VOL IX

Estudos em Ciências Agrárias e Ambientais

Eduardo Spers
(Organizador)

EDITORA ARTEMIS
2026

VOL IX

Estudos em Ciências Agrárias e Ambientais

Eduardo Spers
(Organizador)

EDITORA ARTEMIS
2026

Editora Chefe	Profª Drª Antonella Carvalho de Oliveira
Editora Executiva	M.ª Viviane Carvalho Mocellin
Direção de Arte	M.ª Bruna Bejarano
Diagramação	Elisangela Abreu
Organizador	Prof. Dr. Eduardo Eugênio Spers
Imagem da Capa	Bruna Bejarano, Arquivo Pessoal
Bibliotecário	Maurício Amormino Júnior – CRB6/2422

EDITORA ARTEMIS
2026

Editora Artemis
Curitiba-PR Brasil
www.editoraartemis.com.br
e-mail:publicar@editoraartemis.com.br

EDITORA ARTEMIS
2026

Editora Artemis
Curitiba-PR Brasil
www.editoraartemis.com.br
e-mail:publicar@editoraartemis.com.br

EDITORA ARTEMIS
2026

Editora Artemis
Curitiba-PR Brasil
www.editoraartemis.com.br
e-mail:publicar@editoraartemis.com.br

Dados Internacionais de Catalogação na Publicação (CIP)
(eDOC BRASIL, Belo Horizonte/MG)

E82 Estudos em Ciências Agrárias e Ambientais IX [livro eletrônico] / Organizador Eduardo Eugênio Spers. – 1. ed. – Curitiba, PR: Editora Artemis, 2026.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Edição bilíngue.
Inclui bibliografia.
ISBN 978-65-82858-34-5
DOI 10.37572/EdArt_250926345

1. Ciências agrárias. 2. Ciências ambientais. 3. Agricultura – Pesquisa. I. Spers, Eduardo Eugênio.

CDD 630

Elaborado por Maurício Amormino Júnior – CRB6/2422

EDITORA ARTEMIS
2026

Editora Artemis
Curitiba-PR Brasil
www.editoraartemis.com.br
e-mail:publicar@editoraartemis.com.br

PRÓLOGO

A produção agropecuária contemporânea depende de uma compreensão cada vez mais precisa das relações entre organismos, ambiente, manejo, nutrição e tecnologia. O desenvolvimento de sistemas produtivos mais eficientes exige não apenas aumentar rendimentos, mas compreender os mecanismos fisiológicos que condicionam a adaptação das plantas, aprofundar o conhecimento sobre os fatores relacionados à ocorrência de pragas e doenças, aprimorar a nutrição animal e incorporar práticas capazes de favorecer o bem-estar, a reprodução e a saúde dos animais.

O nono volume de ***Estudos em Ciências Agrárias e Ambientais*** reúne quatorze capítulos organizados em três eixos temáticos: **Produção vegetal, fisiologia e adaptação; Sanidade vegetal e manejo de pragas e doenças; e Produção, nutrição, bem-estar e saúde animal.** Essa organização acompanha diferentes dimensões da produção agropecuária, partindo das respostas fisiológicas e produtivas das plantas, passando pelos fatores que afetam sua sanidade e chegando aos processos relacionados à produção animal.

O primeiro eixo reúne investigações sobre produção vegetal, adaptação e respostas fisiológicas diante de diferentes condições de cultivo e fatores ambientais. A interação entre genótipos de milho e ambientes de produção evidencia a importância de compreender a estabilidade e a adaptabilidade dos materiais vegetais diante de diferentes condições de semeadura e disponibilidade hídrica. Outros estudos analisam os efeitos do estresse salino em oliveiras, as respostas fisiológicas de *Zinnia elegans* cultivada em sistema hidropônico e as alterações fenotípicas e fitoquímicas de Agave salmiana submetido ao estresse por zinco. O eixo contempla ainda a aplicação da técnica in vitro de duplo-haploide como estratégia para acelerar a obtenção de linhagens homozigóticas e apoiar o melhoramento de plantas cultivadas. Em conjunto, esses estudos evidenciam como fatores ambientais, nutricionais, fisiológicos e biotecnológicos podem contribuir para a compreensão e o aprimoramento da produção vegetal.

O segundo eixo concentra-se na sanidade vegetal e no manejo de pragas e doenças. O controle microbiológico da broca-do-café por meio de Beauveria bassiana exemplifica a aplicação de agentes biológicos no manejo fitossanitário. O estudo epidemiológico do manchado de cálice de jamaica investiga as relações entre patógenos, condições climáticas e severidade da doença, enquanto a análise da abundância de *Oebalus spp.* em cultivos de arroz relaciona dinâmica populacional, temperatura e monitoramento como suporte ao manejo integrado de pragas. Esses trabalhos evidenciam a importância de conhecer tanto os agentes causadores de danos quanto as condições ambientais que favorecem sua ocorrência.

O terceiro eixo amplia o olhar para a produção animal, reunindo estudos sobre nutrição, metabolismo, desempenho produtivo, bem-estar, reprodução e saúde. As pesquisas sobre absorção e retenção de fósforo em ovinos e sobre a utilização de fontes gluconeogênicas diante do estresse térmico abordam processos metabólicos associados à eficiência nutricional e à adaptação dos animais. A utilização de cúrcuma na alimentação de cuyes é investigada como alternativa para melhorar o desempenho produtivo, enquanto o estudo realizado com galinhas poedeiras compara sistemas de criação a partir de indicadores de bem-estar animal.

O eixo também incorpora abordagens relacionadas à biotecnologia reprodutiva e à medicina veterinária. A avaliação de um diluidor quimicamente definido, originalmente desenvolvido para sêmen equino, na conservação refrigerada de sêmen caprino evidencia o potencial das tecnologias reprodutivas para o aprimoramento dos programas de inseminação artificial e a conservação de recursos zoogenéticos. Já o estudo clínico e *post mortem de Coendou rufescens* amplia o escopo do volume ao campo da medicina de fauna silvestre, demonstrando a relevância do diagnóstico clínico e anatomopatológico também para espécies não domésticas.

