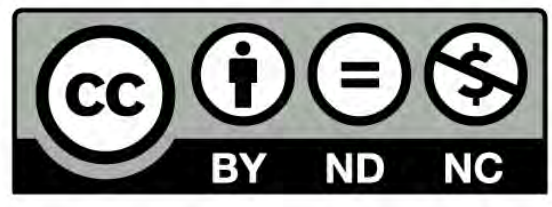
High School Students for Agricultural Science Research

Volume 12 **October 2023**

ISSN: 2340-9746

Published in Granada by Estación Experimental del Zaidín. CSIC



Resilience of olive tree cultivars to intensive salt stress

Lima-Cabello E.[1], de Torres S.[2], Torrellas E.[2], Medina-Correa C.[2], León I.[2], Bejarano P.[2], Rueda-Guzmán E.[2], de la Fuente E.[2], Soto-Pascual A.[2], Salas-Sánchez M.[1], Gázquez, E[1], Castro A.J. [1] & Alché, J.D.[1#]

[1]Plant Reproductive Biology and Advanced Imaging Laboratory (BReMAP). Department of Stress, Development and Signaling in Plants. Estación Experimental del Zaidín. CSIC. Profesor Albareda 1, 18008 Granada, Spain.

[2]IES Jándula, San Vicente Paul 32, 23740 Andújar, Jaén, Spain.

#Corresponding author: juandedios.alche@eez.csic.es

Summary

One of the most important crops in Spain and Mediterranean countries, due to its evolution, extension and social impact, is the olive tree: a plant very well adapted to arid and semi-arid areas. In recent years, increasingly extreme weather conditions, together with high temperatures and lack of water, are increasing salinity levels in numerous aquifers. Although the olive tree is considered tolerant to salt and drought, it is advisable to increase our knowledge and the possibility of selecting the most salt-tolerant varieties. In this work, we studied the effects of continuous irrigation with saline water containing different concentrations (0, 100 and 200 mM) of NaCl on a small selection of young olive trees of varieties with different tolerance degree to salinity, over a period of two months. We analyzed morphological, physico-chemical and biochemical markers on these varieties, and we discussed here the utility of these markers to potentially identify the salinity resistance of the different olive varieties.

Keywords: antioxidant capacity, ions, lipid peroxidation, olive, sodium chloride, total antioxidant capacity, varieties.

INTRODUCTION

Climate change is generating extreme situations where the increase in temperature and lack of precipitation have led to an accelerated soil salinization [1]. Salinity can be caused by natural reasons (e.g. weathering of rocks) or by anthropogenic causes (e.g. excessive use of fertilizers or overexploitation of aquifers). High salinization causes numerous economic losses, and directly influences agricultural production and the environment [2]. The forecast of the evolution of this problem is not promising, and that is why the search for solutions that help us mitigate its effects is urgent.

Salinization can generate various effects on cultivated plants since this process has an impact on almost all stages of the life cycle, making their growth, development and reproduction difficult [3-5]. Thus, salinity has a negative impact on the phenology, physiology, biochemistry, transcriptomics and microbiota of crops.

Roots are considered the most vulnerable part of a plant faced with a salt stress situation since they are in direct contact with the salts in the soil. However, salinity also heavily affects photosynthetic organs and that is why we have selected leaves for the development of the present project.

Salinity affects numerous physiological markers. Thus, conductivity becomes seriously altered in the presence of salinity, as ionic balance (particularly as regard to Na^{2+}, K^{+} and Cl^{-}). High levels of Na+ in plant tissues end up interfering and destabilizing enzymatic activities and protein structures by interfering with their surface charges [6]. Also, salt toxicity will cause osmotic stress, producing reactive oxygen species (ROS) that will damage the lipid membrane, distort protein structures and increase the development of certain cytotoxic metabolites such as methylglyoxal or malondialdehyde (MDA) [7]. Most of these markers have been assessed in the present work.

The olive tree is considered a species tolerant to salt and drought [8, 9]. The olive tree habitat includes calcareous soils, thus available Ca^{2+} can limit the toxicity of Na^{+} on the plasma membrane and prevent the transport of Cl^{-} and Na^{+} to sensitive leaves and shoots [10]. Differential resistance to salinity has been described in different olive varieties [11, 12]. Varieties that are more tolerant to salinity include Arbequina, Frantoio and Picual, whereas more sensitive varieties include Leccino and Pajarero. An early response or osmotic phase has been described in the olive tree, which includes a decrease in the water potential and turgor of the plant, which produces an ABA-dependent stomatal closure in the leaves and a drop in photosynthesis (decrease in photosynthetic pigments) due to NaCl. Subsequently, a late phase of ionic toxicity develops, in which negative effects are seen in the leaves due to the accumulation of Na^{+} and Cl^{-} [9, 13], which can lead to severe defoliation.

