

Greetings

During this whole master I learnt a lot of useful things, from where they are to learn how to understand better the forest functioning and its different processes. But not only that, there is a point in the life where we reach the moment to begin something new and meaningful in our lives.

This master helped me to open my mind and career beyond I used to know, and in this process of transformation, it is not only the opportunity to have the chance to make this master and this project, but also to the people who started the same way beside me, sharing special moment that all together, gather the knowledge of this two years.

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Dissertation topic: Drought Response Assessment of 3 Provenances of *Quercus suber*.

ABSTRACT

Considering that an important source of variation for adaptive traits is at provenance level, four *Quercus suber* contrasting provenances were selected according to their field performance, for growth, phenology and water use efficiency, in a multi-site provenance trial where 35 cork oak provenances covering all the natural distribution area are represented. This study is a first approach to assess provenance effect and acclimation capacity under controlled-environment conditions where drought stress was induced throughout three cycles. During the third drought cycle, three of those contrasting provenances (Haza de Lino, Spain; Ain Rami, Marocco and Landes, France) were submitted to two water regimes: watered at field capacity (Controlled) and drought-induced conditions (Stress). Different strategies were observed among the provenances submitted to drought conditions, such as reducing the number of leaves, height and branches, and increasing the total biomass for the root system. Those plants which were well watered in the first cycle and submitted to drought in the second and third cycles were the most sensitive to drought conditions, particularly the Ain Rami ones. The water relative content as well as the water potential and the photosystem II showed significant differences among the provenances with a general reduction due to drought.

Keywords: *Quercus suber*, drought, provenances, strategies.

Título da Dissertação: Avaliação da Resposta a Seca de 3 Proveniências de *Quercus suber*.

Resumo

Tendo presente que a maior proporção da variação das características adaptativas se encontra ao nível da Proveniência, foram seleccionadas quatro proveniências contrastantes de *Quercus suber*, num ensaio de Proveniências em que estavam representadas 35 Proveniências. Os critérios de selecção foram estabelecidos com base no seu comportamento relativamente ao crescimento, fenologia e eficiência de uso da água. Este estudo corresponde à primeira abordagem em que é avaliado o efeito da proveniência e a capacidade de aclimação após três ciclos de indução de seca. Durante o terceiro ciclo, três destas proveniências contrastantes (Haza de Lino, espanhola; Ain-Rami marroquina e Landes francesa) foram submetidas a dois regimes hídricos: regadas à capacidade de campo (Controlo) e induzidas a condições de seca (Stress). Foram observadas diferentes estratégias entre as proveniências quando sujeitas à seca, tais como redução no número de folhas e ramos, altura, aumento da biomassa total alocada às raízes. As plantas que foram bem regadas no primeiro ciclo e submetidas à seca no segundo foram as que mostraram ser mais sensíveis à seca, principalmente as de Ain-Rami. O teor relativo em água, o potencial hídrico e o fotossistema II mostraram diferenças significativas entre as proveniências com uma redução geral devido à seca.

Palavras chave: *Quercus suber*, seca, proveniências contrastantes, estratégias.

Resumo Extendido

O sobreiro (*Quercus suber* L.) é uma espécie perene da floresta mediterrânica ocidental, com valor ecológico, social e económico. O montado cobre cerca de 25.000 km² espalhados ao redor sudoeste da Europa de acordo com Sibley, (2009). Na verdade, o sobreiro é emblemático na zona do Mediterrâneo, sua existência participa no ciclo de vida de muitos animais contribuindo para a interacção entre as diferentes espécies.

O *Quercus suber* tem uma grande área de distribuição com uma variação substancial das condições ambientais. Assim, espera-se que, através da selecção natural, diferentes proveniências desenvolvam diferenças significativas nas características de fitness, tanto bióticas (por exemplo, resistência a pragas e doenças) como abióticas (por exemplo, tolerância à seca e aos níveis de temperatura variando durante o ano), estes fatores estão envolvidos na capacidade de adaptação a um determinado ambiente. Neste contexto, os ensaios de proveniência são o melhor método para avaliar a variabilidade entre e dentro das populações e fontes de sementes coletadas em uma grande variedade de locais que cobrem a distribuição geográfica das espécies. Assim em 1998, os ensaios de proveniência multi-site foram estabelecidas em três locais em Portugal, como parte da "Rede Europeia de Avaliação de Recursos Genéticos de sobreiro para o uso adequado em Reprodução e Estratégias de Conservação Genética" (Varela, 2000). Os resultados destes testes de campo em sete anos de plantio apontaram que a origem da semente deve ser considerada em programas de reflorestação, já que a sobrevivência, crescimento e características adaptativas (fenologia, eficiência do uso da água - EUA) mostrou diferenças significativas entre as populações.

Portanto, tendo presente que a maior proporção da variação das características adaptativas se encontra ao nível da proveniência, foram seleccionadas quatro proveniências contrastantes de *Quercus suber*, num ensaio de proveniências em que estavam representadas 35 proveniências ao nível de Europa. Os critérios de selecção foram estabelecidos com base no seu comportamento relativamente ao crescimento, fenologia e eficiência de uso da água. Este estudo corresponde à primeira abordagem em que é avaliado o efeito da proveniência e a capacidade de aclimação após três ciclos de indução de seca.

No primeiro ciclo do estudo as plantas foram divididas e submetidas a dois regimes hídricos. Uma metade foi regada à capacidade de campo (Controlo) e a outra submetida a seca (Stress). Durante o segundo ciclo, as plantas foram novamente separadas em mais

dois grupos, onde ficaram plantas que já tinham sido submetidas a seca que mudaram para rega controlada (Stress-Controlo), plantas que mudaram para regime de seca depois de terem sido submetidas a rega (Controlo-Stress) e plantas que continuaram com o mesmo regime de seca (Stress-Stress) ou rega (Controlo-Controlo).

Neste terceiro ciclo, três destas proveniências contrastantes (Haza de Lino espanhola; Ain-Rami de Marrocos e Landes de França) foram submetidas aos mesmos regimes descritos no segundo ciclo repetindo assim os tratamentos.

Os objectivos deste trabalho são averiguar se as proveniências apresentam estratégias diferentes sob condições de seca e se as plantas apresentam qualquer processo de endurecimento ou aclimação após serem submetidas a três ciclos de condições induzidas pela seca.

O estudo foi constituído por 79 plantas, das quais 31 pertenciam a proveniência francesa (Landes), 30 a espanhola (Haza de Lino) e 18 a Marroquina (Ain Rami). Todas as plantas foram plantadas em recipientes de 2,6 litros de volume, do tipo "plug" que contém saliências do lado interior para evitar que as raízes cresçam em espiral e areia usada como substrato.

O terceiro ciclo começou em Janeiro, com um período de estiagem de 80 dias, imposta pela retenção de rega dessas plantas submetidas a stresse. As plantas submetidas ao tratamento de controlo foram regadas à capacidade de campo água, uma vez por semana ou até duas vezes por semana quando era muito quente, com aproximadamente 200 ml por recipiente. As temperaturas médias durante o estudo foram variáveis com máximas que superam os 40 graus nos dias mais quentes até os 20 graus nos dias mais frios.

Os resultados dos experimentos para a taxa de sobrevivência mostraram que a proveniência Ain Rami foi a mais sensível em condições de seca, com o tratamento (CS) totalmente morto antes mesmo do final do estudo e mantendo-se com só uma planta submetida em condições de stresse.

As folhas de Ain Rami mostraram ser as mais compridas e as mais finas, principalmente para os tratamentos submetidos a CS e SS quando em comparação com as de Haza del Lino e Landes que tinham umas folhas mais grossas e pequenas. Ademais, para os tratamentos CS e SS de Ain Rami tiveram maiores quantidades de biomassa total, observando-se que quanto maior era a biomassa total na raiz, maior era a proporção entre a raiz e os ramos.

Relativamente ao desenvolvimento radicular, as plantas sujeitas aos tratamentos SC e CC tiveram uma maior quantidade de raízes desenvolvidas, contrariamente aquelas que foram submetidas a tratamentos sob condições de seca onde o crescimento foi menor, observando-se que as plantas de Haza del Lino tiveram uma maior quantidade de raízes secundárias no tratamento de stresse.

Os tratamentos com maior crescimento da biomassa são os que têm o maior comprimento das raízes mostrando maior eficácia na absorção de água e nutrientes sob condições de seca severa enquanto outras raízes investiram a sua energia em comprimento para a procura de água.

Relativamente a morfologia, a estratégia adaptada sob condições de seca mostrou diferentes respostas para o número de ramos, folhas e diâmetro das plantas entre todas as proveniências.

A altura da planta foi directamente relacionada com o crescimento da planta, onde em condições de seca severa, o crescimento da parte aérea é limitada e acontece uma translocação do crescimento para a estrutura da raiz.

Nos tratamentos de stress foram encontradas a maior quantidade de estomas, verificando-se uma maior eficiência na troca de CO₂ quando submetidas a condições de seca severas, atestando as respostas das curvas de luz. Uma maior quantidade de estomas pode promover o crescimento de mais ramos e folhas de modo que no final há mais fotossíntese e crescimento no geral. As plantas do tratamento CS mostraram uma produção fotossintética mais eficaz do que as do tratamento SS.

Para o teor relativo de água os dados mostraram uma redução geral no volume de água nas folhas, embora estivessem sujeitas a condições óptimas nos tratamentos CC e SC variaram entre 80 e 40%. As plantas de tratamento CS mostraram uma maior sensibilidade geral à seca, respondendo com uma redução maior e crescimento mais rápido do que o tratamento em stresse. Aliás, mostraram-se diferenças significativas no tratamento CC entre as três proveniências.

O potencial de água mostrou uma resposta similar ao teor relativo, porque o tratamento CS também foi o mais sensível a seca e obteve diferenças significativas no tratamento de controlo entre as proveniências. Além de mais, o potencial de água obteve valores mais negativos que o ciclo anterior.

O fotossistema II mostrou uma redução geral, principalmente para os tratamentos submetidos a seca, (CS e SS) para as três proveniências, ainda que Haza del Lino teve

melhores resultados que Landes e Ain Rami.

Baixas intensidades assim como as elevadas intensidades de luz podem significar um stress nas plantas. Um porque limita a fotossíntese e portanto a absorção de carbono bem como o crescimento das plantas e o outro porque favorece a perda de água por evaporação.

Os dados mostraram que as plantas mais sensíveis a seca atingiram o ponto de saturação antes e em taxas anteriores ao PAR máximo, como no caso de Landes para o tratamento de stresse. Valores mais elevados nas curvas de Luz estão relacionados a um menor teor relativo de água, potencial de água e fotossistema II.

Em condições de seca as clorofilas, necessárias para a produção da fotossíntese da planta diminuíram em todos os tratamentos e proveniências. Enquanto é submetida à seca, a quantidade de água está menos disponível, tornando mais difícil a absorção de nutrientes, como o azoto ou magnésio, macronutrientes essenciais para a produção da clorofila e comumente encontrados no solo em forma de compostos complexos, NO_3^+ , NH_3 , etc., e sob condições de seca é ainda mais difícil a sua absorção.

O azoto e o magnésio são elementos vitais para a produção de clorofila e quando são escassos, a fotossíntese não pode ser feita, e o crescimento da planta é interrompido. A sua ausência reduz a área foliar e o crescimento geral da árvore, provocando a translocação do azoto das folhas velhas até as novas. Os valores inferiores a 2 mg/cm^2 de clorofila, mostram uma degradação da clorofila. A proveniência espanhola tem uma menor perda de água por evapotranspiração, o que favorece a absorção dos elementos para realizar a fotossíntese e permanecendo assim viva por mais tempo, apesar de que no final o carbono será usado mesmo para o crescimento das plantas ou para a produção de açúcares e outras substâncias. Contrariamente a proveniência de Ain Rami que obteve folhas maiores e mais finas teve uma perda maior de água reflectindo uma indisponibilidade dos minerais necessários para o processo de fotossíntese.

No caso dos carotenóides quanto maior a quantidade de carotenóides maior a protecção da planta. Além disso, distúrbios fisiológicos aumentam a vulnerabilidade da árvore a tensões secundárias, como danos causados por insectos, geada ou secas repetidas, conforme mencionado em Rouault *et al.*, (2006). Os dados mostraram uma diminuição geral dos carotenos em condições de seca severa para todas as proveniências e tratamentos, com Haza de lino apresentando os valores mais elevados.

Por outro lado, o aumento geral da feofitina (Ppt), ao contrário da clorofila, significa

menor quantidade de magnésio disponível para a produção de fotossíntese. A proveniência espanhola teve valores de Ppt mais elevados que as outras proveniências, podendo significar que em condições de seca moderadas ou graves, pode encontrar o caminho para ter magnésio suficiente para a sua produção de clorofila, mantendo assim as plantas um estado de equilíbrio constante e com um crescimento muito lento ou "estratégias tolerantes" a seca de acordo com a Kozłowski *et al.*, (1991).

Portanto neste estudo confirma-se que as proveniências apresentaram diferentes estratégias contrastantes entre elas, onde a proveniência de Ain Rami mostrou ser a proveniência mais sensível sob condições de seca, em especial as plantas do tratamento CS de acordo com os resultados da taxa de sobrevivência.

As plantas submetidas as condições de controlo que apresentaram diferenças significativas entre as proveniências, como no teor relativo ou potencial de água, nas clorofilas, feofitina, carotenóides e no comprimento da raiz demonstraram que pode ser devido à "proveniência específica", e portanto a uma variação geográfica caracterizada pela evolução individual de cada proveniência sob diferentes condições ambientais. A diminuição da produção de carotenóides nos tecidos refere-se a diminuição de protecção das folhas das plantas favorecendo o ataque as plantas pelos insectos ou agentes externos.

Ain Rami mostrou que a alocação de carbono foi focado principalmente no desenvolvimento das raízes da planta, especialmente no caso do tratamento CS, que apesar de os indivíduos não terem sobrevivido, desenvolveram raízes especializadas com uma maior eficiência na absorção de água e nutrientes. A alta taxa de LA e SLA para a proveniência marroquina, além de ter a maior quantidade de estômas, favoreceu a perda de água por evaporação e secagem das plantas enquanto contrariamente as proveniências espanhola e francesa como tiveram folhas mais pequenas e mais grossas, tiveram menos perda de água por evapotranspiração.

As respostas realizadas pelos tratamentos submetidos a diferentes regimes de seca, seja CS ou SC em todas as proveniências pode ter sido devido a um processo de adaptação ou aclimação das plantas obtido depois dos diferentes regimes consecutivos de seca e rega alternada.

Symbols and abbreviations

Ab	Absorbance
AR	Ain Rami provenance
CC	Control + Control 2 followed regimes under controlled conditions
CCC	Control + Control + Control 3 followed regimes under controlled conditions
Chl _a	Concentration of Chlorophyll a in the leaves
Chl _b	Concentration of Chlorophyll b in the leaves
CS	Control + Stress treatments followed in the two previous years
CSS	Control + Stress + Stress treatments followed in the three years
Dm	Dried mass
Fm	Fresh mass
HL	Haza del Lino provenance
HR	Relative humidity
IRGA	Infra-red gas analysis
Klx	Kilolux
LA	Leaf Area
LD	Landes provenance
LMF	Leaf mass fraction
mM	Milimoles
MMF	N,N-dimethylformamide
Nm	Nanometers
PAR	Photosynthetically active radiation
Ppt a	Pheophytin a
Ppt b	Pheophytin b

PSII	Quantum efficiency of the photosynthetic system II
R	Respiration
RAR	Root/Shoot ratio
Rpm	Revolutions per minute
RWC	Relative water content
S.A.	Surface Area
SC	Stress + Control in the two previous years
SCC	Stress + Control + Control treatments followed in the three years
SLA	Specific leaf area
SS	Stress + Stress in the two previous years
SSS	Stress + Stress + Stress treatments followed in the three years
<i>Tm</i>	Turgid mass
WSM	Water stress moderated
WSS	Water stress severe
WW	Water water (Regime under water field capacity)
μm	Micrometers
Ψ	Leaf water potential

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1. Introduction

1.1. *Quercus suber*, a Mediterranean key specie

Cork oak (*Quercus suber* L.), is a western Mediterranean evergreen forest specie, with ecological, social and economic value. Cork oak forests cover approximately 25,000 km² spreaded around south west of Europe according to Sibley, (2009). Natural stands of cork oak support diverse ecosystems. Indeed, cork oak is emblematic in the Mediterranean area, its existence takes part on the life cycle of many animals as an interaction exchange between different species. For example, in the case of the Iberian Peninsula with an endangered specie such as the Iberian Lynx mentioned in Pereira *et al.* (2008). There, it occurs mostly in savannah-type formations called "montados" in Portugal or "dehesas" in Spain, with sparse tree cover, intermixed with other evergreen oaks, mainly Holm oak.

According to Sibley, (2009) and Hole *et al.*, (2007) cork oak lives about 150 to 250 years. It is slow growing specie; it may reach up to 20m tall in deep soils and with adequate rainfall, although it is typically more stunted in its native environment. Pausas, (1997) showed that the tree forms a thick, rugged bark containing high levels of suberin, an insulating bark that may have been the cork oak's evolutionary answer to forest fires.

One feature that is extremely rare is that the same cork cambium remains active forever, being destroyed only when exposed to air during cork extraction. When the cork is removed from the tree, the cork cambium cell layer dies. A new cork cambium layer is formed in the nonactive part of the underlying phloem tissue. This regenerated cork cambium immediately starts the division of suberized cells, producing a new cork layer (Mendes, Graça, 2009). Year after year the cork cambium of cork oak lays down annual layers of compact cork tissue, made of suberized cells. Over time the cork cambium layer of bark can develop considerable thickness and can be harvested every 9 to 12 years to produce cork. Virgin cork (or "male" cork) is the first cork stripped from generally 25-year-old trees. Rotation period may attain 250-350 years of age. "The trees maintain their ability to produce cork but cork thickness decreases as tress age and 150-200 years seem to be the limit for an industrially useful cork production. Rotation periods between 100 and 150 years, are usually assumed in most management plans" (Pereira and Tomé, 2004).

The tree is cultivated in Spain, Portugal, Algeria, Morocco, France, Italy and Tunisia. Cork oaks are considered to be soil builders and their fruits and leaves have been shown to

have useful insecticidal properties according to Pereira *et al.*, (2008) and Schechter and Haller, (1943). Portugal accounts for around 50% of the world cork harvest.

According to Calheiros and Meneses, (2002), cork is a recyclable, flexible and very useful material, thus its importance is increasing yearly. This material can be used in many different fields and it doesn't pose a waste of many resources, as mentioned in Pereira *et al.*, (2008). Cork is the primary source for wine bottle stoppers and other uses, such as cork flooring. The selection of cork oak forest reproductive material, for new plantations, also longer, more frequent, and more intense drought periods are expected, stress caused by the expansion of arid and semi-arid climate throughout the country might affect the species distribution have encouraged to understand cork oak adaptability to drought conditions. Cork oaks are able to acclimate in response to changes in their environment, as drought regime and shading. Long-term exposure of cork to water deficit and shaded environment might alter their morphology, physiology and anatomy of the plant. Kozlowzski *et al.*, (1991) showed that oaks are plants which strategy under water deficit is displayed in a "tolerant way".

Mediterranean trees are known to typically possess anatomical and morphological structures in their leaves, or other vegetative organs, which provides high resistance to desiccation during high water drought. The accumulation and syntheses of compatible solutes, either in leaf or the root, allow the maintenance of cellular metabolism in conditions under low water potential according to Lassalles and Morillon, (2002). Parry *et al.*, (2003) mentioned that plants use sugars as a strategy of adaptation to drought stress. In general, there is an accumulation of soluble sugars in the leaves in favorable conditions, which favors water uptake from the soil as mentioned in Sharp *et al.*, (2004).

