Drought Resistance in Oat Involves ABA-mediated Modulation of Transpiration and Root Hydraulic Conductivity

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ABSTRACT

Drought is one of the most important constraints to crop productivity worldwide. Control of plant responses to drought is very complex. The mechanisms and their intensity may differ between species and/or genotypes ultimately conditioning tolerance or susceptibility. We explore here the strategy set up by two oat cultivars to cope with drought based on root morphological, anatomical, physiological and molecular studies. A dramatic and rapid abscisic acid increase in the susceptible genotype resulted in a tight and rapid reduction of stomatal conductance. Despite of this, leaf water potential decreased concomitantly due to a decrease in root hydraulic conductivity. By contrast, the resistant genotype, showed a mild and slow increase in abscisic acid that allowed maintaining transpiration longer. This response was linked to an increase in root hydraulic conductance through an increase in total root length and in the length of the thinnest roots as well as a rise in root conductivity. This was also coupled with anatomical changes leading to a reduction of metabolic cost. These changes allowed the resistant genotype to maintain higher water potential reducing drought symptoms and promoting growth under water deficit conditions.

Key words: abscisic acid; drought; hydraulic conductance; oat; root morphology; transpiration.

INTRODUCTION

Drought is an important constraint to agriculture that has significant impacts in both developed and developing countries. Expectedly, there will be an increase of acute drought events under the climate change scenario threatening food production (Lobell and Gourdji, 2012). Therefore, the development of cultivars with better drought adaptation is nowadays a priority in many crop-breeding programs. However, the solution is not simple. Drought tolerance or resistance - as these terms are now commonly used interchangeably (Passioura, 2012) - has several meanings. It ranges from the ability to survive a severe water deficit to, as we consider, the ability to use more efficiently a limited water supply to maintain leaf water status, photosynthesis and yield. Limited water supply may refer to unusual low rainfall during the crop growing season leading to gradual depletion of water from the soil or particular episodes of dryness that may alternate with episodes of rain. Therefore, a particular genotype should not be labelled as tolerant or susceptible in absolute terms, but in relation to the drought stress considered and within a particular agronomic context, since there are no universal genotypes or traits that cover all these possibilities.

Oat (*Avena sativa* L.) is an important cereal crop cultivated for grain, feed, fodder and straw over approximately 10 million hectares worldwide (FAO, 2017). During the last 20 years, there has been a steady increase in the oat cultivated area within the Mediterranean rim (Rispail et al., 2018; Sánchez-Martín et al., 2017). Oat transpiration rates and, hence, water requirements, are usually higher than those of other small grain cereals (Ehlers, 1989). Therefore, one of the challenges faced by this crop in the Mediterranean area is its poor adaptation to drought. Oats are especially susceptible to grain abortion caused by drought, which shows as empty spikelets (Sánchez-Martín et al., 2017). In the Mediterranean agroecosystem, most of the cultivated oats are grown under rainfed conditions. Therefore, the common scenario is the gradual water depletion over time, associated with the increased water demand by the growing crop and higher rates of evapotranspiration as season progresses.

Yield *per se* has very low heritability in drought-prone environments. Thus, yield-based selection has been unsuccessful in the past, except in multi-site and multi-season field trials of highly advanced breeding lines (Rebetzke et al., 2012). Therefore, yield is not a direct target in breeding programs. By contrast, both above- and belowground morphological, physiological and biochemical targets, directly related with growth and yield, have shown to be more heritable and useful. Detailed phenotype information is doubly useful, since it allows a deeper understanding of the functional significance of genes and the development of selection tools for breeding. Recently, the term “phene” has been coined to define the elemental unit of a phenotype (Lynch and Brown, 2012), with the analogy “phene is to phenotype as gene is to genotype” and is replacing the more ambiguously used term ‘trait’ (Violle et al., 2007). It is important to discriminate those phenes that contribute to resistance strategies from those that are reflecting stress

damages observed in susceptible genotypes (Sánchez-Martín et al., 2015, 2017). This trait or phene-based selection or ideotype breeding is generally a more efficient selection strategy, allowing the identification of useful sources of variation (Araus et al., 2002; Lynch, 2011; York et al., 2013). Plant adaptation to water deficit involve several morphological, physiological and molecular changes through which plants increase their ability to avoid damage (avoidance mechanisms) and/or to maintain its metabolic functions under water limiting conditions (tolerance mechanisms). Upon recognition of water stress conditions, many plants react conservatively rapidly reducing transpiration and thus "saving water". However, this promotes oxidative stress and decreases carbon fixation and plant growth. As an alternative, other plant species follow a "water spending" strategy, showing lower sensitivity to evaporative demand and soil moisture through higher stomata control. These plants shows greater fluctuations in leaf potential while they maintain photosynthesis and avoid oxidative stress. However, this strategy may exhibit a higher risk of xylem embolism.

