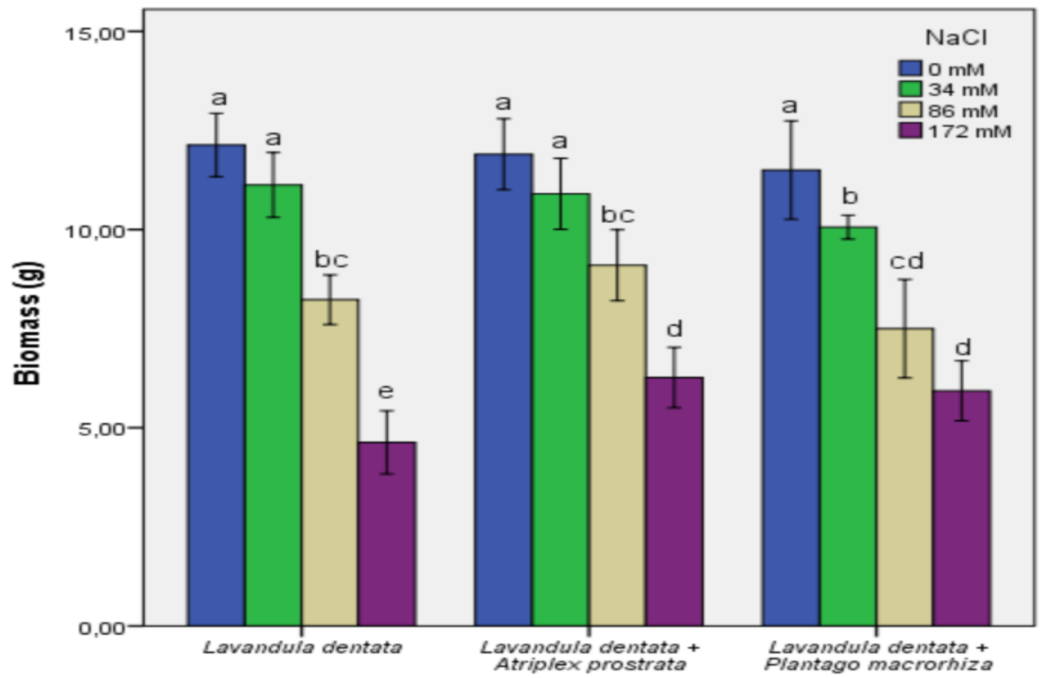

Figure 2: Effect of Salt Stress on the Biomass of *Lavandula Dentata* Alone and in Association with *Atriplex Prostrata* and *Plantago Macrorhiza*

3.2 Length of Aerial Part

The length of the aerial part of *Lavandula dentata* alone also decreases under salt stress, from 26.93 cm at 0 mM to 19.50 cm at 172 mM. However, this reduction is less pronounced when lavender is grown with *Atriplex prostrata* or *Plantago macrorhiza*, with *Atriplex prostrata* showing slightly greater lengths at all NaCl concentrations.

At 34 mM, the length of the aerial part of *Lavandula dentata* alone is 23.6000 cm, 26.1667 cm with *Atriplex prostrata* (10.88% increase), and 24.5667 cm with *Plantago macrorhiza* (4.10% increase). At 86 mM, length is 20.9667 cm alone, 24.1667 cm with *Atriplex prostrata* (15.26% increase), and 23.4333 cm with *Plantago macrorhiza* (11.75% increase). At 172 mM, the length is 19.5000 cm alone, 21.4000 cm with *Atriplex prostrata* (9.74% increase), and 22.2000 cm with *Plantago macrorhiza* (13.85% increase) (Figure 3).