Os capítulos que compõem este volume evidenciam a diversidade de fatores biológicos, ambientais e tecnológicos que interferem na produção vegetal e animal. Ao reunir pesquisas experimentais, revisões e estudos aplicados provenientes de diferentes contextos, Estudos em Ciências Agrárias e Ambientais IX oferece um panorama de diferentes frentes de investigação, desde as respostas fisiológicas das plantas e os desafios fitossanitários até a nutrição, o bem-estar, a reprodução e a saúde animal. Essa diversidade reafirma a importância da produção científica para a compreensão dos processos que sustentam os sistemas agropecuários e para o aprimoramento contínuo das práticas de produção, manejo e conservação.

Boa leitura!

Eduardo Eugênio Spers

SUMÁRIO

CAPÍTULO 2

FOLIAR BEHAVIOR OF OLIVE TREES (*Olea europaea* L.) GRAFTED AND CUT UNDER THE EFFECT OF SALT STRESS

Data de submissão: 01/09/2026
Data de aceite: 14/09/2026

Dhia Gharabi
Laboratory of Molecular and Cellular Biology
Faculty of Nature and Life Sciences
Ibn Khaldoun University
Tiaret, Algeria
https://orcid.org/0000-0002-0194-0091

Maghni Benchohra
Faculty of Nature and Life Sciences
Ibn Khaldoun University
Tiaret, Algeria
https://orcid.org/0000-0002-0980-5679

Muhammad Arif
Department of Plant Protection
Faculty of Agriculture
Sakarya University of Applied Sciences
Sakarya, Turkey
https://orcid.org/0000-0002-8631-4873

Salih Karabörklüb
Department of Plant Protection
Faculty of Agriculture
Sakarya University of Applied Sciences
Sakarya, Turkey
https://orcid.org/0000-0003-4737-853X

Teggar Naima
https://orcid.org/0000-0003-1784-4843

Naceur Benamor
https://orcid.org/0000-0001-5751-0181

Samira Kacha
University Ibn Khaldoun
Tiaret, Algeria
https://orcid.org/0009-0000-7225-7583

Laidia Zerkaoui
Faculty of Nature and Life Sciences
Ibn Khaldoun University
Tiaret, Algeria

Mohamed Abdelhaq Abbes
https://orcid.org/0000-0003-2943-5596

Boulenouar Houari
Houari Boulenouar
https://orcid.org/0000-0001-6976-0655

Malika Zoubeidi
Faculty of Nature and Life Sciences
Ibn Khaldoun University
Tiaret, Algeria

Imane Derouiche
Université IBN Khaldoun
Tiaret, Algeria
https://orcid.org/0000-0002-5295-1241

ABSTRACT: Olive (*Olea europaea* L.) is an important perennial crop in many agricultural regions of the Mediterranean countries but it is planted, often in salty soils. In this context, the objective of this work is to determine the effect of salinity on the morpho-physiological

behavior of young olive plants in cuttings and grafted plants, including two local varieties (Chemlal and Sigoise) and two introduced varieties (Manzanilla and Arbiquina). The experiment was conducted in a laboratory and greenhouse with a well-controlled condition. The experiment was arranged in a completely randomized design of two factors (salinity and varieties) with four replications and two treatments. Not treated plants (without NaCl application) were utilized 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 salinity treatment, the tree seedlings received a nutrient solution fortified by the addition of 100mM of NaCl. Four repetitions are being done according to the field capacity. The study covered the variation of the relative water content in leaves as well as the leaf surface, stomata density, stomatal size and the wax rate on the upper foliar epidermis. According to the achieved results, the RWC leaf area and stomatal density of the treated plants decreased compared to the control. On the other hand, the wax level increases in the case of salt stress compared to the control, both for the grafted plants and for the plants not grafted.
KEYWORDS: salt stress; olive-tree; RWC; stomatal density; wax rate; varieties.

COMPORTAMENTO FOLIAR DE OLIVEIRAS (*Olea europaea* L.) ENXERTADAS E PROVENIENTES DE ESTACAS SOB STRESS SALINO

RESUMO: An oliveira (*Olea europaea* L.) é uma cultura perene importante em muitas regiões agrícolas dos países mediterrânicos, sendo frequentemente cultivada em solos salinos. Neste contexto, o objetivo deste trabalho é determinar o efeito da salinidade no comportamento morfofisiológico de plantas jovens de oliveira – tanto provenientes de estaca como enxertadas –, incluindo duas variedades locais (Chemlal e Sigoise) e duas variedades introduzidas (Manzanilla e Arbequina). A experiência foi conduzida em laboratório e em casa de vegetação, sob condições controladas. O delineamento experimental foi o inteiramente casualizado, com dois fatores (salinidade e variedades), quatro repetições e dois tratamentos. As planta não tratadas (sem aplicação de NaCl) foram utilizadas como controlo. O material vegetal (mudas jovens de oliveira com dois anos de idade) foi selecionado e obtido num viveiro. A experiência iniciou-se com a irrigação das plantas controlo utilizando uma solução nutritiva. Para o tratamento de salinidade, as plântulas receberam uma solução nutritiva enriquecida com a adição de 100 mM de NaCl. Foram realizadas quatro repetições, tendo a rega sido baseada na capacidade de campo. O estudo avaliou a variação do teor relativo de água (TRA) nas folhas, bem como a área foliar, a densidade estomática, o tamanho dos estomas e a quantidade de cera na epiderme foliar superior. Os resultados mostraram que o TRA, a área foliar e a densidade estomática das plantas tratadas diminuíram em comparação com o controlo. Por outro lado, o teor de cera aumentou sob stress salino em relação ao controlo, tanto nas plantas enxertadas como nas não enxertadas.
PALAVRAS-CHAVE: stress salino; oliveira; TRA; densidade estomática; teor de cera; variedades.

1. INTRODUCTION

The olive tree constitutes an economic and food source for the autochthonous inhabitants. The development of olive growing in Algeria was the subject of an extensive program to plant one million hectares of land in olive groves. Thus, new decisions are taken to improve the management of olive growing by extending it to land where intensification of production is possible. 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 (Besnard et al., 2002). It contributes to reducing the soil erosion and the loss of the fertile soil (Álvarez et al., 2007). Otherwise, the recorded salinization of the arid and semi-arid ecosystems is the result of a high evaporation of water from the ground and insufficient and irregular rainfall (Mezni et al., 2002; Munns et al., 2006). This salinization stems also from an inadequately controlled irrigation (Bennaceur et al., 2001; Devkota et al., 2022). Salinity and drought additionally have been shown to affect plant growth, protein accumulation (Zhu, 2001; Balasubramaniam et al., 2023). Therefore, osmotic potential provides a better measure of the effect of salt on plant growth (Ben-Gal et al., 2009) and microbial activity (Chowdhury et al., 2011; Setia and Marschner, 2012) than the EC of a soil extract. The accumulation of salts in the root zone has adverse effects on plant growth (Belkhodja et Bidai, 2004). It is due to the low osmotic potential of the soil solution resulting in decreased availability of water to plants, but also due to the ion imbalance and ion toxicity (Pages et al., 2000; Lindsay et al., 2004; Bartels et Sunkar, 2005). Therefore, it must to understand the mechanisms developed by plants to adapt to changing environmental conditions to maintain their growth and productivity (Szabolcs, 1994; Trinchant et al., 2004). Indeed, according to the salinity in the media, glycophytes are exposed to changes in their morpho-physiological (Bennaceur et al., 2001) and biochemical behavior (Grennan, 2006). So, the plants must modify their growth and development to suit the prevailing environmental conditions.