1.2. Responses to Drought

Plants are subject to constant variation in the supply of carbon (C), including daily alternations between the photosynthetic fixation during the day and respiration growth overnight. Geiger and Servaites, (1994), Stitt and Smith, (2007); Stitt *et al.*, (2007) stated that the majority of the alternations on the supply of carbon induce the plants to accumulate assimilates as starch in leaves which are remobilized overnight to support the metabolic activities in the form of sucrose. The assimilation, accumulation and use of C depend on a complex network, important to sustain the plant growth as a response to changes caused by environmental disturbances like drought conditions according to Smith and Stitt, (2007).

The drought's responses are different depending on the variables of the drought: the intensity of drought (how much overpass the threshold of the water capacity in the soil), the duration of the deficit (in terms of how many days) and intensity (total accumulated of water

deficit over the number of days).

The water balance controls the plant continuum and it is regulated by from soil-root, and from root-leaf-atmosphere interfaces. From soil to root interface controls the water uptake of the plant, defined as water absorption, while the root to atmosphere regulates the water flow along the plant, driven by the evapotranspiration. As more amount of water is evapotranspired, more water is required and thus absorbed by the roots. The evapotranspiration increases when soil dries.

Grote and Suckow, (1998) showed that stored water may contribute to transpiration during diurnal cycles and into the ability of the plants to thrive in dry habitats. Transpired water is moved from soil to leaves and to atmosphere, decreasing gradually its water potential (ψ). Water stress occurs when soil moisture declines, or when transpiration increases, so leaf water potential decreases and tensions in the water capillaries in xylem tissues increases.

Plants used to frequent and severe droughts, sometimes even during the same year, develop a very efficient stomata closure, with few hours of stomata opening in dawn, where the efficiency of uptaking CO_2 is the highest, while in the contrary during the sunset is very inefficient. The process of opening and closing of stomata results from two contradictory demands that pass through the stomata (the carbon assimilation of CO_2 and the water loss to the atmosphere), the coordinated action that controls the turgor of the cells and is regulated by hormones such as ABA, Ethylene, Gibberellins (closure of stomata) and Citocianinas (opening), as mentioned in Wilkinson and Davis, (2002); Viernieri *et al.*, (2001) and Chaves *et al.*, (2003). The hormones responses might not affect in the leaf area growth but influences the flow rate at xylem level, by means, changing the flow and thus the amount of water loss.

According to Cruiziat *et al.*, (2002), stomata closure controls the leaf transpiration and loss of hydraulic conductivity during the course of drought being a tool to reduce the risk of deleterious massive embolism at long period, as it is mentioned in Lemoine *et al.*, (2002); Sperry, (2000); Sperry and Pockman, (1993) and Tyree and Zimmermann, (2002).

Cochard *et al.*, (1999) showed that light exposed branches are less vulnerable than shade ones, submitted to lower transpiration and less negative leaf water potential. The conductivity is restored only the following year by the formation of a new ring of functional xylem. Even though, if the embolism is repaired, it occurs after drought, remaining a costly process, requiring metabolic energy to generate the required positive pressures, and cavitation avoidance remains probably a much more efficient way to cope with reduced soil water, and stomata controlled for transpiration.

In other hand, Arnold and Mauseth, (1999); February and Manders, (1999); Ladjal *et al.*, (2005); Lovisolo and Schubert, (1991) and Schume *et al.*, (2004), mentioned that drought

frequency results in decreases of xylem conduit dimensions in the short term. Moreover, physiological disorders increase tree vulnerability to secondary stresses like insect damage, or frost, as mentioned in Rouault *et al.*, (2006). In long term, plants can reach an irreversible damage with organ dysfunction. The main consequence of drought at tree level is a reduction of sapwood section due to enhanced duraminisation in the sapwood to heartwood driving to the decline of the tree leading to the tree mortality with the only chance to be recuperated from the drought in the following year with a new ring growth.

When stomata are closed, the carbon uptake by the CO₂ exchange will be used either for the development of a better root apparatus, either for the formation of sugars that will be used as a combustible for the plant, or even for the reduction of the water potential value, while helping the tree to recuperate from the drought during the night.

1.3 Geographic variation in natural *Quercus suber* forests and its influence under drought conditions

According to Eriksson *et al.* (2006), provenance is a population or group of individuals of the same species occurring within or originating from one more or less rigorously defined geographic area, these authors recognize that variability for adaptive traits are found at provenance level.

Considering that *Q. suber* has a large distribution area with substantial variation in environmental conditions. Hence, it is expected that, through natural selection, different provenances have developed significant differences in fitness traits, with both biotic (e.g. resistance to pests and diseases) and abiotic (e.g. tolerance to drought and to varying temperature levels during the year) factors being involved in the adaptability to a given environment. In this context, provenance trials are the best resource of material to assess the variability between and within populations from seed sources sampled in a wide range of locations (stands) covering the geographical distribution of the species. Therefore in 1998, multi-site provenance trials were established at three locations in Portugal, as a part of the "European Network for the Evaluation of Genetic Resources of Cork Oak for Appropriate Use in Breeding and Gene Conservation Strategies" (Varela, 2000). Results from these field trials at 7 years from planting pointed out that seed origin must be considered in reforestation programs, since survival, growth and adaptive traits (phenology, water use efficiency - WUE) showed significant differences among populations.

So far, several studies with biochemical and molecular markers showed generally high levels of genetic variation within cork oak populations, but moderate amount of geographic differentiation among them. Although the use of molecular markers make

possible to draw a picture about the genetic structure of the species, it is well known that genetic markers do not reflect the variation in adaptive traits. Therefore, controlled environment studies of plant responses to drought stress in contrasting cork-oak provenances allow to clearly identify adaptive traits and evaluate phenotypic plasticity for this trait, which is a major limitation to cork oak regeneration (Nunes *et al.*, 2008).

Four contrasting provenances considering survival, height growth, leaf phenology and water use efficiency (δC_{13}) were selected according to their field performance in the Portuguese experimental sites of Cork oak provenances trials by the FCT project (PTDC/AGR-AAM/104364/2008). The selected provenances were: Haza del Lino (LD), Spain, for the shortest plants, Landes (LD), France, for the latest flushing, Ain El Rami (AR), Morocco, for the largest plants and Puglia (PG), Italy, for the earliest flushing.

Drought-induced under controlled environmental conditions to these four provenances was initiated in 2011. The study comprised three drought cycles. In the first cycle, the plants from each provenance were randomly assigned to two groups (Control treatment and Stress treatment) In the second cycle, plants were randomly assigned to four groups, again each of them with equal number of plants: i) plants submitted two cycles of Control treatment; ii) plants submitted two cycles of Stress treatment; iii) plants that in the first cycle were submitted to the Stress treatment and in the second cycle were submitted to the control treatment; iv) plants that in the first cycle were submitted to Control treatment and in the second cycle were submitted to the Stress treatment.

This study is a first approach to provide new data to elucidate and show different behaviors between provenances and treatments. For these reasons, the main objectives of this thesis are:

- Whether the provenances display different strategies under drought conditions
- Whether the plants show any process of hardening or acclimation after being submitted to three cycles of drought-induced conditions.

2. Material and Methods

The plants used in this experiment were produced at ISA nursery from acorns collected in autumn 2011 in the same stands, of the four selected provenances Haza del Lino (HL), Landes (LD), Ain Rami (AR) and Puglia (PG), where the seed were harvested in 1996 to establish the multi-site cork oak provenance trial (Varela 2000). Table 1 summarizes information on the location and weather data from these provenances.

Table 1: Provenances Location and weather data for each provenance studied in drought induced experiment

Provenance	Location	Coordinates		Mean Annual Temp. (°C)	Total Rainfall (mm)	Altitude (m)
		Latitude	Longitude			
Haza del Lino (HL)	Granada, Spain	36°49'00"N	31°18'00"W	16-18°C	600-1000mm	1300m
Landes (LD)	Landes, France	48°36'47"N	0°19'14"W	10,8°C	889mm	20m
Ain El Rami (AR)	Morocco	35°7'48"N	5°16'12"W	16,5°C	891mm	300m
Puglia (PG)	Italy	41° 0' 5"N	16° 44' 1"E	13,2	796mm	47m

In the first cycle, each provenance was represented by 90 plants. These plants were randomly assigned to two water regimes:

- 45 to Control treatment "C", in which plants were watered to field capacity
- 45 to Stress treatment "S", in which plants were induced to drought

In the second cycle, the plants from both treatments (Control and Stress) were divided in two new groups. Half of the plants submitted Control treatment ("C") were kept watered to field capacity control treatment (CC= control + control) and the other half were induced to drought (CS= Control + Stress). Similarly half of the plants previously induced to drought ("S"); were watered to field capacity (SC= Stress + Control), while the other half remained with the same stress regime (SS= Stress + Stress).

The present study corresponds to the third cycle of the drought induced to three of the four initial contrasting provenances, where Puglia (PG) won't be represented in the following experiments due to its high mortality in the second cycle. In the third cycle, we kept the plants under the same treatments as in the second cycle: therefore 4 treatments: Plants watered to field capacity (SCC and CCC) and plants induced to drought (CSS and SSS). Table 2 shows the number of plants per provenance and treatment

Table 2: Number of plants per provenance and per treatment (CC=Three regimes of Control; SC=Stress+2 regimes of Control; CS= Control+2 regimes of Stress and SS= Three regimes of Stress) used in the third cycle of drought induced

Provenance	CC	SC	SS	CS	Total
Haza de Lino	8	6	9	7	30
Landes	5	7	10	9	31
Ain Rami	4	3	6	5	18
Total	17	16	25	21	79

All plants were in containers with 2'6 liters of volume, type "plug" planting stock with ribs on the inner side of containers to prevent turning roots around inner side of container (spiral growth), , sand was used as substrate.

The third cycle started in January, with a drought period of 80 days, imposed by withholding watering of those plants submitted Stress treatment. The plants submitted to control treatment were watered to water field capacity, approximately 200 ml per pot once a week or even twice a week when it was too hot.

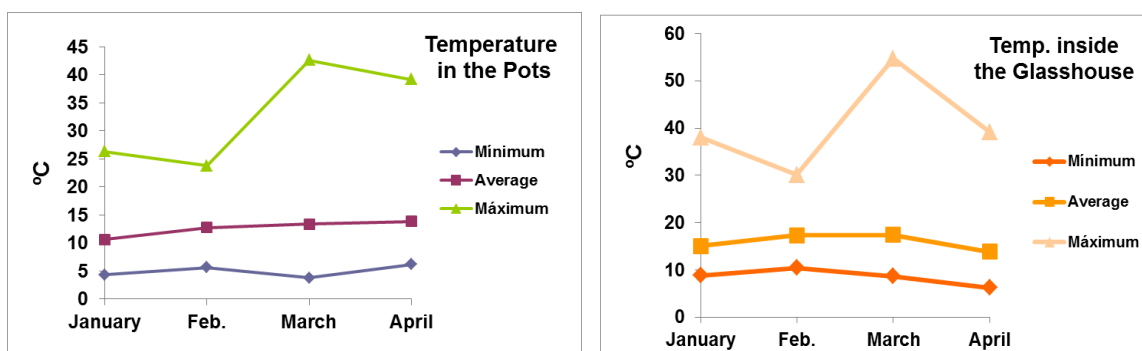


Figure 1: Maximum, minimum and mean temperatures of the pots and inside of the Glasshouse

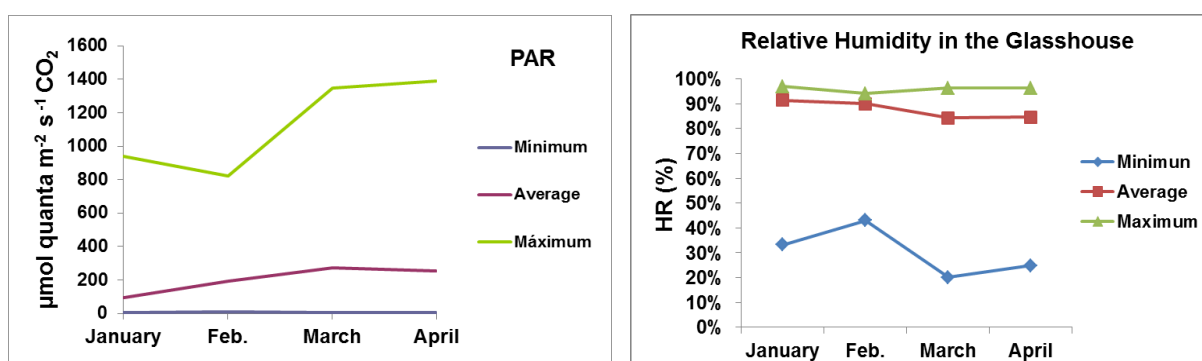


Figure 2: Photosynthetic active radiation (PAR), or spectral range (from 400 to 700 nanometers) and maximum, minimum and mean Relative Humidity (HR) of the Glasshouse, from January to April of the study



Figure 3: General view of the plants during the 3rd cycle of drought. Containers of the plants in the glasshouse, colors and labels of the different provenances and treatments

2.1. Survival Rate

This assessment registers the proportion of plants which last the whole study. It is evaluated through the ratio of the total plants alive and the total plants death per provenances and per treatments in each point of measurement. The data was obtained by counting all the dead plants after 57, 67 days, corresponding to moderate-severe drought-induced conditions and after 80 days, when the study finished.

2.2. Biomass Growth

2.2.1. Leaf Area (LA)

The total leaf (LA) was measured at the end of the drought-induced process, the day 80, after the destruction of the plants, where the total area of the leaves (old and new leaves) in cm² was obtained. Each plant was digitalized using a scanner EPSON expression 1680. Through the program ImageJ the total area of the leaf was obtained, by analyzing the picture of the total area of all leaves. In addition, a convertor was used to convert the pixels to cm². For this method 34 plants were used, 14 from Haza del Lino, 6 from Ain Rami and 14 from Landes provenance.

2.2.2. Specific Leaf Area (SLA)

Specific leaf area is the ratio of leaf area and leaf weight. It is expressed in m² (leaf) kg⁻¹ (leaf). Measured at the end of the study (day 80), after the destruction of the plants. It defines the quantity of leaf area per plant in terms of plant growth by the leaves according to (Rincón-Carruyo X. R. *et al.* 1997; Hunt R. *et al.*, 2002; Villar R. *et al.*, 2004). It was used the same plants mentioned for the LA. It can be expressed as the following equations:

$SLA = \text{Leaves area} / \text{Total dry weight of the leaves (1)}$ or $SLA = LAR * LMF (2)$

Where $LAR = \text{leaf area ratio}$ and LMF is leaf mass ratio.

2.2.3. Total Biomass

The total biomass indicates the growth of the plant. These data were obtained through the destruction of the plants, the day 80, dividing them in the different parts (roots, branches and leaves) follow by the weight of each part separately after a dry process in a stove at 80°C during 72 hours for the roots and 48 hours for the leaves and branches. 34 plants were evaluated, 14 from Haza del Lino, 6 from Ain Rami and 14 from Landes provenances. It is expressed in gr (dry weight).

2.2.4. Root/Shoot Ratio (RAR)

The Root/Shoot ratio is an adimensional measure. It is the proportion of carbon that is used by the plant for the balance of its structure. The data was obtained after the destruction of the plants, the day 80. Once the total biomass is obtained we can proceed to measure the Root/Shoot ratio by the division of the root dry weight / (branches + leaves) dry weight. The amount of plants used for the root/shoot ratio, were the same than in the total biomass.

2.2.5. Root Area and Root Classification

The total root area existent in the plants was measured at the end of the study, day 80. The data obtained by measuring the length, root volume and surface area through the digitalization of each root apparatus from each plant, with a scanner EPSON expression 1680. Followed to the digitalization, with the program winRHIZO software (image analysis system specifically designed for root measurement) was used for the classification of the roots based on 5 different levels of the root diameter ($0 < S.A. \leq 1$, $1 < S.A. \leq 2$, $2 < S.A. \leq 3$, $3 < S.A. \leq 4.5$, $S.A. > 4.5$), from less to higher diameter of the root. It was used a total of 52 plants, 17 from Landes, 21 from Haza del Lino and 14 from Ain Rami.

2.3. Morphological Methods

These methods response the visible transformation performed in the plant, influenced by the geographical variation of the provenances, the environmental conditions or external actions, like pest and deceases, or even animal attack (peacock bites).

2.3.1. Total branches per plant

This method consisted in counting all the branches per plant, by means all the

branches of all the 79 plants. Measure was made in two moments of the project, in the beginning, under controlled conditions, where the plants were still watered until field capacity and 50 days after the study began, where the plants were submitted under moderate-severe drought conditions.

2.3.2. Total leaves of the main branch

This method consisted in counting all the leaves of the main branch per plant, by means all the main branches of all the 79 plants. Measured in two moments of the project, in the beginning, where the plants were watered under field capacity and 50 days after the study began, where the plants were under moderate-severe drought conditions.

2.3.3. Diameter of the main branch

This method consisted in measuring the diameter of the main branch per plant, by means all the branches of all the 79 plants using a Caliper. Measured in two moments of the project, at the beginning, where the plants were watered until field capacity and 50 days after the study began, where the plants were under moderate-severe drought conditions.

2.3.4. Height of the main branch

This method consisted in measuring the height of the main branch per plant, by means all the branches of all the 79 plants using a rule. Measured in two moments of the project, at the beginning, where the plants were watered until field capacity and 50 days after the study began, where the plants were under moderate-severe drought conditions.

2.3.5. Stomata Counting

The stomata counting were made at the beginning of the study. One leaf from 4 plants from each treatment and provenance was used; a total of 16 leaves from Haza de Lino and Landes and 14 from Ain Rami, due to the SC treatment had only 2 plants.

The process consisted in cutting the leaves in the early morning with a cutter, one by one putting them in different glass plates labeled for the recognition of the leaves. Then a transparent polish nail was applied in the downer part of the leaf. Once the polish nail was dried, a piece of transparent tape was stuck on the painted area and then by removing it softly from the leaf, the dry polish nail was glued on the tape. Through this, a footprint of the

leaf was obtained. The tape with the footprint will be stick on a lab glass holder and labeled. Finally, the holder will be putted on the microscope for observing the amount of stomata contained on the observation area. The counting of the stomata was made through the 2 following methods:

Table 3: Variations in average length and frequency of stomata of woody angiosperms, source from Davies et al. (1973). This figure shows the different amount of stomata that can be found in different species

Species	Stomatal length (μm)	Stomatal frequency (number/ mm^2)
<i>Acer saccharinum</i>	17.3	418.8
<i>Acer saccharum</i>	19.3	463.4
<i>Acer negundo</i>	21.6	233.9
<i>Betula nigra</i>	39.4	281.3
<i>Betula papyrifera</i>	33.2	172.3
<i>Catalpa bignonioides</i>	23.2	328.6
<i>Crataegus</i> sp. I.	22.3	399.1
<i>Crataegus</i> sp. II.	37.4	221.4
<i>Fraxinus americana</i>	24.8	257.1
<i>Fraxinus pennsylvanica</i>	29.3	161.6
<i>Ginkgo biloba</i>	56.3	102.7
<i>Gleditsia triacanthos</i>	36.1	156.3
<i>Hamamelis m. Illis</i>	25.3	161.6
<i>Juglans nigra</i>	25.7	242.0
<i>Malus</i> sp.	23.8	219.5
<i>Populus deltoides</i>	30.4	163.4
<i>Prunus serotina</i>	30.5	306.3
<i>Prunus virginiana</i>	27.1	244.6
<i>Quercus rubra</i>	26.7	532.1
<i>Quercus macrocarpa</i>	24.0	575.9
<i>Quercus palustris</i>	30.9	530.4
<i>Rhus typhina</i>	19.4	633.9
<i>Robinia pseudoacacia</i>	17.6	282.1
<i>Salix fragilis</i>	25.5	215.2
<i>Tilia americana</i>	27.2	278.8
<i>Ulmus americana</i>	26.3	440.2
<i>Vitis vinifera</i>	29.7	120.5

^a From Davies et al. (1973).