Previous genotype x environment interaction studies (GxE) based on multi-site field trials of a large oat panel revealed two cultivars with similar flowering time but different adaptation to Mediterranean environments (Sánchez-Martín et al., 2014). Cultivar Patones showed better adaptation presenting higher yield, biomass and resistance to rust than cv. Flega in both the average and the driest environments assessed (Rispail et al., 2018; Sánchez-Martín et al., 2014). These genotypes have been extensively studied under controlled conditions to dissect the particular responses that lead to the observed drought resistance to gradual water depletion (Canales et al., 2019a; Canales et al., 2019b; Sánchez-Martín et al., 2018; Sánchez-Martín et al., 2015; Sánchez-Martín et al., 2012). These studies focused on the plant shoot responses. They revealed an early decrease in leaf turgor in the susceptible genotype followed by an early and tight stomatal closure and the insufficient induction of antioxidant pathways. This leaded to an excessive ROS accumulation that damaged the photosynthetic apparatus and decreased cell membrane stability (Sánchez-Martín et al., 2015). In turn, these studies suggested that the resistant cultivar had a less conservative water use and maintained moderate transpiration for longer. This genotype should engage additional responses/mechanisms allowing the success of its strategy and a better performance under drought conditions. By contrast, the "saving water" strategy of susceptible Flega might have deleterious side effects, even though it is often considered as an efficient drought resistance response (Hepworth et al., 2015; Kholova et al., 2010; Li et al., 2017).

In this work, we explored further the conservative "saving water" strategy in cultivar Flega and a "water spending" strategy in resistant cultivar Patones. To this aim we dissected their biochemical, physiological, morphological and anatomical responses over an imposed water deficit time course under controlled conditions. This revealed the contribution of specific phenes the resistance response that could be useful to improve the oat crop performance under drought conditions.

MATERIALS AND METHODS

1. Plant material, growth condition and sampling

Experiments were carried out with the oat (*Avena sativa*) cultivars (cvs) 'Flega' and 'Patones', which are susceptible and resistant to drought stress, respectively, under controlled and field conditions (Sánchez-Martín et al., 2018; Sánchez-Martín et al., 2015; Sánchez-Martín et al., 2012). Patones exhibits a good adaptation to Mediterranean agro-climatic conditions (Sánchez-Martín et al., 2014). It was developed by 'Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario' (IMIDRA, Madrid, Spain), and 'Plant Genetic Resources Center' (INIA, Madrid, Spain) provided the seeds. Flega was developed by the Cereal Institute (Thermi-Thessaloniki, Greece). These genotypes are not closely genetically related according to previous studies (Montilla-Bascón et al., 2013).

Plants were grown under controlled conditions according to Sánchez-Martín et al., (2018, 2015, 2012) in 0.75 L pots (one plant per pot) filled with peat : sand (2:1), in a growth chamber at 20°C, 65% relative humidity and under 12 h dark/12 h light with 250 μmol m^{-2} s^{-1} photon flux density supplied by white fluorescent tubes (OSRAM, Garching, Germany). During growth, trays carrying the pots were watered regularly with tap water. After three weeks, water was withheld from drought-treated plants (Sánchez-Martín et al., 2018; Sánchez-Martín et al., 2015; Sánchez-Martín et al., 2012) producing a gradual soil water depletion until plants were around 38 days old. Control plants were watered regularly throughout the experiment. During the drought treatment, the relative soil water content (sRWC) was monitored gravimetrically daily, reaching a level of approximately 15–20% by the end of the experiment (18 days withholding water) which is consistent with previous drought-related studies on oat (Gong et al., 2010). This ensured that during the whole drought time course Flega and Patones plants were subjected to similar sRWC and hence to similar stress doses as previously observed (Sánchez-Martín et al., 2018; Sánchez-Martín et al., 2015; Sánchez-Martín et al., 2012).

Sampling times were chosen to cover different levels of sRWC: still-sufficient water (approximately 6 days after water withholding (daww), 55-60% sRWC), mild water deficit (9 daww, 40-45% sRWC), moderate water deficit (12 daww, 30-35% sRWC), high water deficit (15 daww, 20-25 % sRWC) and severe water deficit (18 daww; 15-20% sRWC). At each sampling date, leaves and roots of five oat plants per cultivar and treatment (well-watered and droughted) from each independent experiment were harvested, washed out under tap water to remove soil residues and stored appropriately or immediately used according to the different experiments (see below). At the latest time-point plants were 38 days-old and droughted plants had not reached the wilting point.

