Title: Phenotypic and molecular traits determine the tolerance of olive trees to drought stress

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Abstract

Olive trees are known for their capacity to adapt to drought through several phenotypic and molecular variations, although this can vary according to the different provenances of the same olive cultivar. We confronted the same olive cultivar from two different location in Spain: Freila, in the Granada province, with low annual precipitation, and Grazalema, in the Cadiz province, with high annual precipitation, and subjected them to five weeks of severe drought stress. We found distinctive physiological and developmental adaptations among the two provenances. Thus, trees from Freila subjected to drought stress exhibited increasing root dry weights and decreasing leaf numbers and relative stem heights. On the other hand, the treatment with drought in Grazalema trees reduced their leaf chlorophyll contents, but increased their relative stem diameter and their root hydraulic conductivity. The physiological responses of Freila tree roots to drought were linked to different molecular adaptations that involved the regulation of genes related to transcription factors induced by ABA, auxin and ethylene signaling, as well as, the action of a predicted membrane intrinsic protein (MIP). On the other hand, the responses of Grazalema trees were related with different root genes related to oxidation-reduction, ATP synthesis, transduction and posttranslational regulation, with a special mention to the cytokinins signaling through the transcript predicted as a histidine-containing phosphotransfer protein. Our results show that olive trees adapted to dry environments will adjust their growth and water uptake capacity through transcription factors regulation, and this will influence the different physiological responses to drought stress.

Keywords: olive, drought, transcription factors, membrane intrinsic proteins.

Introduction

The domesticated olive tree has been historically a key species in the economical development of rural areas all along the Mediterranean basin as it has been able to adapt and grow in very diverse environments, from dry to heavy rain areas (Palese et al., 2010). Olives are known for their resistance to water stress especially during summer, when they face scarce precipitation and high temperatures (Connor, 2005). The need of novel strategies to understand and improve olive trees towards dry conditions is a major issue in order to avoid the decline of their productivity in the context of climate change predictions of water scarcity (Lesk et al., 2016). Plants fight back the detrimental effects of drought by a series of physiological adjustments to avoid desiccation, such as altering transpiration and regulating root water uptake (Perrone et al., 2012; Calvo-Polanco et al., 2016), inducing antioxidant activity and osmotic adjustments (Reddy et al., 2004), and altering hormonal contents (Peleg and Blumwald, 2011). The transfer of water from the soil to the xylem is mainly limited by the root radial water transport. Under drought conditions, when transpiration is minimized, the main contributions to water uptake will be attributed to the symplastic path, which will use water channel proteins called aquaporins at the cell membranes to transfer water. While the apoplastic path through cell walls, where the transpiration stream will act as the main driving force, will be reduced (Verdoucq and Maurel, 2018).

Physiological and morphological plant adaptations to drought are regulated by several stress perception and transduction pathways, which interconnect at various steps inducing or repressing different genes (Joshi et al., 2016). The use of transcriptomics, proteomics and metabolomics have allowed the identification of various drought stress responsive genes in crops, corresponding to genes coding for proteins that have either metabolic or regulatory roles (revised in Joshi et al., 2016). Among the different regulatory networks, which

synchronize signal transduction and expression of genes during stress, a special attention has been paid to the regulatory class of genes that are grouped into transcription factors (AREB, DREB, MYB, WRKY, NAC, and bZIP), basic helix-loop-helix (MYC,MYB), signaling protein kinases (MAPK, CDPK, receptor protein kinases) and protein phosphatases (Wani et al., 2013). Transcription factors (TFs) have gained attention for their significant role in plant stress tolerance (Shao et al., 2015), as they act as molecular switches in the regulation of the expression of associated genes with subsequent consequences in plant development (Franco-Zorrilla et al., 2014; Joshi et al., 2016). In plants, up to 10% of the genes in the genome potentially encode TFs (Franco-Zorrilla et al., 2014) and represent one of the most critical steps in the signaling cascades toward physiological and morphological adaptation of plants (Shao et al., 2015). Different TFs are induced or repressed by the action of hormones. Under drought, abscisic acid (ABA) biosynthesis and accumulation within the plant tissues has been commonly observed (Zhang and Davies, 1987), and it has been pointed as the main responsible for inducing several stress-related genes (Yang et al., 2011). However, ABA likely does not act alone but in combination with other hormones as cytokinins, auxin and ethylene (revised in Rowe,2016).

