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Effect of the exposure period to different water salt levels on the morphological behavior of olive plants

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Abstract— *The increasing salinity of water and soil is one of the environmental factors that most threatens the sustainability of olive cultivation systems in the Mediterranean basin. The identification of plant material with high tolerance to this stress would be one of the ways to solve this challenge, but it is generally a slow and expensive process. The selection of the most reliably parameters involved in the response of the plant to salinity and that are easy to evaluate, could help to speed up the identification of the most tolerant genotypes. The objective of this study is to determine the most interesting morphological characters which could be used in future as early criteria in the selection process of olive tolerant genotypes. For that, young plants, three-month-old, were exposed to salinity (0, 4 and 8 g/L NaCl) during different periods of time (30, 50 and 70 days), and several morphological parameters were assessed. The obtained results showed that most of the parameters were affected by the concentration 8 g/L of NaCl after a treatment period of 50 days. The most affected parameters by this level of salinity were the plant height, the leaf number and the number of lateral shoots. The PCA analysis showed that the number of lateral shoots was poorly correlated with the other parameters, but the height and the leaf number were highly correlated with each other.*

Keywords— **Salt stress, Olea europaea, early selection, salt tolerance, olive growth parameters.**

I. INTRODUCTION

The olive tree, producing the famous olive oil known for its very beneficial chemical properties to human health, is one of the oldest crops in the Mediterranean region (Maazoun et al., 2021). The importance of the olive tree in this region is not only economic, but also social and environmental, due to its great ability to adapt to the semi-arid and arid climate and its ability to use efficiently the few available resources (Hammami and Ben Mimoun, 2015).

Since one of the most limited resources in the area of olive cultivation is water (Milano et al., 2013), several studies proposed the use of brackish water to maintain this crop and improve its productivity (Melgar et al., 2009, Chartzoulakis and Bertaki, 2015). The olive tree is known for its moderate tolerance to salinity, but it presents a great varietal diversity that could serve as a reserve for the selection of tolerant genotypes among the existing varieties or through genetic improvement. The selection of tolerant varieties is a laborious, long and expensive process due to the difficulties related to the evaluation of many parameters in a large number of plants (Hammami et al., 2011).

One of the solutions proposed to solve this impediment is to determine the parameters that are the best indicator of sensitivity to saline stress (that is, the first and the most ones that are affected by this stress) (Bernstein et al., 2001; Soltabayeva, 2021). The number of parameters to be evaluated can also be reduced, limiting the use of those that are not correlated with each other (Hammami et al., 2012). However, there is little information on the time sensitivity of morphological parameters affected in the olive tree upon salt stress. On the other hand, the level and speed of the response of the olive tree to salinity also depends on the dose of salt in the water and the duration of exposure to this stress (Goreta et al., 2007; Melgar et al., 2009). Indeed, it is also important to determine the dose to use on young olive trees to observe a rapid response without causing irreversible damage to the plants.

The objective of the present work is to identify the most relevant morphological parameters which could be used in future as an early marker in the a selection process for salt tolerance.

II. MATERIAL AND METHODS

2.1. Plant material and experiment design

The trial was conducted on a pool of young olive plants (three-month-old) of different cultivars, which have a very similar initial height. These plants were re-potted in polyethylene pots with a volume of 5 liters containing a mixture of silty-clay soil and peat (2:1, v/v). They were grown in a greenhouse and irrigated at full capacity once or twice a week for 70 days. For the design of the experiment, a randomized complete block with twenty replicates and three blocks (sixty plants per treatment) was installed. The plants were irrigated with three concentrations of salt water: 0, 4 and 8 g/L NaCl, as a salt treatment.

2.2. *Morphological parameters assessment*

In order to study the impact of irrigation with brackish water at different concentrations, on the growth of young olive plants, several morphological parameters were assessed: plant height, node number of the main stem, length of the internodes (ratio of plant height/node number), diameter of the main stem (measured at 5 cm from the ground), number of leaves and number of lateral shoots. These measurements were recorded at 30, 50 and 70 days after irrigation with different salt water concentrations.