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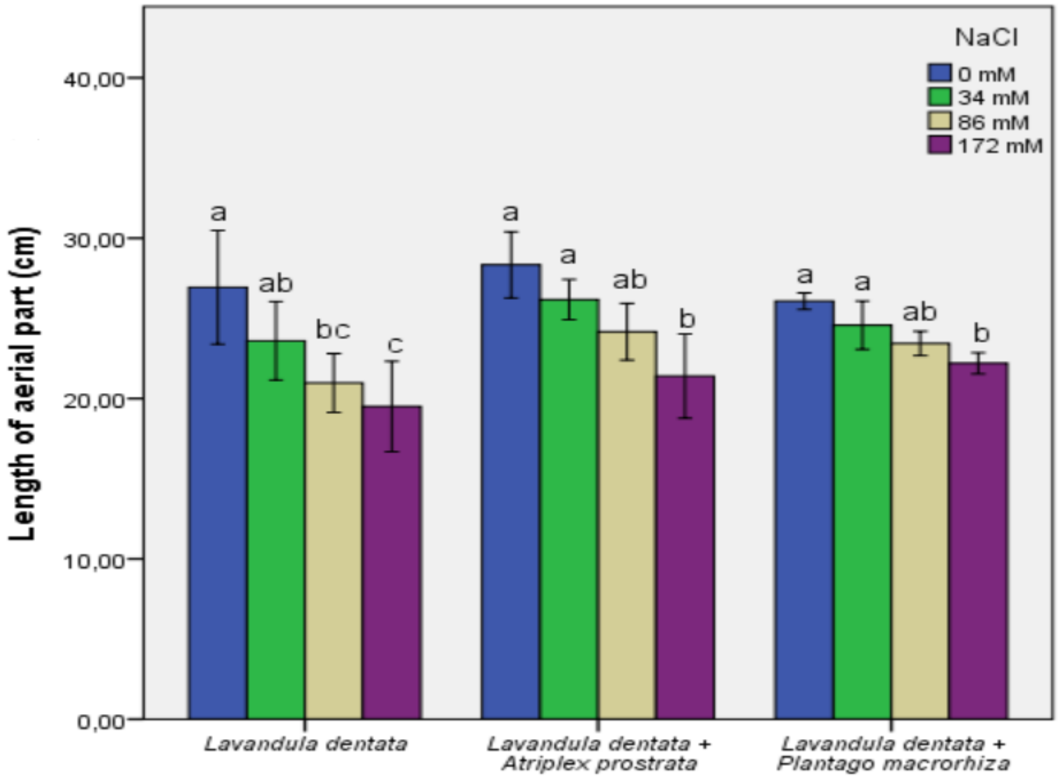

Figure 3: Effect of Salt Stress on the Length of Aerial Part of *Lavandula Dentata* alone and in Association with *Atriplex Prostrata* and *Plantago Macrorhiza*

3.3 Root Length and Root Volume

Root length and root volume of *Lavandula dentata* alone decrease with salinity. However, these parameters are significantly better when lavender is combined with halophytic plants, *Atriplex prostrata* being more effective than *Plantago macrorhiza*.

At 34 mM, the length of the root section of *Lavandula dentata* alone is 21.4000 cm, 21.8333 cm with *Atriplex prostrata* (2.02% increase), and 22.4667 cm with *Plantago macrorhiza* (4.99% increase). At 86 mM, length was 19.8000 cm alone, 22.2000 cm with *Atriplex prostrata* (12.12% increase), and 22.1000 cm with *Plantago macrorhiza* (11.62% increase). At 172 mM, the length is 18.1333 cm alone, 21.2833 cm with *Atriplex prostrata* (17.38% increase), and 21.2100 cm with *Plantago macrorhiza* (16.97% increase).

At 34 mM, the root volume of *Lavandula dentata* alone is 3.3667 ml, 5.2667 ml with *Atriplex prostrata* (56.43% increase), and 5.1333 ml with *Plantago macrorhiza* (52.51% increase). At 86 mM, the volume is 2.9500 ml alone, 4.6500 ml with *Atriplex prostrata* (57.63% increase), and 4.6167 ml with *Plantago macrorhiza* (56.52% increase). At 172 mM, the volume is 2.6000 ml alone, 3.9000 ml with *Atriplex prostrata* (50.00% increase), and 3.5000 ml with *Plantago macrorhiza* (34.62% increase) (Figure 4).

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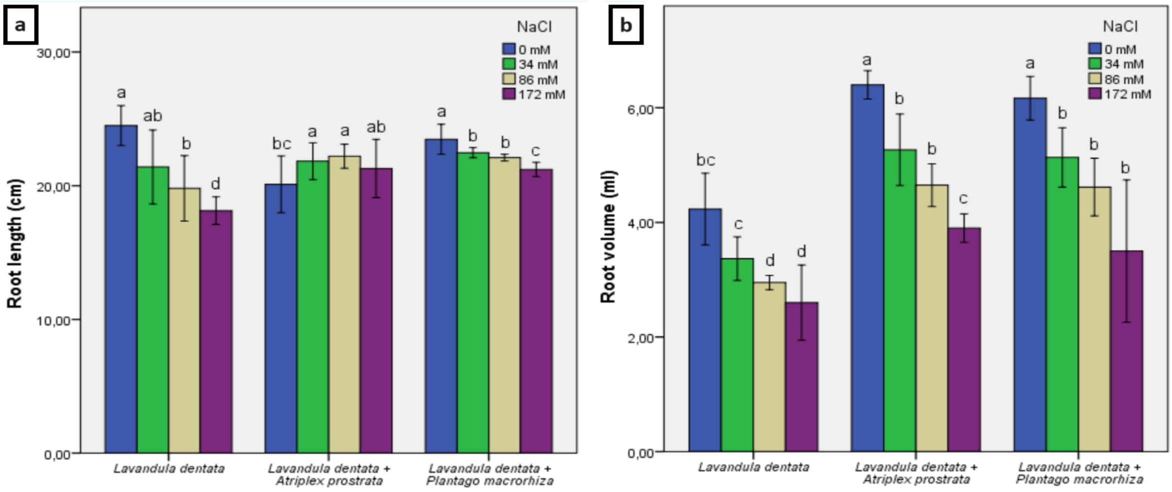