2.3.5.1. By Microscope

After putting the holder with the leaf footprint in the microscope, the counting of the stomata was made within an 8x8 mm² area of the microscope in 4 different points of the footprint to get an approximated value. Once we knew the total leaf area we could estimate the total amount of stomata per leaf. The leaf area was obtained by the digitalization of the same leaves and analyzed by ImageJ, through a picture of 1596x1196 pixels, the equivalent to 1756,44 μm .

2.3.5.2. By HD Photographs

In this method, the holder with the footprint was used in a High Definition (HD) microscope called Olympus SC20 u-CMAD3 Japan, Cell^A program, which will take a picture of 1596*1196pixels as area, which its equivalent is 1756,44 μm . Through the program ImageJ, the picture was analyzed, measuring and counting the total area covered by all the

stomas in the picture. Once the total leaf area is known, the total amount of stomata is obtained.

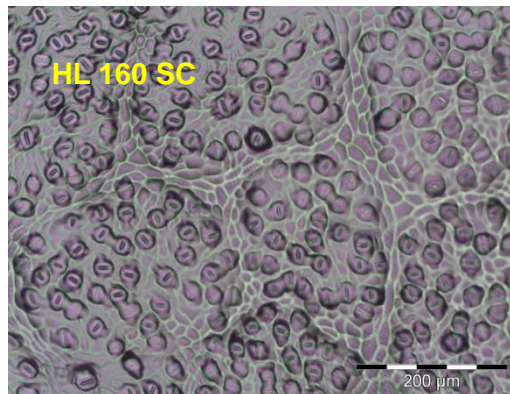


Figure 4: HD microscope picture of Haza del Lino under one regime of Stress followed by two regimens of Control (HL 160 SC)

2.4. Physiological Methods

2.4.1. Relative Water Content (RWC)

This method measures the water status of the tissues of the plants, intimately related to several leaf physiological variables, such as leaf turgor, growth, stomata conductance, transpiration, photosynthesis and respiration according to (Kramer and Boyer, 1995).

The measurements were made at the beginning of the study under water field capacity and after 80 days, where the plants were submitted under severe drought conditions and performed on full expanded leaves collected before sunrise. RWC of a plant tissue is expressed by the following equation:

$$RWC (\%) = [(Fm - Dm)/(Tm - Dm)] * 100$$

Where: *Fm* (Fresh mass), *Dm* (Dried mass), and *Tm* (Turgid Mass) of the tissue.

From each leaf, we got 2 discs that were immediately weighted for the Fresh mass (*Fm*) data. In order to obtain the Turgid mass (*Tm*), the discs were hydrated by putting them in distilled water floating, inside a closed Petri dish during 6 to 24 hours in environmental temperature till they became turgid according to (Yamasaki and Dillenburg, 1999). In last, for getting the Dried mass (*Dm*), after the discs were weighted turgid mass data, they were replaced inside the stove (*memmet* 854 schwabach, Germany) at 80°C, during 24 hours, and then weighted. All mass measurements were obtained using an analytical scale, with precision (METTAER AE 260 Delta Range®). For RWC 32 total plants were used, where 14 were from Haza del Lino, 6 from Ain Rami and 12 from Landes.

2.4.2. Leaf Water Potential (Ψ)

The leaf water potential is the potential energy of water per unit volume relative to pure water in reference conditions (Curtis and Barnes, 1985).and quantifies the tendency of water to move from one area to another by osmosis, gravity, mechanical pressure, or matrix effects caused by surface tension.

The measurements were made at the beginning of the study and after 80 days. It was measured in the predawn in fully expanded leaves with a Scholander pressure chamber (PMS Instrument Co., Corvallis, USA) with the tap of corks. The data was obtained from 32 plants in total, where 14 were from Haza del Lino, 6 from Ain Rami and 12 from Landes.

2.4.3. Photosynthetic Light Response Curve

The photosynthetic light response curve is an analytical method that measures the amount of photosynthesis (En) through CO₂ exchanges and light exposition. It can be measured while environmental conditions are varying. The light curve response follows a rectilinear line that grows as much as the amount of light is increased, till the leaf reaches its saturation point, where the photosynthetical response will be constant or it starts declining.

Photosynthetic light response curves were performed on fully expanded leaves using an Infra-Red Gas Analysis (IRGA and Li-COR 6400 and Li-COR – NE) under medium severe drought conditions. Photosynthetic active radiations intensities were as follows: 0, 25, 50, 75, 100, 200, 400, 800, 1600 and 2000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1} \text{CO}_2$.

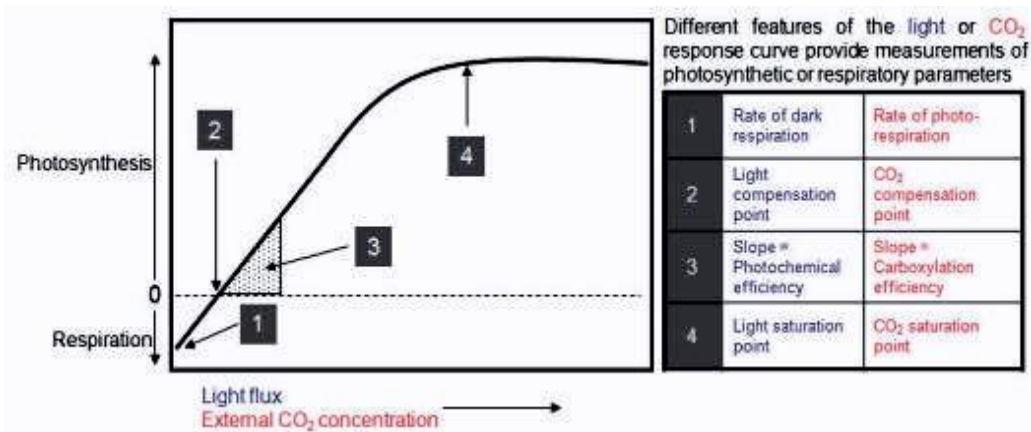


Figure 5: Image IRGA's Light Curve

explanation. <http://www.sidthomasnet/SenEssence/Genes/photosynthesis.htm>

Measured during the morning in the glasshouse with a previous stabilization of the machine with CO₂ calibration in the lab and followed adjustment of the leaf area in the case that the leaf area was smaller than the area of the chamber of the IRGA. The procedure

consisted, in enclosing a leaf in a chamber with a small bulb attached. By changing the amount of light emitted by IRGA to the leaf, it will measure the amount of photosynthesis, starting from the respiration (R) with negative values, where there is no light exposition. At the same time, humidity (about 50%) and the temperature (from 20°C to 25°C) were controlled. It was used the same plants than for the stomata counting.

2.4.4. Chlorophyll Fluorescence Parameters

This is an optical method that detects the fluorescence emitted by the green plants (alive). The energy required to activate the photosynthetic system II (PSII) is a photon with the wavelength of 6800nm. Chlorophyll fluorescence parameters were measured in three moments of the study, at the beginning under water controlled conditions, after 22 days and 63 days after the study began by using a portable Chlo fluorometer (Fluor Pen FP 100). Leaves were kept in dark during 30 minutes before the measurement. Saturated flash intensity was 8000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The parameters were calculated following formula Fv/Fm (Fv: variable fluorescence and Fm: maximum fluorescence). For this method, we used 3 leaves from 4 plants (12 leaves by treatment), of the same treatment and provenance, by means 34 plants, 14 from Haza del Lino, 6 from Ain Rami and 14 from Landes provenances.

2.5. Biochemical composition of the leaves

2.5.1. Chlorophylls and Carotenoids Contents

The chlorophyll content measures the change in the photosynthesis production by the increase, decrease or degradation of detrimental components for its functioning. The changes in the chlorophyll content of leaves during the water deficit treatment were determined by following N, N-dimethylformamide (DMF) extraction (Wellburn, 1994).

For the procedure, we used the same leaves than in the PSII experiment. The measurements were made at the beginning, under water conditions (WW), under moderate drought conditions (WSM) and under severe drought conditions (WSS) at the end of the study. Two discs (0.7 mm diameter) from each leaf were collected with a cork borer. The pigments were extracted adding 2 ml of DMF. After 24 hours, we made the lectures of the absorbance of the samples through a spectrophotometer (UWR UV-1600 RC spectrophotometer), using 663 and 645 nm for the chlorophylls a and b wavelength respectively, and for the carotenoids 480 nm. For the chlorophyll and total carotenes calculations, it was applied the following equations:

$$\text{Chlorophyll a } (\mu\text{g}/\text{cm}^2): (12 \times \text{Abs at } 663,8 \text{ nm} - 3,11 \times \text{Abs. at } 646,8 \text{ nm})/\text{area}$$

Chlorophyll b ($\mu\text{g}/\text{cm}^2$): $(20,78 \times \text{Abs. at } 646,8 \text{ nm} - 4,88 \times \text{Abs. at } 663,8 \text{ nm})/\text{area}$

Total Carotenoids ($\mu\text{g}/\text{cm}^2$): $((1000 \times \text{Abs. at } 480 \text{ nm} - 1,12 \times \text{Chlo}_a - 34,07 \times \text{Chlo}_b)/245)/\text{area}$

2.5.2. Pheophytin Content

The pheophytin content measures indirectly the degradation of the chlorophyll of the plant. Under drought conditions the viability of the Magnesium (Mg^{2+}) in the soil decrease, when this occurs, the chlorophyll of the plant is depredated at the same time that the pheophytin content increases. For obtaining the pheophytin content was used the same process described in the Chlorophyll with the exception that the lectures were 666 nm and 654 nm of the wavelength. The measures of the Pheophytin content were obtained at the same time than the Chlorophyll and Carotenoids contents and with the same leaves. For the pheophytin, it was applied the following equations:

$$Ppta (\mu\text{g}/\text{cm}^2) = (23,91 * A_{666} - 7,22 * A_{654}) / (\text{Area } (\text{cm}^2))$$

$$Pptb (\mu\text{g}/\text{cm}^2) = (-16,38 * A_{666} + 37,41 * A_{654}) / \text{Area } (\text{cm}^2)$$

$$Ppt \text{ total } (\mu\text{g}/\text{cm}^2) = (7,53 * A_{663,8} + 30,19 * A_{646,8}) / \text{Area } (\text{cm}^2)$$

2.6. Data Analyses

The statistical test used is called *t*-test of two-samples assuming unequal variances, also known as the Welch–Satterthwaite equation. This theorem can be used to test the difference between sample means even when the population variances are unknown and unequal, where tails = 1 (one-tailed) or 2 (two-tailed). This t-test follows the statements:

- There are two samples from two populations. (The samples can be different sizes.)
- The two samples are independent.
- Both populations are normally distributed or both sample sizes are large enough that the means are normally distributed.
- Population's standard deviations are unknown, but are assumed to be not equal.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{s_{\bar{X}_1 - \bar{X}_2}} \quad s_{\bar{X}_1 - \bar{X}_2} = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

Figure 6: Equations of the *t*-test two-sample assuming unequal variances, \bar{x}_1 and \bar{x}_2 = the sample means and s_{x1} and s_{x2} = the sample standard deviations of two sets of data of size n_1 and n_2 respectively

For the analysis of the data of the results, we used the R program. The Data Analyses were made with 95% confidence interval.

3. Results

3.1 Survival Rate

To understand provenances' behaviour, it is necessary to point out the survival rate as a preview look of the results through the total death plants by date and provenance.

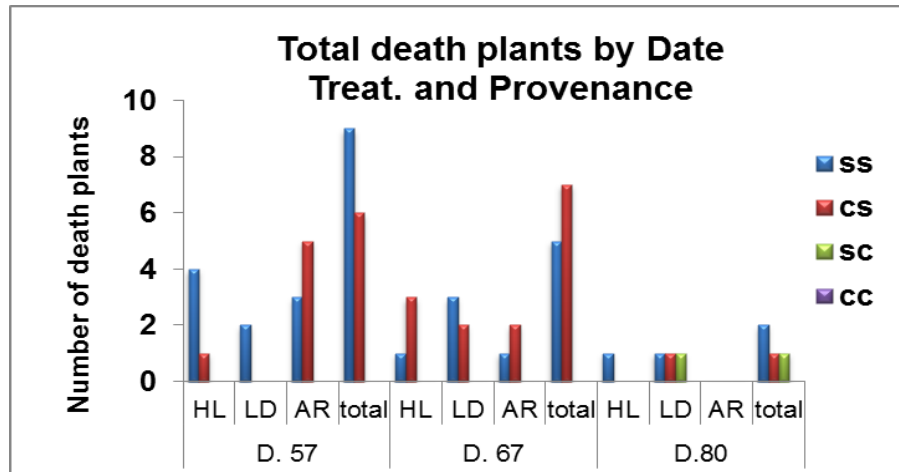


Figure 7: Total number of death plants per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress, measured in the days 57, 67 80 of the study

Based on these results, we can see that mainly the plants from CS and SS treatments died after being submitted under water drought conditions. Any plant submitted under controlled conditions died, with the exception of one plant from the SC treatment belonged to Landes provenance that died under severe drought conditions and at the end of the study.

The first signs of water deficit were shown around 57 days after the study started. In this first point, a total of 15 plants were death. The provenance more affected was Ain Rami with 8 death plants, where 5 were from the CS treatment and 3 from SS. Haza del Lino (HL) had 5 dead plants, 4 from SS and 1 from CS treatment. In the contrary, Landes (LD) had only 2 dead plants from the SS treatment. 67 days after the beginning of the third drought cycle there is some signs of water deficit, with 12 more death plants. In this date, the most affected provenance was Landes with 5 dead plants, 3 from SS and 2 from CS treatments, followed by 4 plants from Haza del Lino, 3 from CS and 1 from SS treatments, and in last Ain Rami with 3 plants death, 2 from CS and 1 from the SS treatment.

In the last date, Day 80, it was found 1 dead plant from Haza del Lino from the SS treatment and 3 plants of Landes, 1 from SS, SC and CS treatments respectively, while Ain Rami that all the plants of the CS treatment were died in the day 67, didn't have any death plant from the SS treatment having still 1 plant left as its survival rate. The total dead plants

by the end of the study were 31 plants. The plants from the CS treatment of Ain Rami were completely dead and only 1 plant from the SS treatment remained, besides the ones under Control conditions. Based on these results, the plants from Ain Rami were the most sensitive ones to die after being submitted under drought-induced conditions (Table 4).

Table 4: Survival rate from the CS and SS treatments out of the total plants per provenance, final survival rate and final plants alive from both mentioned treatments. Measured (D57, D 67 and D80)

Provenance	Total death plants (CS and SS treat.)			Survival rate (%)	Plants Alive
	D57	D67	D80		
Haza del Lino (HL)	5/16 (31,25%)	+4 (56,25%)	+1 (62,5%)	37,5%	6
Landes (LD)	2/19 (10,52%)	+5 (36,84%)	+2 (47,37%)	52,63%	10
Ain Rami (AR)	8/11 (72,73%)	+3 (100%)	-	0%	0

3.2. Biomass Growth

3.2.1. Leaf Area (LA)

From this measure was obtained quantitative and qualitative data. The qualitative (see ANNEXES), was the digitalization of the total amount of the leaves (old and new), from where we for the quantitative data was measured (see Figure 8 and Table 5).

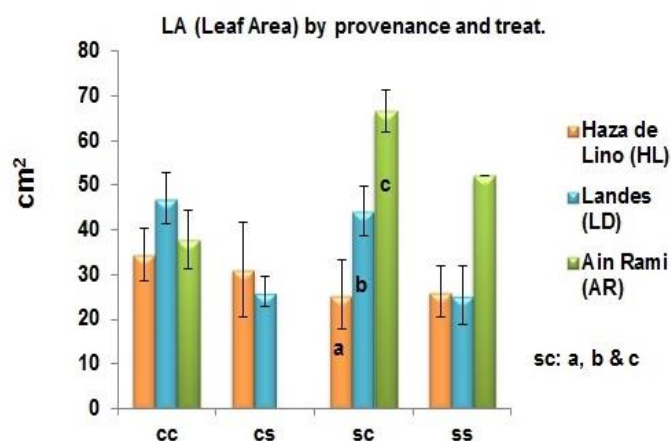


Figure 8: LA (cm²) per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC 3 regimes of Controlled conditions, SS 3 regimes of Stress, SC first under Stress and the 2 last ones of Controlled and CS first under Controlled and 2 of Stress. Measured Day 80

Table 5: T-test of two-samples assuming unequal variances per provenance for the LA

Provenance	Treat.	t-test	df	p-value	95% conf. Int.
HL-AR (a & c)	SC	-4,53356	4	0,005274	2,131847 2,776445
LD-AR (b & c)	SC	-3,01903	3	0,028402	2,353363 3,182446

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value.

The data analysis showed significant differences between Haza del Lino and Ain Rami and between Landes and Ain Rami for the SC. Although, we can say that Landes got the higher leaves for the CC treatment, while Haza del Lino for the CS and Ain Rami in both SC and SS treatments.

3.2.2. Specific Leaf Area (SLA)

Through this method we can explain the thickness of the leaves. As higher the SLA is, thinner the leaf is, usually linked with a fast growth, less fiber and protection, so therefore, more fragile to environmental factors like pest and deceases, as well as, more powerless to climate change (cold and hot in a very drastic way).

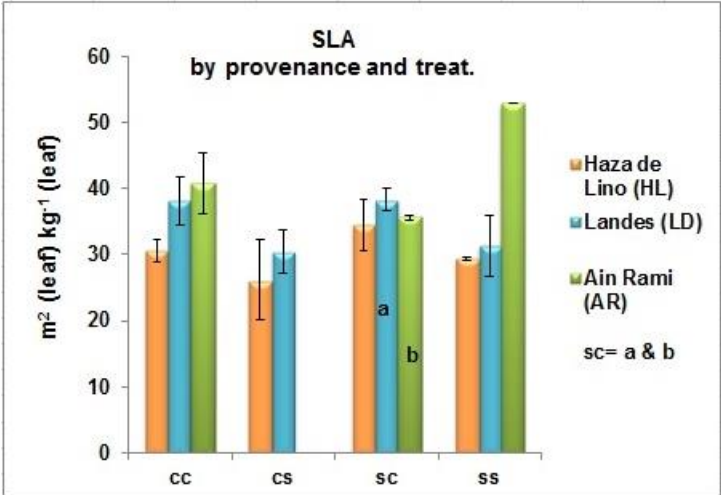


Figure 9: SLA per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress, measured the day 80

Table 6: T-test of two-samples assuming unequal variances per provenance for the SLA

Provenances	Treat.	t-test	df	p-value	95 % conf. interval	
LD-AR (a & b)	SC	-14,8961*	1	0,04267	-10,62689*	-0,84311*

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value.

The data analyses showed significant differences for the SC treatment between the Landes and Ain Ram (Figure 9 and Table 6), as for the LA. In other hand, based on these results, it can be seen that the leaves of the Moroccan provenance for the SS treatment had the higher SLA values, in comparison with Landes and Haza del Lino.