2. Visual assessment of drought symptoms

To confirm the response of the two cultivars under drought stress, drought symptoms were assessed in five replicates per genotype, treatment and sampling time and in two independent experiments according to previous work (Sánchez-Martín et al., 2018; Sánchez-Martín et al., 2015; Sánchez-Martín et al., 2012). Briefly, drought severity values were assessed daily according to a 0-5 scale where 0 = vigorous plant, with no leaves showing drought symptoms; 1 = one or two leaves show mild drought symptoms (less turgor) but most leaves remain erect; 2 = most leaves show mild levels of drought stress, however one or two leaves still show no drought symptoms; 3 = all leaves show mild drought symptoms; 4 = all leaves show severe drought symptoms including incipient wilting; 5 = the whole plant is wilted with all leaves starting to dry appearing rolled and/or shrunken (for pictures see (Sánchez-Martín et al., 2012).

3. Physiological Measurements

3.1. Transpiration

Transpiration was calculated per time and leaf area in five replicates per genotype, treatment and sampling time gravimetrically. To fulfill this objective, both ends of pots were covered with polythene bags fixed to the pot with adhesive tape. A small slit was made in the top of the bag to allow the plant to pass through it. Control pots without plants showed minimum water loss. The initial and final (after 8 hours in the central time of photoperiod) pot weight was taken and transpired water was calculated by subtracting the final from the initial pot weight using a three decimal precision balance (Kern PLJ model PLS 420-3F, Germany). Plant leaf area was calculated by analyzing the scanned leaves (Epson Perfection V370 Photo scanner) with ImageJ software (Schneider et al., 2012) to normalize transpiration.
Plant transpiration were calculated by the formula:

$$\left(\frac{\text{Final pot weight} - \text{Initial pot weight}}{Time * Leaf\ area}\right) * 10E9$$

3.2. Stomatal conductance

Stomatal conductance was measured in ten plants per cultivar, treatment and sampling time with an AP4 cycling porometer (Delta-T Devices Ltd, Cambridge, UK). The porometer allows rapid measurement of a high number of samples with a relatively large leaf area (17.5 x 2.5 mm) in a non-destructive manner. It was used on the mid of the adaxial surface of leaf laminae. Measurements were carried out in the second leaves at each sampling time and were taken at midday.

3.3. Leaf water potential

Leaf water potential (Ψ_{leaf}) was measured at midday and one hour before the light period (pre-dawn) on the second leaf of four replicate plants per cultivar, treatment and sampling time using a pressure chamber (Model 3000F01 Plant Water Status Console; Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Leaves were detached with a razor blade from the plants and the cut surface was cleaned with deionized water and filter paper to remove cellular debris. Then the leaf was introduced in the chamber with the cut end exposed at atmospheric pressure. Excess pressure was applied slowly and carefully by forcing compressed nitrogen into the pressure chamber until xylem sap bubbles could be seen with a magnifying glass on the cut surface. The pressure at which this occurred was recorded.

3.4. Root hydraulic conductance

Root hydraulic conductance was measured, according to Garcia-Tejera et al. (2016), with some modifications, in four replicate plants per cultivar, treatment and sampling time using a pressure chamber (Model 3000F01 Plant Water Status Console; Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Plants were extracted from their pots and immersed in water to remove part of the substrate, taking extreme care not to disturb the root system. Then the upper part of the plant was cut 5 cm above the plant collar; the remaining shoot stub was covered with paraffin from the collar to 1 cm below the cut in order to avoid any radial water flux into it. The detached root system was then fixed to the specimen holder of the pressure chamber using an appropriate rubber seal, in such a way that after the chamber closure, the root system was completely immersed in water. All the measurements were performed inside the growth chamber at 20°C (±0.5 °C) to avoid any thermal shock to the root system.

Three different pressures were applied: 0.05, 0.1 and 0.15 MPa. The xylem sap flowing through the root system at each pressure was collected during periods of at least 10 min using cotton-filled sample tubes previously weighted in a three decimal precision balance (Kern PLJ model PLS 420-3F, Germany). The weight of the sample tube plus the xylem sap after 10 min was obtained with the same precision balance. The flux was then calculated by dividing the difference of dry and wet weight of the tube by the time interval. The process was repeated several times until the flux difference between measurements was <0.5‰, then, the flux was considered to be constant and the measurement was recorded. Root hydraulic conductivity (conductance per root unit) was calculated following morphological root trait assessment (see below).