Indeed, given the salt constraint, activity and morphology of the leaves play a role in plant resistance to stress. According to Garg et al. (2002), Moinuddin et al. (2005) and Hassani et al. (2008), the plant expressed itself by maintaining turgor by reducing transpiration. Therefore, this experiment was conducted to examine the influence of NaCl or the effect of salinity on morpho-physiology of the leaf for four varieties of olive trees derived from cuttings or grafted Including two local varieties (Chemlal and Sigoise) and two introduced varieties (Manzanilla and Arbiquina) (Gharabi et al., 2022). The analysis focused on changes in the relative water content of leaves, area, density of stomata, rate of wax and the internal structure. The induction of salt stress is achieved by adding a NaCl to the nutrient solution

2. MATERIALS AND METHODS

2.1. THE EXPERIMENTAL DEVICE

The plantlets used in the experiment were 18-month-old obtained by cutting rooted under nebulization (crop nursery). 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, soil and an organic matter at proportions of four volumes from sand / one volume from soil / one volume from manure or an organic matter. The experiment was conducted in a greenhouse in the Ibn Khaldoun University of Tiaret (Algeria) 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 three factors experiment was laid out in Randomized Complete Block Design (RCBD) with four replications (four blocks) were set up to study the different parameters that demonstrate the physiological effects of salt water on the olive plants tested. The experiment is carried out on four varieties, two of which are to produce table olives (Sigoise and Manzanilla) and two for olive oil production (Chemlal and Arbiquina). The cuttings of the local varieties (Sigoise and Chemlal) come from Algiers and the introduced plants (Manzanilla and Arbiquina) come from Spain.

The induction of the salt stress of the four varieties was achieved by adding NaCl to the nutrient solution. Each plantlet received a standardized and a balanced nutrient solution as Hoagland and Arnon (1938). Treated plants were watered with the nutrient solution and twice a week with 300ml of 100 mM NaCl solution. Control plants received only the nutrient solution. Analyzes and measurements were done after twelve (12) weeks of saline stress.

2.2. THE RELATIVE WATER CONTENT (RWC)

It was determined by method of Barrs and Weatherley, (1962) according by the formula of Clarck and McCaig (1982) and used by Rascio et al. (1988).

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 4 ºC for 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 80 ºC. The relative content water of the leaves is estimated by the equation:

$$\mathbf{RWC\ (\%) = [\ ifw - dw\ /\ wft - dw] \times 100\ \%}$$

2.3. THE LEAF AREA

The leaf area is directly measured by electronic planimeter LICOR-3000A (Cheverry, 1995) with a resolution of 1 mm2.

The Wax Rate. The excised sheet at its base is introduced into a washed and weighed test tube (P1) to which chloroform is added to extract the wax. The test tube with the wax and the chloroform is dried in an oven for 24 h at 45 °C and then weighed (P2). The wax ratio is determined according to the formula:

$$\textbf{Wax Rate in mg.cm}^{-2} = \textbf{P2-P1 / SF}$$

2.4. DENSITY AND SIZE OF THE STOMATA

The stomata density, according to the method of Dohman et al. (1991) adopted by Denden and Lemeur (1999). 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, the varnish layer is removed 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 to determine the number and the size of the stomata (length and width). The measurements are carried out using a ZEISS type microscope (with microscope and OPTIKA photo tube).

The structure of the leaf. 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 (Martoja et Martoja, 1968). Fragments with a length of two centimeters taken from young leaves were placed in a fixative based on alcohol, and formalin, and acetic acid for twenty-four hours. Then the samples were washed and dehydrated with increasing doses of the ethanol (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 obtain lices of 12 µm thicknesses. They placed on to a slide in gelatin water then colored, fixed and using a microscope with an ocular micrometer ZEISS type.

2.5. STATISTICAL ANALYSIS

The obtained data were processed with *Statistica* 12.0, by analyzing the variance and the correlation matrix. The analysis of variance and the means were compared to the Tukey test at 5% probability level.

3. RESULTS

3.1. THE RELATIVE WATER CONTENT

Table 1. Analysis of the variance of the Relative Water Content (RWC) and the leaf area of stressed and unstressed olive trees.

Variable	Variety effect (F1)	Plant effect (F2)	Stress effect (F3)	F1*F2	F2*F3	F1*F3	F1*F2*F3
RWC	0.026	0.221	0.630	0.573	0.855	0.763	0.098
Leaf area	0.007	0.074	0.462	0.132	0.726	0.942	0.588

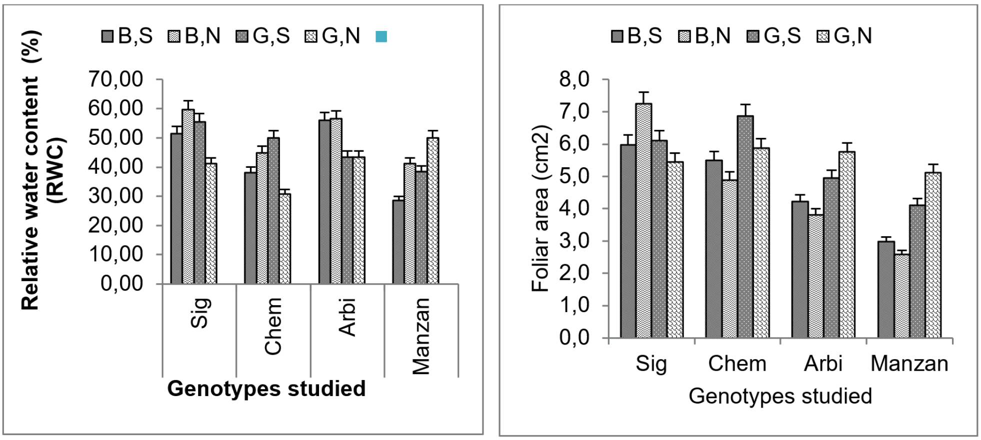
Our statistical results are significant for the varietal effect on the relative water content and nonsignificant for the effect of the plant type (the bit error rate threshold of 5%). Indeed, it shows that after six weeks of treatment with 100 mM.l-1 of NaCl, most of the genotypes studied did not show a significant decrease in the relative water content. The reduction of the RWC of all the tested plants fluctuates between 1% and 2% compared to the control (Fig. 1a).