3.2.3. Total Biomass

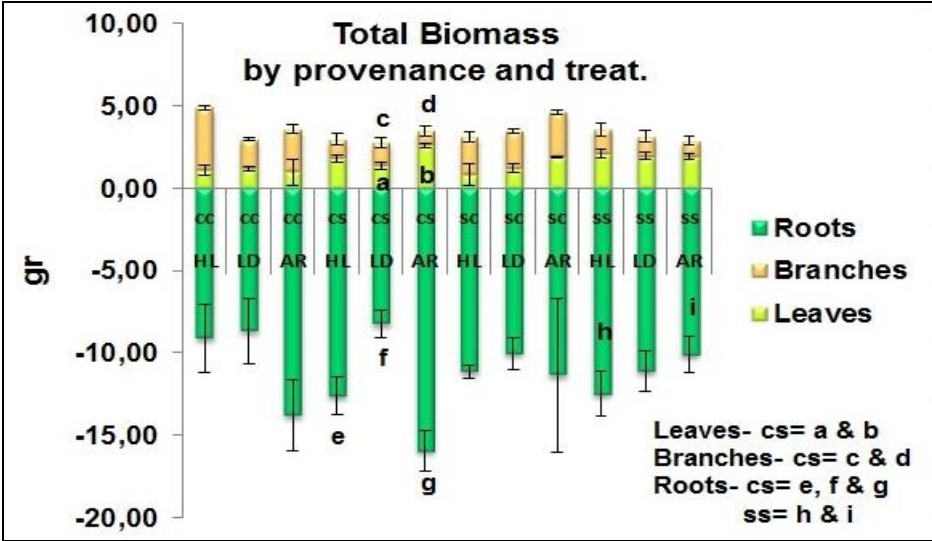


Figure 10: Total Biomass of the Leaves, Branches, and Roots, per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress

Table 7: T-test of two-samples assuming unequal variances by provenance for the Total Biomass (Leaves, branches and roots)

Provenances (Leaves)	Treat.	t-test	df	p-value	95 % conf. interval	
LD-AR (a & b)	CS	3,8195	5	0,01238	0,449993	2,302374
Provenances (Branches)	Treat.	t-test	df	p-value	95 % conf. interval	
LD-AR (c & d)	CS	4,3465	3	0,02249	0,5255731	3,399277
Provenances (Roots)	Treat.	t-test	df	p-value	95 % conf. interval	
HL-LD (e & f)	CS	7,3703	5	0,000723	7,175089	14,86061
LD-AR (f & g)	CS	12,0317	5	0,0007	11,43707	17,65197
HL-AR (h & i)	SS	8,6046	4	0,001003	7,438774	14,52623

The leaves, branches and roots showed significant differences for the CS treatment between Landes and Ain Rami provenance. Besides, for the roots, the data analyses showed differences for the CS treatment also between Haza del Lino and Landes. Besides, it was found significant differences between Haza del Lino and Ain Rami for the SS treatment (Figure 10 and Table 7). Nevertheless, with these results we can say that Ain Rami provenance, especially for the CS provenance, even its plants were dead before the ending the study, they had the highest carbon efficiency as a bigger total biomass.

3.2.4. Root/Shoot Ratio (RAR)

This ratio relates the growth of the plant from the shoot and the root.

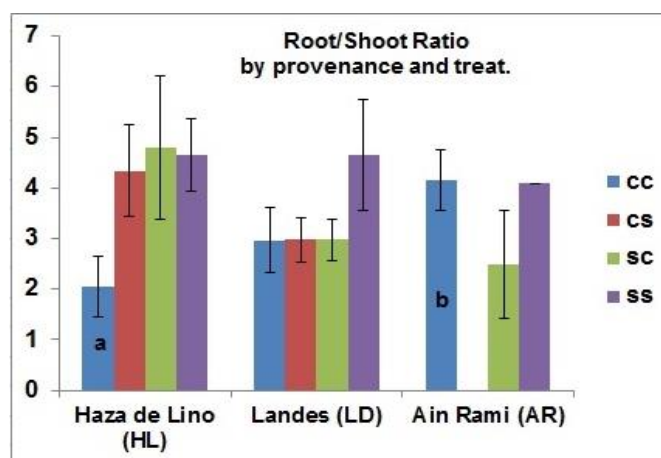


Figure 11: Root/Shoot ratio per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress

Table 8: T-test of two-samples assuming unequal variances per provenance for the Root/Shoot ratio

Provenances	Treat.	t-test	df	p-value	95 % conf. interval
HL-AR (a & b)	CC	-5,1144*	2	0,03617	-4,82417* -0,41583*

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value.

The data analyses showed significant differences in the CC treatment between Haza del Lino with both Landes and Ain Rami provenances, where Haza del Lino showed the smallest values and Ain Rami the biggest (Figure 11 and Table 8).

3.2.5. Root Area and Root Classification (type, area)

In the root classification we got qualitative and quantitative data. The qualitative data was the digitalization of the roots by a scanner. From the roots scanned we analyzed them by Winrhizo discomposing the root in the different diameter types. In addition, in some cases, at the moment to make the classification of the roots we found out diverse characteristics in the root development (see ANNEXES), where we can appreciate differences between the provenances and treatments, as in the case of HL 111 CC plant, where it can be appreciated that the main root continues growing, instead of reaching a point where the secondary roots and the fine root developed. Or in some cases, from Ain Rami, plant where the principal root

has a double main root, like for AR 59 CS plant or three main roots instead of only one root like for AR 156 SC plant. Or the case of in the case of AR 213 CS where it appeared high specialized roots (which are highly efficient roots for absorbing water from the soil-to root interface), as it can be observed in the following pictures:

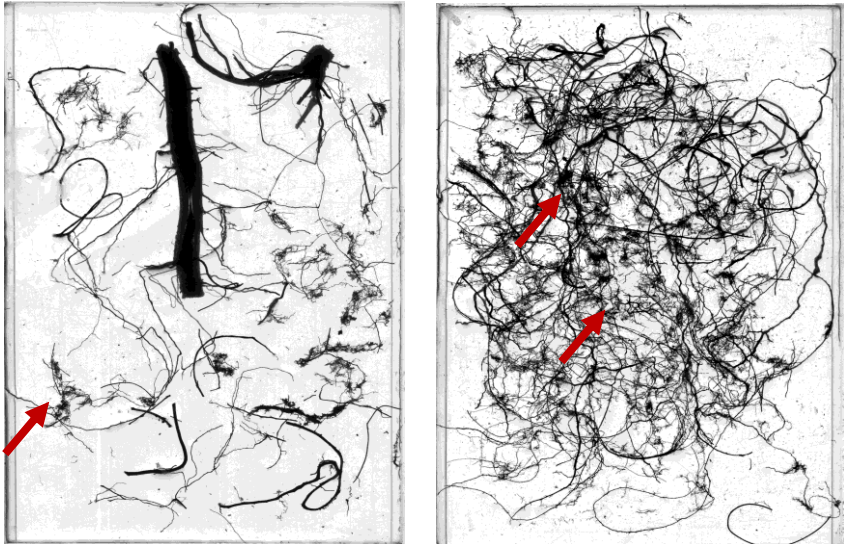


Figure 12: AR 213 CS photos divided in main root and secondary roots, where it can be appreciated the appearance of the highly specialized roots in the picture in the right side



Figure 13: Highly specialized roots founded in AR 213 CS plant

The quantitative data: Length (mm)

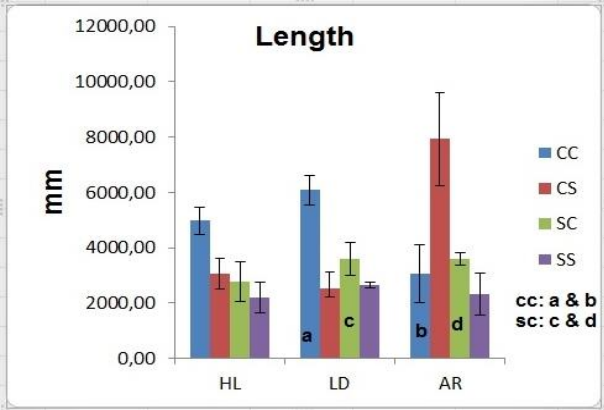


Figure 14: Length of the roots (mm) per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress,

SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress

Table 9: T-test of two-sample assuming unequal variances per provenance for the Root length

Provenances	Treat.	t-test	df	p-value	95 % conf. interval	
LD-AR (a & b)	CC	4,7958	2	0,04083	242,1203	4467,046
LD-AR (c & d)	SC	-42,0451*	1	0,01514	-1158,812*	-620,958*

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value

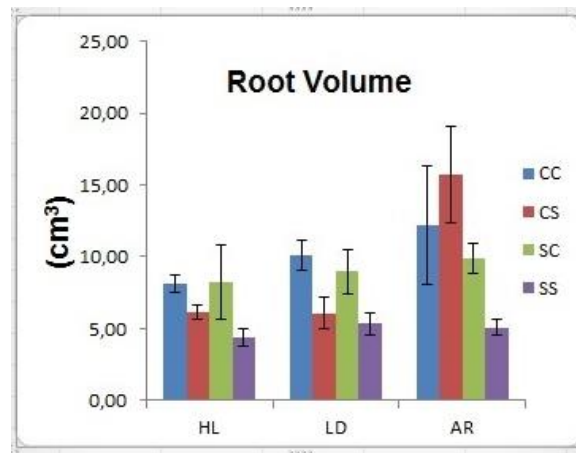


Figure 15: Root Volume (cm³) per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled conditions and CS means first regime under Controlled and the two last ones under Stress conditions

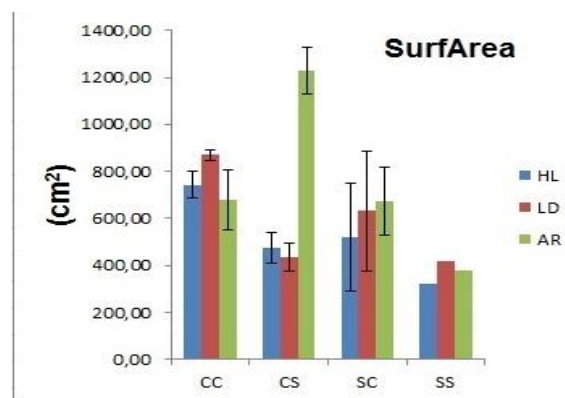


Figure 16: Surface area (cm²), by diameter of the root, per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress

Data analyses didn't show significant differences for the root volume nor for the root surface area. However, it was found significant differences significant for the length between Landes and Ain Rami for both CC and SC treatments (Figure 15 and Table 8).

In other hand, for the classification of the roots, we divided the roots in 5 different classes by the diameter, from $0 < S.A. \leq 1$, $1 < S.A. \leq 2$, $2 < S.A. \leq 3$, $3 < S.A. \leq 4.5$, $S.A. > 4.5$, where S.A. means Surface Area from thinner to thicker respectively. For making this classification, we did it independently per provenance and treatment, starting showing the figures from Haza del Lino, Landes and in last Ain Rami:

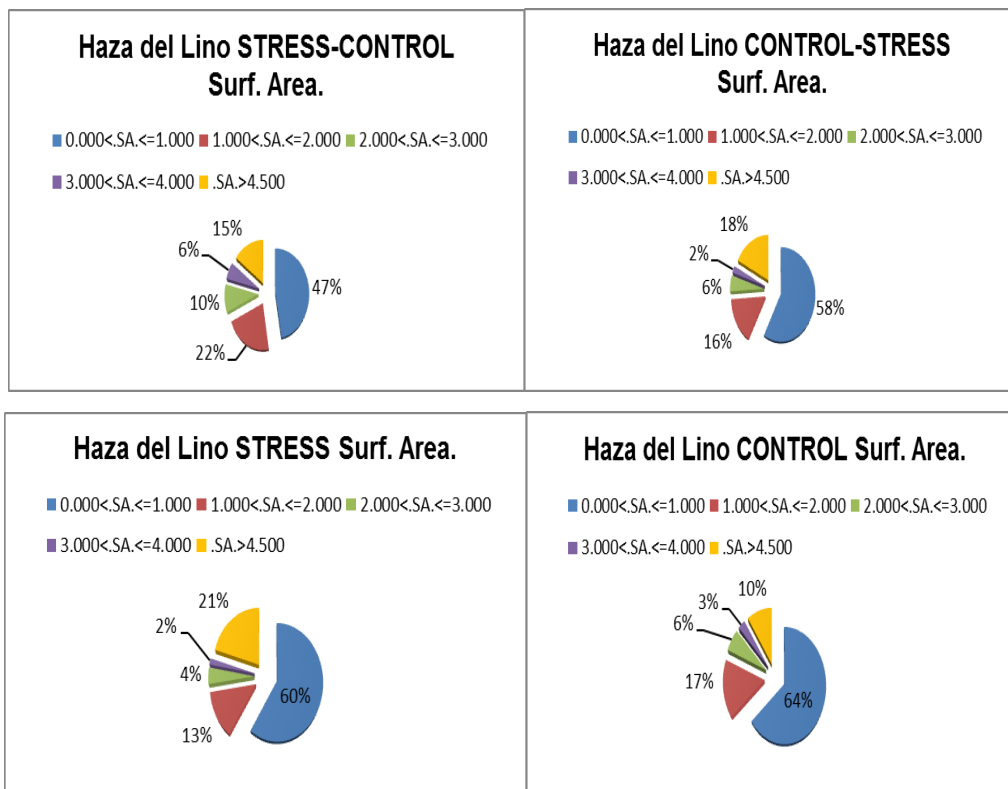


Figure 17: Surface area (S.A.) of Haza del Lino disposed by treatment: CC=Control, SC, Stress+Control, CS= Control+Stress and SS Stress regimes and by percentage of type of the diameter of the root, from $0 < S.A. \leq 1$, $1 < S.A. \leq 2$, $2 < S.A. \leq 3$, $3 < S.A. \leq 4.5$, $S.A. > 4.5$ in %

From the classification of the roots, the CC treatment had the higher amount of thinner roots with 64%; SC treatment is characterized by having the less percentage in the finest roots, while in the contrary it has the highest amount of medium roots $1 < S.A. \leq 2$, $2 < S.A. \leq 3$, $3 < S.A. \leq 4.5$ in comparison to the other treatments. The SS treatment had the highest production of the biggest roots with 21%.

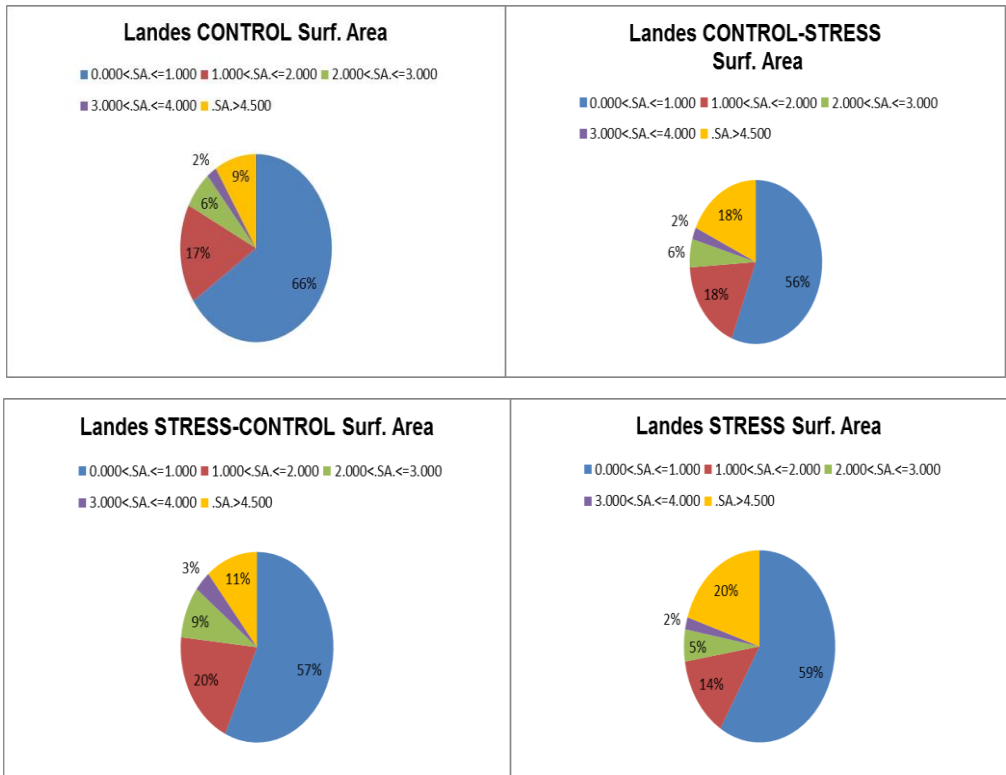
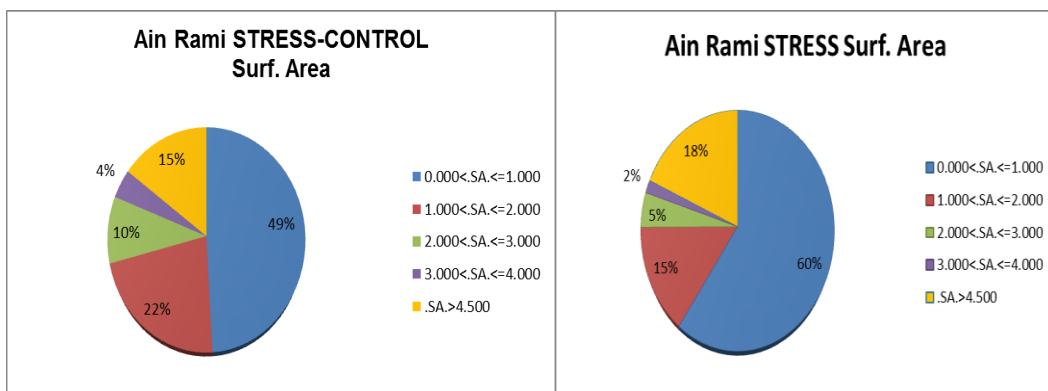


Figure 18: Surface area (S.A) of Landes disposed by treatments CC=Control, SC, Stress+Control, CS= Control+Stress and SS Stress regimes and by percentage of type of diameter of the root, from $0 < S.A. <= 1$, $1 < S.A. <= 2$, $2 < S.A. <= 3$, $3 < S.A. <= 4.5$, $S.A. > 4.5$ in %

From the CC treatment had the higher amount of finest roots even if we compare this amount between the provenances with 66%; SC treatment is characterized for having the biggest amount of medium roots $1 < S.A. <= 2$, $2 < S.A. <= 3$, $3 < S.A. <= 4.5$. The SS treatment had the highest production of the thickest roots with 20%. While in the contrary the CS treatment is characterized for having the fewer amounts of the finest roots.



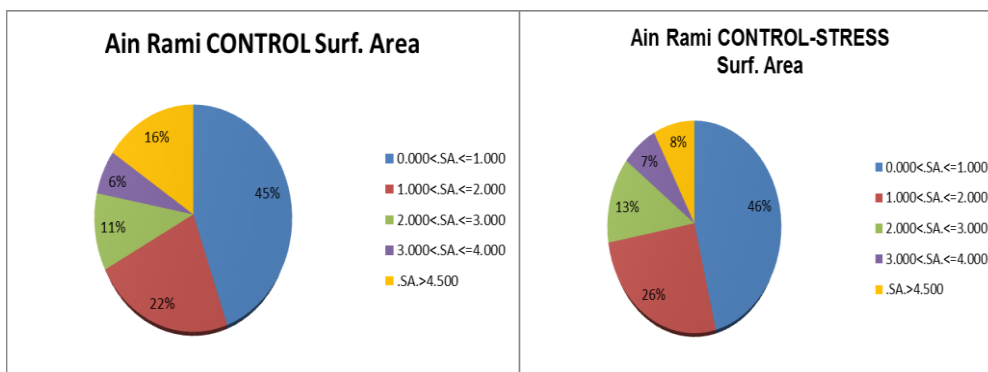


Figure 19: Surface area (S.A) of Ain Rami disposed by treatments CC=Control, SC, Stress+Control, CS=Control+Stress and SS=Stress and by % of type of root by diameter, from 0<S.A.<= 1, 1<S.A.<= 2, 2<S.A.<= 3, 3<S.A.<= 4'5, S.A. > 4'5

Ain Rami for the SS treatment had the higher amount of finest roots 60%, in addition it contains the highest amount of the biggest roots with 18%; CC treatment is characterized by having the less percentage in the finest roots, while in the contrary it had the second biggest amount of medium roots 1<S.A. <= 2, 2<S.A. <= 3, 3<S.A. <= 4'5. The CS treatment had the highest production in the 3<S.A. <= 4'5 roots with 26%, and 2<S.A. <= 3 with 13%.