Figure 4: Effect of Salt Stress on a: Root Length part and b: Root Volume of *Lavandula Dentata* alone and in Association with *Atriplex Prostrata* and *Plantago Macrorhiza*

3.4 Leaf Water Content

We observed a significant decrease in the water content of *Lavandula dentata* leaves under salt stress. At 34 mM NaCl, the water content of *Lavandula dentata* leaves grown alone was 58.4%, while it was 65.83% in combination with *Atriplex prostrata*, an increase of 12.72%, and 64.5% in combination with *Plantago macrorhiza*, an increase of 10.46%. At 86 mM, water content was 50.53% alone, 63.07% with *Atriplex prostrata* (24.79% increase), and 62.33% with *Plantago macrorhiza* (23.37% increase). At 172 mM, water content is 40.87% alone, 61.13% with *Atriplex prostrata* (49.59% increase), and 61.5% with *Plantago macrorhiza* (50.49% increase) (Figure 5).

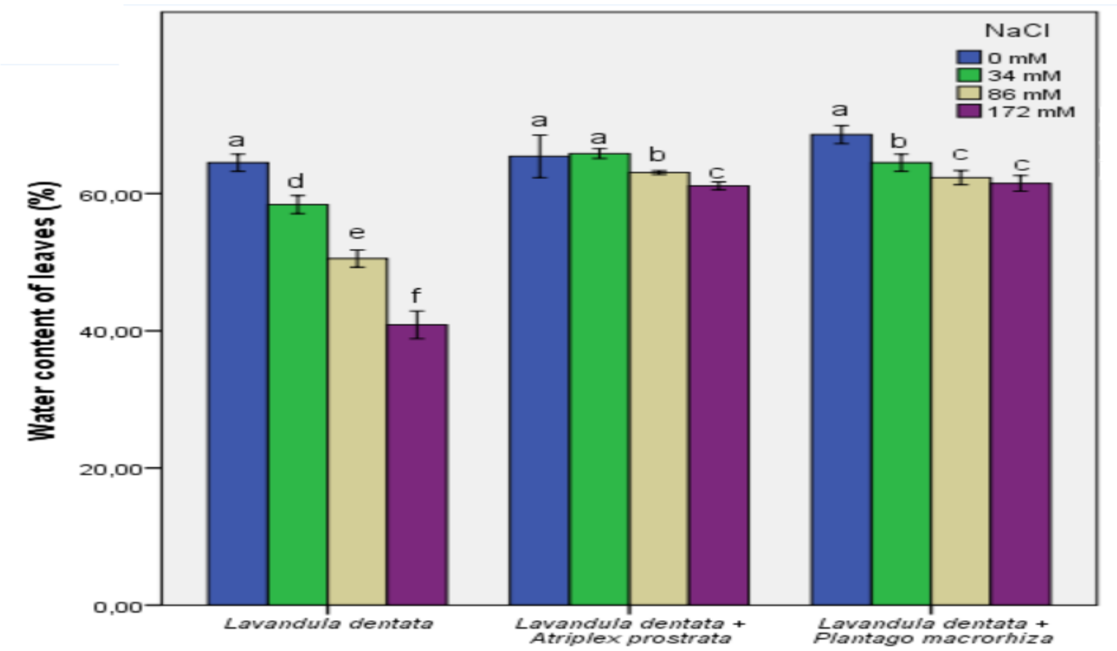

Figure 5: Effect of Salt Stress on the Leaf Water Content of *Lavandula Dentata* Alone and in Association with *Atriplex Prostrata* and *Plantago Macrorhiza*

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3.5 Chlorophyll Content

At 34 mM, the chlorophyll content of *Lavandula dentata* grown alone is 1.61 mg/g MF, while it is 1.73 mg/g MF in combination with *Atriplex prostrata,* an increase of 7.55%, and 1.82 mg/g MF in combination with *Plantago macrorhiza*, an increase of 13.05%. At 86 mM, chlorophyll content was 1.27 mg/g MF alone, 1.58 mg/g MF with *Atriplex prostrata (*24.41% increase), and 1.54 mg/g MF with *Plantago macrorhiza* (21.26% increase). At 172 mM, chlorophyll content was 0.63 mg/g MF alone, 1.48 mg/g MF with *Atriplex prostrata* (133.08% increase), and 1.48 mg/g MF with *Plantago macrorhiza* (134.15% increase) (Figure 6).