Thus, the varieties of olive trees studied (local and introduced) have retained a high RWC in the presence of salt stress, indicating that the olive tree is of the “stay green” type (Liu et al., 2024). The olive tree retains green leaves and photosynthetically active allowing having reasonable yields even in the presence of abiotic stresses. However, a high content can be found in local varieties of olive trees compared to introduced varieties. For the local plants treated with 100 mM NaCl and in absence of salinity, the sheet exhibited a RWC of 70% and 60% respectively for Chemlal and Sigoise, and 50% on average for the introduced varieties.

3.2. THE LEAF AREA

The same effect (Table 1) is observed when analyzing the variance of the leaf area. statistical calculations appear to be significant for the varietal effect on the leaf area and not significant for the effect of the plant type under the saline treatment effect and the various interactions at the 5% error threshold. Saline stress affects the leaf area slightly (p = 7.4%). Indeed, the foliar area recorded in the control plants is 49.34 cm2 and then it drops to 27.19 cm2 for the leaves of the plants treated with 100 $mM.l^{-1}$ NaCl, (Fig. 1b). Except for the effect of the plant type, the impact of salinity is very highly significant on the stomatal density (p=0%), as well as the varietal effect and the various interactions, particularly at leaf level of the plants in 100 $mM.l^{-1}$NaCl, (Table 2).

Figure 1. Relative water content (RWC) (a) and the leaf area (cm2) (b) as measured from the penultimate leaf of the studied plantlets (SC: Stressed Cut, CC: Cutting Control, SG: Stressed Graft, GC: Grafted Control).



(a) (b)

3.3. THE WAX RATE

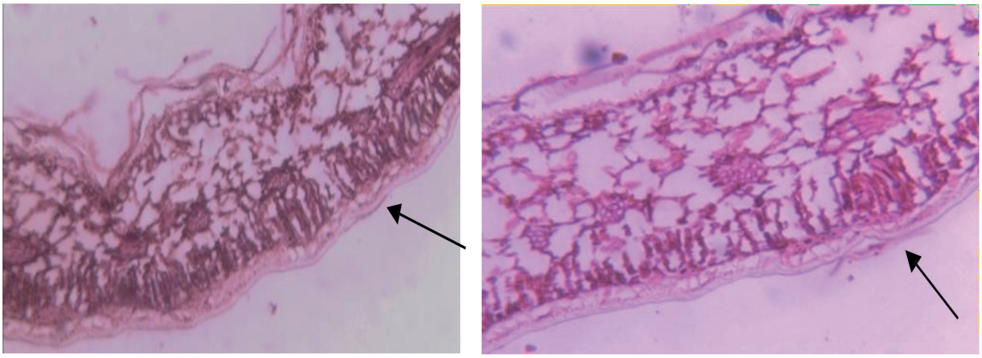
Table 2. Analysis of the variance of the wax rate of the stressed and unstressed olives genotypes.

Variable	Variety effect (F1)	Plant effect (F2)	Stress effect (F3)	F1*F2	F2*F3	F1*F3	F1*F2*F3
The wax rate	0 .012**	0.000***	0.000***	0.000	0.000	0.001	0.004**

***p<0.001, **p<0.01, *p<0.05; insignificant: p>0.05

The analysis of wax variance shows a very highly significant influence for all factors tested at p<0.05, the varietal effect on stomatal density, “plant type” effect, Saline treatment effect and plant genotype-type interactions, genotype-stress, genotype-treatment and genotype-type plant-treatment (Table 2). The histograms displayed in Fig. 3a show that all the genotypes studied (local and introduced) developed a cuticle by the deposition of wax.

Figure 2. The foliar cuticle in olive tree “Sigoise” BS: Stressed Cut (a) and Stressed Graft (b) (photo by Gharabi, Hassani, 2015).



(a) (b)

In addition, Hopkins, (2003), have established the relationship between transpiration and stomatal resistance in Arabidopsis thaliana, by increasing this resistance during salt stress to minimize water loss and sweating, (Cocozza et al., 2020), becomes more important in case of thin cuticle leaves. According to our results, the stressed genotypes have cuticles thicker than those under normal conditions; this can explain the resistance of our plants by keeping their leaves throughout the duration of the stress applied (Fig. 2).

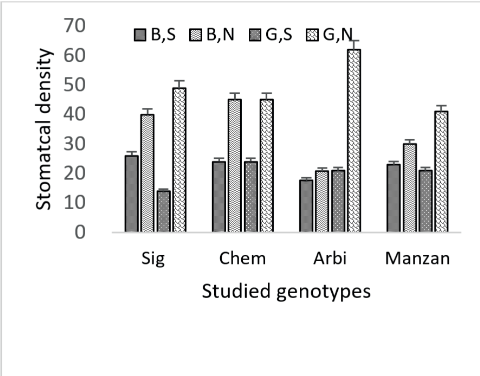
3.4. THE STOMATA DENSITY

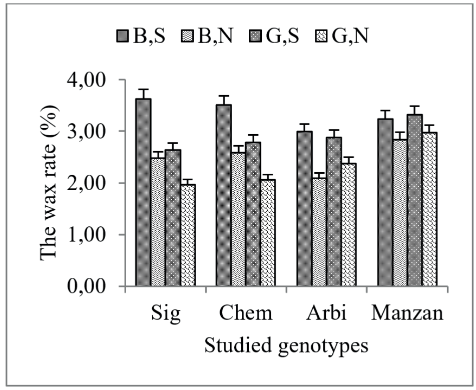
Table 3. Analysis of the variance of the stomatal density (cm2), the length and width of the stomata (µm) of stressed and unstressed olive trees.