2.3. Statistical analysis

Analysis of variance and the Tukey's test at $P< 0.01$ were used to assess differences between treatments. The main component analysis was performed to assess the relationships between morphological parameters. All statistical analyzes were performed using *Statistix 8.0* and the *Unscrambler v 9.7*.

III. RESULTS

Results showed that most of the vegetative parameters were affected by the concentration of 8g/L NaCl, and at very lesser degree by 4g/L NaCl upon 50 days of treatment. Toxicity symptoms were also observed in most of the plants irrigated with the highest dose of salt. However, no death of treated plants has been recorded. These results indicated that the use of 8g/L seems to be efficient to cause saline stress without causing irreversible damage to young olive plants, when applied for 50 to 70 days.

The height of the plant has been used in different works as an efficient criterion of selection for earliness of production and drought tolerance in improvement programs (Gashaw et al., 2007; Hammami et al., 2021). The evaluation of these parameters in young olive plants exposed to various levels of water salinity during different periods showed that the first differences between the control and the treated plants were observed 50 days after the beginning of the trial (Fig. 1). At this period, the high salinity level (8g/L NaCl) significantly reduced plant height, by approximately 23%, while the moderate salinity level had no significant effect. Upon three weeks later, the differences became clearer and more significant for the two salinity treatments, but at different levels. Indeed, compared to control, plant height decreased with 15% by 4g/L NaCl and with 32% by 8g/L NaCl (Fig. 1). The negative effect of irrigation water salinity on plant height has been observed in other plant species (Heuer and Nadler, 1995; Dolatabadian et al., 2011). These results indicated that plant height is sensitive to stress and could serve as a criterion for evaluating the impact of this factor.

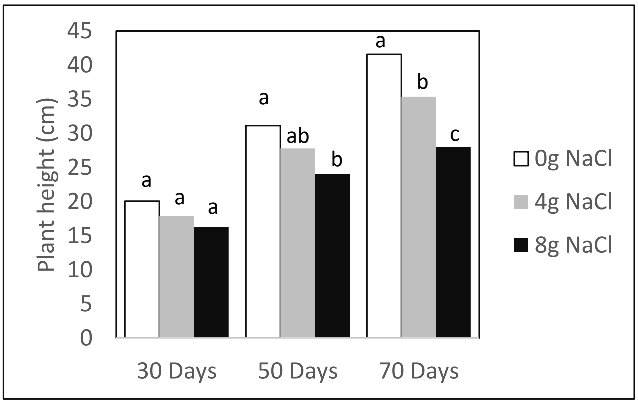

Fig. 1: Plant height for different water salinity concentrations and recorded during different dates after the beginning of the treatments. Different letters indicate significant difference among salt treatments (0, 4 and 8 g/L NaCl) for the same period at P<0.01.

The stem diameter represents the secondary growth in plants (Niklas, 1993). In different studies carried out on olive trees and other species, it has been shown that the diameter of the trunk or the main axis was generally sensitive to abiotic stress (Moriana and Fereres, 2002; Kant et al., 2002; Ohashi et al., 2006;). The obtained results showed a negative effect of water salinity on this parameter only with the high concentration of 8g/L of NaCl and at the end of the experiment (i.e., at 70 days) (Fig. 2). Despite this result, the stem diameter seemed to be less sensitive to salt stress than primary growth represented by plant height.

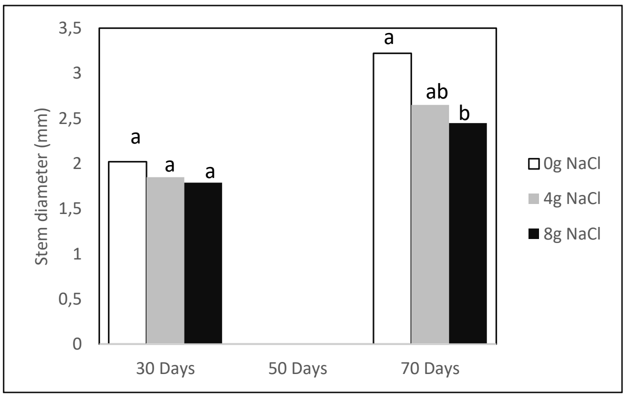

Fig. 2: Stem diameter for different water salinity concentrations and recorded during different dates after the beginning of the treatments. Different letters indicate significant difference among salt treatments (0, 4 and 8 g/L NaCl) for the same period at P<0.01.

