

# Response to salinity in young olive trees of three Iberian varieties

Coelho R<sup>1,2</sup>, Sousa A<sup>1</sup>, Rato A<sup>1</sup>, Vaz M<sup>1</sup>

<sup>1</sup> ICAAM, Universidade de Évora, Apartado 94, 7002-554 Évora, Portugal

<sup>2</sup> rcoelho@uevora.pt

(ISBN 978-989-8096-52-4)

## Abstract

Soil salinization is a problem in the Mediterranean region. This paper reports a research on the response to salt in two year-old olive trees (*Olea europaea* L.) of three Iberian varieties: Arbequina, Cobrançosa and Galega Vulgar.

Plants were grown in plastic pots containing approximately 9 Kg of a sandy granitic soil, on a greenhouse at the University of Évora since February 2010. The experiment went from February to April 2012. As a rule, plants were watered every other day alternating salt solution (0 mM, 80 mM or 200 mM NaCl) or tap water.

After three months irrigation with the different NaCl solutions, soil electric conductivity and soil water content were significantly higher on salt-irrigated pots. Salt also decreased significantly stomatal conductance ( $g_s$ ) and mid-day leaf water potential ( $\Psi$ ), Cobrançosa having in general higher  $g_s$  and  $\Psi$  but lower SLA than the two other varieties. Chlorophyll content of leaves was not affected by salt after this three months exposure to NaCl but was significantly higher on Arbequina and lower on Cobrançosa.

In general, hyperspectral reflectance indexes did not show significant correlations with salt irrigation, except for the Photochemical Reflectance Index (PRI) which was clearly lower on plants of all three varieties irrigated with salt. Interestingly, Cobrançosa showed frequently vegetation indexes different from the other two varieties.

## Introduction

The problem of soil salinity has been an increasingly important issue due to the decrease in good quality irrigation water. The Mediterranean region is no exception.

Salinity affects plant growth by osmotic stress, ion toxicity or both (Munns and Tester, 2008). The area occupied by intensive and hedgerow olive orchards in the Mediterranean region has greatly increased over the last years (Santos et al., 2013). These orchards need frequently high levels of irrigation during summer which may lead to an increase in soil salinity due to the high evaporative demand. Therefore, investigation on the tolerance to salinity of the different olive-tree varieties is of particular importance.

This paper reports a research on the response to salt in two year-old olive trees (*Olea europaea* L.) of three Iberian varieties: Arbequina, Cobrançosa and Galega Vulgar.

## Materials and methods

Plants were grown in 10 L plastic pots containing approximately 9 Kg of a sandy granitic soil, on a greenhouse at the University of Évora since February 2010. The experiment went from February to April 2012. As a rule, plants were watered every other day alternating salt solution or tap water. There were three levels of salinity in the irrigation water, 0 mM, 80 mM or 200 mM NaCl, corresponding to about 0,05 dS m<sup>-1</sup>, 7,3 dS m<sup>-1</sup> or 19 dS m<sup>-1</sup>, respectively (6 plants per salinity level in a total of 18 plants of each variety). After 3 months, several parameters were assessed. Soil water content and salinity were measured with a portable conductivity meter (Hanna HI9835, USA). In addition, mid-day leaf stomatal conductance ( $g_s$ ) was measured with a AP4 porometer (Delta-T Devices, Uk), mid-day leaf water potential ( $\Psi$ ) with a pressure chamber (PMS Instrument Company, USA), percentage of water, relative water content, specific leaf area (SLA), leaf chlorophyll content measured with a CL-01 chlorophyll meter (Hansatech Instruments, UK) and different indices derived from hyperspectral leaf signature (Table 1), measured with a FieldSpec Spectroradiometer (ASD Inc., USA). **Table 1.** List of vegetation indexes analysed in this work.