MATERIALS AND METHODS

Olive cultivars analyzed. Commercial young olive trees (3-4 years old) were purchased from "viveros Silvia" (Guadalcázar, Córdoba). The following varieties were analyzed:

-Changlot Real (though to be highly resistant to salinity)

-Picual (though to be moderately resistant to salinity)

-Arbequina (though to be moderately resistant to salinity)

-Cobrançosa (though to be sensitive to salinity)

-Pico-Limón (undetermined resistance to salinity)

-Cornezuelo (undetermined resistance to salinity)

The degrees of sensitivity to salinity indicated above correspond to previously reported evaluations.

Plants were acclimatized for one month (February 2023) prior to the beginning of the stress experiments using a standard watering rate with Hoagland modified medium. Then, three olive tree samples of each variety were used as a control and were maintained with the standard watering rate of Hoagland modified medium (#cultivar# C), whereas three olive tree samples of each variety were subjected to watering with Hoagland medium supplemented with either 100 mM NaCl (#cultivar# C1) or 200 mM NaCl (#cultivar# C2), following the same watering rate

in all cases (2 L/week in two separate watering days). Such treatments were maintained for two months (March/April 2023).

Determination of morphological and growth parameters. At the beginning of the experiment and 46 days after treatment, for each plant, the basal diameter, total height, number of lateral shoots, total length of lateral shoots, lateral shoots (after removing the leaves) and the stems (main axis) were measured.

Physico-chemical parameters. Conductivity, pH, and K^+ and NO_3 concentrations were determined in olive leaf crude extracts of each sample after two months of treatment by using the corresponding LAQUAtwin electrochemical measuring devices (HORIBA Scientific) previously calibrated with the standard solutions provided.

Biochemical assays

Total antioxidant activity

Total antioxidant capacity was measured using the "Total antioxidant Capacity Assay" kit (ref. MAK187-1KT, Sigma-Aldrich), following the manufacturer's instructions. First, a standard curve was generated from the Trolox standard solution. In the case of leaf samples, 0.25 g was mixed with 500 µL of the "Protein mask" solution from the kit and the mixture was homogenized in a vortex. Finally, 100 µL of each standard and sample were mixed with 100 µL of the Cu^{2+} working solution previously prepared according to the manufacturer's instructions. The mixtures were incubated for 90 min at room temperature in the dark in a 96-well plate and, finally, the absorbance was measured at 570 nm in an "iMark Microplate Absorbance Reader" (Bio-Rad). To determine the antioxidant concentration of each sample/standard, the following calculation was performed: C= Sa/Sv, where C is the concentration of total antioxidant in the sample (nM/µL or equivalents of Trolox mM), Sa is the unknown concentration of Trolox (nM) of each of the samples and points of the standard curve, and Sv is the volume of sample added to the wells.

Lipid peroxidation assays

This marker was determined using the "Lipid Peroxidation" kit (ref. KB03002, BQC). First, a standard curve was generated with the standard solution from the kit itself. Next, 100 µL of the samples/standards were mixed with 325 µL of solution A and 75 µL of reagent B from the kit. After incubating the mixture for 40 min at 40ºC, the absorbance was measured at 586 nm in a plate reader. The content of MDA + HNE (µM) was calculated using the following formula: MDA + HNE= (As- intercept)/slope. Slope and intercept values are obtained from the standard curve.

RESULTS & DISCUSSION

Effects of salt stress on stem length

As an example of the different morphological parameters analyzed, Figure 1 shows the changes in the length of the main stem of the different varieties and treatments over six periods of watering with different levels of salinity. A first approach shows that some varieties display longer stems (e.g. Picual, Pico-Limón and Cobrançosa) than others (e.g. Arbequina and

Changlot real) as a typical feature of these varieties. Most cultivars showed some degree of growth even after five watering periods and even in the presence of saline conditions, with the exception of Pico-Limón and Cornezuelo, which experimented nearly zero growth. These differences, although noticeable, cannot be considered statistically significant due to both the low number of samples and the limited growth of the stems, as the period of analysis was very short.