Variable	Variety effect (F1)	Plant effect (F2)	Stress effect (F3)	F1*F2	F2*F3	F1*F3	F1*F2*F3
Stomatal Density	0.000	0.378	0.000	0.000	0.003	0.041	0.036
length of stomata	0.000	0.481	0.001	0.000	0.000	0.020	0.037
width of stomata	0 .000	0.239	0.000	0.007	0.024	0.000	0.000

According to the obtained analysis (Table 2) the reaction of the plants followed the same tendency about the expression of this parameter. The Figure 3b shows a significant variation in the number of stomata. In fact, Sigoise cuttings have 40 stomata (control) versus 26 (stressed) stomata. The Chemlal presents 45 against 24, the Arbiquina 44 against 24, and the Manzanilla – 30 against 23 stomates/cm2. For the genotypes grafted on oleaster the fall is more important. According to Fig. 5, the values were 49 (control) versus 14 for the Sigoise, 45 against 24 for Chemlal, 62 against 21 for Arbiquina, and 48 against 21 stomata for the variety Manzanilla.

Figure 3. The wax rate of the upper surface of the leaves(a) and stomatal density (/mm^2) of lower surface (b). (SC: Stressed Cut, CC: Cutting Control, SG: Stressed Graft, GC: Grafted Control).





Otherwise, the impact of salinity on the length of stomata, particularly on the leaves of plants treated with 100 mM.l^{-1} NaCl except for the plant type, is highly significant (p = 0%). It is the same for the varietal effect and the different interactions. The width of the stomata had the same fate (Table 3). Indeed, the values of these parameters, shows that the length and width of stomata in the leaves of plants treated with 100 mM·1^{-1} NaCl react differently.

The results presented in this study reveal a clear and consistent effect of salinity on wax deposition on the upper leaf epidermis, but with a strikingly different response depending on the mode of propagation (cutting vs. grafting) and the variety considered. This duality in response is not only biologically intriguing but also provides valuable insights into the adaptive strategies deployed by the olive tree, a species renowned for its hardiness and resilience in Mediterranean environments.

In non-grafted plants (cuttings), salinity induced a systematic increase in wax deposition across all varieties, although the magnitude of this increase varied considerably. The local varieties, Sigoise (+95 units) and Chemlal (+80 units), exhibited the most pronounced increases, suggesting a stronger capacity for cuticular reinforcement in response to osmotic stress. By contrast, the introduced varieties, Arbequina (+10 units) and Manzanilla (+5 units), showed only marginal increases, indicating a more limited capacity for this particular adaptive response.

This marked accumulation of epicuticular wax in local varieties under salt stress is consistent with a well-documented adaptive mechanism in woody perennials: the reinforcement of the cuticular barrier to limit non-stomatal water loss (Hopkins, 2003; Hassani et al., 2014). In arid and semi-arid environments, where water availability is scarce and evaporative demand is high, the deposition of wax on the leaf surface constitutes a physical shield that reduces cuticular transpiration and helps maintain leaf hydration. This is particularly relevant in our study, as the same stressed cuttings also maintained relatively high relative water content (RWC), suggesting that the increase in wax is part of a coordinated water-saving syndrome.

The fact that local varieties (Sigoise and Chemlal) showed the strongest wax increase is noteworthy. These varieties have been cultivated for generations in Algerian conditions and have likely undergone natural and human selection for traits conferring resilience to local environmental constraints. Their ability to rapidly deposit wax under salt stress may reflect a genetic predisposition for cuticular reinforcement, making them particularly well-suited for cultivation in marginal or saline soils.

In stark contrast, grafted plants exhibited a systematic and substantial decrease in wax deposition under salinity stress, across all varieties. The reductions were considerable: Sigoise: (–75), Chemlal: (–70), Arbequina: (–45), Manzanilla:(–35).

At first glance, this result may seem counterintuitive. If salt stress induces water deficit, why would the plant not reinforce its cuticle, as observed in cuttings? The answer, we argue, lies in the biological function of grafting and the physiological signals transmitted from the rootstock to the scion.

In grafted plants, the rootstock (oleaster, in this case) is the first organ to perceive the osmotic stress caused by salinity. Upon perception, the root system can generate long-distance signals – including abscisic acid (ABA), hydraulic signals, and ionic signals – that travel through the xylem to the shoot (Hassani et al., 2014; Liu et al., 2024). These signals can redirect the plant's metabolic priorities. Instead of investing energy in the biosynthesis of wax (a costly process involving fatty acid elongation and transport), the plant may shift towards more rapid and reversible mechanisms, such as:

Stomatal closure, which provides an immediate reduction in transpiration.

Osmotic adjustment through the accumulation of compatible solutes (e.g., proline, sugars), which helps maintain cellular turgor without requiring morphological changes.

In this context, the reduction in wax deposition in grafted plants may be interpreted not as a failure to adapt, but rather as a strategic reallocation of resources towards more dynamic, physiologically based mechanisms. This interpretation aligns with recent findings in other woody species, where grafting has been shown to modulate stress responses by altering hormone balance and gene expression profiles (Shahid et al., 2021; Cocozza et al., 2020).

A consistent pattern emerges when comparing the two groups of varieties. The local cultivars (Sigoise and Chemlal) exhibit greater amplitude of variation in wax deposition, regardless of the propagation method. Whether increasing wax in cuttings or decreasing it in grafted plants, the local varieties show stronger responses to salinity than the introduced ones. This suggests a higher phenotypic plasticity, which is a valuable trait in variable and unpredictable environments. It allows the plant to fine-tune its morphology and physiology to match the prevailing stress intensity.

In contrast, the introduced varieties (Arbequina and Manzanilla) show more moderate variations. This relative stability may indicate a different adaptive strategy, possibly based on constitutive tolerance mechanisms that require less morphological adjustment. However, it may also reflect a narrower genetic background or a lower capacity for rapid acclimation to the specific saline conditions of the Algerian semi-arid environment.

It is worth noting that Manzanilla showed the smallest variation in wax deposition in both cuttings (+5) and grafted plants (–35), suggesting a particularly stable cuticular phenotype. Whether this stability represents an advantage or a limitation depends on the severity and duration of the stress. In mild or episodic stress, such stability may suffice; in chronic stress, greater plasticity may be more advantageous.

One might ask: is it normal that grafted plants reduce wax deposition under salt stress, given the olive tree's reputation for hardiness? The answer is yes, and this is precisely what makes the olive tree such a resilient species.