<b>Vegetation Index</b>		<b>Equation</b>
<b>SIPI</b>	Structure Insensitive Pigment Index	$R800-R445/R800-R680$
<b>MSI</b>	Moisture Stress Index	$R1599/R819$
<b>NDII</b>	Normalized Difference Infrared Index	$(R819-R1649)/(R819+R1649)$
<b>NPQI</b>	Normalized phaeophytinization index	$(R415-R435)/(R415+R435)$
<b>WBI</b>	Water Band Index	$R900/R970$
<b>WI</b>	Water Index	$R900/R1600$
<b>NDVI</b>	Normalized Difference Vegetation Index	$(R750-R705)/(R750+R705)$
<b>NDVI<sub>680</sub></b>	Normalized Difference Vegetation Index	$(R810-R680)/(R810+R680)$
<b>PRI</b>	Photochemical Reflectance Index	$(R531-R570)/(R531+R570)$
<b>REIP</b>	Red Edge Inflexion Point Index	$R700+40((Re-R700)/(R740-R700))$
<b>CRI<sub>2</sub></b>	Carotenoid Reflectance Index 2	$(1/R510) - (1/R700)$
<b>ARI<sub>2</sub></b>	Anthocyanin Reflectance Index 2	$R800 [(1/R550) - (1/R700)]$
<b>NDLI</b>	Normalized Difference Lignin Index	$(\log R1754)-(\log R1680)/(\log R1754)+(\log R1680)$
<b>PSRI</b>	Plant Senescence Reflectance Index	$(R680 - R500)/R750$

## Results

After three months irrigation with the different NaCl solutions, soil electric conductivity and soil water content (Table 2) were significantly higher on salt-irrigated pots.

**Table 2.** Salinity and water content in the soil after 90 days irrigation with different NaCl solutions. Values are mean  $\pm$  SE ( $n = 6$ ). For each parameter, different letters indicate statistical difference ( $p < 0,05$ ).

	NaCl	Arbequina	Cobrançosa	Galega
<b>Soil CE</b> (dS m <sup>-1</sup> )	<b>0 mM</b>	0,18 $\pm$ 0,02 <sup>e</sup>	0,18 $\pm$ 0,01 <sup>e</sup>	0,17 $\pm$ 0,02 <sup>e</sup>
	<b>80 mM</b>	1,02 $\pm$ 0,10 <sup>cd</sup>	0,62 $\pm$ 0,13 <sup>de</sup>	1,02 $\pm$ 0,11 <sup>cd</sup>
	<b>200 mM</b>	1,88 $\pm$ 0,27 <sup>ab</sup>	1,47 $\pm$ 0,09 <sup>bc</sup>	2,06 $\pm$ 0,18 <sup>a</sup>
<b>Soil water content</b> (%)	<b>0 mM</b>	0,12 $\pm$ 0,01 <sup>bc</sup>	0,13 $\pm$ 0,03 <sup>bc</sup>	0,11 $\pm$ 0,02 <sup>c</sup>
	<b>80 mM</b>	0,12 $\pm$ 0,01 <sup>bc</sup>	0,13 $\pm$ 0,01 <sup>bc</sup>	0,14 $\pm$ 0,02 <sup>bc</sup>
	<b>200 mM</b>	0,22 $\pm$ 0,07 <sup>a</sup>	0,17 $\pm$ 0,02 <sup>b</sup>	0,17 $\pm$ 0,04 <sup>b</sup>

Table 3 shows the effects of NaCl on plants. Salt irrigation decreased significantly stomatal conductance ( $g_s$ ), Cobrançosa having in general higher  $g_s$  than the two other varieties. Mid-day leaf water potential ( $\Psi$ ) was also significantly lower on salt-irrigated plants and again Cobrançosa exhibited slightly higher values compared with the other two varieties.

Relative water content was not significantly affected by salt irrigation. Salt had also no clear effect on the specific leaf area (SLA), but Cobrançosa had on average higher SLA than the two other varieties. Chlorophyll content of leaves was not affected after this three months exposure to NaCl but was significantly higher on Arbequina and lower on Cobrançosa.

**Table 3.** Mid-day stomatal conductance ( $g_s$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ,  $n = 48$ ), water potential ( $\Psi$ , MPa,  $n = 6$ ), relative water content (RWC,  $n = 6$ ), specific leaf area (SLA,  $n = 6$ ) and relative chlorophyll content (arbitrary units,  $n = 30$ ) of plants after 90 days irrigation with different NaCl solutions. Values are mean  $\pm$  SE. Different letters indicate statistical difference ( $p < 0,05$ ).