3.3 Morphological measures

3.3.1 Total branches per plant

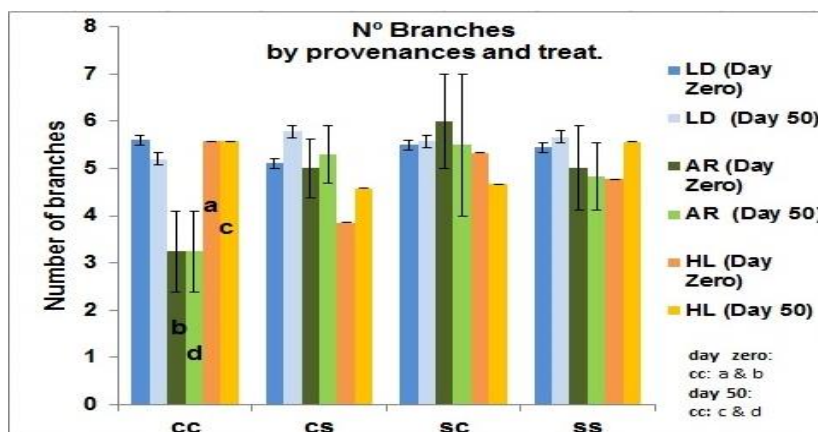


Figure 20: Total Number of branches per provenances: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means 3 regimes under Controlled conditions, SS 3 regimes under Stress, SC first under Stress and 2 under Controlled and CS first Controlled and 2 under Stress. Measured Day Zero and Day 50

Table 10: T-test of two-sample assuming unequal variances per provenance for the Number of branches

Date	Provenance	Treat.	t-test	df	p-value	95% conf. Interval
Day Zero	HL-AR (a & b)	CC	3,2205	3	0,04857	0,034691 5,467531
Day 50	HL-AR (c & d)	CC	3,2205	3	0,04857	0,0324691 5,467531

The data analyses showed significant difference for the plants under CC conditions for both measurements (Day Zero and D50 after the study began). The provenances that showed differences were Haza del Lino and Ain Rami. In other hand, it can be seen a general tendency from the first to the second measurement of an increase of number of branches for all the provenances and treatments except for Ain Rami for the SC and SS treatments and Landes for CC treatment.

3.3.2 Total leaves of the main branch per plant

The leaves are used for the growth of the plant, through the photosynthesis of the plant, while taking CO₂ and exchanging water to the atmosphere. As more leaves the plant has, more the plant can achieve photosynthetically.

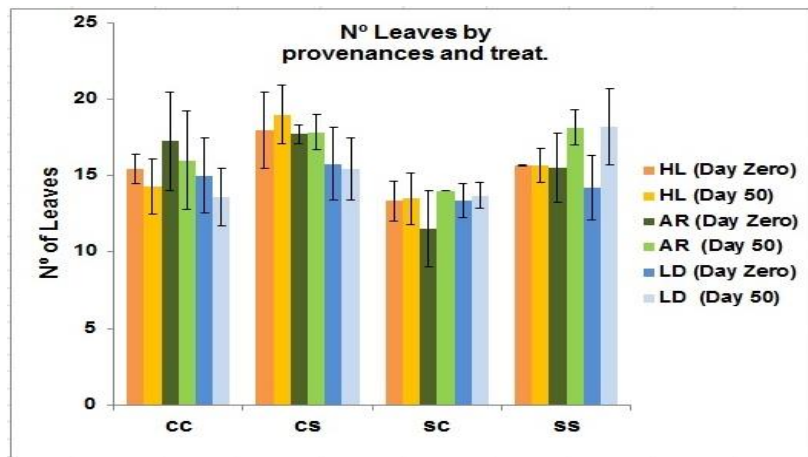


Figure 21: Total Number of leaves per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress Measured Day Zero and Day 50

The data analysis didn't show any significant differences in any of the days of measurement. However, we can say for the CC treatment the number of leaves decreased in the second measurement, while for the CS treatment, the number of leaves increased in both Haza del Lino and Ain Rami, when Landes had slightly less number of leaves. In the case of the SC and SS treatment for all the provenances number of leaves increased.

3.3.3 Diameter of the branches

The diameter of the branch, tell us about the water potential pressure, smaller sapwoods, higher pressure. It is linked to the development of the main root.

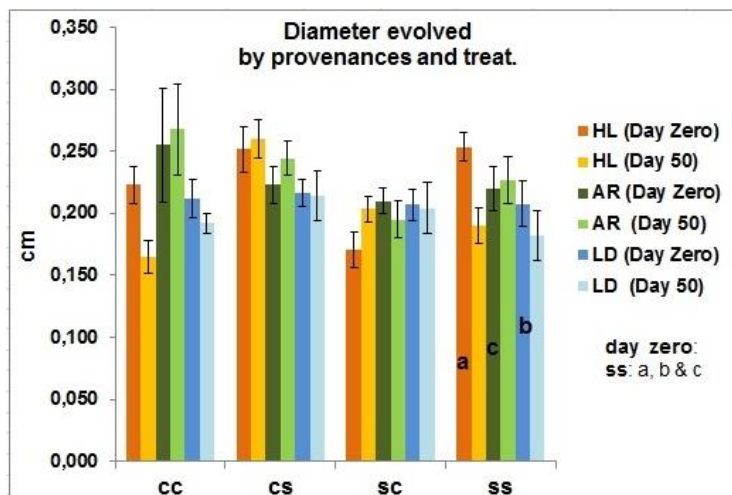


Figure 22: Diameter of the main branch per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and per treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS with first regime under Controlled and the two last ones under Stress. Measured Day Zero and Day 50

Table 11: T-test of two-samples assuming unequal variances per provenance for the Diameter of the main branch

Date	Provenance	treat.	t-test	df	p-value	95% conf. Interval	
Day Zero	HL-LD (a & b)	SS	-12,444	7	4,89E-19	-6,53471	-4,44779
Day Zero	HL-AR (a & c)	SS	-12,3221	7	5,32E-06	-6,60015	-4,47485

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value

The data analysis showed significative differences for the diameter of the branches for the SS treatment between Haza del Lino and both Landes and Ain Rami provenances in the day zero, at the beginning of the study. For the Day 50, it was not found significative differences between the provenances for any treatment. However, we can appreciate that the provenances displayed different responses after being submitted under drought conditions, for the different treatments, where Haza del Lino increased its diameter for CS and CS treatment but it decreases in SS, while Ain Rami increased its diameter for CS and SS, but decreased in SC treatment and Landes remained similar for SC and CS treatments but decreased its diameter for SS treatment.

3.3.4 Height of the plant

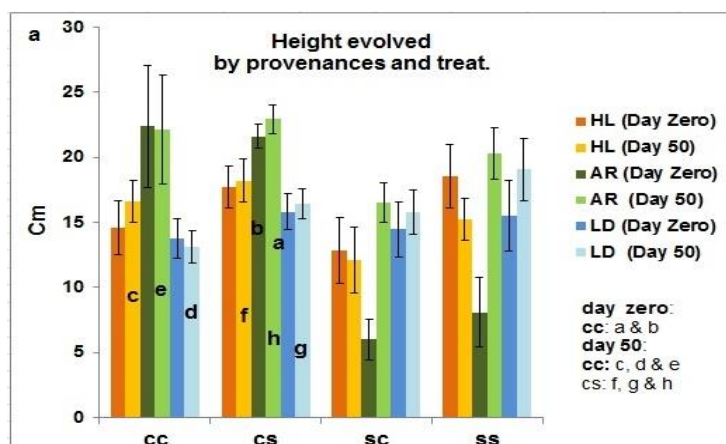


Figure 23: Height of the main branch per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured Day Zero and Day 50

Table 12: T-test of two-samples assuming unequal variances per provenance for the Height of the plant

Date	Provenance	treat.	t-test	df	p-value	95% conf. Interval	
Day Zero	LD-AR (a & b)	CS	-3,1708	6	1,93E-02	-8,327	-1,073
Day 50	HL-LD (c & d)	CC	3,0394	4	3,84E-02	0,472744	10,44726
Day 50	HL-AR (c & e)	CC	-3,2555	3	0,04624	-17,9147	-0,28533
Day 50	HL-AR (f & g)	CS	-2,7065	6	0,03527	-8,97642	-0,04522
Day 50	LD-AR (g & h)	CS	-2,8892	6	0,02772	-10,6858	-0,88565

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value

The data analysis showed significant differences for the height of the branches between the provenances for both moments of measurements. The treatment CS had significant differences for both moments of measure between Landes and Ain Rami. In the day 50, Haza del Lino and Ain Rami also had significant differences for the CS treatment. For the CC treatment was found differences after 50 days, under moderate drought conditions between Haza del Lino and both Landes and Ain Rami provenances.

3.3.5. Stoma Counting

3.3.5.1. By Microscope

Through this experiment we obtained the number of stomas per leaf and the total stomas contained in 1cm² of the leaf (Figure 24, 25 and Table 13).

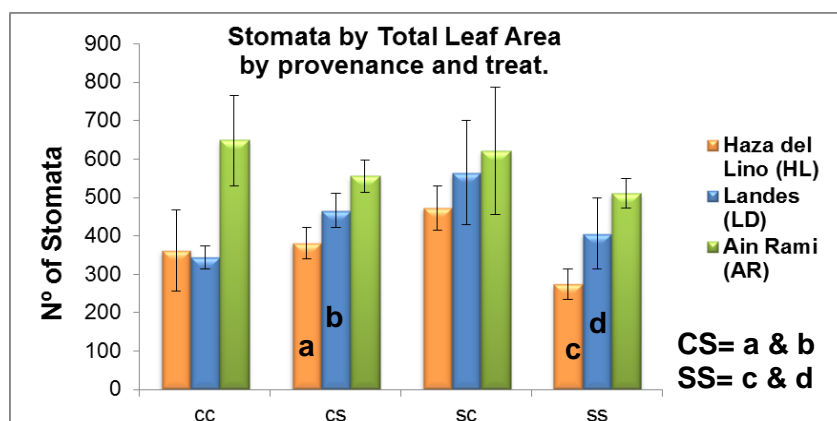


Figure 24: Total Stomata by total leaf area per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured at the beginning of the study

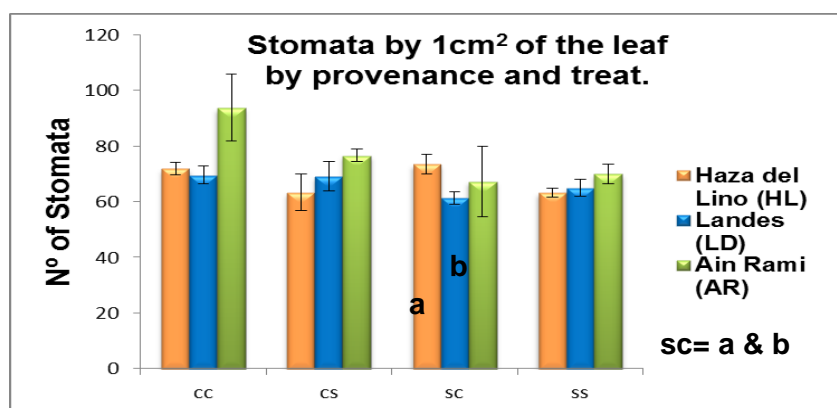


Figure 25: Total stomata contained in 1 cm² of the leaf per provenance and treatment. Measured at the beginning of the study

Table 13: T-test of two-samples assuming unequal variances by provenance for the Total amount of Stomata measured by the Total Leaf Area and by 1 cm of leaf Area

Provenances (Total Area)	Treat.	t-test	df	p-value	95 % conf. interval	
HL-LD (a & b)	CS	-5,0858*	3	0,01469	-281,4049*	-64,7801*
HL-LD (c & d)	SS	-3,3963*	3	0,04258	-456,9295*	-14,8505*
Provenances (1 cm ² leaf)	Treat.	t-test	df	p-value	95 % conf. interval	
HL-LD (a & b)	SC	5,8972	3	0,009735	5,573591	18,64141

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value

Ain Rami leaves had a bigger size than Haza del Lino and Landes, thus this influences in the total amount of stomata. The data analysis showed significative differences between the provenances for the CS and SS treatments between Haza del Lino and Landes

for the leaf area (Figure 25). In the case of 1cm² of the leaf area was shown statistical differences between Haza de Lino and Landes for the SC treatment (Table 13). In addition, it can be seen that the CC treatment, Ain Rami showed higher amount of stomata than Haza del Lino and Landes, moreover in the graph of total leaf area, this is due to the big variety of within the provenance that it raises the value, but it is not significative according to our statistical analyses.

3.3.5.2. By Photographs

Through this method we got qualitative and quantitative data. The qualitative data obtained were the pictures taken by the HD Microscope. Through the analyses of the pictures the quantitative data was obtained:

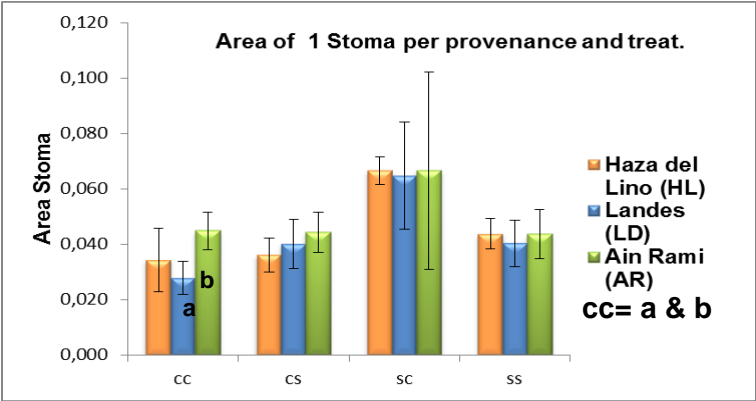


Figure 26: Average of the area of 1 stoma per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Data obtained at the beginning of the study

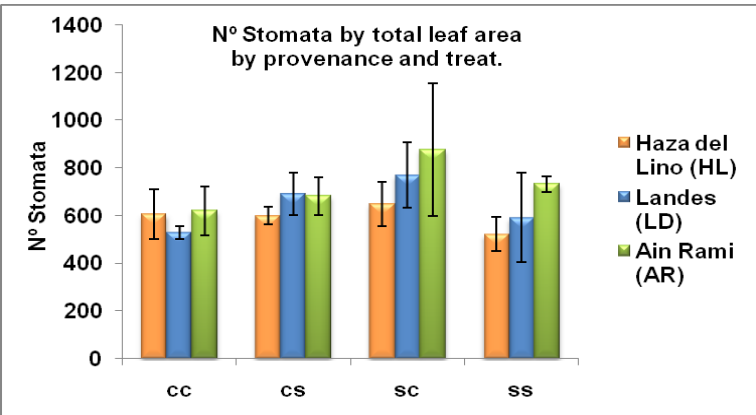


Figure 27: Total Stomata by Total Leaf Area per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, three regimes under Controlled conditions (CC), three regimes under Stress (SS), SC one under Stress and two last ones under Controlled (SC) and one under Controlled and two last under Stress (CS)

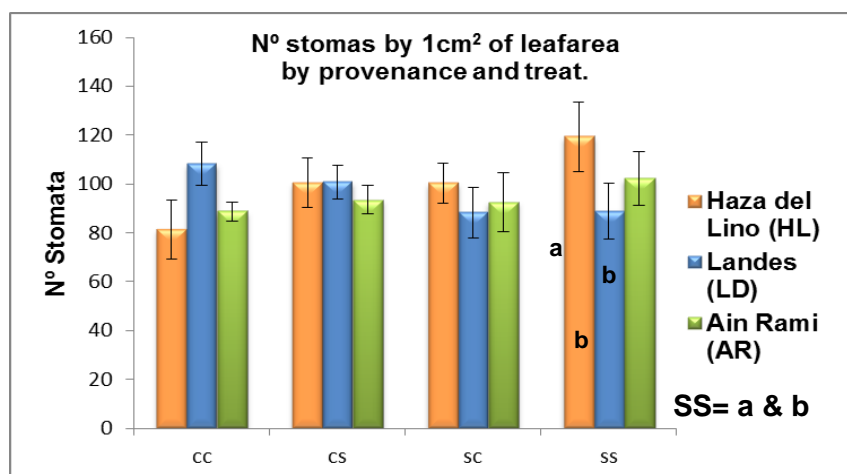


Figure 28: Total Stomata contained in 1 cm² of the per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress

Table 14: T-test of two-samples assuming unequal variances per provenance for the stomata in 1 cm² of the leaf

Provenances (Area 1 Stoma)	Treat.	t-test	df	p-value	95 % conf. interval	
LD-AR (a & b)	CC	-3,8173*	3	0,03163	-0,031173*	-0,00283*
Provenances (1 cm ² leaf)	Treat.	t-test	df	p-value	95 % conf. interval	
HL-LD (a & b)	SS	5,3441	3	0,01281	174,9429	690,0571
HL-AR (a & b)	SS	6,8223	3	0,006443	223,8141	615,1859

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value

Data analyses didn't show significant differences for the total stomata by total leaf area. However, it was found differences significant for the area of one stoma between Landes and Ain Rami for the CC treatment and in Total amount of stomata by 1 cm² of the leaf area, between Haza del Lino and both Landes and Ain Rami, where Haza del Lino had the higher amount of stomata (Table 14).

3.4. Physiological Methods

3.1.1 Relative Water Content (RWC)

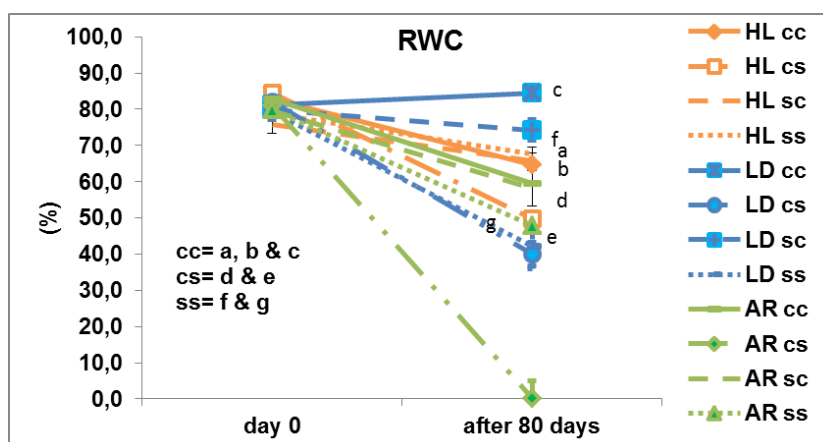


Figure 29: Relative Water Content (%) per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured at the beginning and 80 days after the study began

Table 15: T-test of two-samples assuming unequal variances per provenance for the WRC (%)

Provenances	Treat.	t-test	df	p-value	95 % conf. interval	
HL-AR	CC	-4,6041*	2	0,04408	-46,1733*	-1,5114*
LD-AR	CC	-4,5618*	2	0,04485	-48,2363*	-1,41035*
HL-LD	CS	-6,1705*	2	0,02527	-17,6462*	-3,14714*
HL-LD	SS	13,1827	2	0,005705	17,12324	33,71676

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value.