3.5. Dry mass

Root and shoot dry weight were measured in five plants per cultivar, treatment and sampling time. Roots were thoroughly washed out to remove plant substrate. Roots and shoots were then dried

in an oven at 70ºC during four days. Dried root and shoot biomass were weighted in a three decimal precision balance (Kern PLJ model PLS 420-3F, Germany).

4. Abscisic acid quantification

Before ABA extraction, tissue previously frozen in liquid nitrogen and stored at -80ºC were lyophilized. ABA was extracted from leaves and roots of three samples per genotype, treatment and sampling time. Each sample consisted of a pool of two leaves or two complete root systems from independent plants. Samples were quantified as previously described (De Ollas et al., 2013) with slight modifications. Briefly, 0.2 g of dry plant material was extracted in 2 mL of distilled H_2O after spiking with 25 µL of a 2 mg L^{-1} solution of d6-ABA as internal standard. After centrifugation (10.000 × g at 4°C), supernatants were recovered and pH adjusted to 3.0 with 30% acetic acid. The acidified water extract was partitioned twice against 3 mL of diethyl ether. The organic layer was recovered and evaporated under vacuum in a centrifuge concentrator (Speed Vac, Jouan, Saint Herblain Cedex, France). The dry residue was then resuspended in a 9:1 H_2O: MeOH solution by sonication. The resulting solution was filtered and directly injected into a UPLC system (Waters Acquity SDS, Waters Corp., Milford, MA) interfaced to a TQD triple quadrupole (Micromass Ltd., Manchester, UK) mass spectrometer through an orthogonal Z-spray electrospray ion source. Separations were carried out on a Gravity C18 column (50 × 2.1 mm, 1.8-µm, Macherey-Nagel GmbH, Germany) using a linear gradient of MeOH and H_2O supplemented with 0.1% acetic acid at a flow rate of 300 µL min^{-1}. Transitions for ABA/d6-ABA (263>153/269>159) were monitored in negative ionization mode. Quantitation was achieved by external calibration with known amounts of pure standard using Masslynx v4.1 software.

5. Morphological root trait assessment

Roots were thoroughly washed out and stained with an abundant volume of 0.01% neutral red (Sigma Chemical Co.) for 24 hours to increase contrast for further analysis (Schumacher et al., 1983). The stained roots were placed in a transparent tray with a thin layer of water and scanned using a commercial scanner (Epson Perfection V370 Photo) at a resolution of 600 pixels per mm. Root images were analyzed using WinRHIZO (Regent Instruments Inc., Québec City, QC, Canada) as described by Himmelbauer et al. (2004) and total root length, surface, volume, average diameter, number of forks and root length per diameter class were recorded.

6. Root anatomy

To investigate root anatomical features, root samples stored in 70% (v/v) ethanol were dissected following the procedure described by Pregitzer et al.(2002) in order compare roots from same order. The most distal root tips were labeled as the first order, two first order roots joined to form

a second order root, and two second order roots joined to form a third order root, and so on. At the different sampling time only first, second and third order roots had been developed.

Samples from the three root orders of four plants per cultivar, treatment and sampling time were fixed in FAA (50 % ethanol + 5 % formaldehyde + 10 % glacial acetic acid in water) for 48 h and embedded in synthetic resin (Historesin; Leica Microsystems GmbH). Cross sections (2 μm thickness) were obtained using a rotary microtome (RM 2245; Leica Microsystems) with carbon-tungsten blades (TC-65; Leica Microsystems). Sections were placed on slides, stained with 0.1% toluidine blue-o (TBO) solution in citrate buffer (pH 5) and sealed with mounting medium (Entellan; Merck). Samples were observed using an optical microscope (Eclipse 50i; Nikon Instruments Inc.), and images were acquired with a digital camera (DS-Fi1; Nikon Instruments Inc.) connected to a computer through the control unit DS-U2 (Nikon Instruments Inc.). Cortex, stele and total xylem areas were measured using NIS Elements v4.5 software for Windows (Nikon Corporation). In addition, number of cortex rows and xylem vessels were also recorded. Following preliminary observations, we found that anatomical root structures had not been completely developed in first and second order roots, so only third order roots were assessed.

7. Statistical analysis

All experiments followed completely randomized designs. The number of replications ranged between 3 and 10 as specified for each recorded data. Four main independent experiments (plus additional preliminary experiments to set up experimental conditions) were carried out. The first was conducted to record stomatal conductance, the second to record visual symptoms, transpiration, leaf area and fresh and dry weight, the third to measure visual symptoms and hormones, and the last one to record leaf water potential, hydraulic conductivity, root morphology and anatomy. For stomatal conductance and visual symptoms, measurements were taken on the same plants all along the drought time course. For the rest of experiments only the second leaves and/or the complete root system was measured/harvested at each time point and the rest of the plant was discarded.