	NaCl	Arbequina	Cobrançosa	Galega
<b>Stomatal conductance</b> ( $g_s$ )	<b>0 mM</b>	$63 \pm 7^b$	$109 \pm 9^a$	$63 \pm 6^b$
	<b>80 mM</b>	$27 \pm 3^{cd}$	$36 \pm 5^c$	$18 \pm 3^{cd}$
	<b>200 mM</b>	$13 \pm 2^d$	$17 \pm 3^{cd}$	$9 \pm 1^d$
<b>Mid-day leaf water potential</b> ( $\Psi$ )	<b>0 mM</b>	$-1,33 \pm 0,05^a$	$-1,12 \pm 0,05^a$	$-1,29 \pm 0,07^a$
	<b>80 mM</b>	$-1,94 \pm 0,13^{bc}$	$-1,34 \pm 0,07^a$	$-1,57 \pm 0,05^{ab}$
	<b>200 mM</b>	$-2,18 \pm 0,25^c$	$-1,72 \pm 0,07^{abc}$	$-2,28 \pm 0,26^c$
<b>Relative water content</b> (RWC)	<b>0 mM</b>	$0,84 \pm 0,02$	$0,81 \pm 0,03$	$0,84 \pm 0,02$
	<b>80 mM</b>	$0,79 \pm 0,03$	$0,82 \pm 0,03$	$0,78 \pm 0,02$
	<b>200 mM</b>	$0,82 \pm 0,02$	$0,82 \pm 0,01$	$0,79 \pm 0,01$
<b>Specific leaf area</b> (SLA)	<b>0 mM</b>	$3,02 \pm 0,16^b$	$3,96 \pm 0,37^{ab}$	$3,26 \pm 0,17^{ab}$
	<b>80 mM</b>	$3,12 \pm 0,24^b$	$4,28 \pm 0,53^a$	$3,45 \pm 0,17^{ab}$
	<b>200 mM</b>	$3,69 \pm 0,39^{ab}$	$3,19 \pm 0,18^b$	$3,47 \pm 0,17^{ab}$
<b>Relative chlorophyll content</b>	<b>0 mM</b>	$139 \pm 5^{abc}$	$129 \pm 5^{bc}$	$146 \pm 5^{ab}$
	<b>80 mM</b>	$153 \pm 7^a$	$145 \pm 4^{ab}$	$128 \pm 6^{bc}$
	<b>200 mM</b>	$136 \pm 6^{abc}$	$121 \pm 5^c$	$144 \pm 7^{ab}$

In general, hyperspectral reflectance indexes (Table 4) did not show significant correlations with salt irrigation. From all the fifteen vegetation indexes analysed (Table 1.), only the Photochemical Reflectance Index (PRI) was clearly lower on plants of all three varieties irrigated with salt (Table 4).

Interestingly, Cobrançosa showed frequently vegetation indexes different from the other two varieties, higher Moisture Stress Index (MSI), Normalized Phaeophytinization Index (NPQI) and Normalized Difference Vegetation Index (NDVI) but lower Normalized Difference Infrared Index (NDII), Water Index (WI), Structure Insensitive Pigment Index (SIPI), Carotenoid Reflectance Index (CRI<sub>2</sub>), Anthocyanin Reflectance Index (ARI<sub>2</sub>) and Plant Senescence Reflectance Index (PSRI).

**Table 4.** Vegetation indexes measured on individual leaves after 90 days irrigation with different NaCl solutions. Values are mean  $\pm$  SE (n = 6). Different letters indicate statistical difference ( $p < 0,05$ ).