The outcome of the olive genome in 2016 (Cruz et al., 2016), with about 1.38 Gb total length, opened new opportunities in the study of the different molecular traits and phenotypic variations within this species. The aim of this study is to identify distinctive response genes to drought stress in the olive tree Picual cultivar from two different locations Freila, a Mediterranean location with low-average annual precipitation (380 mm), and Grazalema, with high-average annual precipitation (2223 mm). Based on studies in other tree species where the origin of the same populous clone determined the transcriptional outcome upon drought exposure (Raj et al. 2011), We-we anticipate that the trees from Freila will have a high diverse

intrinsic regulation of the plant gene expression under drought conditions and that the olive trees from Grazalema will not be able to handle long-term exposure to drought stress, and they will have a contrasting gene expression regulation. Therefore, olive trees from both provenances were subjected to a severe drought stress and suppression-subtractive gene hybridization was carried out comparing both provenances in root tissues under drought conditions. Several morphological, growth and physiological parameters were determined at the same time.

Materials and Methods

Olive cuttings production and growth conditions

Olive cuttings (*Olea europaea* L. cv. Picual) were produced from mature olive trees growing at two locations of the south of Spain that are distinctively characterized for their pluviometry, a drought area close to Freila (37°31'43"N, 2°54'34"W), with an annual precipitation of 380 mm, and another in Grazalema (36°46'4"N, 5°21' 57" W) with an annual precipitation of 2223 mm. The olive branches were removed and transported in a humid piece of cloth to a greenhouse and the cuttings were produced as described in Suárez et al. (1999). Briefly, 18cm length and 4–6 cm diameter cuttings were treated with indolbutyric acid (IBA) 3500 ppm by immersing the cutting base for 10 s in a 1:1 (v:v) IBA hydro-alcoholic solution. The cuttings were immediately transferred to a mist propagation system for 90 d on a perlite substrate at 25 °C basal heating for rooting. Once the cuttings were rooted, they were transferred into 2L pots using a 2:1 (w:w) soil: quartz-sand (<1 mm) mixture. The soil was sieved (2 mm), sterilized by steaming (100 °C for 1 h on 3 consecutive days). The soil had a pH of 8.1 (water); 1.5% organic matter, nutrient concentrations (g kg⁻¹): total N, 1; total P, 1 (NaHCO₃-extractable P);

total K, 11. The soil texture comprised 38.3% sand, 47.1% silt and 14.6% clay. Plants were then transferred to a greenhouse at 22/18 °C, 65% relative humidity and were irrigated with 50% modified Hoagland's solution (Epstein 1972) once per week: 2.5mM KNO₃, 0.5mM KH₂PO₄, 2.5mM Ca(NO₃)₂, 1mM MgSO₄, 23µM H₃BO₃, 5 µM MnCl₂, 0.3µM ZnSO₄, 0.2µM CuSO₄, 0.01 µM (NH₄)₆Mo₇O₂₄ and 90µM EDTA-Fe.

Six months after planting, trees from each location were separated into two groups and subjected to drought stress (55% field capacity) for 5 weeks, while the other half were left as well-watered (95% field capacity). Soil moisture was controlled using a ML2 ThetaProbe (AT Delta-T Devices Ltd., Cambridge, UK) and the water content of the soil was maintained by weighing the pots every day and replacing the water lost to recover the desired level of soil water content (Calvo-Polanco et al., 2016).

Growth and physiological determinations

Leaf and root dry weights were determined at harvest time by detaching the shoots from the roots and after oven-dry for 72 hours at 65°C, in 10 trees (n=10) per treatment combination. Relative height, diameter and number of leaves were determined from the measurements taken at the beginning and at the end of the drought treatment and expressed as percentage: [(final value-initial value) / initial value] x 100.

