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Mechanism of Salinity Tolerance in Oleaster and Cultivated Olive Tree: Physiological and Morphological Characterization of the Foliar System

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Authors' contributions

This work was carried out in collaboration between all authors. Author HA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author SD managed the literature searches, analyses of the study performed the spectroscopy analysis. Authors KM and BF managed the experimental process and Authors HB and MB identified the species of plant. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The olive tree has a positive impact on the environment and help to the conservation of the landscapes. Better adapted to aridity conditions around the Mediterranean, it is a fastening element for the rural population. Oleaster (the wild olive

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tree) represents a valuable phyto-resource for the improvement of the olive trees varieties. This work focuses on determining the physiological and morphological response of the foliar system in cultivated olive tree (variety Sigoise) and Oleaster in condition of different salinity level of the irrigation water (by increasing NaCl concentration).
Methodology: The experiment took place in a greenhouse with a well controlled condition. The experimental plan is in a complete randomization of two factors (salinity and species) and three salinity levels (treatments); plants without NaCl application were used as control. The plant material (young 2-year-old olive seedlings) was selected and brought from a crop nursery. The experimentation started by the irrigation of the control plant with a nutrient solution. About the other treatments, the tree seedlings received a nutrient solution fortified by the addition of the NaCl with increasing doses of 75,100 and 150mM of the NaCl. Four repetitions are being done according to the field capacity.
Results and Conclusion: The study covered the variation of the relative water content in leaves as well as the leaf surface, stomata density, the wax rate on the upper foliar epidermis and their internal anatomy. According to the achieved results, the relative water content of the treated plants decreased when compared to the control plants. The same was found for leave surface and stomata density. On the other hand, the wax rate increased in the salt stress situations compared to the control plant and to the bulbous form cells' number and the wood vessels.

Keywords: Olive tree; salt stress; relative water contain; wax rate; internal anatomy.

1. INTRODUCTION

The process involved in drawing up the crop's yield is influenced not only by genetic factors but also by environmental factors [1]. Thus, the behavior of the plants is under a continuous stress effect of an osmotic and ionic type [2,3]. The recorded salinisation of the arid and semi-arid ecosystems is the result of a high evaporation of water from the soil [4] and of a regular and an insufficient rainfall [5]. This salinisation stems also from an inadequately controlled irrigation [6]. Every year the losses surfaces due to soil salinity are considered to be in the range of twenty million hectares in the world.

The agricultural spaces affected by the salinity in the world will be three hundred and forty million, around 23% of all the cultivated land [7].

Therefore, these changes require a comprehensive analysis on strategies that will be taken, in order to understand the mechanisms developed by the plants to be adapted to new environmental conditions and to maintain their growth and their productivity [8]. Indeed, according to the salinity degree in the area, the glycophyte are exposed to a change of their morpho-physiological behavior [6] and biochemical [9].

In the face of this constraint, vigorous plants trigger mechanisms of resistance including the activity and the morphology of the foliar organs. In fact, they play a significant role of the plant resistance to the salt stress. Tolerance, in case of a reduction of the water losses by the plant, is expressed via the maintenance of the turgor thanks to a reduction of the transpiration [10,11]. This phenomenon appears today as a major mechanism of adaptation to ionic and osmotic stress which is expressed by the capacity of a plant in maintaining its water status at such level that permits it a normal metabolism [12]. This respect, the study of the plant attitude to face the salinity is extremely important to streamline the interventions that target the improvement of its level of water. Olive *(Olea europaea* L.) is an important perennial crop in many agricultural regions of the Mediterranean countries. By adaptation to arid conditions, it has a positive impact on the environment and the economy of the region [13]. When is arranged as terrace (a bench), it helps to reduce erosion and loss of soil fertility [14,15].

In this context, our work aimed at studying the physico-morphologic and anatomical behavior of two olive genotypes conducted under an increasing NaCl supply. Our analysis deals with the variations of the relative water content in the leaves of plants then their surfaces, their stomatadensity, their wax rate and their internal structure. We opted for the olive tree because of its high feeding value and that contains the highest rate of the unsaturated fatty acids "Oméga 3".

2. MATERIALS AND METHODS

2.1 Experimental Device and Plants

The experiment was conducted in a greenhouse with a daytime temperature of 18ºC and nocturnal of 10ºC, the relative humidity of the air was 70% and the photoperiod of 10-12 h.