For statistical analysis, data recorded as percentages were transformed to arcsine square roots (transformed value = 180/π x arcsine [√(%/100)]) to normalize data and stabilize variances throughout the data range. However, for ease of understanding means of raw percentage data are presented in figures. Data were subjected to three-factor analysis of variance (ANOVA) with genotype, treatment and time as factors using SPSS software and residual plots were inspected to confirm normality of the distribution. In addition, significance of differences between means at each time point was determined by contrast analysis (Scheffe's).

RESULTS

1. Quick and tight stomatal closure associated with fast increases of abscisic acid rapidly reduced total transpiration in the susceptible oat genotype

In the present work, Flega and Patones behaved as susceptible and resistant to drought respectively. Accordingly, Flega showed earlier and stronger drought symptoms in terms of loss of turgor and early senescence (Supplemental Fig 1). As showed in Figure 1, the susceptible cv Flega closed its stomata earlier than the resistant Patones under drought. These results confirmed those previously obtained in similar experiments (Canales et al., 2019b; Sánchez-Martín et al., 2018; Sánchez-Martín et al., 2015; Sánchez-Martín et al., 2012). Under well-watered conditions, both genotypes showed similar behavior. However, we observed that Flega started earlier to significantly reduce stomatal conductance when subjected to gradual water depletion. Overall, significant differences between genotypes were detected under water deficit conditions (P<0.001). Stomatal conductance of droughted Flega plants was significantly lower than its well-watered control from approximately 60% of sRWC (6 daww) onwards, whereas in the resistant Patones it decreased significantly from 40% sRWC (10 daww). Measurements of total plant transpiration recorded in a different experiment, confirmed these data (Fig. 2). Accordingly, Flega showed faster and tighter reduction of transpiration from approximately 60% sRWC, whereas it started at 40% sRWC in Patones. At 15-20% sRWC (18 daww) transpiration was negligible in both genotypes (Fig 2) that had similar stomatal conductance during light and dark periods (Fig 1, Sánchez-Martín et al., 2012). This indicated that plants were already suffering severe water deficit at this time despite they had still not reached the wilting point that occurred in the susceptible genotype more than one week later. A detailed study of hourly transpiration at 45% of sRWC showed that transpiration level of droughted plants were lower than those of well-watered plants reaching a steady state 4-5 hours after the onset of the light photoperiod. At this sRWC, a significant difference between Flega and Patones was detected with Flega transpiring less than Patones during the complete steady state period (Supplemental Fig 2).

Further analysis showed that the early stomatal closure in Flega was associated with a strong and early accumulation of ABA in root and leaves (Fig 3 a, b). Overall, ABA production in both genotypes followed a similar trend although the amount of ABA produced differed. In roots, ABA increased slightly earlier than in leaves and maintained a steady increase proportional to water depletion in the soil (Fig 3b). In leaves, ABA concentration dramatically increased at an early stage reaching a plateau, from 30-35% or 20-25% sRWC in Flega and Patones respectively, which is maintained throughout the remaining drought time course (Fig 3a). The genotypic differences in ABA concentration under drought were highly significant (P<0.001) even at the earliest sampling time (6 daww) in roots. Levels of ABA were almost 2-fold higher in the susceptible Flega compared with the resistant Patones in all plant organs. In addition, ABA concentration was correlated with the level of transpiration (Fig 3c) with levels of around 35-40 ng ABA mg^{-1} DW associated with a reduction of more than 80% of the maximum transpiration. Similar level of transpiration was achieved in both genotypes at similar level of ABA (Fig 3c).

However, similar levels of sRWC induced higher production of ABA in Flega than in Patones (Supplemental Fig. 3). Since transpiration was almost completely inhibited at the lowest level of sRWC in both genotypes and ABA concentration in Flega almost doubled that of Patones at that time, a question remains about the possible role of the additional ABA production in Flega.

ABA levels have been reported to refine root development and modify leaf elongation and expansion during water deficit (Farooq et al., 2013; Reddy et al., 2014). Thus, root/shoot dry mass ratio was measured (Fig 4). Overall, well-watered Flega showed a significant higher root/shoot dry weight ratios than well-watered Patones. By contrast, under drought conditions, root/shoot dry weight ratio was significantly higher in Patones overall.