Data analyses showed statistical differences between Haza del Lino and Ain Rami and Landes with Ain Rami for the CC treatment. In addition, it showed significant differences between Haza del Lino and Landes for the both CS and SS treatments.

The Relative Water content graphic showed that after 80 days the water content in the leaves mainly decreased for the 3 provenances, for all the treatments, with the exception of the CC plants from the French provenance that increased its relative water content.

The general tendency showed a reduction of the water capacity in the leaves even though they were in optimal conditions like in the case of the CC and SC treatments where the RWC at the beginning of the study ranged about 80% and decreased till 40%. The CS and SS treatments were more sensitive to water loss, and therefore they had a smaller RWC.

3.1.2. Leaf Water Potential

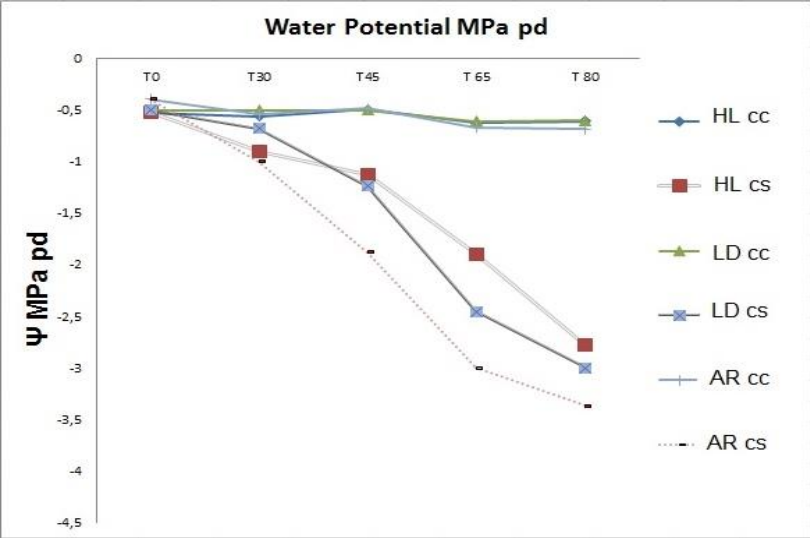


Figure 30: Leaf water potential from the second cycle per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured in 5 moments T0, T30, T45, T65 and T80

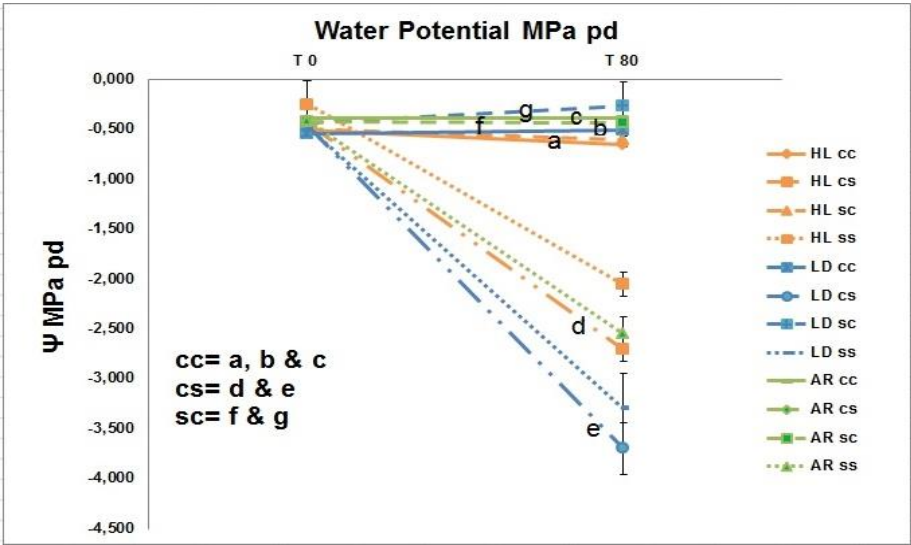


Figure 31: Leaf water potential of the third cycle per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured at the beginning and 80 days after the study began

The data analyses showed significant differences (Table 16) for the CC treatment between all the provenances. In addition, Haza del Lino showed significant differences between Landes and Ain Rami for the CS treatment, where Haza del Lino showed higher values under severe drought conditions than the other two provenances (Figure 30). Landes

showed significant differences with Ain Rami for the SC treatment after 80 days of the study, where Landes had higher values.

Table 16: T-test of two-samples assuming unequal variances per provenance for the Leaf Water Potential (MPa)

Provenances	Treat.	t-test	df	p-value	95 % conf. interval	
HL-AR (a & b)	CC	-5,7446*	3	0,01048	-0,213674*	-0,06133*
HL-LD (a & c)	CC	-6,1482*	3	0,008657	-0,398377*	-0,12662*
LD-AR (b & c)	CC	-5,0000*	3	0,01539	-0,204561*	-0,04544*
HL-LD (d & e)	CS	4,8990	3	0,01628	0,3503859	1,764961
LD-AR (f & g)	SC	-3,8060*	3	0,03178	-0,298377*	-0,02662*

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value

For the water potential happened similarly in the responses than in the RWC. The CS treatments of all the provenances were the ones that showed a drastic decline for its water potential, followed by the SS treatments. This means that the CS treatment for all the provenances were more sensitive to drought than in any other treatment.

In addition, in the water potential of the second cycle (Figure 12), it can be observed that indeed, the treatments CS and SS were dropping when they were submitted under severe drought conditions.

3.1.3. Chlorophyll Fluorescence Parameters (PSII)

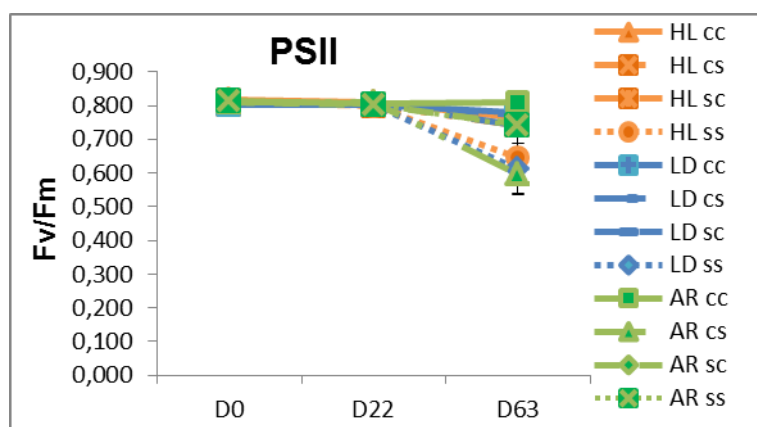


Figure 32: PSII data per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured in D0 and D22 and D63 of the study

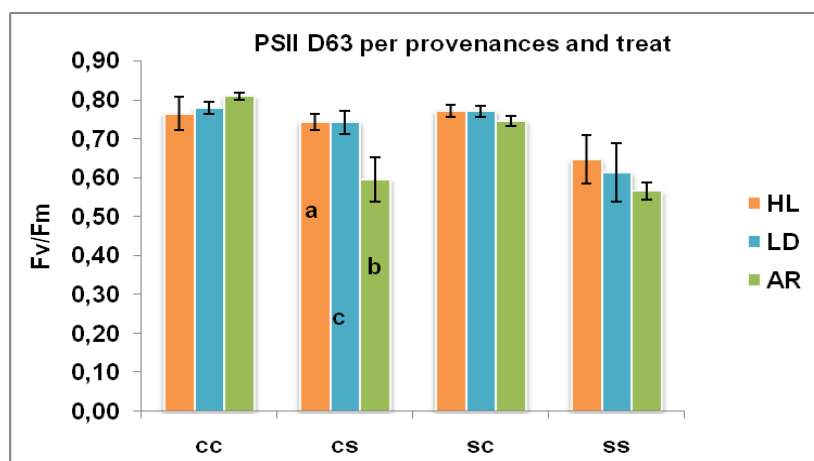


Figure 33: PSII by columns of the D63 of measured, represented per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress

Table 17: T-test of two-samples assuming unequal variances per provenance for the PSII at D63

provenances	treat.	t-test	df	p-value	95% int. Conf.	
HL-AR (a & b)	CS	2,46183075	6	0,02449881	1,94318027	2,44691185
LD-AR (c & b)	CS	2,2854322	8	0,02581584	1,85954803	2,30600413

Data Analyses showed significant differences, hence based on these results, it can be said that 22 days after the study began, the values were very similar and with a very effective photosynthetic rate. Only after 63 days the study started, when the plants were under drought conditions, it could be seen significant differences between the provenances.

After 63 days appeared clear signs of drought by dryness of the leaves or death plants. The 0.7 value for the Fv/Fm is the threshold of the photosynthesis production, and for the treatments CS and SS their PSII decreased under that value, thus its photosynthetic production and so its growth.

3.1.5. Photosynthetic Light Response Curves

The light is a complex factor that affects growth, mainly in the intensity, duration and quality. To ensure optimal plant growth, the light source must provide sufficient energy input for photosynthesis. Optimal light intensity is 23-35 Kilolux (klx). Duration of light is directly related to the day length. The quality of light is expressed by its wavelength, where 440-620 nm have the most significant impact on growth. CO₂ concentration in the air has decisive influence on the extent of photosynthesis. Natural CO₂ concentration level is about 390 ppm.

The Gas Exchange and Light Curve were obtained while the water deficit was moderated, around 45 days after the study started. Through the figures, we observe the

relation of higher light, higher photosynthetic production.

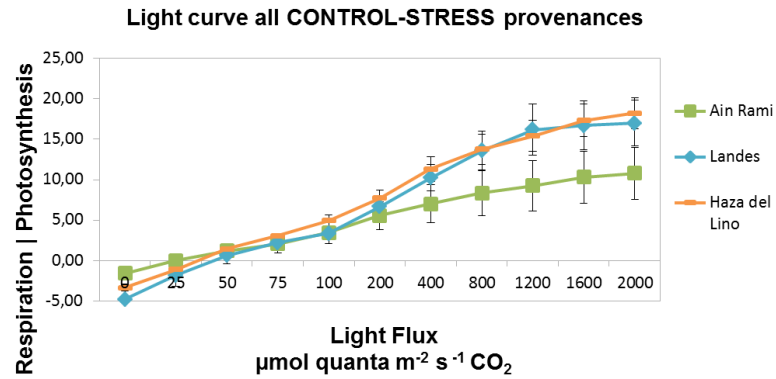


Figure 34: Light curve of the Infra-Red Gas Analysis of all the control-stress plants per provenance, where it is measured photosynthetic active radiations (PAR) of: 0, 25, 50, 75, 100, 200, 400, 800, 1600 and 2000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1} \text{CO}_2$ intensities

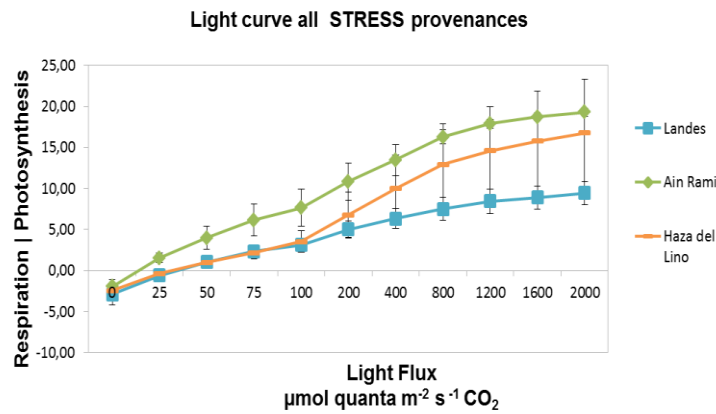


Figure 35: Light curve of the Infra-Red Gas Analysis of all the stress plants per provenance, where it is measured photosynthetic active radiations (PAR) of: 0, 25, 50, 75, 100, 200, 400, 800, 1600 and 2000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1} \text{CO}_2$ intensities

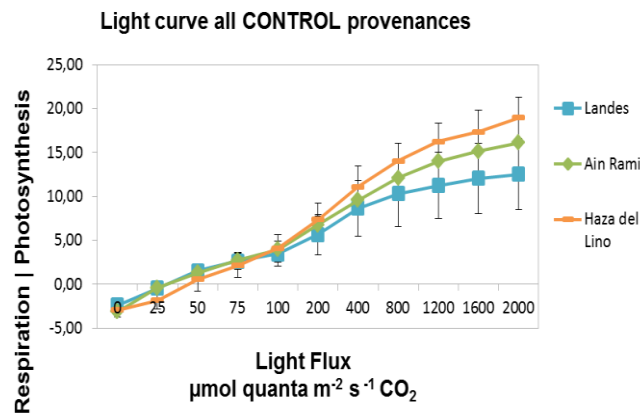


Figure 36: Light curve of the Infra-Red Gas Analysis of all the control plants per provenance, where it is measured the (PAR) of: 0, 25, 50, 75, 100, 200, 400, 800, 1600 and 2000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1} \text{CO}_2$ intensities

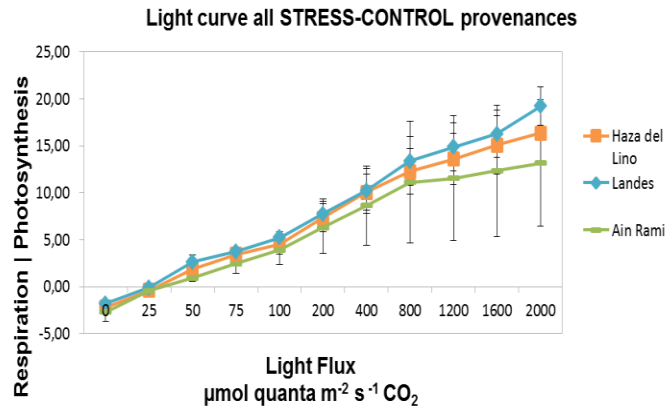


Figure 37: Light curve of the Infra-Red Gas Analysis of all the stress-control plants per provenance, where it is measured photosynthetic active radiations (PAR) of: 0, 25, 50, 75, 100, 200, 400, 800, 1600 and 2000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1} \text{CO}_2$ intensities

For the CS light curve, the water deficit led to a reduction of the photosynthetic rate for Ain Rami provenance, when Landes and the Haza del Lino obtained similar responses between them and higher than Ain Rami. In the case of the light curve of the SS, the French provenance had the less photosynthetic production and Ain Rami with the higher photosynthetic rate. In the CC Light curve, all the provenances follow a similar tendency with a clear separation at the end. Haza del Lino provenance had the best photosynthetic efficiency and Landes was the one with the lowest values reaching the saturation point very early in comparison with the other two provenances that even at PAR 2000 didn't reach that point yet. In the other hand, for the SC treatment, the three provenances follow a similar tendency, but Landes is the provenance with a better response to the light exposure, being more efficient and Ain Rami the less efficient.

3.5. Biochemical composition of the leaves

3.5.1. Chlorophylls and Carotenoids Contents

Based on the results of the chlorophylls it was obtained the following data of Chlo a, Chlo b, Total Chlo, relation Chlo a/Chlo b, Total Carotenoids.

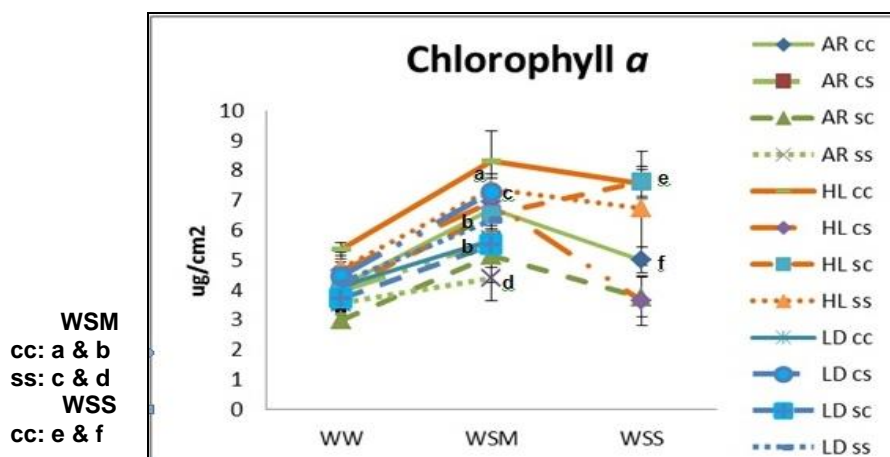


Figure 38: Chlorophyll a per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured where the plants were subjected under WW (Water Conditions), WSM (Water Stress Moderate) and WSS (Water Stress Severe)

Table 18: T-test of two-samples assuming unequal variances by provenance for the Chlo a

Date	Provenances	Treat.	t-test	df	p-value	95 % conf. interval
WSM	HL-LD (a & b)	CC	3,8567	5	0,01192	1,249427 6,243906
WSM	HL-AR (a & b)	CC	7,3223	3	0,005262	1,348423 3,421577
WSM	HL-AR (c & d)	SS	3,8346	7	0,00642	1,053706 4,443794
WSS	HL-AR (d & e)	CC	14,0137	2	0,005053	1,963412 3,703255

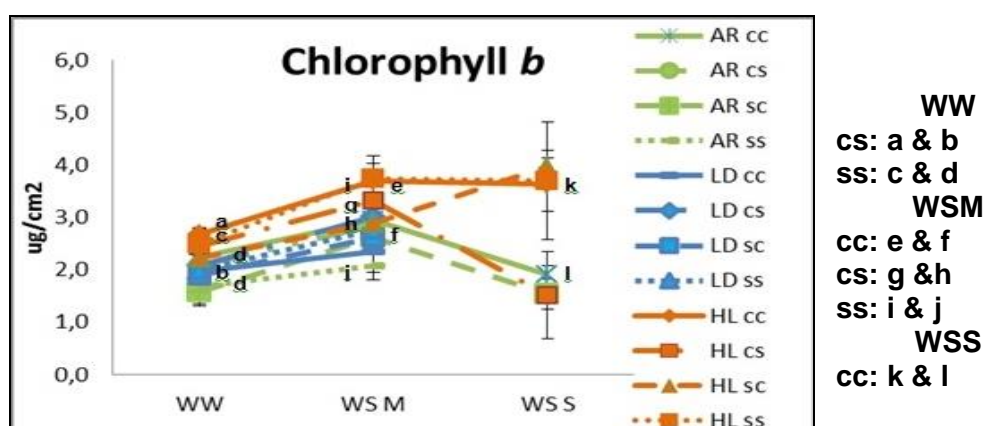


Figure 39: Chlorophyll b per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured where the plants were subjected under WW (Water Conditions), WSM (Water Stress Moderate) and WSS (Water Stress Severe)

Table 19: T-test of two-samples assuming unequal variances by provenance for the Chlo b

Date	Provenances	Treat.	t-test	df	p-value	95 % conf. interval
WW	HL-LD (a & b)	CS	3,3061	6	0,01628	0,105812 0,708474
WW	HL-LD (c & d)	SS	2,7006	6	0,03283	0,05162 0,856952
WSM	HL-LD (e & f)	CC	3,5090	5	0,01712	0,504113 3,265887
WSM	HL-AR (g & h)	CS	2,6634	6	0,03735	0,072812 1,718617
WSM	HL-AR (i & j)	SS	3,7870	2	0,006829	0,612701 2,649799
WSS	HL-AR (k & l)	CC	4,9608	2	0,03831	0,275064 3,871604