The number of main axis nodes showed a relatively similar behavior to that observed for the diameter of the stem. After one month of irrigation, the number of nodes formed was very similar among all the treatments. In the two following evaluation periods, 50 and 70 days, only the 8g/L NaCl concentration was able to significantly reduce the number of nodes with approximately 24% (Fig. 3). The negative impact of salt stress on the number of nodes has been also shown in other olive cultivars (Perica et al., 2003).

The elongation of the internodes is considered an energetically costly process for the plant (Anandan et al., 2012). In different species, even in olive trees, it has been seen that this parameter was sensitive to salt stress (Wang et al., 1997; Khorasaninejad et al., 2010; Kchaou et al., 2010; Alizadeh et al., 2011). Our observations indicated a relatively low sensitivity of this parameter to salt stress compared to the other vegetative parameters. In fact, a negative effect of salinity on the length of the internode was only seen at the end of the test with a concentration of 8g/L of NaCl and with a small reduction of 12% (Fig. 4). These results confirm that this parameter could not be considered as a useful early selection criteria, since it is difficult to be measured and have a low sensitivity.

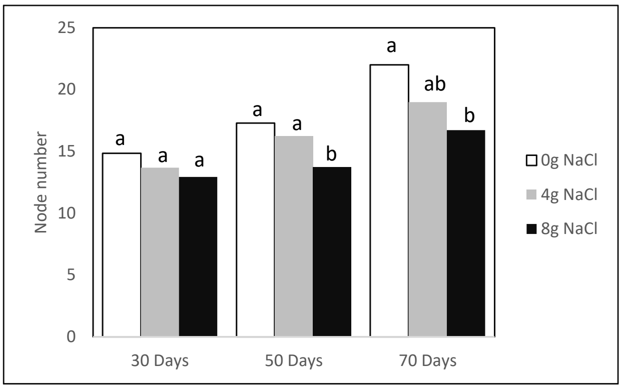

Fig. 3: Node number of main stem for different water salinity concentrations and recorded during different dates after the beginning of the treatment. Different letters indicate significant difference among salt treatments (0, 4 and 8 g/L NaCl) for the same period at P<0.01.

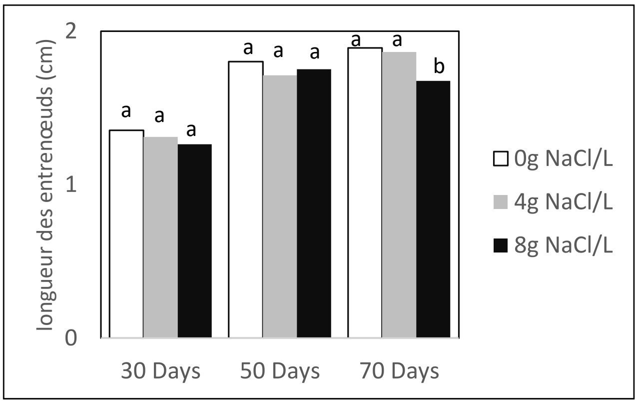

Fig. 4: Internode length of main stem for different water salinity concentrations and recorded during different dates after the beginning of the treatment. Different letters indicate significant difference among salt treatments (0, 4 and 8 g/L NaCl) for the same period at P<0.01.

One month of irrigation with saline water of 8g/L NaCl has decreased the number of leaves significantly by approximately 15%, compared to the control. This decrease has worsened over time to reach 36% at the end of the trial (Fig. 5). The negative impact of salinity on the number of leaves can be caused by growth inhibition but also by leaf drop (Chartzoulakis et al., 2002).

Like the number of leaves, the number of lateral shoots was affected only one month after watering the plants with 8g of NaCl, and it worsened with time. The reduction was the most notable of all the parameters, reaching 55% compared to the control (Fig. 6). This parameter appears to be the most sensitive to salinity. Branching is closely related to the physiological state of the axillary buds

(Leyser, 2009). It is likely that salt stress negatively affects this state and consequently its ability to sprout.