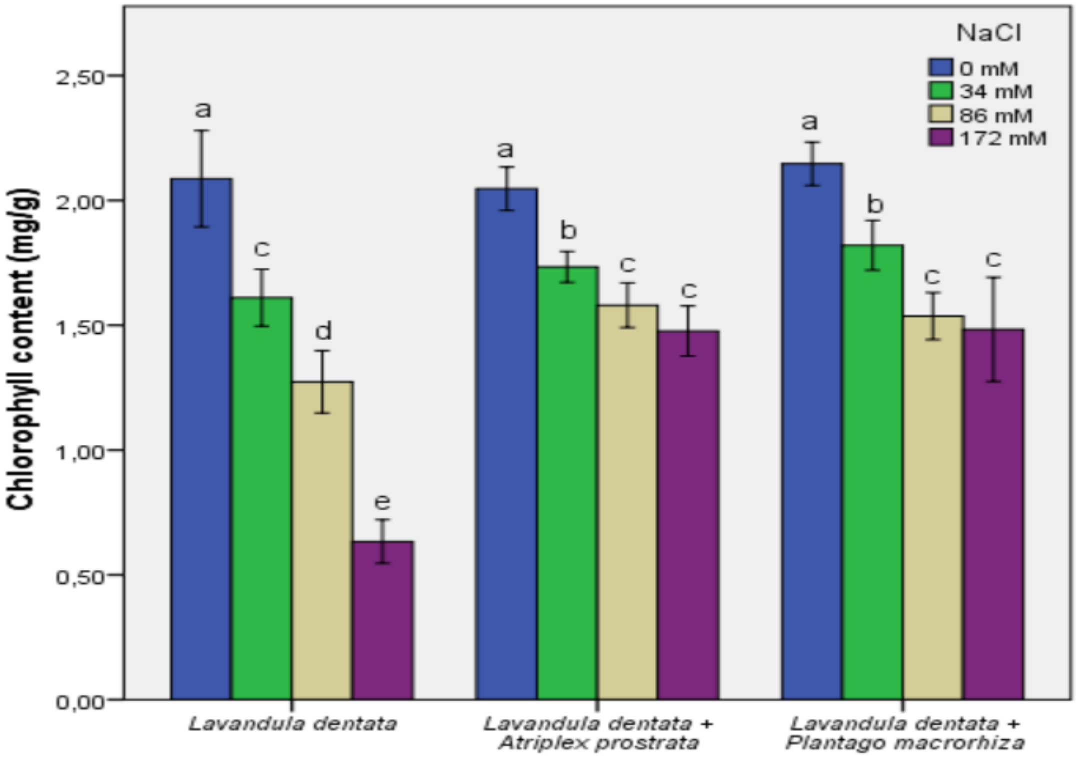

Figure 6: Effect of Salt Stress on the Chlorophyll Content of *Lavandula Dentata* Alone and in Association with *Atriplex Prostrata* and *Plantago Macrorhiza*

3.6 Proline Content

We observe a significant increase in proline content, indicating increased secretion in response to salt stress. At 34 mM NaCl, the proline content of *Lavandula dentata* grown alone is 4.11 mg/g MS, while it is 2.76 mg/g MS in combination with *Atriplex prostrata*, a reduction of 32.77%, and 2.79 mg/g MS in combination with *Plantago macrorhiza*, a reduction of 32.09%. At 86 mM, proline content was 6.81 mg/g MS alone, 5.5 mg/g MS with *Atriplex prostrata* (19.24% reduction), and 5.47 mg/g MS with *Plantago macrorhiza* (19.72% reduction). At 172 mM, proline content was 10.21 mg/g MS alone, 7.33 mg/g MS with *Atriplex prostrata* (28.19% reduction), and 7.39 mg/g MS with *Plantago macrorhiza* (27.61% reduction) (Figure 7).