Rusticity in olive does not mean invulnerability or a single universal response. Rather, it signifies a broad repertoire of adaptive mechanisms that can be deployed depending on the plant's physiological state, developmental stage, and environmental context. The olive tree does not rely on a single trait to tolerate salinity; it possesses a multi-layered defense system that includes:

Morphological adjustments (leaf area reduction, cuticle thickening, stomatal regulation).

Physiological adjustments (osmotic adjustment, ion compartmentalization).

Biochemical adjustments (antioxidant enzyme activity, compatible solute accumulation).

The fact that grafted plants reduce wax deposition does not indicate vulnerability; it indicates that they have switched strategies. They rely more heavily on stomatal control and osmotic adjustment, while reducing investment in the cuticular barrier. This flexibility is a hallmark of a truly resilient species – one that can adjust its resource allocation to achieve the most cost-effective stress mitigation under given conditions (Liu et al., 2024).

Moreover, the choice of oleaster as rootstock may itself play a role in this shift. Wild olive relatives are often more tolerant to abiotic stresses and may transmit to the scion a set of stress-related signals that favour physiological over morphological responses. This opens interesting perspectives for breeding programs aiming to improve salinity tolerance through rootstock selection.

Taken together, our results demonstrate that the response of olive leaves to salinity is not uniform but depends critically on the mode of propagation and the genetic background of the variety. Cuttings respond by reinforcing their physical barrier (wax deposition), while grafted plants adopt a more physiological strategy, reducing wax investment in favour of other stress-mitigation mechanisms. This dual strategy is consistent with the known rusticity of the olive tree and highlights the adaptive plasticity that makes this species so successful in the challenging environments of the Mediterranean basin.

Future research should investigate the hormonal and molecular signals underlying these divergent responses, particularly the role of ABA and other stress-related phytohormones in mediating the rootstock–scion communication. Additionally, the long-term performance of these different strategies under field conditions, where salinity may be combined with other stresses (drought, heat), deserves further attention to guide breeding and agronomic decisions in the context of climate change.

Table 4. Correlation effect between salinity and the leaf physio-morphological parameters.

Variable	Variété	plant	Salinity	RWC	Leaf area	Number stomata	Wax rate
Variety	1.000						
Type de plant	0.000	1.000					
Salinity	0.000	0.000	1.000				
RWC	-0.249*	0.147	0.058	1.000			
Leaf area	-0.421*	0.211	0.086	0.268*	1.000		
Number stomata	-0.022	0.043	0.825***	-0.088	-0.011	1.000	
Wax rate	-0.178	0.269*	0.383*	0.033	0.169	-0.221	1.000

***p<0.001, **p<0.01, *p<0.05; insignificant: p>0.05.

4. DISCUSSION

Our study focuses on the irrigation of native and introduced (Spanish) olive trees with salt water at the rate of 6g / l of soluble salts, i.e. approximately 100 mM.l-1 of NaCl for 6 weeks. It enabled us to determine certain characteristics of the morpho-physiological behavior of this species under the salt stress. The water state of the plant, expressed by the relative water content, was sensitive to the applied treatments (r = -0.2). Indeed, salt stress causes a decrease in the values of the relative water content. This parameter, is one of the criteria for evaluating abiotic stress tolerance because it indicates the state of turgescence of the plant tissues and its ability to maintain a level of hydration of the tissues. The last could guarantee the continuity of its metabolism and the water state of a plant can be expressed by its RWC (Morant-Manceau et al, 2004; Mehani et al., 2012, Liu et al., 2024).

The results obtained during our study demonstrate that the RWC of all the stressed varieties changed with the salinity. However, the reduction was quite small compared to the control (between 1 and 2%). Local varieties retained a higher RWC than the introduced ones. We also noted that saline treatment did not cause a significant reduction in water content. The analysis of the relative water content is a good indicator of the water status of the plant.

On the other hand, salinity is a complex phenomenon that often leads to osmotic stress due to reduced amounts of water available at the root level, due to the reduced ability of plants to absorb water. The immediate response to salt stress is expressed by a reduction in leaf area as was noted by (Wang and Nil, 2000) and that decreased vegetative growth, expressed as leaf area reduction. Leaf area is generally the first response of glycophytic exposed to saline stress (Munns et al., 2006).

We observed that the decrease in the leaf area of all the genotypes studied shown only a slight drop which was between 1 and 2% compared to the control. This was confirmed by the results of the analysis of the correlation matrix between variety and leaf area (p = -0.421, see Table 4). For cuttings and grafted plants, it was found that grafted and stressed local ecotypes showed an increase in leaf area compared to the control, and cuttings and stress decreased leaf area compared to control (less 1.1 cm2 at the control). Thus, decreasing of leaf area was considered as a form of adaptation to salt stress, by reducing water losses through perspiration, but it may also cause a decrease in yields due to the reduction of photosynthesis (Acevedo, 1991; Hassani et al., 2008).

The stomatal density of leaves of all stressed genotypes decreased compared to that of no salt (Fig. 4). Moreover, this decrease was greater in the ecotypes obtained from herbaceous cutting (it was 65% for Sigoise, 52.78% for Chemlal, 54.29% for Arbiquina, and 75% for Manzanilla) than those grafted (69% for Sigoise, 34.01% for Chemlal, 45.31% for Arbiquina, and 43.2% for Manzanilla). According to Guyot (1998), stomatal transpiration accounts for 90% of total sweating for 24 hours.

The opening and closing of the stomata are controlled by the turgidity of their guard cells, which depend on soil and air moisture, sheet temperature, incident radiation, wind and concentration CO_2 in the air as well as in the chamber under stomatal conditions (Hassani et al., 2014). However, whenever the plant reduces its transpiration by closing the stomata, it causes the reduction of the production of dry matter following the reduction of the chlorophyll assimilation (Zhang and Shi, 2013).

Moreover, obtained results show a strong positive relationship between salt stress and stomatal density in the lower leaf epidermis. Indeed, the high correlation obtained between salinity and stomatal density ($r = 0.825$, $p<0.001$) shows that the presence of NaCl leads to an increase in the number of stomata, which is not logical when we know that they are small stomata to reduce water losses and increase the RWC.

According to Erchidi et al. (2000), plants that live in dry environments have many stomata with small sizes and that the presence of small and large stomata allows a much more effective regulation of sweating than that of large and small stomata and the increase in the number of stomata per unit area could be one of the factors of resistance to water deficit if accompanied by a good physiological activity (Slama et al., 2005).