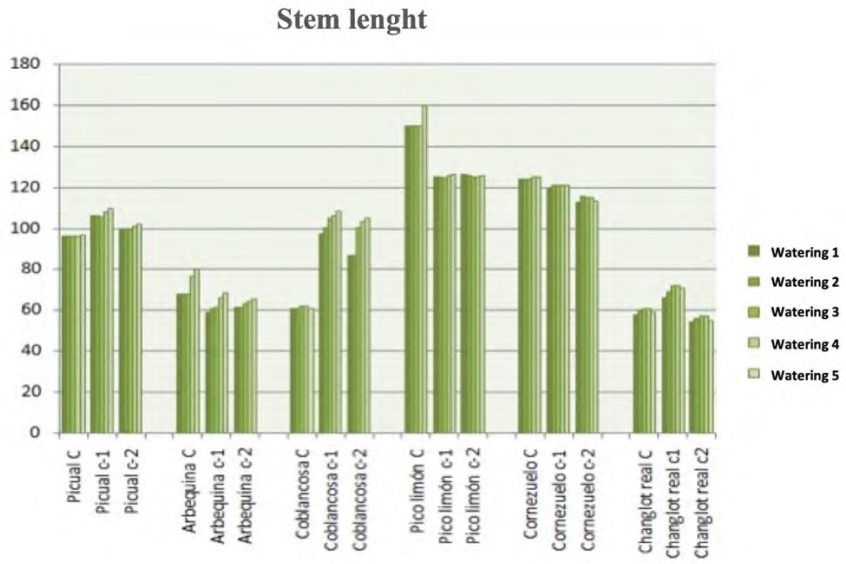


Figure 1. Stem length of the olive trees subjected to the treatments over 6 watering periods (6 weeks).

Effects of salinity over conductivity

Conductivity analysis of the samples corresponding to olive leaf crude extracts is shown in Figure 2. All olive leaf extracts analyzed after two months of treatment with NaCl presented higher conductivity than the control samples. Moreover, and with the exception of Cobrançosa, olive varieties treated with 200 mM NaCl showed higher conductivity than those treated with 100 mM NaCl.

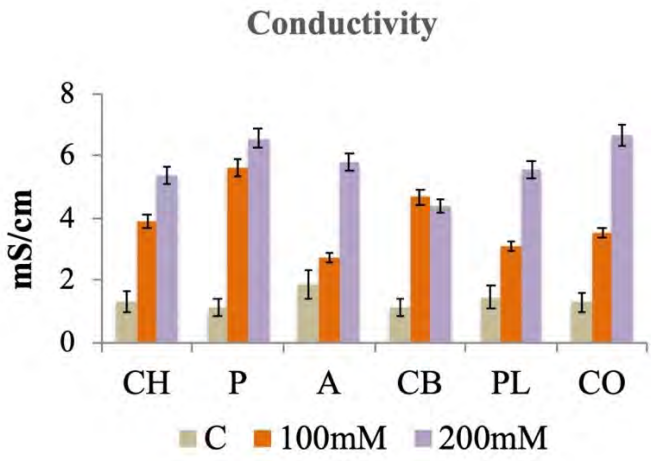


Figure 2. Effect of salinity over leaf extract conductivity. CH: Changlot real; P: Picual; A: Arbequina; CB: Cobrançosa; PL: Pico-Limón; CO: Cornezuelo.

The conductivity values observed here can be extrapolated to field cultivation. For example, in

a field study, a high concentration of salt was observed in the substrate at the end of the summer period, with conductivity values of up to 11.7 dS/m in the most superficial layer (0 -30 cm) of the soil, as a result of continued irrigation with saline water during the summer months [14]. Likewise, [15] observed that the use of saline water with a conductivity of 7.5 dS/m for irrigation induces a significant increase in salinity in the soil in a field study with the Chemlali olive tree variety. However, contrary to what happens in pot trials, the toxic effect of salt in field conditions is dependent on the climatic conditions of the crop. For example, the high conductivity values observed by [14] at the end of the summer period decreased significantly after autumn, due to the leaching process of soil salts by the rainwater that fell during that period.

Effects of salinity on pH

Figure 3 shows the pH of the leaf extracts corresponding to the different samples analyzed. No significant differences in the pH were detected within a given variety, independently of the salt treatment applied. Slight differences of pH were detected in some varieties, compared to other varieties, which were significant particularly in the case of Arbequina.