2. Reduction of transpiration in the susceptible genotype do not contribute to improve water status

It might be expected that the reduction of transpiration observed in Flega, could "save water" in this genotype improving its water status. To assess the plant water status, leaf water potential was evaluated during the steady state at midday and pre-dawn. The steady state was characterized in a preliminary experiment taking hourly measurements of leaf water potential in both genotypes (Supplemental Fig. 4). Pre-dawn leaf water potential is representative of soil water status. Accordingly, it was less negative than midday leaf water potential (Fig. 5). Midday and pre-dawn leaf water potential were similar in the two genotypes under well-watered conditions (Fig. 5). As sRWC reduced, leaf water potential progressively decreased, although it was maintained consistently higher during pre-dawn period compared with midday measurements. The later was true for all measurements except for the most limiting sRWC in Flega. At that stage, midday and pre-dawn leaf water potential reached similar high negative values, supporting the severe drought symptoms observed in this genotype at that time. Despite its reduced transpiration, Flega showed more negative leaf water potential than Patones throughout the assessed sRWC levels. The statistical analysis showed a highly significant interaction between genotype and treatment ($P<0.001$) indicating that although the two genotypes responded with a similar pattern, they showed very different kinetic with Flega reducing earlier and faster its water potential (Fig. 5).

3. Improved water status in the resistant genotype correlated with an increase of hydraulic conductance and conductivity coupled with growth of fine roots.

As shown above, Patones maintained a better water status at limiting sRWC than Flega, despite its higher transpiration rate. An explanation for this might be a higher root conductance (conductance of the whole root system) in the resistant genotype that, by improving water transport could maintain the leaf water content together with moderate transpiration levels. To test this hypothesis, we studied root conductance through steady state experiments in which we

measured the volume exuded through the neck of the plants at increasing hydrostatic (pneumatic) pressure gradient. In a preliminary experiment, we established the pressures at which the water volume increased linearly over time (Supplemental Fig. 5).

Data showed similar total root conductance and conductivity (conductance per root unit) for Flega and Patones cultivars under well-watered conditions. Interestingly the total conductance of control plants slightly increased over the experiment whereas hydraulic conductivity slightly decreased. The former might reflect the increasing root volume whereas the later might reflect the higher proportion of older roots with lower conductivity. Under limited water access, the two genotypes showed a different response. The resistant Patones showed a significant and higher root conductance (P<0.001; Fig. 6a) and conductivity (P<0.001; Fig. 6b) than the susceptible Flega. Flega hydraulic conductance and conductivity decreased concomitantly with gradual water depletion. By contrast, Patones showed an initial increase of conductance and conductivity that coincided with an increase in root density, arising from the intense formation of fine roots with diameters smaller than 0.5 mm (Fig 7). Accordingly, the average root diameters of the resistant genotype were significantly smaller than those of the susceptible Flega (Fig 7). At root level, Flega was more vigorous than Patones under well-watered conditions. However water stress completely arrested root growth in this genotype whereas it was maintained in Patones, which could be an important adaptive response to water deprivation.

These data suggested that an increase of root conductance in the resistant genotype might contribute to the better water status observed allowing the maintenance of moderate transpiration levels. This is supported by the highly significant correlation between root conductance and transpiration (r=0.60; P<0.001; Table 1). Root conductance was also significantly correlated with root length and leaf area (Table 1), indicating, as expected, the importance of leaf turgor in leaf expansion.

4. Early root anatomical changes in the resistant genotype favored root growth and hydraulic conductivity

The higher hydraulic conductivity observed in the resistant genotype compared with Flega might be explained by the higher proportion of fine roots detected in Patones. However, it has been reported that specific root anatomical changes might also contribute to increase the hydraulic conductance per root unit favoring water transport from soil to xylem vessels. In order to explore this possibility, we compared several root anatomical parameters between susceptible and resistant genotypes (Supplemental Fig. 6). Overall, water deficit reduced root diameter (Fig. 8) confirming previous results. Gradual soil water depletion also increased lignification of stele cells and vessels as suggested by the stronger blue turquoise staining observed in this area. However, the experimental setup hampered the detection of differences in lignification between genotypes (Fig 8). Interestingly, Patones showed early changes in several anatomical traits, including a

significant reduction of the number of cortical cell layers, of the stele area and of the metaxylem area as compared with susceptible Flega (P<0.001 for all mentioned traits; Fig 9). Gradual water depletion also reduced the cortex area in both cultivars. The reduction initiated earlier in the resistant Patones. At the latest sampling times, reduction of the cortex area was similar in both genotypes. Drought treatment also induced the formation of aerenchyma areas (P=0.03) decreasing the living cortex area. However, no significant differences were observed between genotypes in the extent of aerenchyma formation. No interaction was observed between genotypes and treatments for several of the assessed parameters, indicating the induction of a general drought response common to both genotypes. However, this drought response was generally stronger and faster in resistant Patones as shown by the stronger reduction detected for most anatomical traits (i.e. cortical cell number, stele area, and xylem area; Fig 9).