Stomatal conductance (*gs*) was measured at harvest time, 3 hours after sunrise in fully developed mature leaves of ten plants (n = 10) per treatment combination, with a portable AP4 Porometer (Delta-T Devices Ltd). Leaf relative water content (RWC) was determined in eight mature leaves per treatment combination (n = 8), after the *gs* measurements. Mature, fully developed leaves were excised from the main shoot, weighted (*W*₀) and introduced into 15 ml centrifuge tubes (BD Falcon, Fisher Scientific) with a piece of moist cotton for 24

hours at 4°C. Leaves were weighted again (W_h) and dried at 72°C for 2 days (W_d). Leaf RWC was calculated as $RWC = (W_0 - W_d)/(W_h - W_d) \times 100$. Leaf chlorophyll contents were extracted in 100% methanol and concentration calculated using the coefficients and equations reported in Lichtenthaler (1987).

Root hydraulic conductance (K_r) was measured after g_s determinations using a high pressure flow meter (HPFM, Dynamax Inc., Houston) in eight roots per treatment combination ($n=8$) (Calvo-Polanco et al., 2016). Detached roots were connected to the HPFM using compression couplings, and water was perfused at increasing pressures ranging from 0 to 500 kPa. Root volume was calculated after the measurements and the hydrostatic root hydraulic conductivity determined by dividing K_r by the root volume.

Root poly(A)+RNA extraction and cDNA suppression-subtractive hybridization

Total RNA was first isolated from roots by a phenol/chloroform extraction method followed by LiCl precipitation (Kay et al., 1987). After mRNA extraction we extracted the poly-A RNA using the Promega Protocol PolyATtract mRNA Isolation System (Promega, USA) following the manufactured instructions. We got a total of 6 different polyA mRNA extractions per olive genotype under drought conditions. cDNA was synthesized from 2 µg of poly-A RNA using oligo(dT)12–18 as a primer and M-MLV as reverse transcriptase (Invitrogen).

To identify sequences putatively regulated by drought stress in the ~~low~~-two genotypes we constructed a subtractive cDNA library using a PCR-Select™ cDNA subtraction kit (Clontech, CA, USA), following the manufacturer protocols. The cDNA of the two olive genotypes under drought stress were either used for tester and later as driver in order to determine the characteristic differences of their behavior under drought stress. Subtracted

cDNA products were ligated into the pGEM[®] T-easy vector (Promega, WI, USA) and transformed into *Escherichia coli* DH5 α cells. The presence and size of the inserts were determined by direct amplification from crude bacterial lysate using the nested PCR primers 1 and 2R from the PCR-selected cDNA subtraction kit.

Sequences analyses

The positive clones were sequenced at the Sequencing Service of the Estación Experimental del Zaidín (CSIC, Granada), and all unique ESTs were annotated on the basis of the existing annotation of non-redundant databases at the NCBI using BLASTN. Sequences were further analyzed by using the SRS database from the OLEAGEN consortium (Muñoz-Mérida et al., 2013). Homologies with e-values lower than $1e^{-05}$ for more than 100 nucleotides were considered significant. Functional classification of the ESTs was performed according to the functional categories of *Arabidopsis thaliana* proteins (<http://mips.helmholtz-muenchen.de/proj/funcatDB/>) (Ruepp et al., 2004). Blast2GO was used to identify gene ontology (GO) terms associated with the identified genes (Conesa et al., 2005).