The increase in stomatal density can increase the net assimilation of CO_2 and decrease the loss of water. In fact, many small stomates are fast closing (Heller et al., 2004).

In addition, Hopkins (2003) established the relationship between transpiration and stomatal resistance in Arabidopsis thaliana by increasing this resistance during salt stress, to minimize water loss and sweating, becomes more important in the case of thin cuticle leaves.

According to our results, the stressed genotypes have cuticles thicker than those under normal conditions that can explain the resistance of our plants by keeping their leaves throughout the duration of the stress applied.

5. CONCLUSION

The morpho-physiological parameters of the leaf, retained in this study, would be closely involved in the regulation of the water state of the leaf tissues. Salt resistance is a polygenic character that can be controlled at different levels of organization, from the cell to the entire plant. However, the diversity of salt effects on plants offers a wide range of physiological and biochemical criteria that can be the basis for rapid testing for large-scale selection. Application of salt water resulted in a reduction in RWC (relative water content), but relatively higher compared to other plants subjected to the same salt stress. This characteristic can be attributed to the osmotic fit of the stressed plant. This control of the hydration reveals a good ability to adjust the osmotic potential in the olive tree in general and especially in the varieties studied. For this purpose, it can be deduced that the varieties of olive trees studied have retained a high RWC in the presence of saline stress, indicating that this plant is of the “stay green” type, which keeps synthetically active green and photo leaves, have reasonable yields even in the presence of abiotic stress.

Under arid climates and in conditions of ionic stress accompanied by osmotic stress, the plant must maintain a dynamic balance between the opening and closing of the stomata. This activity allows it to increase the fixation of the carbon and a better transpiration thus avoiding the overheating of the plant. In Mediterranean regions, physiological drought (due to excess salinity), is often chronic, resulting in a decrease in photosynthesis. Our results showed that all the varieties studied under the effect of salinity, developed a thick cuticle compared to those under normal conditions, that decreasing leaf transpiration. This develops the resistance of the olive tree in general and especially the studied genotypes that keep their leaves during the applied stress. The results obtained in this study show that the olive tree is a salinity-resistant plant that is a characteristic of arid and semi-arid areas. Olive trees, then, is a promising alternative to improve the productivity of marginalized lands.

REFERENCES

Álvarez, S., Soriano, M.A., Landa, B.B., Gómez, J.A. (2007). Soil properties in organic olive groves compared with that in natural areas in a mountainous landscape in southern Spain. Soil Use and Management, 23, 404-416. https://doi.org/10.1111/j.1475-2743.2007.00104.x

Balasubramaniam, T., Logeswaran, J., Pandiaraj, K., & Veerasamy, R. (2023). Plants’ response mechanisms to salinity stress: An update. Frontiers in Plant Science, 14, 1016272. https://doi.org/10.3389/fpls.2023.1016272

Barrs, H.D. & Weatherley, P.E. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences, 15, 412-428. https://doi.org/10.1071/BI9620413

Bartels, D. & Sunkar, R. (2005). Drought and Salt Tolerance in Plants. Critical Reviews in Plant Sciences, 24, 23-58. https://doi.org/10.1080/07352680590910410

Belkhodja, M. & Bidai, Y. (2004). Réponse de la germination des graines d’Atriplex halimus L. sous stress salin. Sécheresse, 15(4), 331-335.

Ben-Gal, A., Borochov, H., Yermiyahu, U., Shan, I.U. (2009). Osmotic potential is a more appropriate property than EC for evaluating whole-plant response to salinity. Environmental and Experimental Botany, 65, 232-237. https://doi.org/10.1016/j.envexpbot.2008.09.006

Bennaceur, M., Rahmoun, C., Sdiri, H., Medahi, M., Selmi, M. (2002). Effect of salt stress on the germination, growth and yield of wheat. Sécheresse, 12(3), 167-174.

Besnard, G., Khadari, B., Baradat, P., Bervillé, A. (2002). Olea europaea (Oleaceae) phylogeography based on chloroplast DNA polymorphism. Theoretical and Applied Genetics, 104, 1353-1360. https://doi.org/10.1007/s00122-001-0832-x

Chowdhury, N., Marschner, P., Burns, R. (2011). Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant and Soil, 344, 241-254. https://doi.org/10.1007/s11104-011-0743-9

Clarke, J.M. & McCaig, T.N. (1982). Excised-leaf water retention capability as an indicator of drought resistance of Triticum genotypes. Canadian Journal of Plant Science, 62, 571-578. https://doi.org/10.4141/cjps82-086

Cocozza, C., Brilli, F., Pignattelli, S., Pollastri, S., Brunetti, C., Gonnelli, C., Tognetti, R., Centritto, M., & Loreto, F. (2020). The excess of phosphorus in soil reduces physiological performances over time but enhances prompt recovery of salt-stressed Arundo donax plants. Plant Physiology and Biochemistry, 151. https://doi.org/10.1016/j.plaphy.2020.04.011

Denden, M. & Lemeur, R. (1999). Mesure de la transpiration par le modèle de Penman-Monteith. Sécheresse, 10, 39-44.

Devkota, K., Devkota, M., Rezaei, M., & Oosterbaan, R. (2022). Managing salinity for sustainable agricultural production in salt-affected soils of irrigated drylands. Agricultural Systems, 196, 103345. https://doi.org/10.1016/j.agsy.2021.103345

Dohman, A.J., Gash, J.H.C., Roberts, J., James, W. (1991). Stomatal and surface conductance of tropical rainforest. Agricultural and Forest Meteorology, 54, 303-318. https://doi.org/10.1016/0168-1923(91)90011-E

Erchidi, A.E., Benbella, M., Talouizte, A. (2000). Relation entre des paramètres contrôlant les pertes en eau et le rendement grain chez neuf variétés de blé soumis au stress hydrique. Cahiers Options Méditerranéennes, Série A, 40, 279-282.