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value

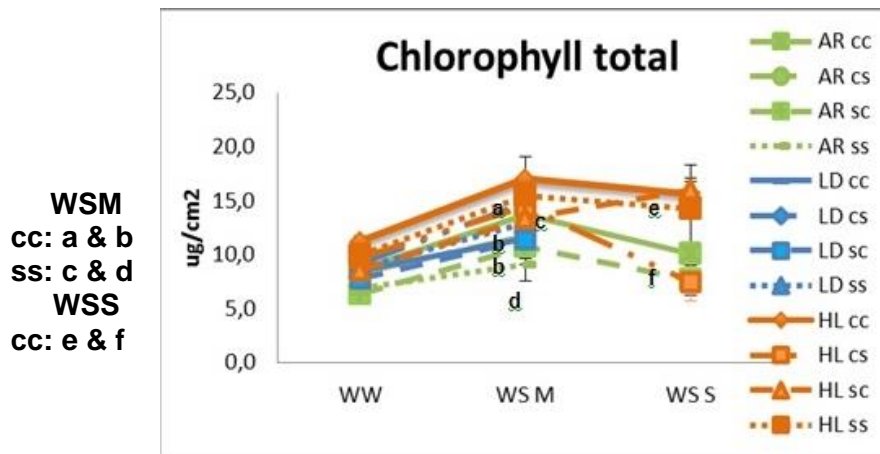


Figure 40: Total Chlorophyll per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured where the plants were subjected under WW (Water Conditions), WSM (Water Stress Moderate) and WSS (Water Stress Severe)

Table 20: T-test of two-samples assuming unequal variances per provenance for the Total Chlo

Date	Provenances	Treat.	t-test	df	p-value	95 % conf. interval
WSM	HL-LD (a & b)	CC	3,8262	5	0,0123	2,562956 13,05704
WSM	HL-AR (a & b)	CC	5,6955	3	0,011	2,161493 7,748507
WSM	HL-AR (c & d)	SS	4,0625	7	0,004795	2,461644 9,318356
WSS	HL-AR (e & f)	CC	10,0535	2	0,009749	3,620923 9,039077

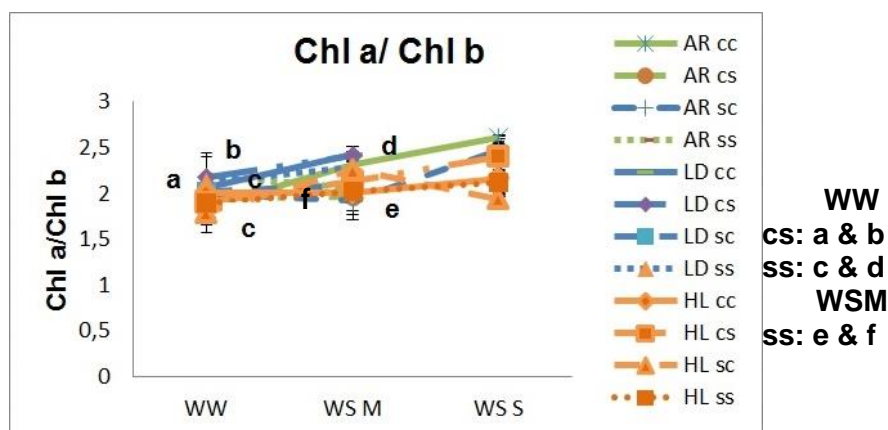


Figure 41: Chlorophyll a/chlorophyll b per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under conditions and CS means first regime under Controlled and the two last ones under Stress. Measured where the plants were subjected under WW (Water Conditions), WSM (Water Stress Moderate) and WSS (Water Stress Severe)

Table 21: T-test of two-samples assuming unequal variances per provenance for the relation of Chlo a/ Chlo b

Date	Provenances	Treat.	t-test	df	p-value	95 % conf. interval	
WW	HL-LD (a & b)	CS	-3,4002*	6	0,01449	-0,44956*	-0,07329*
WW	HL-LD (c & d)	SS	-4,5068*	6	0,004074	-0,50256*	-0,14887*
WSM	HL-LD (e & f)	SS	-2,3760*	7	0,04917	-0,50877*	-0,00123*

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value

The data Analyses showed significance differences for Chlo a under moderate and severe drought-induced conditions (Figure 38 and Table 18). It was found differences in the CC treatment between Haza del Lino and both Landes and Ain Rami. In the case of Chlo b, there was found significance differences under water conditions (WW), between Haza del Lino and Landes for the CS and SS treatments. Besides, there are significant differences between Landes and Ain Rami for the SS treatment (Table 19). Under Moderate Stress conditions (WSM) it was found significative differences for the CC treatment between Haza del Lino and Landes, in addition to differences between Landes and Ain Rami. CS treatment showed differences significant between Haza del Lino and Ain Rami provenances. In last, SS treatment also showed differences significant between Haza del Lino and both Landes and Ain Rami. For the last regime, Water Severe Stress (WSS), it showed differences for the CC treatment between Haza del Lino and Ain Rami. For the Total Chlo, it was found significative differences under moderated drought between Haza del Lino and both Landes and Ain Rami for the CC treatment. For the SS treatment, there were significative differences between Haza del Lino and Ain Rami. In other hand, under water severe stress (WSS), it was found

significant differences between Haza del Lino and Ain Rami for the CC treatment (Figure 40 and Table 19). For the relation of Chlo a/Chlo b was shown differences significant under water conditions for the CS and SS treatments between Haza del Lino and Landes and under moderated stress (WSM) it was found significant differences for the SS treatment for the same provenances, (Figure 41 and Table 20).

Based on these results, we can see a general tendency of decrease under severe drought conditions for the chlorophyll production. Less chlorophyll production means less photosynthesis, which means less plant growth. The values under $2 \mu\text{g}/\text{cm}^2$ means that there is a degradation of the chlorophyll a, provoking an increase of the chlorophyll b, or the apparition of the pheophytin.

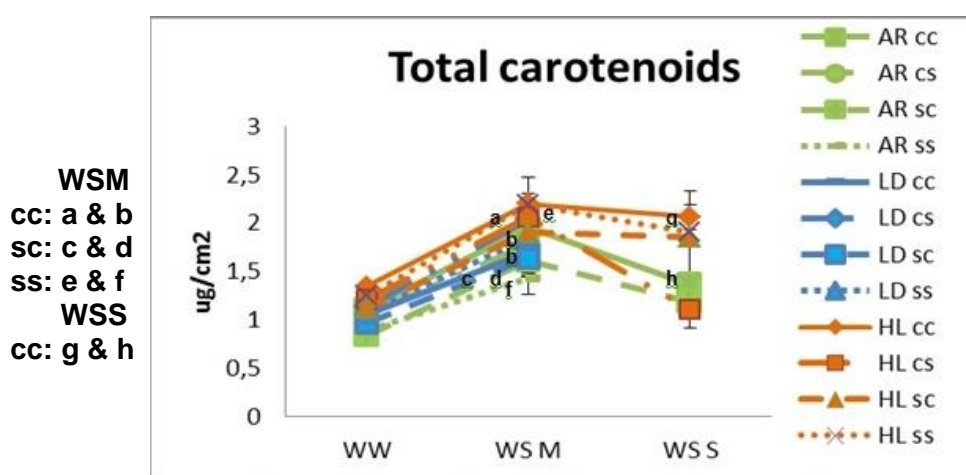


Figure 42: Total Carotenoids per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured where the plants were subjected under WW (Water Conditions), WSM (Water Stress Moderate) and WSS (Water Stress Severe)

Table 22: T-test of two-samples assuming unequal variances per provenance for the Carotenoids

Date	Provenances	Treat.	t-test	df	p-value	95 % conf. interval
WSM	HL-LD (a & b)	CC	2,8466	5	0,03597	0,077083 1,512917
WSM	HL-AR (a & b)	CC	3,8177	3	0,03162	0,073212 0,806788
WSM	LD-AR (c & d)	SC	21,5000	1	0,02959	0,175876 0,684124
WSM	HL-AR (e & f)	SS	4,2853	7	0,003631	0,325509 1,126991
WSS	HL-AR (g & h)	CC	24,0420	2	0,001726	0,667776 0,958891

Data analyses showed significative differences for the total carotenoids under moderated stress (WSM) for the CC between Haza del Lino and both Landes and Ain Rami provenances, where Haza del Lino had the higher carotenoids production. SC also showed

significance differences between Landes and Ain Rami under WSM. In addition, between Haza del Lino and Ain Rami showed significative differences for the SS treatment.

Under water severe stress (WSS) for the CC treatment was shown significative differences between Haza del Lino and Ain Rami, where Haza del Lino remained close to the 2 $\mu\text{g}/\text{cm}^2$, and the Moroccan provenance dropped under 1,5 $\mu\text{g}/\text{cm}^2$.

3.5.2 Pheophytin (Ppt) Content

Under drought conditions, the magnesium is scarce, provoking the production of the element called pheophytin. The compound pheophytin (ppt) occurs when it is not found magnesium or any other metallic element in the center of the chain to produce the chlorophyll, thus its increase in the plant it is related with the absence of Magnesium that is needed for the photosynthesis. The production of pheophytin is a result of the degradation of the chlo a, and it is compound by 4 atoms of Nitrogen and one of H^+ in the middle, as a substitution of the Mg^{2+} .

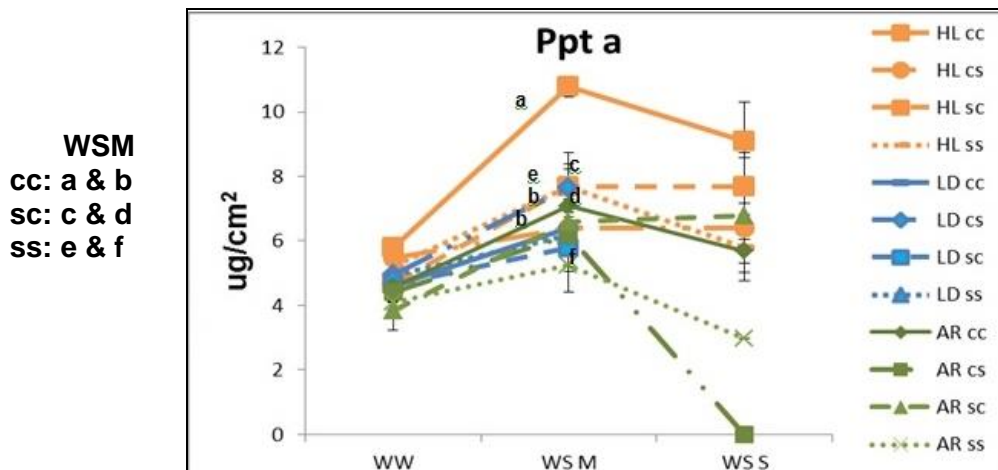


Figure 43: Ppt a per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress conditions, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured where the plants were subjected under WW (Water Conditions), WSM (Water Stress Moderate) and WSS (Water Stress Severe)

Table 23: T-test of two-samples assuming unequal variances per provenance for the Pheophytin a

Date	Provenances	Treat.	t-test	df	p-value	95 % conf. interval
WSM	HL-LD (a & b)	CC	4,3156	5	0,007602	1,826321 7,207013
WSM	HL-AR (a & b)	CC	11,6856	3	0,001346	2,439486 4,265514
WSM	HL-AR (c & d)	SC	19,7500	1	0,03221	0,563504 2,596496
WSM	HL-AR (e & f)	SS	3,1983	7	0,0151	0,696609 4,648391

WW
cs: a & b
WSM
cc: c & d
sc: e & f

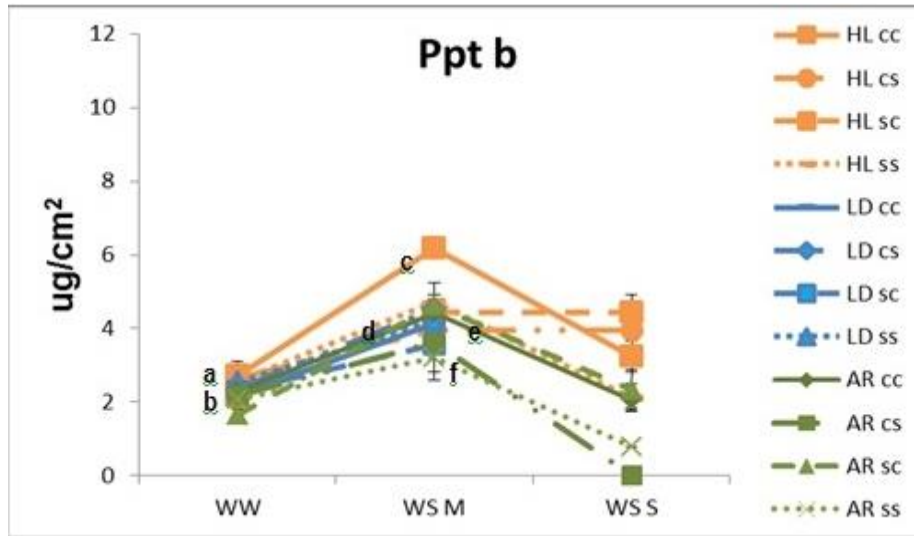


Figure 44: Ppt b per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured where the plants were subjected under WW (Water Conditions), WSM (Water Stress Moderate) and WSS (Water Stress Severe)

Table 24: T-test of two-samples assuming unequal variances per provenance for the Ppt b

Date	Provenances	Treat.	t-test	df	p-value	95 % conf. interval	
WW	HL-LD (a & b)	CS	2,5420	6	0,04396	0,01346	0,70654
WSM	HL-LD (c & d)	CC	3,6384	5	0,01493	0,618263	3,595071
WSM	HL-AR (e & f)	SC	12,0974	3	0,001216	1,25094	2,14406

WW
cc: a & b
sc: c & d
WSM
cc: e & f

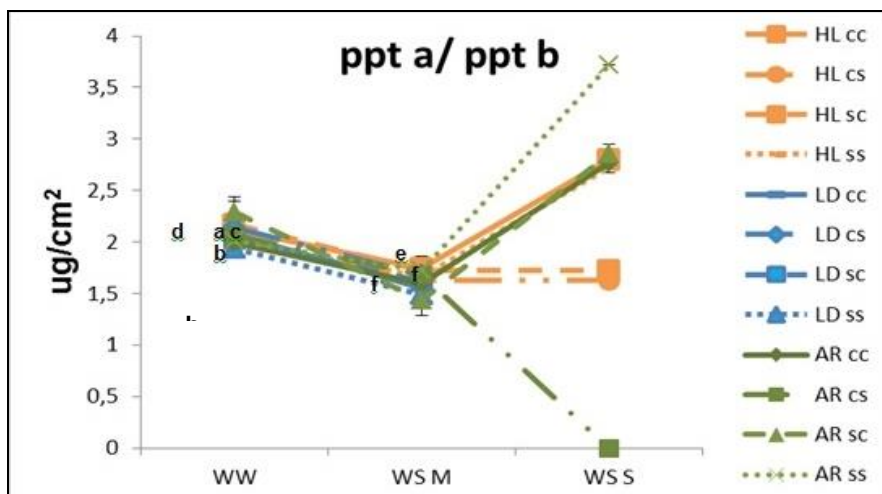


Figure 45: Relation of Ppt a/ Ppt b per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) and treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under

Controlled and the two last ones under Stress. Measured where the plants were subjected under WW (Water Conditions), WSM (Water Stress Moderate) and WSS (Water Stress Severe)

Table 25: T-test of two-samples assuming unequal variances per provenance for the relation of Ppt a/ Ppt b

Date	Provenances	Treat.	t-test	df	p-value	95 % conf. interval	
WW	HL-AR (a & b)	CC	5,8985	3	0,009728	0,102453	0,342547
WW	HL-AR (c & d)	SC	-25,5000*	1	0,02495	-0,75412*	-0,25588*
WSM	HL-LD (e & f)	CC	3,1104	5	0,02654	0,034134	0,359199
WSM	HL-AR (e & f)	CC	5,6955	3	0,01073	0,047433	0,167567

* T-values can be negative, but p-values are always positive. The p-value for a negative t-value is the same as that for the positive version of that t-value

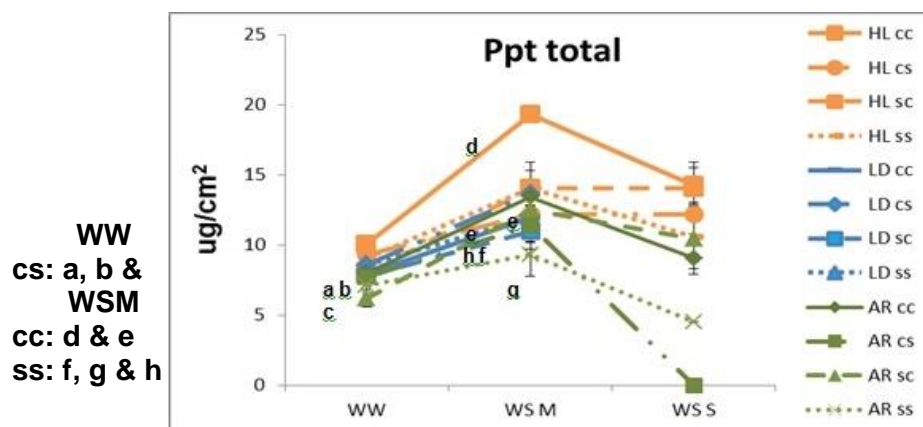


Figure 46: Total Ppt per provenance: Haza del Lino (HL), Landes (LD) and Ain Rami (AR) treatment, which CC means three regimes under Controlled conditions, SS means three regimes under Stress, SC means first regime under Stress and the two last ones under Controlled and CS means first regime under Controlled and the two last ones under Stress. Measured where the plants were subjected under WW (Water Conditions), WSM (Water Stress Moderate) and WSS (Water Stress Severe)

Table 26: T-test of two-samples assuming unequal variances per provenance for the Total Ppt

Date	Provenances	Treatment	t-test	df	p-value	95 % conf. interval	
WW	HL-LD (a & b)	CS	12,2727	6	0,00001783	5,807936	8,700636
WW	HL-AR (a & c)	CS	9,8323	6	0,00006378	5,41784	9,007874
WW	LD-AR (b & c)	CS	18,1003	6	0,00000183	5,421148	7,115995
WSM	HL-LD (d & e)	CC	4,0692	5	0,009642	2,77913	12,2814
WSM	HL-AR (d & e)	CC	26,2100	3	0,0001218	4,76629	6,08371
WSM	HL-AR (f & g)	SS	10,3670	7	0,00001685	10,2849	16,3626
WSM	LD-AR (h & g)	SS	10,2035	7	0,0001873	7,978313	12,79169

Data analyses showed significant differences for Ppta (see Figure 43 and Table 23) under moderated water drought conditions (WSM), between Haza del Lino and both Landes

and Ain Rami for the CC treatment and between Haza del Lino and Ain Rami for the SC and SS treatments, where Haza del Lino showed higher values in all measurements.

For the Ppt b, data analyses showed differences between Haza del Lino and Landes for the CS treatment under water conditions (WW). Under moderated water deficit, it was found significant differences between Haza del Lino and Landes for the CC treatment and Haza del Lino and Ain Rami for the SC treatment (Figure 44 and Table 24). Haza del Lino had higher concentrations of Pptb under WSM and WSS regimes.

For the relation of ppta/pptb it was found differences significant under water conditions (WW) between Haza del Lino and Ain Rami for CC and SC treatments. In other hand Haza del Lino had significant differences with Landes and Ain Rami for the CC treatment under moderate drought conditions (see Figure 45 and Table 25).