Real-time quantitative PCR

The expression pattern of several genes was confirmed using RT-qPCR using the RNA from the roots of both the olive trees locations. RNA was extracted as explained in Calvo-Polanco et al. (2016) using the phenol-chloroform protocol. For expression of the genes we used the primers described in Supplemental TableS1, and was determined using a RT-qPCR (iCycler-Bio-Rad, Hercules, CA). Each 23 μ l reaction mixture contained 1 μ l of cDNA (80 ng), 10.5 μ l of Master Mix (Bio-Rad Laboratories S.A.), 8.6 μ l of deionized water and 0.45 μ l of each primer pair at a final concentration of 0.2 mM. The PCR program consisted of 3 min

incubation at 95 °C, followed by 32cycles of: 30 s at 95 °C, 30 s of annealing temperature of 58 °C and 72 °C for 30 s. We tested several housekeeping genes (actin, tubulin and the elongation factor) and the elongation factor was chosen with the normfinder algorithm (Andersen et al. 2004) (<https://moma.dk/normfinder-software>) as the most stable one in all the treatments. Three different root RNA samples for each treatment were used for the analysis (n=3), with each of them repeated three times. Negative controls without cDNA were used in all the PCR reactions.

Statistical analyses

Physiological data were analyzed using analysis of variance (ANOVA) with the Proc MIXED procedure in SAS (version 9.2, SAS institute Inc., NC, USA) together with the post hoc Tukey's test to detect significant differences among treatment means.

Results

Olive trees growth and physiological parameters

Plant development followed different strategies according to the origin of the trees. Under well-watered conditions, Freila trees had higher leaf number than Grazalema trees, while they have similar values for the other physiological parameters studied, (Figure 1b, Figure 2, Table 1). Once drought was applied, Freila trees significantly reduced their relative leaf number and shoot heights (Figure 1a; Table 1), however, they increased their root dry weights when compared with well-watered conditions (Figure 1b). In the case of Grazalema trees, the drought treatment did not have an effect on root dry weight (Figure 1b), relative leaf number (Figure 1a), leaf dry weight and shoot to root ration (Table 1), but induced a reduction in leaf chlorophyll content and relative stem diameter (Table 1).

Both Freila and Grazalema trees reduced their g_s under drought (Figure 2a), although Grazalema trees increased their root hydraulic conductivity under drought when compared with well-watered conditions (Figure 2b).

Differential regulation of transcripts between both provenances under drought stress

Two cDNA libraries enriched in genes that are induced under drought stress were constructed in olive trees from both provenances, Freila and Grazalema. The aim of the study was to identify genes responsible for the differential root development and hydraulic conductivity phenotypes that we could find under drought stress. Olive trees from the dryer area in Freila showed seven differentially-expressed genes that could be classify into four functional groups (Table 2): 1) Posttranslational proteins: with two transcription factors (PosF21, and RAP2-12-like) and one prenyl protease (CaaX-Abi family); 2) One transport related protein belonging to the plasma membrane intrinsic proteins (MIP); 3) Two biosynthesis proteins and 4) One senescence proteins.

Roots from Grazalema trees were characterized by seven differentially-expressing genes that were classif~~ied~~^{iedy} into seven functional groups: transduction, biosynthesis, postranslational regulation, oxidation-reduction processes, energy, and mRNA processing, and one protein that was not characterized (Table 2). In order to test the efficacy of the test, we run RT-qPCR with some of the characterized genes in roots of Freila and Grazalema trees under drought (~~Supplemental Fig. S1~~^{Figure 3})

Discussion

Our study has shown different physiological responses to drought of the same genotype of olive trees from different locations, reflecting the plasticity of this species to adapt to the

environment and that growing in different areas modifies the plant response to drought. Similar results were found previously in populous trees because epigenomic processes (Raj et al. 2011). Drought induced an increase on root DW and a reduction on relative leaf number on Freila trees (dry area) that did not affect root hydraulic conductivity. On the other hand, in Grazalema trees (wet area), drought did not affect root dry weight and leaf relative number, but affected root hydraulic conductivity by increasing it. Trees growing under drought usually do not have a balanced allocation of the dry matter among the different plant organs (Hartmann et al., 2015), and specifically in olive trees growing in semi-arid conditions, it has been related with a reduction of the vegetative growth in favor of the root system development (Palese et al., 2010). This general trend can be easily assumed in the Freila trees, however the trees from Grazalema seems to be more balanced in the allocation of the resources as can be seen in their shoot to root ratio. It is plausible that the roots of Grazalema trees have different conformation, extension and density compared to the ones of Freila (Masmoudi-Charfiet al., 2011) that did not affect their dry matter, and this allowed Grazalema trees to be more effective accessing the water resources of the soil and to sustain a higher number of leaves at the shoot level.