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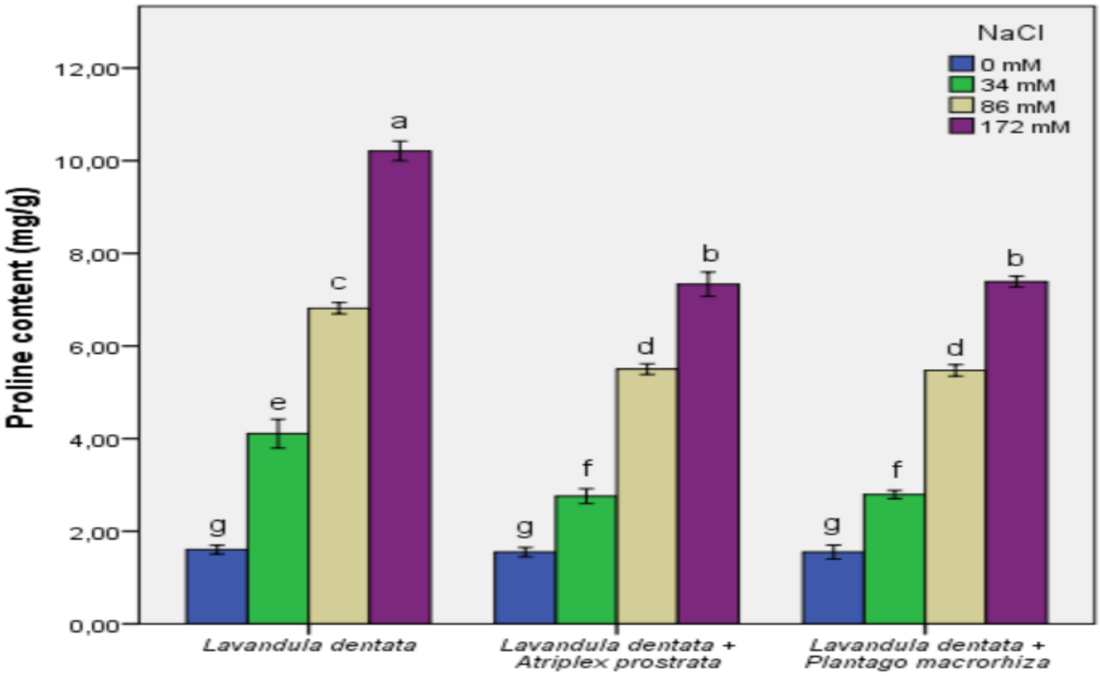

Figure 7: Effect of salt Stress on the Proline Content of *Lavandula Dentata* Alone and in Association with *Atriplex Prostrata* and *Plantago Macrorhiza*

3.7 Total Sugar Content

We observe a significant increase in total sugar content in response to salt stress. At 34 mM NaCl, the sugar content of *Lavandula dentata* grown alone is 1.22 mg/g MS, while it is 0.88 mg/g MS in combination with *Atriplex prostrata*, a reduction of 28.16%, and 1.12 mg/g MS in combination with *Plantago macrorhiza*, a reduction of 8.20%. At 86 mM, the sugar content was 3.75 mg/g MS alone, 0.98 mg/g MS with *Atriplex prostrata* (73.87% reduction), and 1.44 mg/g MS with *Plantago macrorhiza* (61.60% reduction). At 172 mM, the sugar content was 5.12 mg/g MS alone, 1.98 mg/g MS with *Atriplex prostrata* (61.44% reduction), and 1.85 mg/g MS with *Plantago macrorhiza* (63.88% reduction) (Figure 8).

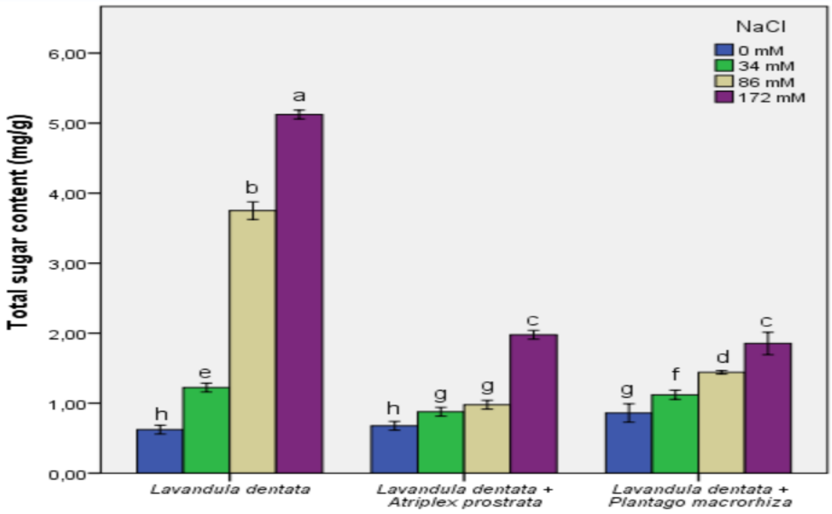

Figure 8: Effect of Salt Stress on the Proline Content of *Lavandula Dentata* Alone and in Association with *Atriplex Prostrata* and *Plantago Macrorhiza*