Data analyses showed differences for the Total ppt (Figure 47 and Table 26) under water (WW) conditions, right at the beginning of the study and under moderated drought conditions (WSM). Under water conditions, the differences found were in the CS treatment between Haza del Lino and both Landes and Ain Rami and Landes with Ain Rami and under moderated drought between Haza del Lino and Ain Rami for the CC and SS treatments and between Ain Rami and Landes for the SS treatment.

4. Discussion

Considering the Survival rate, Ain Rami provenance showed to be the most sensitive provenance when submitted to drought conditions, with a whole treatment (CS) dead during the study, remaining with only 1 plant under SS conditions. In other hand, Haza del Lino responded similarly to Landes but when the drought conditions were severe, Landes increased in dead plants.

Concerning Leaf Area and SLA, Ain Rami showed that the plants from the SC and SS treatments had the biggest and thinner leaves in comparison with Haza del Lino and Landes that had in average smaller a thicker leaves.

Desiccation may influence water uptake in two ways: firstly, desiccation can kill roots. A reduction in functional root will decrease the surface area for water uptake from the existing root system and also in number of root initials for root production. Secondly, desiccation by reducing leaf water potential cause stomata closure as mentioned in McKay and White, (1997). In addition, the Cork Oak leaves are, however, less dense and less tolerant to climatic extremes than the leaves of other evergreen trees, for example, Holm Oak (*Quercus rotundifolia* = *Quercus ilex* subsp. *ballota*) that frequently coexists with the Cork Oak, according to Pereira *et al.*, (2008).

In the total biomass the plants contained in the CS treatment performed different responses, where Ain Rami roots had the higher amount of total biomass even when the plants died before than the ones from the other provenances. In opposite, Landes had the less amount of total biomass in the roots but the higher amount of biomass in the branches. In the case of Haza del Lino performed an average response for both provenances. Bigger the total biomass on the root is, the bigger the root/shoot ratio is. The plants belonged to CS treatment were the ones with higher root/shoot ratio.

For the root classification, the plants belonged to SC and CC treatments had a higher amount of roots developed, probably due to a non-restriction on the root growth because of the absence of water drought, when the treatments submitted under drought conditions their root apparatus was smaller. It can be seen how being all under the same environmental conditions, the presence of the water deficit affected differently making different strategies between the provenances.

For the surface area, the CC treatment had the higher amount of the finest roots where Landes had the higher % between all the provenances and Ain Rami had the less amount of finest roots were found in the Control treatment. Haza del Lino had the higher amount of biggest roots were found in the Stress treatment,

It is curious how the treatments with higher biomass growth are the ones that have the less length, surface area and volume. This means, that their roots are more effective in the water and nutrients uptake under severe drought conditions, while the other roots invest its energy in the length of the root apparatus for the search of water.

For the morphological measures, the strategy displayed under drought conditions is different for all the provenances. It can be found different responses for the number of branches, leaves, and diameter and even in the height of the plant. Because when in one provenance the growth increases for one treatment for another provenance decreases, when in the contrary another provenance increases while the other one decreases.

The loss of leaves could be due to the environmental alterations, changes on the temperatures increasing the water loss. Under drought conditions, usually the plants response eliminating by excision parts of its body such as the leaves decreasing the number of leaves as a try to avoid the water loss, or reducing the leaf area.

Higher amount of leaves is linked with a bigger diameter of the branch. This means, more leaves the plants have, bigger diameter is needed to supply them, and in another way, more leaves produce more photosynthesis, which is needed to be transported to the roots for the sugar production and in growth favor of the plants, so a higher conduct to transport through the xylem and phloem is needed, explaining the size of the diameter of the branches.

The height of the plant is directly related with the growth of the plant. Under severe drought conditions, the shoot growth is limited translocating its growth to the root apparatus for maintaining the sugar production for the survival of the plant, as it happens in slow growing plants, like in the Spanish provenance, which it is a small plant for the shoot and root system part. In the case of the Moroccan provenance the growth was focused in the development of a root apparatus, while for the SC treatment, the plant continued growing in the upper part having the tallest plants.

The greatest amount of stomata was found in the SS treatments, due to the increased of the efficiency of its CO₂ exchange under the severe water deficit conditions, certifying the photosynthetical light response curves. A higher amount of stomata may promote its growth to more branches and more leaves so more photosynthesis, for developing its root apparatus to a deeper and more specialized root for higher water uptake efficiency and nutrients. The plants that belong to CS showed a more effective photosynthetic production than the SS, probably due to drought modified the carbon allocation among the leaves and root, for carbohydrate storage and fruiting. Under drought conditions, seed production may be increased, but often drops during the two to three years following a drought (Hendry S.J. et al., 2000, 2001; Innes J.L., 1994).

Regarding the WRC the general tendency showed a reduce of the water capacity in the leaves even though they were in optimal conditions like in the case of the CC and SC treatments, that ranged from 80 to 40%. Drastic picks of temperature that occurred during the months of the study increased this fact happen. CS showed in general a higher sensitivity to water drought, responding with a higher and faster decrease than the SS treatment.

In the leaf water potential the CS treatments of all the provenances were the ones that showed a very drastic decline of its water potential, followed by the SS treatments, with even higher values than in the water potential of the previous cycle. This means that after previous cycles of drought, the CS treatment for all the provenances were more sensitive to drought declining before and leading in some cases to death.

The small photosynthetical rates of the PSII for the treatments CS and SS in the provenances could explain the decrease in the light curves, and the increase of the total biomass in the root system for these treatments, as we have less photosynthesis, we have less chlorophyll, and therefore less growth but an allocation of the carbon to the roots for a better and deeper root apparatus. However, Haza del Lino had better PSII results probably due to their leaves area is minor than the ones from Landes or Ain Rami, and therefore their leaves have less water loss.

Low light intensities can mean a stress on the plants because irradiance limits photosynthesis and thus the carbon uptake and plant growth. In the contrary, high amount of light can mean a stress for the plants as well, hence long exposition to light leads to water loss by evaporation. "At high irradiance, photosynthesis become light-saturated and is limited by the carboxylation rate which is governed by some combination of CO₂ diffusion into the leaf and carboxylation capacity" (Monsi and Saeki, 1953). It is showed that the plants that were more sensitive to drought conditions reached the saturation point in smaller rates of PAR, like in the case of Landes for the SS provenances. The highest values in the light curves are linked with a less RWC (%).

The Chlorophyll is needed for the photosynthesis production, and water is vital for the internal processes of the plant. Under drought conditions, the amount of water in the soil is less available making more difficult the uptake of nutrients, overall the nitrogen making hard its absorption, because nitrogen is commonly found in the soil as a complex compound, NO₃⁺, NH₃, etc. Nitrogen is a detrimental element for the chlorophyll production, and when this is scarce, the photosynthesis cannot be made, and the growth of the plant is stopped. Its absence reduces the leaf area and the general growth of the tree, provoking a possibly translocation of the Nitrogen from the new to the old leaves.

Values less than 2 µg/cm² of chlorophyll a, means that there was a degradation of the

chlorophyll a to produce chlorophyll b. As the Spanish provenance has less water loss by evapotranspiration, the water in the soil remains longer favoring the elements uptake to make the photosynthesis, even though at the end it won't be use that carbon for the plant growth, but rather, for the sugar production and other substances. While in contrary, Ain Rami with bigger and thinner leaves will have a higher water loss that will provoke the unavailability of the minerals needed for the process of photosynthesis.

In the case of the carotenoids, higher amount of carotenoids corresponds to a higher protection of the plant. Moreover, physiological disorders increase tree vulnerability to secondary stresses like insect damage, frost or another repeated drought, as mentioned in (Rouault *et al.*, 2006); this could explain the appearance of red spider and other damages in the beginning of the study. Based on these results, we can see a general tendency of decrease under severe drought conditions in the production of the chlorophyll.

An increase of the pheophytin means lower magnesium available for the photosynthesis production. The Spanish provenance had higher ppta and pptb, which means that under moderate and severe conditions, it can find the way to have enough Magnesium for its chlorophyll production, by means of maintaining the plants in a constant steady state and a very slow growth or "tolerant strategy" to drought according to Kozlowzski *et al.*, (1991).

5. Conclusions

- Ain Rami showed to be the most sensitive provenance under drought conditions, specially the plants submitted under CS treatment, according to the survival rate results, while Landes plants reacted later being submitted under drought conditions, and Haza del Lino under severe drought had less mortality
- The plants submitted under CC (control) conditions that showed significant differences between the provenances as it is showed for the RWC, water leaf potential, chlorophylls, pheophytin, Carotenoids and in the Root Length showed that it could be due to provenance specific, geographic variation characterized by the individual evolution under different environmental conditions
- Haza del Lino showed very efficient photosynthesis, where the carbon will be rather used for the sugar production, and remaining the smallest plant; When in the contrary Landes showed averaged values between Haza del Lino and Ain Rami
- The decrease of the Carotenoids production in the leaves tissues relates the diminish protection of the plants by toxins and favoring the plant attack
- Ain Rami showed that the carbon allocation was focused in the developing of the roots of the plant such in the CS treatment case, that died before the study finished but had high specialized roots
- The high LA and SLA, in the Moroccan provenance, besides the higher amount of stomata, favored the water loss by evaporation and dryness of the plants, while in the contrary, the Spanish and French provenances remained with smaller and thicker leaves with less water loss
- The decrease of the Chlorophyll production relates the efficiency of the photosynthesis production, where Haza del Lino was more efficient under drought conditions
- The responses performed by the treatments submitted under different drought regimes in a row (CS and SC) in all the provenances, might be due to the process of hardening or acclimation of the plants.

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7. ANNEXES

Qualitative data: Digitalization of the leaves of the plants. Example of the data collected.

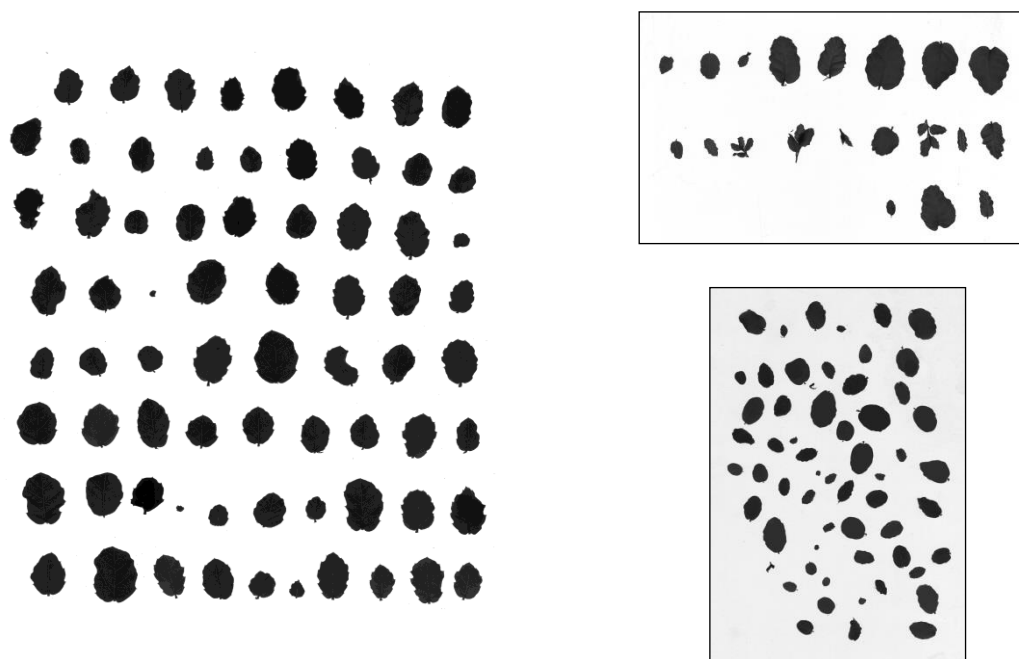


Figure 47: Example of qualitative data of a total leaves of a plant (HA 1 CC). New leaves scanned of the AR 145 CC plant and old leaves scanning of the LD 110 SC plant

Qualitative data: Roots digitalization by provenance and treatment.

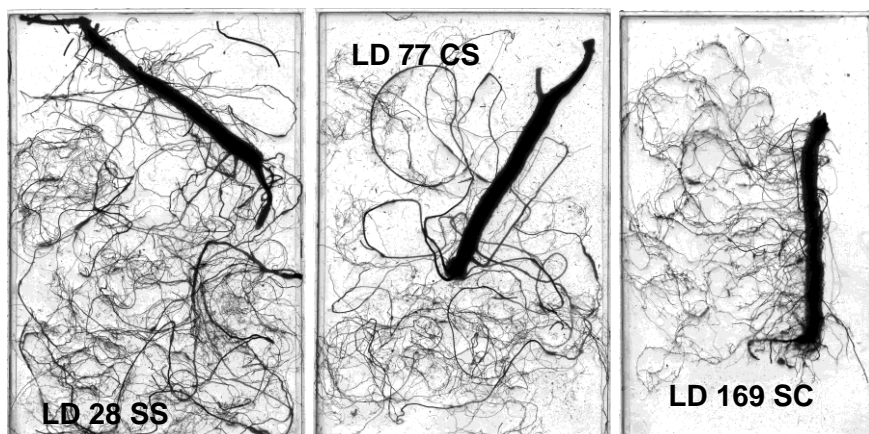


Figure 48: Digitalization of the plant LD 28 SS, LD 77 CS and LD 169 SC for the root classification and analysis

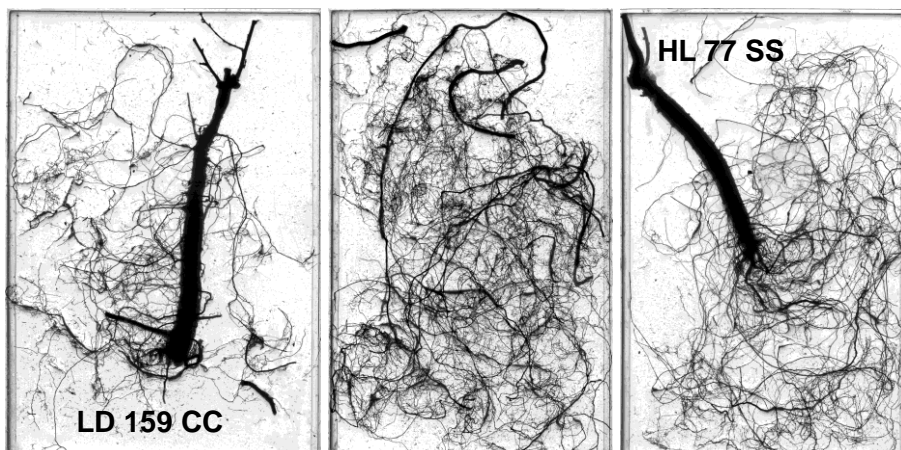


Figure 49: Digitalization of the plant LD 159 CC and HL 77 SS for the root classification and analysis

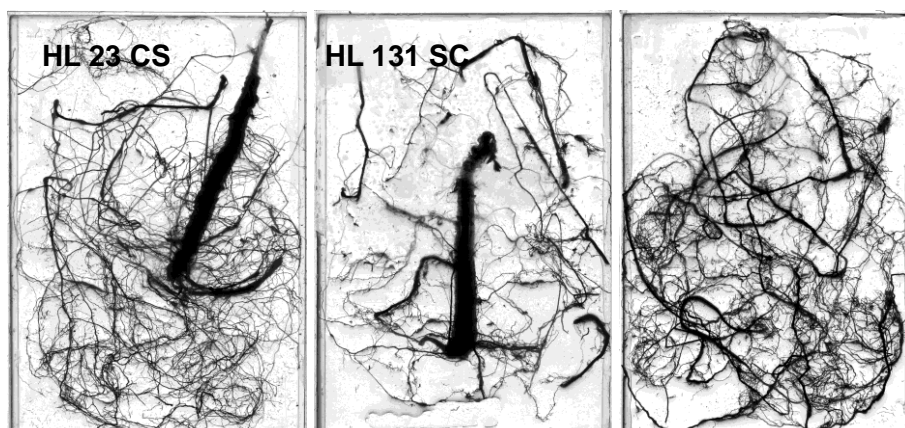


Figure 50: Digitalization of the plant of HL 23 CS and HL 131 SC for the root classification and analysis

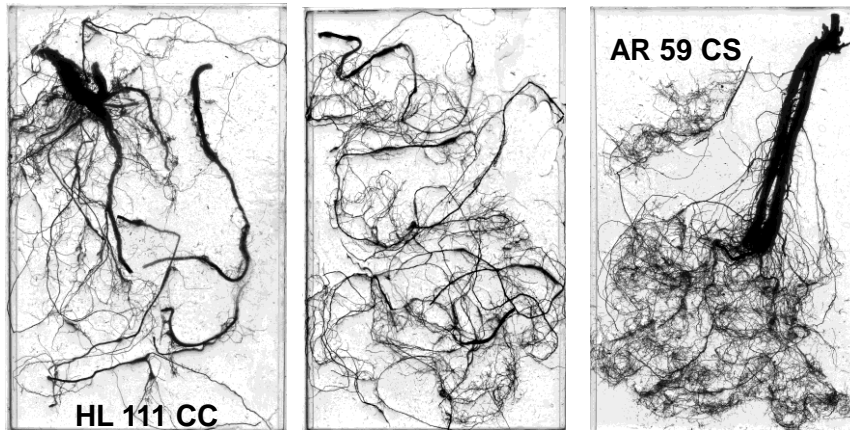


Figure 51: Digitalization of the plant of HL 111 CC and AR 59 CS for the root classification and analysis

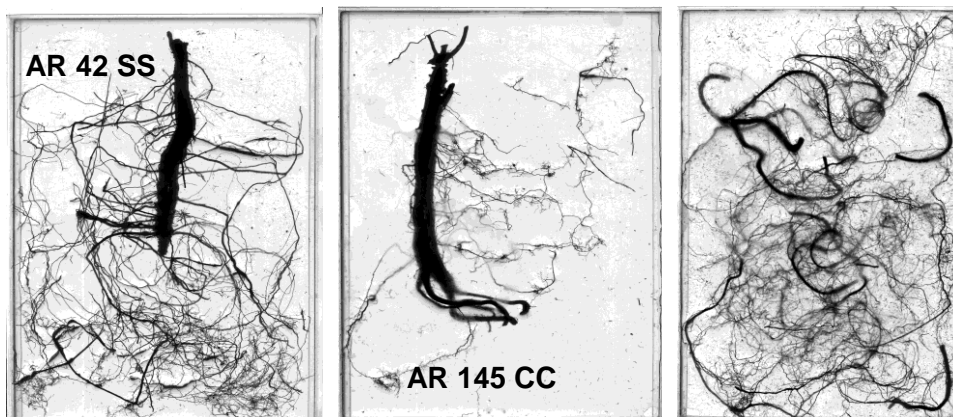


Figure 52: Digitalization of the plant of AR 42 SS and AR 145 CC for the root classification and analysis

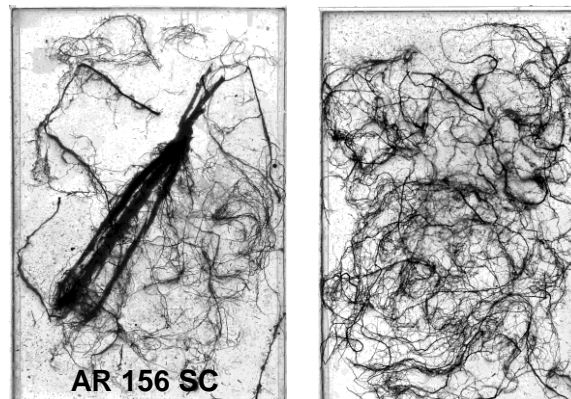


Figure 53: Roots of MA 156 SC plant divided in main root and secondary roots, where it can be appreciated the appearance of the three main roots