The plant material was composed of a genotype of a self-sown olive tree called Oleaster [wild *Olea europea* L.] and a genotype of a cultivated olive tree [*Olea europea* var Sigoise]. The plantlets used in the experiment were 8-month-old and were obtained by cutting rooted under nebulization'(crop nursery). The initial growing substrate was replaced and the roots of the olive plants were placed into a vinyl polychloride (VPC) cylinders (sixty centimeters long, and twenty centimeters diameter) filled with a mixture of soil made by sand, and soil and an organic matter at proportions of four volumes from sand / one volume from soil and one volume from manure or an organic matter.

The experimental design was completely randomized by four repetitions. For each repetition, three treatments had been applied (75, 100 and 150 mM). Some plantlets subjected to 0mM were used as control. The induction of the salt stress was achieved by adding NaCl to the nutrient solution. Each plantlet received a standardized and a balanced nutrient solution as Hoagland and Arnon (1938) [16].

2.2. The Work Methodology

2.2.1 The relative water content (RWC)

It's determined by method of Barrs and Weatherley (1962) according by the formula of Clarck and McCaig [17].

After excising the leaf, the initial fresh weight (ifw) was determinated. Then, the leaf introduced into a test tube containing of distilled water. The unit is placed at the darkness at 4ºC during 12 hours. The leaves were weighed again (weight in full turgidity, wft). The dry weight (dw) is obtained by drying in the oven during 48 hours at 80ºC. The relative content water of the leaves is estimated by the equation:

$$RWC\ (\%) = [\ ifw - dw\ /\ wft - dw]\ .\ 100$$

- **Ifw**: initial fresh weight;
- **dw**: dry weight;
- **wft**: weight at full turgidity.

2.2.2 The leaf area

The leaf area is directly measured using an electronic planimeter(cm^2) (7).

2.2.3 The stomata density

The stomata density is evaluated on the lower and the upper surface in the central portion of the penultimate leaf that was dusted off and, after removing the epidermal hairs by applying an adhesive tape, thin layer of a clear nail varnish was applied. After two minutes, we removed this varnish layer with the stomata print by using another diaphanous adhesive tape, then spreading it on the microscope slide which has been beforehand washed and dried, and we observed it using an optical microscope in order to determine the number and the size of the stomata.

2.2.4 The internal structure of the leaf

That operated on limiting the water loss were assessed by performing an anatomic cut of the leaves and the histological study was carried out by the method of [18]. Fragments with a length of two centimeters taken from young leaves were placed in a fixative based on alcohol, and formalin, and acetic acid during twenty-four hours. Then the samples were washed and dehydrated with increasing doses of the ethanol (50º, 70º and 100º).

We went ahead with an impregnation of the samples: toluene then “paraffin-toluene” (V/V) during four hours at 60ºC before including them in a pure and warm paraffin. The use of the microtome (type LEICA RM 2145) allowed to obtains lices of 12 µm thicknesses. They placed onto a slide in gelatin water then colored and fixed. The achieved measures and observations using a microscope fitted with an ocular micrometer ZEISS type, were focused on the xylem vessels and the bulliform cells.

2.3 Statistical Analysis

The obtained data were processed by Statistica Software Version 2.0, by analyzing the variance and the correlation matrix. The obtained values were statistically averaged (four repetitions) with a confidence interval calculated at the threshold of 5%.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1 The relative water content

The above statistical results are seen more significant (Table.1), at the bit error rate threshold of 5%, of the salinity effect on the relative water content (RWC). Indeed, this content decreased from 83.26% (control plant) to 48.25% (treatment at 150 mM of NaCl) for the cultivated genotype Sigoise and it decreased from 75.93% (control plant) to 46.58%

(treatment at 150 mM of NaCl) for the self-sown genotype (Oleaster). Moreover, we noticed that the self-sown genotype showed a lower RWC than the cultivated genotype (Fig.1a).

Table 1. Analysis of the variance of the relative water content of the olive trees genotype

Variable	Varietal Effect (F1)	Saline effect (F2)	Interaction effect (F1* F2)
RWC	0.000	0.000	0.330

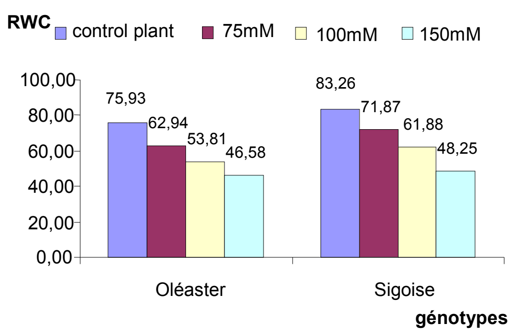
3.1.2. The leaf area

The salt stress reduced the leaf area and increased the stomata density. Our results were highly significant (p=0%) as well as the “genotype” effect and the interaction «salinity* genotype» (p=0%) (Table 2).