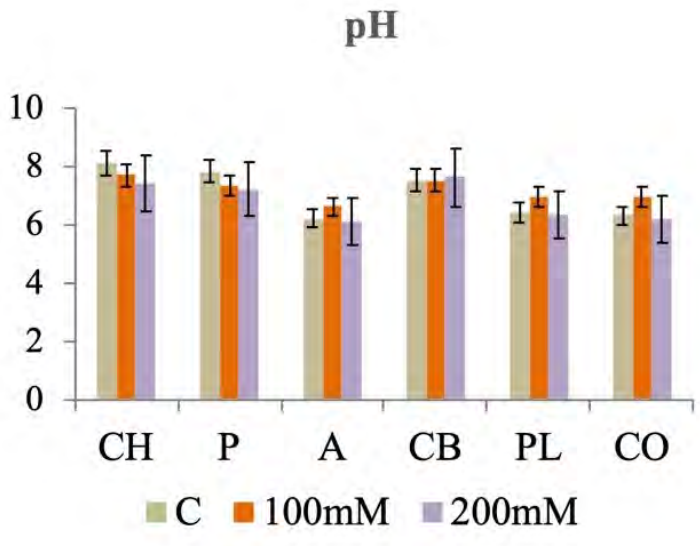


Figure 3. Effect of salinity on pH. CH: Changlot real; P: Picual; A: Arbequina; CB: Cobrançosa; PL: Pico-Limón; CO: Cornezuelo.

Effects of salinity on the presence of K+

Figure 4 shows the content in K^+ of the leaf extracts corresponding to the different samples analyzed. No common trends were detected among the varieties, with some of them significantly increasing K+ concentration in the samples subjected to salt watering (e.g. Changlot real and Cobrançosa), whereas in other varieties the opposite trend was detected (e.g. Picual, Pico-Limón or Cornezuelo).

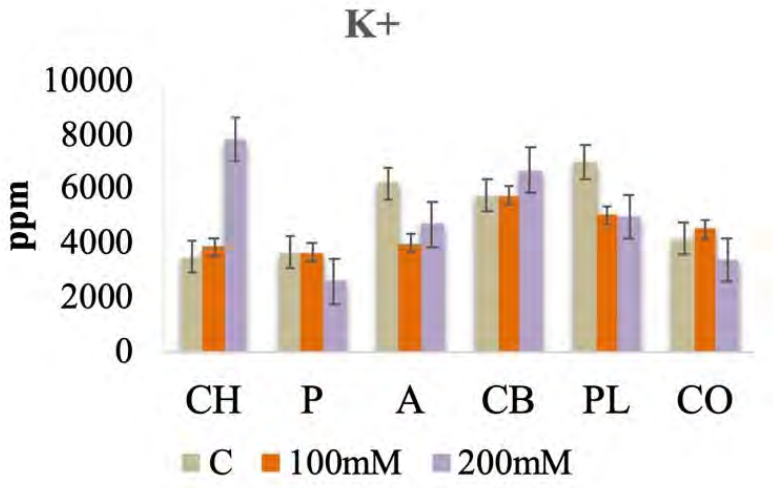


Figure 4. Effect of salinity on K+ concentration. CH: Changlot real; P: Picual; A: Arbequina; CB: Cobrançosa; PL: Pico-Limón; CO: Cornezuelo.

Under salt stress conditions, Na+ absorption competes with the K+ ion. This generates an increase of Na+ in the cytoplasm instead of the accumulation of Cl- inside the cell. The increase in Na+ generates, in turn, a decrease in the absorption of K+, Mg2+ and Ca2+, therefore affecting the K+/Na+ ratio [16]. For this reason, we believe that in future studies, we will need to determine Na+ ion as well, and then analyze the K+/Na+ ratio in order to produce a better and clearer picture of ion interchanges.

Effects of salinity on the concentration of NO_3^-

NO_3^- concentration in the different samples analyzed is shown in Figure 5. Although the differences can not be considered statistically significant, all varieties other than Changlot real (which is considered the most resistant variety), display higher concentrations of NO_3^- in the samples treated with either 100 mM or 200mM NaCl in comparison with the control samples. This might indicate that NO3- concentration would represent a marker for salinity resistance. Values of Picual variety under 200 mM NaCl irrigation are likely to represent an error, that should be fixed in future repeats of the experiment.