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3.8 Analysis of Variance (ANOVA)

Analysis of variance (ANOVA) revealed significant effects on multiple morphological and physiological parameters in the case of lavender cultivation alone and in the case of cultivation in association with the two halophytic plants under NaCl concentrations. Biomass, above-ground length, root length, root volume, leaf water content, chlorophyll content, proline content and total sugar content all showed significant variations influenced by both plant species and salt stress levels, as well as their interaction. These observations highlight the differentiated responses of *Lavandula dentata* when grown alone and in association with *Atriplex prostrata* and *Plantago macrorhiza* to salt stress, indicating potential avenues for enhancing the plants' ability to cope with saline environments (Table 1).

Table 1: Analysis of Variance (ANOVA) of for the Parameters Studied

Source		df	Mean square	F-value	P-value
Cultivation in association with *Atriplex prostrata* and *Plantago macrorhiza* (A)	Biomass	2	1,940	15,837	0,000
	Length of aerial part	2	15,548	23,361	0,000
	Root length	2	5,802	14,037	0,000
	Root volume	2	11,231	216,503	0,000
	Leaf water content	2	439,306	1297,376	0,000
	Chlorophyll content	2	,432	185,208	0,000
	Proline content	2	7,734	1667,163	0,000
	Total sugar content	2	8,607	7042,445	0,000
NaCl (B)	Biomass	3	68,778	561,605	0,000
	Length of aerial part	3	61,147	91,874	0,000
	Root length	3	9,754	23,597	0,000
	Root volume	3	8,163	157,353	0,000
	Leaf water content	3	232,440	686,452	0,000
	Chlorophyll content	3	1,311	561,959	0,000
	Proline content	3	79,602	17159,748	0,000
	Total sugar content	3	9,387	7680,397	0,000
(A) * (B)	Biomass	6	1,158	9,457	0,000
	Length of aerial part	6	2,540	3,816	0,008
	Root length	6	8,723	21,102	0,000
	Root volume	6	,184	3,541	0,012
	Leaf water content	6	62,801	185,468	0,000
	Chlorophyll content	6	,136	58,338	0,000
	Proline content	6	1,310	282,296	0,000
	Total sugar content	6	2,820	2307,197	0,000

3.9 Correlation Analysis

The correlation in Table 2 reveals significant associations among several studied parameters. Biomass shows positive and significant correlations with aerial part length ($r = 0.856$, $p < 0.01$), root length ($r = 0.567$, $p < 0.01$), and root volume ($r = 0.663$, $p < 0.01$), highlighting their interdependence in overall plant growth. Similarly, leaf water content exhibits strong positive correlations with chlorophyll content ($r = 0.910$, $p < 0.01$) and negative correlations with proline ($r = -0.802$, $p < 0.01$), indicating complex relationships between leaf hydration and cellular physiology under stress.

Furthermore, total sugar content is strongly negatively correlated with leaf water content ($r = -0.957$, $p < 0.01$) and positively correlated with biomass ($r = 0.745$, $p < 0.01$), suggesting a link between sugar accumulation and overall plant growth. Lastly, the strong inverse correlation between proline and biomass ($r = -0.948$, $p < 0.01$) indicates a potential role of proline in stress response affecting plant growth.