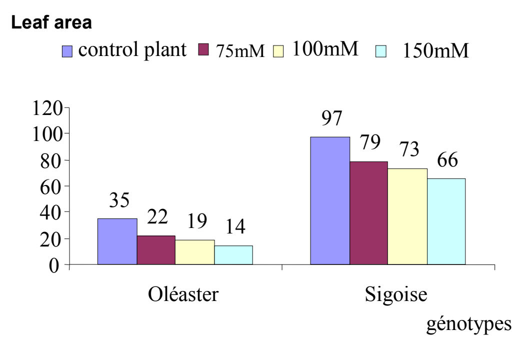
Table2. Analysis of the variance of the studied foliar parameters of the olive trees genotypes

Variable	Varietal Effect (F1)	Saline effect (F2)	Interaction effect (F1* F2)
Leaf area / cm^2	0.004	0.000	0.000
Stomata density /mm^2	0.000	0.000	0.010

The leaf area regression operated with the sense of the increasing salinity which is 75mM to 150mM of NaCl. The leaf area noticed on the control plantlets was 35mm^2 then it falled to 14mm^2 in the leaves of the plants treated with 150 mM of NaCl in Oleaster. The same was seen for the cultivated olive trees, the leaf area was 97mm^2 in the control plantlets then it falled to 66mm^2 in the plantlets treated with 150 mM of NaCl, (Fig. 1b).



(a)



(b)

Fig. 1. Relative water content (a) and the leaf area (mm^2) (b) as measured from the penultimate leaf of the studied plantlets

3.1.3. The stomata density of lower surface of the leaf

The same effect (Table 2) was seen for the impact of salinity on the stomata density in particular on the leaves of the plants that received an equal or higher salinity to100 mM of NaCl. Indeed, the later brought an important variation of the stomata numbers/ mm^2 with forty-six stomata/mm^2 (control plantlets) against thirty-one stomata/mm^2 (treatment with 150 mM of NaCl). For the self-sown genotype, and for the cultivated genotype the values are sixty stomata/mm^2 (control plant) against forty-five stomata/mm^2 (treatment with 150 mM of NaCl. (Fig. 2a).

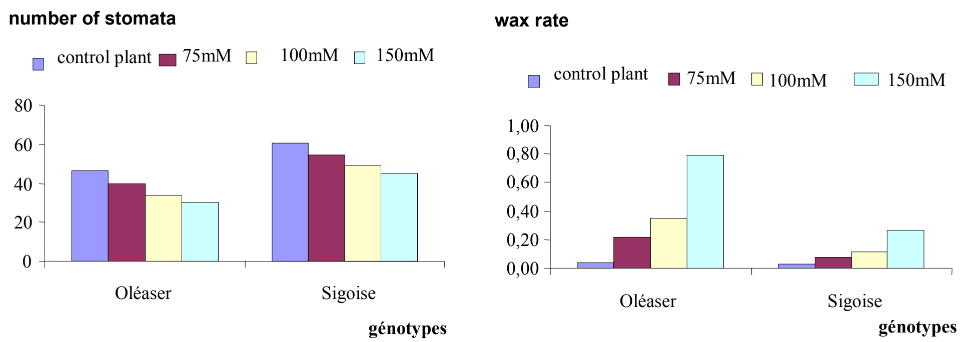


Fig 2. The stomata density (/mm^2) of lower surface of the leaves (a) and the wax rate of the upper surface of the leaves

3.1.4. The number of the bulliforms cells

According to the obtained analysis (p=2%), (Table 3) the reaction of the plants followed the same tendency about the expression of this parameter. As well as its elaboration that intensified with the acuteness of the salinity degree. In this context it appeared that the salt stress induced a limited water supply, which was interpreted by the density transformation and the volume of this epidemic cells type.