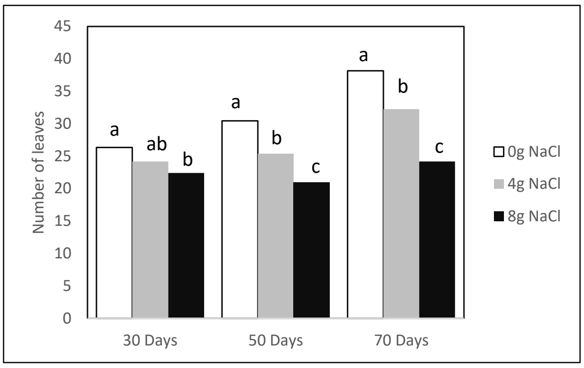

Fig. 5: Number of leaves of main stem for different water salinity concentrations and recorded during different dates after the beginning of the treatment. Different letters indicate significant difference among salt treatments (0, 4 and 8 g/L NaCl) for the same period at P<0.01.

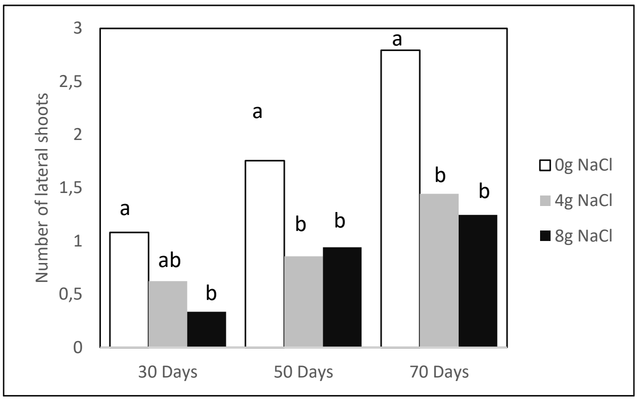

Fig. 6: Number of lateral shoots for different water salinity concentrations and recorded during different dates after the beginning of the treatment. Different letters indicate significant difference among salt treatments (0, 4 and 8 g/L NaCl) for the same period at P<0.01.

The PCA analysis of the correlations showed three groups of parameters highly correlated with each other. The first group is composed of the height, number of nodes and leaves, the second contains the length of the internodes and the diameter of the axis, while the number of lateral shoots is separated from them in group three (Fig. 7). Based on this analysis, we can recommend keeping the evaluation of the height by the first group and the diameter of the stem in the second group, because they are the easiest parameters to measure, in addition to keeping the number of lateral shoots. The high correlation observed between height and the other growth parameters has also been observed in olive seedlings previously (Hammami et al., 2011).

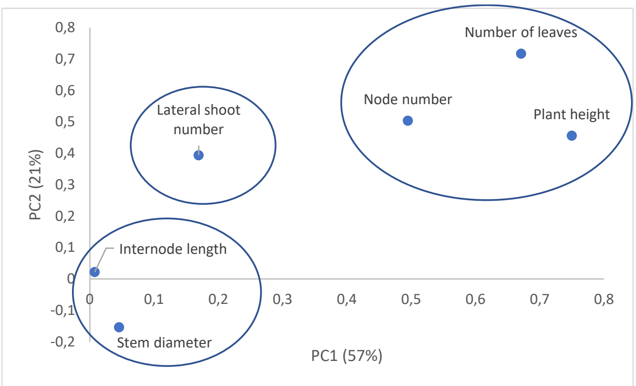

Fig. 7: Principal analysis of component for the evaluated growth parameters of young olive plants growing under different salt stress conditions (0, 4 and 8 g/L NaCl).

IV. CONCLUSION

In conclusion, the plant height, the number of leaves and of lateral shoots were the most sensitive parameters to salinity. Based on the PCA analysis, we can discard the number of leaves that is highly correlated with the plant height, and kept only the plant height parameter, the easy one to determine, as a marker of selection. Consequently, we can propose the plant height and the number of lateral shoots as criteria for evaluating the sensitivity of young olive plants to salinity and use the dose of 8g/l NaCl for a period of 50 days. However, comparing them with more parameters and in other plant ages and crop conditions would be necessary to check and confirm the efficiency of these parameters.

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