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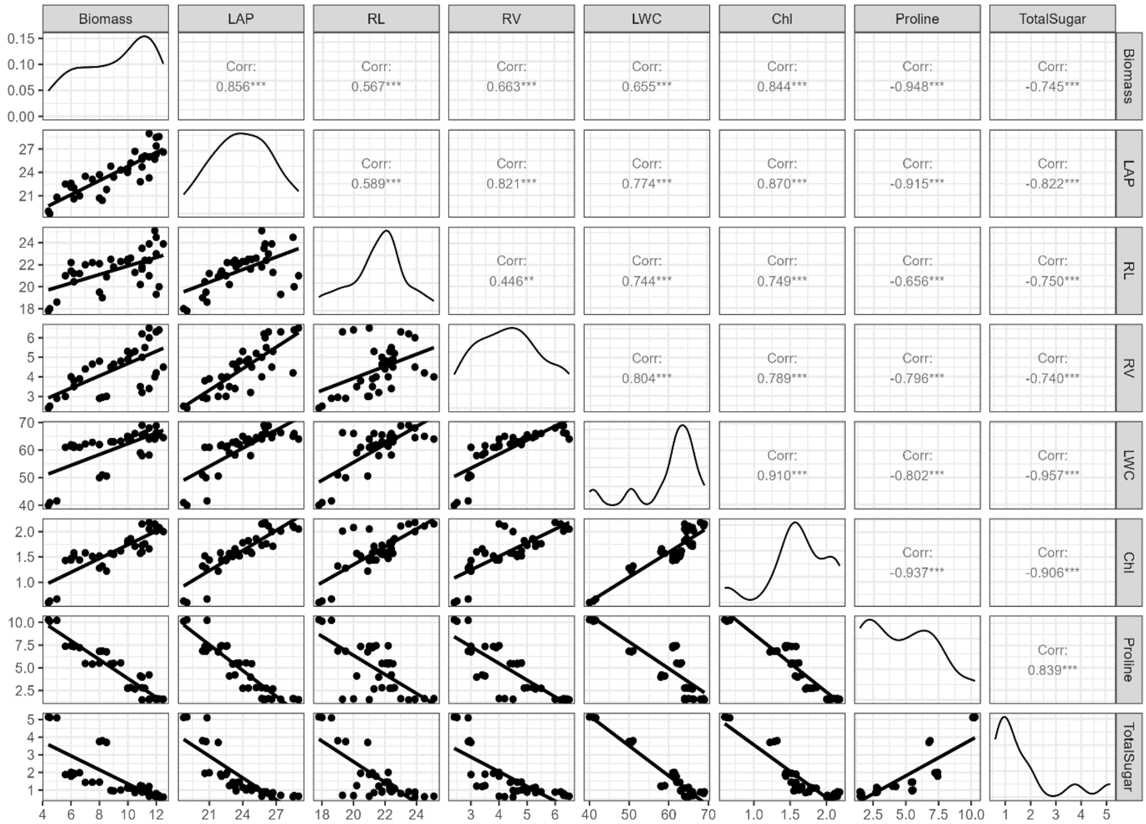
These results underscore the complex interrelationships among the various physiological parameters studied, highlighting their importance in understanding plant adaptive responses to diverse environmental conditions, particularly saline stress.

Table 2: Correlation Analysis of Parameters Studied in *Lavandula Dentata* under Saline Stress



3.10 Principal Component Analysis

Principal component analysis shows that the first principal component (Prin1) explains 81.4% of the total variance, mainly influenced by biomass, length of aerial part, and chlorophyll content. The second principal component (Prin2) explains 8.2% of the variance, mainly influenced by biomass, length of aerial part, and leaf water content. The results of principal component analysis (PCA) indicate that *Lavandula dentata*, when associated with the halophytes *Atriplex prostrata* and *Plantago macrorhiza*, shows significant correlations between various physiological parameters. The main observations show strong positive correlations between biomass and above-ground length, chlorophyll content and leaf water content, between above-ground length and biomass, root volume, chlorophyll and leaf water content, and between root length and above-ground length, root volume and leaf water content. These results suggest that associations with halophytes improve the performance of *Lavandula dentata* under salt stress, by increasing growth parameters such as biomass, length of aerial part, and chlorophyll content. Interactions with halophytes can therefore potentially improve plant resilience under saline conditions (Figure 9).

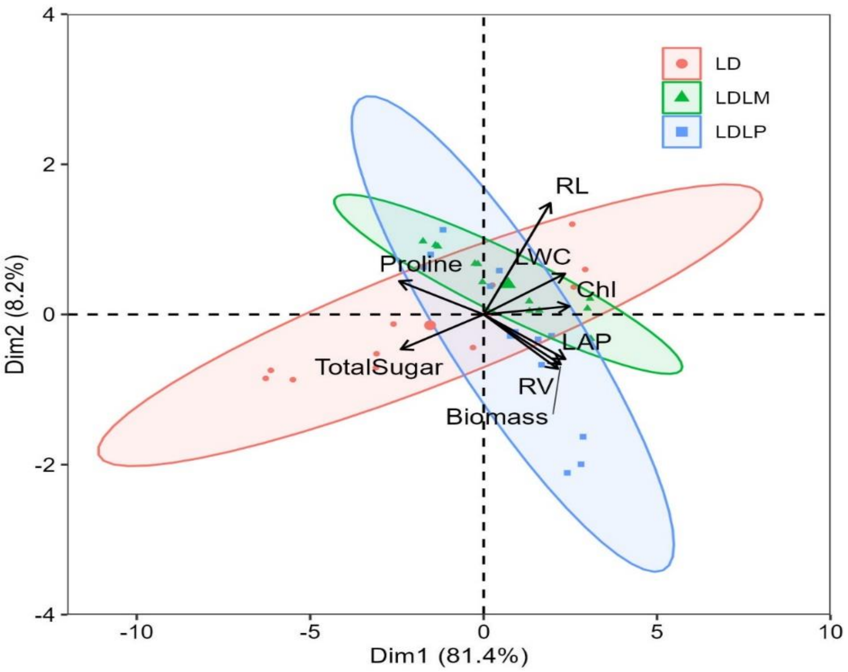


Figure 9: Principal Component Analysis of Parameters Studied in *Lavandula Dentata* under Saline Stress Alone and with *Atriplex Prostrata* and *Plantago macrorhiza*