	NaCl	Arbequina	Cobrançosa	Galega
Photochemical Reflectance Index (PRI)	0 mM	0,026 $\pm$ 0,003 <sup>a</sup>	0,024 $\pm$ 0,003 <sup>ab</sup>	0,027 $\pm$ 0,002 <sup>a</sup>
	80 mM	0,019 $\pm$ 0,005 <sup>abc</sup>	0,018 $\pm$ 0,003 <sup>abc</sup>	0,009 $\pm$ 0,006 <sup>c</sup>
	200 mM	0,005 $\pm$ 0,012 <sup>bc</sup>	0,009 $\pm$ 0,003 <sup>c</sup>	0,011 $\pm$ 0,009 <sup>c</sup>
Moisture Stress Index (MSI)	0 mM	0,49 $\pm$ 0,04 <sup>b</sup>	0,61 $\pm$ 0,04 <sup>a</sup>	0,49 $\pm$ 0,03 <sup>b</sup>
	80 mM	0,49 $\pm$ 0,05 <sup>b</sup>	0,58 $\pm$ 0,04 <sup>ab</sup>	0,52 $\pm$ 0,02 <sup>ab</sup>
	200 mM	0,57 $\pm$ 0,05 <sup>ab</sup>	0,49 $\pm$ 0,03 <sup>b</sup>	0,52 $\pm$ 0,02 <sup>ab</sup>
Normalized Phaeophyt. Index (NPQI)	0 mM	0,02 $\pm$ 0,01 <sup>b</sup>	0,05 $\pm$ 0,01 <sup>a</sup>	0,02 $\pm$ 0,01 <sup>b</sup>
	80 mM	0,02 $\pm$ 0,01 <sup>b</sup>	0,06 $\pm$ 0,01 <sup>a</sup>	0,02 $\pm$ 0,01 <sup>b</sup>
	200 mM	0,03 $\pm$ 0,01 <sup>b</sup>	0,06 $\pm$ 0,01 <sup>a</sup>	0,02 $\pm$ 0,01 <sup>b</sup>
Normalized Difference Vegetation Index (NDVI)	0 mM	0,64 $\pm$ 0,02 <sup>ab</sup>	0,66 $\pm$ 0,02 <sup>ab</sup>	0,70 $\pm$ 0,02 <sup>a</sup>
	80 mM	0,62 $\pm$ 0,04 <sup>b</sup>	0,66 $\pm$ 0,02 <sup>ab</sup>	0,61 $\pm$ 0,04 <sup>b</sup>
	200 mM	0,61 $\pm$ 0,02 <sup>b</sup>	0,67 $\pm$ 0,01 <sup>ab</sup>	0,66 $\pm$ 0,04 <sup>ab</sup>
Normalized Difference Infrared Index (NDII)	0 mM	0,27 $\pm$ 0,03 <sup>a</sup>	0,18 $\pm$ 0,03 <sup>b</sup>	0,27 $\pm$ 0,03 <sup>a</sup>
	80 mM	0,27 $\pm$ 0,04 <sup>a</sup>	0,20 $\pm$ 0,03 <sup>ab</sup>	0,24 $\pm$ 0,02 <sup>ab</sup>
	200 mM	0,21 $\pm$ 0,04 <sup>ab</sup>	0,27 $\pm$ 0,02 <sup>a</sup>	0,24 $\pm$ 0,02 <sup>ab</sup>
Water Index (WI)	0 mM	2,14 $\pm$ 0,15 <sup>ab</sup>	1,70 $\pm$ 0,10 <sup>c</sup>	2,14 $\pm$ 0,14 <sup>ab</sup>
	80 mM	2,19 $\pm$ 0,23 <sup>a</sup>	1,80 $\pm$ 0,14 <sup>bc</sup>	1,96 $\pm$ 0,10 <sup>abc</sup>
	200 mM	1,87 $\pm$ 0,15 <sup>abc</sup>	2,09 $\pm$ 0,11 <sup>ab</sup>	1,98 $\pm$ 0,09 <sup>abc</sup>
Structure Insensitive Pigment Index (SIPI)	0 mM	1,00 $\pm$ 0,00 <sup>ab</sup>	0,99 $\pm$ 0,00 <sup>c</sup>	1,00 $\pm$ 0,00 <sup>ab</sup>
	80 mM	1,00 $\pm$ 0,00 <sup>a</sup>	0,99 $\pm$ 0,00 <sup>c</sup>	1,00 $\pm$ 0,00 <sup>a</sup>
	200 mM	1,00 $\pm$ 0,00 <sup>ab</sup>	0,99 $\pm$ 0,00 <sup>c</sup>	1,00 $\pm$ 0,00 <sup>b</sup>
Carotenoid Reflectance Index (CRI <sub>2</sub> )	0 mM	16,1 $\pm$ 1,4 <sup>a</sup>	12,8 $\pm$ 1,2 <sup>ab</sup>	15,1 $\pm$ 2,3 <sup>ab</sup>
	80 mM	16,8 $\pm$ 1,3 <sup>a</sup>	11,1 $\pm$ 1,8 <sup>b</sup>	16,7 $\pm$ 2,8 <sup>a</sup>
	200 mM	17,5 $\pm$ 1,6 <sup>a</sup>	13,5 $\pm$ 2,3 <sup>ab</sup>	15,1 $\pm$ 1,9 <sup>ab</sup>
Anthocyanin Reflectance Index (ARI <sub>2</sub> )	0 mM	0,26 $\pm$ 0,08 <sup>a</sup>	-0,05 $\pm$ 0,09 <sup>b</sup>	0,23 $\pm$ 0,12 <sup>a</sup>
	80 mM	0,46 $\pm$ 0,13 <sup>a</sup>	-0,09 $\pm$ 0,04 <sup>b</sup>	0,39 $\pm$ 0,10 <sup>a</sup>
	200 mM	0,29 $\pm$ 0,17 <sup>a</sup>	-0,02 $\pm$ 0,11 <sup>b</sup>	0,47 $\pm$ 0,05 <sup>a</sup>
Plant Senescence Reflect. Index (PSRI) $\times 10^3$	0 mM	2,5 $\pm$ 0,6 <sup>a</sup>	-0,6 $\pm$ 0,8 <sup>bc</sup>	2,0 $\pm$ 1,1 <sup>a</sup>
	80 mM	3,5 $\pm$ 0,2 <sup>a</sup>	-1,5 $\pm$ 0,1 <sup>c</sup>	2,0 $\pm$ 0,1 <sup>a</sup>
	200 mM	2,0 $\pm$ 0,2 <sup>a</sup>	-1,5 $\pm$ 0,1 <sup>c</sup>	1,6 $\pm$ 0,1 <sup>ab</sup>