The differences in root hydraulic conductivity did not represent differences in g_s under drought for both genotypes. Olive trees are well-recognized for their ability to control g_s at different water regimes (Torres-Ruiz et al., 2013), while maintaining a suitable hydraulic efficiency to yield water towards the photosynthetic apparatus (Raimondo et al., 2009). Furthermore, the suppression-subtractive hybridization (SSH) study pointed to aquaporins (MIP) to have a role in the water uptake in the roots of Freila trees. In olive trees, there are twelve aquaporins (Secchi et al., 2007; Lovisolo et al., 2007; Calvo-Polanco et al., 2016), with some of them identified to have water transport functionality (Secchi et al., 2007; Lovisolo et

al., 2007). Although aquaporins are known to have different functions and to be regulated differently as to the intensity and duration of the drought (Secchi et al., 2007; Calvo-Polanco et al., 2016), the distinctive presence of this predicted MIP in the roots of Freila trees will indicate, in principle, a higher dependency of Freila trees on the symplastic pathway and in particular of aquaporins activity for root water transport. However, we also need to take into account that, under long-term drought stress conditions, posttranslational regulation of aquaporins such as phosphorylation will be one of the main regulation processes taking place to determine the activity of aquaporins related with water transport (Calvo-Polanco et al., 2016). Thus, even though we do not have a distinctive aquaporin gene in Grazalema trees, they could have other mechanisms for a right functioning in the system in order to increase their root hydraulic conductivity. On the other hand, Tataranni et al. (2015) showed the close correlation among root hydraulic conductivity and the amount of suberin and root cell density in olive trees, giving a main role to the apoplastic path. Lo Gullo et al.(1998) found a differentiation at the endodermis level of the suberized tissue in response to water stress, what will alter how the water is transported to the main cylinder. Taking into account the deep transformation that take place in roots under drought, we expect a close relation among plant suberin development and aquaporins activity, and this will be a key element in the adaptation of plants to stress.

In order to get a deeper insight into the different genes regulating root growth and development under drought conditions, we further studied the responses of Freila and Grazalema roots to drought using a SSH test. Among the genes differentially expressed, it is remarkable the role of transcription factors, especially in Freila trees (Table 2), and how these TFs may be related with different hormonal signaling. TFs represent one of the most extended functional proteins classes in eukaryotes, with roles in plant development (van Leene et al.,

2016). We identified two potential TFs differentially expressed in the roots of Freila trees (bZIP59/PosF21 and RAP2.12) and one in Graza lema roots (AHPs). The TFs found in Freila roots are related to the pathway signaling of auxin, ethylene and ABA. Hence, the bZIP59/PosF21 found in Freila roots, from the basic region leucine zipper (bZIP) group I belongs to the family bZIP. These TFs are known for their role in plant development (Corrêa et al., 2008) as well as for being crucial in the response to abiotic stresses such as drought and for being ABA inducible (Llorca et al., 2014). In the specific case of bZIP59/Post21 it was found to be expressed in the vascular tissue (Dai et al., 2004) and root meristem (Pyo et al., 2006) interacting with several members of the bZIP group I subfamily (van Leene et al., 2016), and promoting a cascade of genes of auxin signaling pathway affecting root development (van Leene et al., 2016). Further, the related to apetala 2.12 (RAP2.12) gene is an ethylene responsive transcription factor VII (ERF-VII subfamily). It belongs to a group of TFs that participate in several hormone-signaling pathways such as the ethylene, jasmonic acid and salicylic acid pathways (Mantiri et al., 2008) and that have been shown to improve the tolerance of plants against drought (Li et al., 2017). In particular, RAP2.12 can be also involved in plant growth and development (Paul et al., 2016), and in the responses to oxidative and osmotic stresses (Papdi et al., 2015).