Table 3. The variance analysis of the foliar anatomic parameters

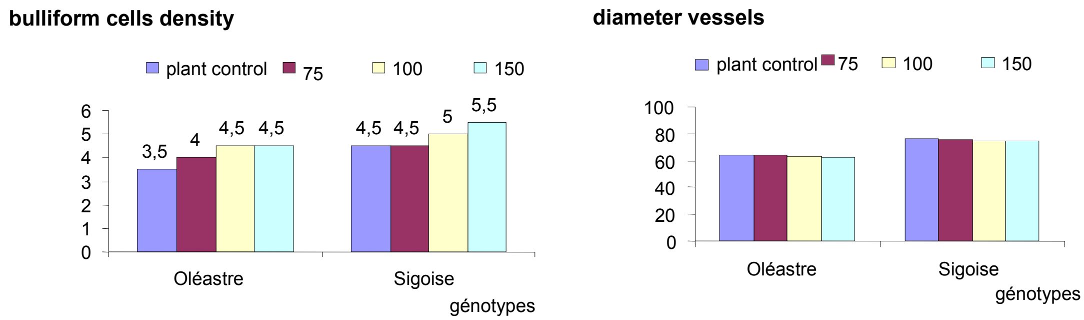
Variable	Varietal Effect (F1)	Saline effect (F2)	Interaction effect (F1* F2)
Bulliform cells density (mm^{-2})	0.025	0.002	0.152
Diameter vessels (µm)	0.105	0.005	0.045

Therefore, the introduced constraint went in one way with a substantial increase of their number per site at the level of the leaf lower surface (Fig. 3a). The achieved measures (Table 3), showed that the density of the bulliform cells at the level of the leaf reached a minimal average value estimated in 4.5±1.01 at the level of the conducted treatment without salt, while the irrigation with the salty water (150mM of NaCl,) produced increases of this estimated number to 6.00±1.00 and this is for the two genotypes of an effective increase of 21.73% at the scale of the conducted treatments of the control plantlets to 150 mM of NaCl. (Fig. 3a).

3.1.5. The diameter of the xylem vessels

The Table 3, shows significant results of the salinity at this parameter. Indeed, we noticed that plantlets subjected to the treatment with 150 mM of NaCl, showed a thin diameter of 62.13µm. At the level of the control plantlets, the thickness was 63.96µm in Oleaster. For the Sigoise variety, the tendency was the same but the values were very high with a diameter of 74.88µm in150 mM of NaCl treatment and of 76.38µm in the control plantlets (Fig. 3b).

It should be noted that the increase of salinity level reduced the xylematic vessel diameter. The reduction was of the 10% for Oleaster and of the 18% for the cultivated genotype.



(a) **(b)**

Fig 3. Density of the bulliform cells (a) and diameter Vessels (b) in the leaves

3.2. DISCUSSION

In the control plantlets, the water availability was higher than in the treatment with 150mM of NaCl. Thus the salinity action reflected in the reduction of the available water for the plant, due to the increase of the osmotic pressure subtract. This decrease caused a turgor loss. Indeed, the high negative correlation obtained between the salinity and the stomata density (r=0.781) means that an increase of the NaCl rate leads to a reduction of the stomata number and consequently reduce water losses and increasing the RWC, (Table 4) [19].

Table 4. Correlation effect between salinity and the foliar morphological parameters (P =5%)

	Salinity	RWC	Leaf area	Number of stomata	Wax rate
Salinity	1				
RWC	-0.370*	1			
Leaf area	-0.281ns	-0.570**	1		
Number of stomata	-0.781**	-0.441**	0.650**	1	
The wax rate	0.366*	-0.767**	-0.601**	-0.250 ns	1

*Significant * : p < 0.05; more significant ** : p < 0.01; no significant or " ns" : p > 0.05*

The study of the leaf area reduction and the increase of the stomata density demonstrates that this last increase is relative. These results are in line with those of Acevedo (1991) [20] showing that the reduction in leaf area, limits the perspiration. Those transformations permit to the leaf to preserve water [21] which promotes an optimum physiological functioning. This reaction seems obvious because of the water content tissues depletion resulted from a variations of the osmotic substrate potential due to a high salt content corresponding to the stomata closure that tend to inhibit the stomata perspiration [22]. It is obvious that in the case of our experimentation, if the basic nutrition formula was constituted by the ideal balance for those varieties, the area increase should not appear and the increase curve would have been decreasing exponentially.