## Discussion

As expected, soil salinity reduced significantly water potential ( $\Psi$ ) and stomatal conductance ( $g_s$ ) of all plants. The fact that Cobrançosa showed higher  $\Psi$  and  $g_s$  than the other two varieties can not be attributed to differences in irrigation, as soil water content was similar in pots of all three varieties. Also, total leaf area of plants of Cobrançosa was just marginally lower than that of the other varieties. One possible explanation is that Cobrançosa shortened the time span of their stomatal opening therefore reducing water lost, despite having higher  $g_s$ , and thus increasing leaf water potential.

Hyperspectral reflectance index PRI showed a positive correlation with chlorophyll content (0,55,  $p < 0,001$ ) but a negative correlation with salinity (-0,44,  $p < 0,001$ ). However, the relationship between salinity and chlorophyll was not significant (-0,1) which suggests that PRI may have been affected directly by salt in the leaves as pointed out in previous studies (Zinnert et al. 2012). There were also clear differences in vegetation indexes between Cobrançosa and the other two varieties, Arbequina and Galega. Focusing on water-related indexes, Cobrançosa showed higher Moisture Stress Index (MSI) and lower Water Index and Normalized Difference Infrared Index (NDII), all these suggesting lower water content. Nevertheless the relative water content of leaves was not significantly different from one variety to the other, and the water percentage was even slightly (but not significantly) higher in Cobrançosa. There seems to be some characteristics of Cobrançosa leaves that mask the reading of these indexes.

Pigment-related indexes were also determined. NPQI, the Normalized Phaeophytinization Index, which is an indicator of chlorophyll degradation, was much higher in Cobrançosa than on the other two varieties. Actually, Cobrançosa showed significantly lower chlorophyll content than the other two varieties, somehow contradicting the higher NDVI found in this variety. SIPI was also lower on Cobrançosa, suggesting lower carotenoid content, also supported by the lower  $CRI_2$  observed on this variety but in some way contradicting the lower Plant Senescence Reflectance Index (PSRI). Strangely, Anthocyanin Reflectance Index ( $ARI_2$ ) showed negative values in Cobrançosa.

Determination of actual pigment content in the leaves would be necessary to verify these data. The response of Cobrançosa to salt, clearly different from that of Arbequina or Galega, needs further investigation.

## References

- Munns, R and Tester, M (2008) Mechanisms of Salinity Tolerance. *Annu. Rev. Plant Biol.* 59:651–81
- Parida AK and Das AB (2005) Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety* 60 324–349
- Santos *et al.* (2013) Water use, transpiration and crop coefficients for irrigated hedgerow olives grown in Southern Portugal. *Actas do VII Congresso Ibérico de Agro Engenharia e Ciências Hortícolas*, Madrid
- Zinnert JC *et al.* (2012) Effects of salinity on physiological responses and the photochemical reflectance index in two co-occurring coastal shrubs. *Plant Soil* (2012) 354:45–55