~~The interaction of stress signals as TFs and hormones is of great importance for the adaptation, tolerance and development of plants under stress (Montiel et al., 2004). In general, ABA is considered the main hormone controlling the responses of plants to drought, although the mechanisms responsible for such effects involve the interactions through other hormones (Rowe et al., 2016). Within this scheme, the reduction of root growth has ABA as the central stress hormone, and it is coordinated with ethylene and auxin signaling. Although direct ABA-ethylene crosstalk is not clear (Cheng et al., 2009), ethylene regulates root growth by affecting~~

~~auxin transport and biosynthesis and directly altering root growth (Thole et al., 2014). The links among the differential TFs in Freila roots and their effect on hormonal signaling may be one of the main responsible for the increase of root biomass in our experiments, making them key target genes that may improve tolerance of plants to drought.~~

The system seems to be working differently in Grazalema roots under drought, as the expressed related genes found in this genotype have general roles of plant transduction, biosynthesis and energy and mRNA processes. Among them, there is one related with gene transduction, a histidine-containing phosphotransfer protein 1-like(AHPs). AHPs belong to the htp superfamily and take part in hormone signal transduction as mediator between cytokinin sensor histidine kinases and response regulators (Ruszkowski et al., 2014). They are known to be strongly expressed in roots and to be induced by salt, cold and drought stress (Miyata et al., 1998). ~~Under drought, ABA reduces trans zeatin type cytokinin concentrations by modulating the expression of cytokinin biosynthesis metabolic enzymes (Nishiyama et al., 2011). Cytokinins affect many aspects of plant development as root elongation and lateral root formation (Laplaze et al., 2007), with AHPs acting as positive regulators of cytokinins signaling (Hutchison and Kieber, 2007).Cytokinins inhibit root growth by antagonizing auxins, to modulate the rate of cell division and differentiation in the root apical meristem (Su et al., 2011). For the roots from Grazalema trees, this seems to be the main mechanisms as for the plants to restrict the growth of the roots, as well as, for balancing the distribution of biomass along the shoot and the roots of the plants.~~

In conclusion, we have found two different mechanisms in response to drought stress that induce a cascade of interconnected network in order to adapt physiologically and morphologically the development of the same olive cultivar from two different provenances. The results of this research ~~is~~are of high interest as there is no information available of such

ecologically and commercial species in the Mediterranean area. Genetic manipulation of these multiple stress-responsive genes stands to be a powerful approach for improving plant tolerance, especially the ones that control the perception and transduction of stress signals.

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Table 1. Leaf relative water content (RWC, %), leaf chlorophyll (mg g⁻¹FW), relative height increments(%),relative stem diameters (%), leaf dry weights (g) and shoot to root ratio in olive trees from two different locations, Freila and Grazalema. The trees were grown under well-watered conditions and under drought conditions for five weeks. Different letters indicate significant differences among treatment means after Tukey's test at p=0.05.

	Treatment	Freila	Grazalema
Leaf RWC	Well-watered	91,1 ± 2,3 a	91,2 ± 2,3 a
	Drought	86,6 ± 1,0 a	86,7 ± 1,0 a
Leaf chlorophyll	Well-watered	14,9 ± 0,9 a	13,2 ± 1,1 a
	Drought	12,7 ± 1,7 ab	9,9 ± 1,0 b
Relative shoot height increments	Well-watered	13,4 ± 1,9 a	11,2 ± 3,3 ab
	Drought	3,4 ± 1,4 c	5,7 ± 1,9 bc
Relative stem diameter increments	Well-watered	7,8 ± 1,7 a	7,4 ± 1,8 a
	Drought	5,5 ± 1,6 ab	1,7 ± 1,8 b
Leaf dry weights	Well-watered	4,5 ± 0,5 a	4,5 ± 0,3a
	Drought	4,2 ± 0,2a	3,8 ± 0,3a
Shoot: root ratio	Well-watered	4,9 ± 0,4a	4,4 ± 0,2 ab
	Drought	3,9 ± 0,3b	5,5 ± 0,5a

499 Table 2. Functional classification of the selected candidate genes induced during drought treatment in roots of olive trees from Freila
500 and Grazalema.