A detailed correlation between morphological and anatomical traits showed that under drought, total root conductance and root conductivity were negatively correlated with the number of cortical cell layers (Fig 10). Root conductance was positively correlated with root hydraulic conductivity and with morphological and anatomical phenes. Under well-watered conditions, root length and finest root length were positively correlated with morphological and anatomical phenes. However, they were negatively correlated with these phenes under drought conditions. Average root diameter was significantly correlated with most of the anatomical traits under both well-watered and drought conditions, and most anatomical phenes were correlated among them (Fig 10).

DISCUSSION

Plants develop a variety of mechanisms to successfully adapt to harsh environments. The success of a particular strategy relies on the intensity and/or seasonal distribution of the stress and should be considered within the agronomic context (Passioura, 2012). Under particular drought conditions reduction of stomatal conductance/transpiration is a mean to save water and resist drought (Hepworth et al., 2015; Kholova et al., 2010; Li et al., 2017). However, stomatal closure is generally a negative response from an agronomic point of view, whose interest is to maximize CO_2 fixation under drought stress and not so much survival under severe drought (Blum, 2009, 2015; Galmes et al., 2007; Galmes et al., 2013). Thus, higher yielding wheat, rice or cotton genotypes under drought stress had greater stomatal conductance (Araus et al., 2002; Blum et al., 1982; Izanloo et al., 2008). Transpiration maintenance *per se* is not enough to preserve photosynthesis under drought, as it must be coupled with a variety of responses/mechanisms to maintain the water status necessary for cell functioning and photosynthesis. Identification of these particular responses and phenes that contribute to a successful drought adaptation would be valuable to improve drought resistant crops. In this work, we focused on two previously characterized oat genotypes with markedly different behavior as shown in field G x E studies (Sánchez-Martín et al., 2017; Sánchez-Martín et al., 2014). Patones is a genotype well-adapted to dry Mediterranean environments while Flega is susceptible as

shown by its lower yield under field drought or severe drought symptoms in seedlings under drought controlled conditions (Canales et al., 2019b; Sánchez-Martín et al., 2018; Sánchez-Martín et al., 2015; Sánchez-Martín et al., 2012). In this study, we explored the strategy followed by the better drought-adapted genotype to maintain transpiration and leaf water status. We performed these studies under controlled conditions as previously (Canales et al., 2019a; Canales et al., 2019b; Sánchez-Martín et al., 2018; Sánchez-Martín et al., 2015; Sánchez-Martín et al., 2012). Yield and its components are best phenotyped in field trials. However the measurement of plant secondary traits under controlled conditions allows accurate control of the main environmental parameters, such as moisture stress, air humidity, temperature, or light, necessary for exhaustive phene dissection, which is more difficult under field conditions (Tuberosa, 2012). This exhaustive phene dissection may offer focused targets for further validation under field conditions.

Maintenance of leaf turgor is an important adaptive mechanism that plays a key role in stomata regulation and photosynthetic activities under water stress conditions (Lipiec et al., 2013). A variety of determinants may drive stomatal closure, ABA production being one of the most important, particularly under drought. ABA accumulation is a universal response observed in plants subjected to drought and other abiotic stresses (Quarrie, 1991; Setter, 2006). Therefore, ABA has been widely considered as a fundamental component of the mechanisms allowing the plant to match water demand with water supply and to optimize growth and survival under water deficit conditions (Borel et al., 2001; Xiong et al., 2007; Zhang and Davies, 1990). Additionally, ABA modulates the expression of a large number of genes whose products protect the cell from the harmful effects of dehydration (Bray, 2002; Seki et al., 2007). ABA has been shown for instance to induce proline accumulation (Pál et al., 2018; Stewart, 1980). In agreement with this, our results showed that accumulation of ABA in roots and leaves was among the earliest responses observed during gradual water depletion in both genotypes (Fig 3). However, the comparison of the response of susceptible and resistant accessions during gradual water depletion revealed a novel differential ABA modulation between genotypes. Strikingly, a higher and faster increase in ABA concentration was detected in the susceptible Flega. At the lowest sRWC, ABA in Flega doubled Patones ABA content, despite transpiration was almost completely inhibited in both genotypes. This larger ABA accumulation in the susceptible cultivar might play additional roles. On one hand, similar to the ABA accumulation, an increase in proline was observed in this genotype (Sánchez-Martín et al, 2015). On the other hand, it might be linked to non-desirable collateral effects considering the susceptible phenotype of Flega. In cereals, ABA accumulation has been highlighted as one of the factors influencing reproductive fertility (Boyer and Westgate, 2004; Landi et al., 2001; Saini and Westgate, 2000; Setter and Flannigan, 2001; Tang et al., 2008; Yang et al., 2007; Zhang et al., 2009) and endosperm development (Mambelli and Setter, 1998; Ober et al., 1991; Seiler et al., 2011; Setter et al., 1996; Tuberosa et al., 1992). The higher ABA concentration in Flega might, thus, be related with an attempt to preserve fertility under stress. The potential effect of ABA on additional responses is still under debate since