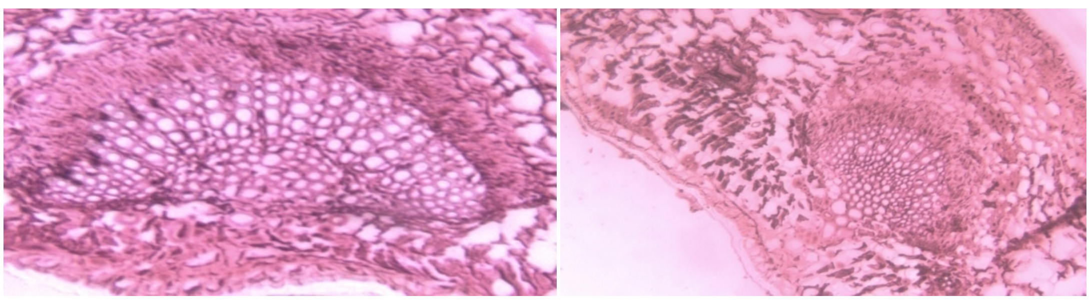
The structural parameters of the used leaf in this study should be closely involved in the water regulation of the foliar tissues. The obtained results indicate that the salt stress leads to a water stress that is accompanied by an increase of the bulliform cells number of the ventral side (r=-0.770**). (Table 5). The bulliform cells dry out and become narrower and causing a leaf curl thus exposing a lower leaf area to the air, and reducing its perspiration. The multiplication of those cells constitutes a parameter of the reference adaptation. The anatomical study of the leaf demonstrates that the characteristic related to the xylematic vessel diameter undergo to a negative correlation with the number of the bulliform cells (r = -0.452**).

Table 5. Correlation effect between salinity and the foliar morphological parameters (P=5%)

	Salinity	Xylem diameter (µm)	Number of bulliform cells
Salinity	1.000		
Xylem diameter (µm)	0.452**	1.000	
Number of the bulliform cells	0.770**	0.335*	1.000

*Significant * : p < 0.05; more significant ** : p < 0.01; no significant or " ns" : p > 0.05*

In contrast, a xylematic diameter reduction that allowed the maintaince of an optimum state of turgidity thus a moderate water loss and helps to maintain a high water potential (Fig. 4).



(a) **(b)**

Fig 4. Diameter of the xylematic vessels of the cultivated olive tree's leaves in salt stress situation (a) and of the self sown studied plants (b)
(*Photo Seddiki and Hassani, 2012***)**

However, the effect is clearly attenuated on those anatomic parameters whenever the plants are irrigated by a non-saline solution. In this case, structural transformations consist probably of the reduction of the protoplasmic content sensitive to dehydration. Such transformations intervene in intensifying the sap conduction and in adjusting the absorption capacities related to the substrate moisture characterized by the high salt concentrations. In arid climate and in ionic stress situation accompanied by the osmotic stress, the plant must maintain a dynamic balance between the opening and the closure of the stomatato increase the carbon fixation and allowing transpiration to avoid the heating of the leaf. In Mediterranean regions, the physiological drought (due to excess salinity) is often chronic, thus a reduction in the photosynthesis results [23].

Genetic variations in salt tolerance exist, and the degree of salt tolerance varies with plant species and varieties. In the last two decades sumptuous amount of research has been done in order to understand the mechanism of salt tolerance in plant [24]. For improving salinity tolerance, genetic transformation technology enables scientists to achieve gene transfer in precise and predictable manner for maintaining protein structure and functions among the genetic engineering strategies [25].

4. CONCLUSION

Salinity tolerance involves a complex of responses at metabolic, physiological, morphological and whole-plant level. The fundamental research, done under controlled conditions, relating to some studied physiological and morphological parameters (the relative water content, the leaf area, the stomata density, the wax rate, the foliar structural parameters) allowed us to achieve the following results: the studied parameters, had undergone a decrease during the period of restraint except the wax rate that had increased.

Concerning the cultivated olive variety (Sigoise), the salt restraint has begotten an important decrease at the level of the studied physiological parameters. While at the level of the self-sown olive variety (Oleaster), the decrease is less acute. Concerning the structural foliar parameters, this difference of the physiological behavior shows that the self-sown variety is more adapted to salinity than the cultivated one.

To respond to the saline constraint, the two olive varieties have reacted with a decrease of the RWC. This decrease is accompanied by a closure of the stomata in order to limit the water loss via transpiration**.**

The stomata closure diminishes the gaseous exchange between the leaf and its surrounding environment where a decline in return and consequently a decline of the photosynthesis activity and of the catalytic capacity of the carboxylation can be observed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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