Garg, A.K., Kim, J., Owens, T., Ranwala, A., Choi, Y., Kochian, V., Wu, R.J. (2002). Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proceedings of the National Academy of Sciences USA, 99, 15898-15903. https://doi.org/10.1073/pnas.252637799

Gharabi, D., Bellhabib, A., & Hassani, A. (2022). Morphological and biochemical behavior of the olive tree in semi-arid areas of Algeria. South Asian Journal of Experimental Biology, 12(3). https://doi.org/10.38150/sajeb.12(3).p398-406

Grennan, A.K. (2006). High Impact Abiotic Stress in Rice. An "Omic" Approach. Plant Physiology, 140, 1139–1141. https://doi.org/10.1104/pp.104.900188

Hassani, A., Dellal, A., Belkhodja, M., & Kaid-Harch, M. (2008). Effet de la salinité sur l'eau et certains Osmolytes chez l'Orge (Hordeum vulgare). European Journal of Scientific Research, 23(1), 61-69.

Hassani, A., Gharabi, D., Kouadria, M., Bounaceur, F., Sehari, N., Belkhodja, M. (2014). Impact of salt stress on morphological and physical behavior of the foliar system by cultivating olive and wild olive trees. International Journal of Plant & Soil Science, 3(12), 1542-1551.

Heller, R., Esnault, R., Lance, C. (2004). Physiologie végétale, Tome 1 – Nutrition. Dunod, Paris.

Hoagland, D.R. & Arnon, D.I. (1938). The water culture method for growing plants without soil. University of California, Agricultural Experimental Station Circular, 347, 1-39.

Hopkins, W.G. (2003). Physiologie végétale (Traduction de la 2e édition américaine par S. R.). De Boeck, p. 66-81.

Lindsay, M.P., Lagudah, E., Munns, R. (2004). A locus for sodium exclusion (Nax1), a trait for salt tolerance, mapped in durum wheat. Functional Plant Biology, 31, 1105-1114. https://doi.org/10.1071/FP04111

Liu, X., Elzenga, J. T. M., Venema, J. H., & Tiedge, K. J. (2024). Thriving in a salty future: morpho-anatomical, physiological and molecular adaptations to salt stress in alfalfa (Medicago sativa L.) and other crops. Annals of Botany, 134(7). https://doi.org/10.1093/aob/mcae152

Martoja, M. & Martoja, R. (1968). Initiation aux techniques d'histologie. Masson, Paris.

Mehani, M., Bissati, S., Djeroudi, Q. (2012). Effet de l'eau de mer sur deux paramètres hydriques de jeunes plants d'Atriplex canescens. Journal of Materials and Environmental Science, 5, 840-845.

Moinuddin, A., Fischer, R., Savre, K., Reynolds, M.P. (2005). Osmotic Adjustment in Wheat in Relation to Grain Yield under Water Deficit Environments. Agronomy Journal, 97, 1062-1071. https://doi.org/10.2134/agronj2004.0152

Morant-Manceau, A., Pradier, E., Tremblin, G. (2004). Osmotic adjustment, gas exchanges and chlorophyll fluorescence of a hexaploid triticale and its parental species under salt stress. Journal of Plant Physiology, 161, 25-33. https://doi.org/10.1078/0176-1617-00963

Munns, R., Richard, A.J., Lauchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. Journal of Experimental Botany, 57(5), 1025-1043. https://doi.org/10.1093/jxb/erj100

Pages, L., Asseng, S., Pellerin, S., Diggle, A. (2000). Modelling root system growth and architecture. In Root Methods: A Handbook (pp. 113-146). Springer, Berlin. https://doi.org/10.1007/978-3-662-04188-8_4

Rascio, A., Cedola, M., Sorrentino, G., Wittmer, G. (1988). Pressure volume curves and drought resistance in two wheat genotypes. Physiologia Plantarum, 73, 122-127. https://doi.org/10.1111/j.1399-3054.1988.tb09202.x

Setia, R. & Marschner, P. (2012). Carbon mineralization in saline soils as affected by residue composition and water potential. Biology and Fertility of Soils, 48(4), 475-479. https://doi.org/10.1007/s00374-011-0643-4

Slama, A., Ben Salem, M., Ben Naceur, M., Zid, E. (2005). Les céréales en Tunisie : production, effet de la sécheresse et mécanismes de résistance. Sécheresse, 16, 225-229.

Szabolcs, I. (1994). Soils and salinization. In Handbook of Plant and Crop Stress (pp. 3–11). M. Pessarakali (Ed.). Marcel Dekker, New York.

Trinchant, J.C., Boscari, A., Spennato, G., Van de Sype, G., Le Rudulier, D. (2004). Proline-Betaine Accumulation and Metabolism in Alfalfa Plants under NaCl Stress. Exploring Its Compartmentalization in Nodules. Plant Physiology, 135, 1583-1594. https://doi.org/10.1104/pp.103.037556

Wang, Y. & Nil, N. (2000). Changes in chlorophyll, ribulose biphosphate carboxylase oxygenase, glycine betaine content, photosynthesis and transpiration in Amaranthus tricolor leaves during salt stress. Journal of Horticultural Science and Biotechnology, 75, 623-627. https://doi.org/10.1080/14620316.2000.11511297

Zhang, J.L. & Shi, H. (2013). Physiological and molecular mechanisms of plant salt tolerance. Photosynthesis Research, 115, 1-22. https://doi.org/10.1007/s11120-013-9813-6

Zhu, J.-K. (2001). Plant salt tolerance. Trends in Plant Science, 6(2), 66-71. https://doi.org/10.1016/S1360-1385(00)01838-0

SOBRE O ORGANIZADOR

Eduardo Eugênio Spers realizou pós-doutorado na Wageningen University (WUR), Holanda, e especialização no IGIA, França. Possui doutorado em Administração pela Universidade de São Paulo (USP). Foi Professor do Programa de Mestrado e Doutorado em Administração e do Mestrado Profissional em Comportamento do Consumidor da ESPM. Líder do tema Teoria, Epistemologia e Métodos de Pesquisa em Marketing na Associação Nacional de Pós-Graduação e Pesquisa em Administração (ANPAD). Participou de diversos projetos de consultoria e pesquisa coordenados pelo PENSA e Markestrat. É Professor Titular no Departamento de Economia, Administração e Sociologia, docente do Mestrado em Administração e Coordenador do Grupo de Extensão MarkEsalq no campus da USP/Esalq. Proferiu palestras em diversos eventos acadêmicos e profissionais, com diversos artigos publicados em periódicos nacionais e internacionais, livros e capítulos de livros sobre agronegócios, com foco no marketing e no comportamento do produtor rural e do consumidor de alimentos.

ÍNDICE REMISSIVO

A

B

C

D

E

F

G

H

M

N

O

P

R

S

U

V

W

Z


EDITORA
ARTEMIS
2026