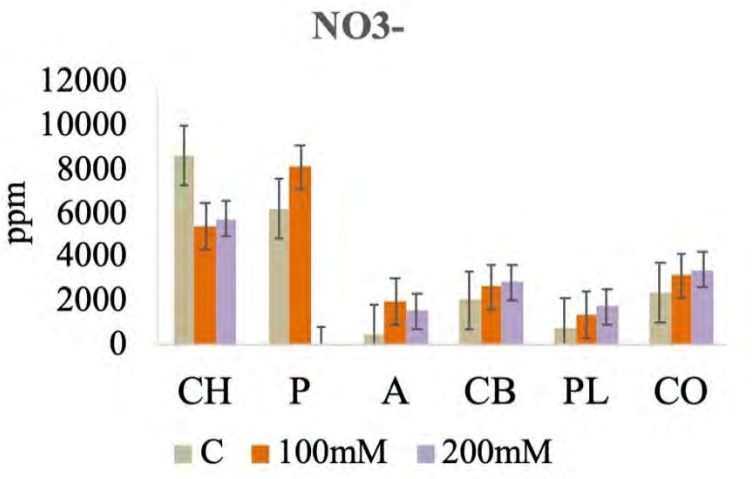


Figure 5. Effect of salinity on NO_3^- concentration. CH: Changlot real; P: Picual; A: Arbequina; CB: Cobrançosa; PL: Pico-Limón; CO: Cornezuelo.

When cultivars of different olive varieties are subjected to increasing concentrations of NaCl, a concomitant reduction in the concentration of nitrates (NO_3^-) takes place in the leaves [17]. The decrease in NO_3^- is explained because Cl- is an antagonist of the NO_3^- ion and, therefore, the levels of the latter can decrease due to the absorption of Cl^- through the root. In our analyses, we observed that NO_3^- levels are higher in the leaves of Arbequina, Cobrançosa, Pico-Limón and Cornezuelo plants treated with 100 and 200 mM NaCl than in the respective control plants, therefore in good agreement with the observations of [17]. However, Changlot real displays an opposite trend, and results concerning Picual samples are doubtful as mentioned above. In order to better explain the described changes, experiments should be repeated, and if possible, combined with Cl^- determinations, which will be now possible at the EEZ after the recent acquisition of a Cl^- dedicated electrode.

Effects of salinity on the concentration of total antioxidant capacity of the samples

As shown in Figure 6, total antioxidant capacity (TAC) of the samples corresponding to either treatment with 100 mM and 200 mM NaCl was significantly lower than the control samples for all cultivars except Cornezuelo. The differences are much more impressive in the varieties Changlot real, Picual and Arbequina, those considered highly resistant, moderately resistant and moderately resistant to salinity, respectively (note circle in Fig. 6). Again, this marker could be considered proportional to the salinity resistance.

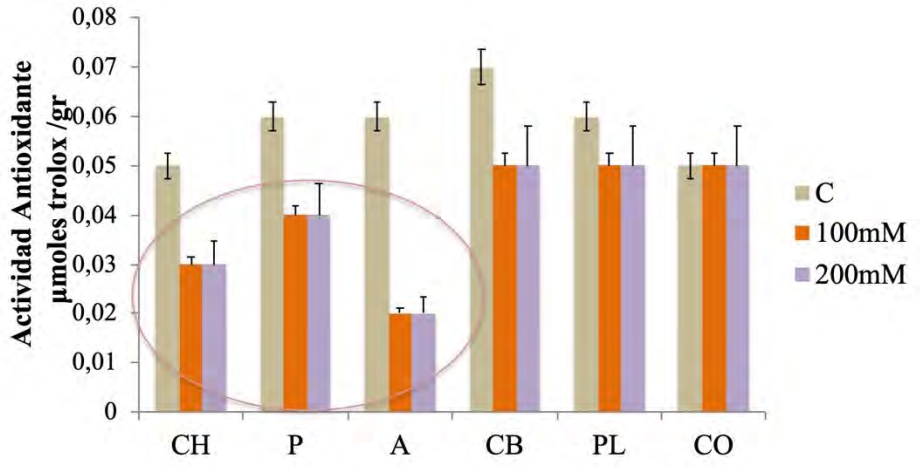


Figure 6. Effect of salinity on total antioxidant capacity. CH: Changlot real; P: Picual; A: Arbequina; CB: Cobrançosa; PL: Pico-Limón; CO: Cornezuelo.

The level of TAC in the leaves is dependent on the genotype (i.e. cultivar) and salt concentration. Thus, in a previous study, no significant increase in antioxidant capacity was observed in Arbequina olive plants treated with 75 mM NaCl compared to control plants without salt [18]. In other varieties, however, antioxidant activity doubled compared to the control under the same experimental conditions [18]. The increase in phenolic compounds can generate an increase in total antioxidant activity [19]. At this regard, the analysis of the concentration of total phenols in leaves is proposed for future studies, as this data may help us to understand the observed changes in TAC.