Soil location	ID	Functional Group	Database	Sequence bp	Sequence #	Annotation
Freila	PosF21	Postranslational regulation	Genbank	310	XM_023003691.1	<i>Olea europaeavar.</i> sylvestristranscription factor PosF21 (LOC111380199)
	CAAX	Postranslational regulation	Genbank	136	XM_023042285.1	<i>Olea europaeavar.</i> sylvestris CAAX prenylprotease 1 homolog (LOC111411716)
	RAP2-12	Postranslational regulation	Genbank	444	XM_022988652.1	<i>Olea europaeavar.</i> sylvestris ethylene-responsive transcription factor RAP2-12-like (LOC111367665)
	RPC5	Biosynthesis	Genbank	296	XM_023014840.1	<i>Olea europaeavar.</i> sylvestris DNA-directed RNA polymerase III subunit RPC5-like (LOC111389862)
	SAM2	Biosynthesis	SRS	660	OLroot.13_2A12.F.ab1	S-adenosylmethionine synthase 2-like isoform 2 [<i>Fragaria vesca</i> subsp. <i>vesca</i>]
	MIP	Transport	SRS	442	OLroot.13_2D01.F.ab1	MIP plasma membrane intrinsic protein (<i>Olea europaea</i>)
	Senescence	Senescence	SRS	337	OLroot.19_2B05.F.ab1	Senescence associate protein
Grazalema	AHP1	Transduction	Genbank	279	XM_022993498.1	<i>Olea europaeavar.</i> sylvestris histidine-containing phosphotransfer protein 1-like (LOC111371478)
	ADI1	Biosynthesis	Genbank	474	XM_023019359.1	<i>Olea europaea</i> var. sylvestris 1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase 2 (LOC111393695)
	E2 38	Postraslational regulation	Genbank	314	XM_023006746.1	<i>Olea europaea</i> var. sylvestris putative ubiquitin-conjugating enzyme E2 38 (LOC111382707)
	NADH	Oxidation-Reduction	SRS	510	OLroot.27_2K06.F.ab1	NADH-ubiquinone oxidoreductase 11 kDa subunit [<i>Gossypium arboreum</i>]
	RBP45	m-RNA processing	SRS	478	OLroot.10_2O09.F.ab1	Polyadenylate-binding protein RBP45-like [<i>Sesamum indicum</i>]
	ATP5B	Energy	SRS	434	OLroot.24_2G22.F.ab1	ATP synthase subunit beta [<i>Medicago truncatula</i>]
	C16E8.02	Not characterized	SRS	258	OLroot.19_2M02.F.ab1	Uncharacterized endoplasmic reticulum membrane protein C16E8.02-like [<i>Nicotianatomentosiformis</i>]

501
502 Species or genus names from the column under 'ID' were assigned to sequence homology with sequences from Genbank or SRS as
503 indicated in the column 'Database'. The number of base pairs representing each gen described in the column 'Sequence bp' and the
504 assigned accession number of sequences submitted to each database is listed under 'Sequence #'. The identified name in the different
505 databases is listed under 'Annotation'.

Figure Captions

Figure 1. Relative leaf number (%) (a) and root dry weight (g) (b) in *Olea europaea* trees from different locations, Freila with low annual precipitation, and Grazalema with high annual precipitation. The trees were grown either under well watered conditions or under drought stress for five weeks. Different letters above the bars indicate significant differences among treatment means after Tukey's test at $p=0.05$.

Figure 2. Stomatal conductance (g_s) (a) and root hydraulic conductivity (L_{pr}) (b) in *Olea europaea* trees from different locations, Freila with low annual precipitation, and Grazalema with high annual precipitation. The trees were grown either under well-watered conditions or under drought stress for five weeks. Different letters above the bars indicate significant differences among treatment means after Tukey's test at $p=0.05$.

Figure 3. Relative gene expression in roots of olive trees from different locations, Freila with low annual pluviometry, and Grazalema, with high annual pluviometry. The data were analyzed using t-test. Asterisks over the bars indicate significant differences at $\alpha=0.05$