4. DISCUSSION

To understand how *Lavandula dentata* responds to salinity, this study examines a range of morphological, physiological, and biochemical parameters under the impact of sodium chloride (NaCl). Plants are known to adjust their growth and physiology in response to environmental stresses, and salinity is particularly critical in arid and coastal areas where it can severely compromise the growth and survival of plant species. By exploring these responses, we aim to better understand the adaptive strategies of *Lavandula dentata* to this environmental challenge. Additionally, the use of halophytic plants like *Atriplex halimus* and *Plantago maritima* in association with lavender could provide complementary strategies to enhance the salinity tolerance of *Lavandula dentata*. These halophytic species are known for their ability to thrive in high saline conditions due to specific physiological and biochemical mechanisms that could benefit the survival and growth of lavender in similar environments.

The results of our study, as well as those of other researchers such as Zouaoui et al. (2018), and Dubey and Singh (1999), show that the growth of *Lavandula dentata* is significantly affected by salinity [16, 17]. This reduction in growth, according to Xiong and Zhu (2002) and several other authors is a key adaptive response of plants to saline conditions, although the effects on plant height may vary depending on salt concentrations [18]. Studies indicated that prolonged exposure to salinity leads to a decrease in aboveground plant parts, resulting from disruption in cell division and elongation. Additionally, salinity negatively affects root development, as observed by Tavili and Biniaz (2009), where significant reductions in root length and dry matter production are noted under saline stress [19]. These effects are supported by studies demonstrating a decrease in root system volume and overall inhibition of glycophyte plant growth in response to osmotic stress, involving complex mechanisms including alterations in cell structure, hormonal metabolism, and membrane stability. Thus, plant adaptations to salinity reflect a series of crucial physiological and biochemical responses essential for their survival in fluctuating saline environments.

According to Brugnoli and Lauteri (1991), water stress induced by salinity primarily affects photosynthesis by causing stomatal closure, while relatively preserving chloroplast reactions as long as other plant processes are not severely affected [20]. Our study confirmed that salinity has a negative effect on the physiological parameters of lavender when cultivated alone. However, when lavender is grown in association with halophytic plants such as *Atriplex* and *Plantago*, the effects of saline stress on these parameters are amplified. We observed a significant increase in water and chlorophyll content in this context, illustrating the beneficial role of halophytic plants in reducing the effects of saline stress on lavender. Consistent with our findings, Zhang et al. (2021) have highlighted a decrease in SPAD values (chlorophyll) with increasing saline concentration [21].

Halophytes, due to their unique ability to thrive in saline environments, offer promising opportunities for the rehabilitation and exploitation of saline lands, which are essential for food security, theray and environmental preservation [21,22]. Our results clearly demonstrate the significant impact of halophytic plants such as *Atriplex* and *Plantago* on the biochemical parameters of lavender cultivated under saline stress. When cultivated alone, lavender showed a marked increase in proline and soluble sugars content in response to saline stress, indicating an adaptation to mitigate osmotic stress effects. However, when lavender was cultivated with halophytic plants, the levels of proline and sugars were comparatively lower. This observation suggests that halophytic plants, while enhancing saline stress tolerance in this context, also regulate the levels of these crucial biochemical compounds. Thus, association with halophytic plants could be a promising strategy to optimize lavender cultivation in saline soils by beneficially modulating the biochemical responses necessary for resilience to environmental stresses.

5. CONCLUSION

This study examined the effects of salt stress on *Lavandula dentata*, both alone and in association with the halophytes *Atriplex prostrata* and *Plantago macrorhiza*. The results show that salt stress induced by NaCl significantly affects various physiological parameters of *Lavandula dentata*, such as biomass, length of the aerial part, root length, root volume, leaf water content, chlorophyll content, proline content, and total sugar content. The correlation analysis revealed significant relationships between these parameters, while the principal component analysis (PCA) identified key components influencing the total variance. In particular, the association with *Atriplex prostrata* and *Plantago macrorhiza* showed beneficial effects, improving the resilience of *Lavandula dentata* to salt stress.

These results highlight the potential of *Lavandula dentata* associations with halophyte plants to develop adaptation and sustainable management strategies for crops in saline environments. Integrating these associations into agricultural practices could offer promising solutions for growing plants under increased salinity conditions, thus contributing to more resilient and sustainable agriculture.

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