different effects were observed depending on the ABA concentration and stress situation. For instance, ABA content have been positively correlated with root hydraulic conductivity (Glinka, 1977; Morillon and Chrispeels, 2001; Thompson et al., 2007). However, Markhart et al., (1979) observed the opposite trend with a marked reduction of the root system conductivity at high ABA concentrations. Concentrations applied by Glinka (1977) were about 10-fold lower than those applied by Markhart et al. (1979), suggesting that ABA action might depends on its concentration range (Beaudette et al., 2007). Markhart et al. (1979), demonstrated that root hydraulic conductivity was determined by the root membrane lipid characteristics, and that ABA interacts with or alters the membrane that is rate-limiting to water flow. Our previous results showed that Flega and Patones membrane lipid content was differentially affected during gradual soil water depletion (Sánchez-Martín et al., 2018) supporting the role of membrane lipids on conductivity (Scoffoni et al., 2017). Markhart et al. (1979) and others (i.e. Guschina et al., 2002) proposed that ABA could stabilize membranes of damaged cells by reducing the mobility of the hydrocarbon chain which would affect the conductivity. Thus, early and moderate ABA accumulation would be responsible of stomatal regulation and/or hydraulic conductivity increase. By contrast, the dramatic ABA accumulation observed in Flega, which correlated with an early decrease in cell membrane stability, might be a consequence of the damage, rather than an indication of tolerance (Ashraf and Foolad, 2007; Sánchez-Martín et al., 2015). ABA effect on hydraulic conductivity could also be exerted through, for instance, changes in aquaporin expression, which are altered by abiotic factors, such as drought (Parent et al., 2009; Sanchez-Romera et al., 2018). The importance of aquaporins in hydraulic conductivity has been previously demonstrated (Maurel et al., 2008). Different studies suggested that high abundance of aquaporins increases hydraulic conductivity whereas low abundance reduces water permeability of biological membranes (Martre et al., 2002). In addition aquaporin activity could be regulated by their phosphorylated/dephosphorylated state (Aroca et al., 2005). However, the effect of ABA on hydraulic conductivity have not been clearly demonstrated with studies showing no or only transient effect (Hose et al., 2000; Aroca et al., 2003; Wan and Zwiazek, 2001), which might be explained by differences in ABA concentrations.

The Flega reduction of stomatal conductance could be a way of saving water. However, it did not improve its water status due to the sharp decrease in its root hydraulic conductance. By contrast, the resistant genotype increased root hydraulic conductance maintaining longer an adequate leaf water status. The increase in hydraulic conductance was correlated with transpiration (R=0.60). Similar or higher correlation coefficient were also reported in Arabidopsis, grapevine, eucalyptus or sugarcane ((Franks, 2006; Meinzer and Grantz, 1990; Vandeleur et al., 2009; Maurel et al., 2010). These reports point to a strong link between root conductance and integrated carbon fixation in shoots and suggest that optimized water transport could facilitate plant growth at low transpiration (Maurel et al., 2010). The significant correlation that we observed between root conductance, leaf area and root length also support this hypothesis. Indeed, it has been shown that reduction in leaf water potential slows down leaf expansion, and limits radiation capture

(Ehlert et al., 2009). Thus, the higher root conductance observed in Patones might facilitate water transport to leaves even at low transpiration allowing maintenance of photosynthesis and growth.

Different root morphological and developmental characteristics favored the resistant genotype to cope with water deficit. The observed increase in hydraulic conductance in Patones was associated to an increase of the total root density and hydraulic conductivity (referred as the conductance per root unit). ABA has also been associated with root growth, but this too is under debate. In artificially-induced water deprived maize seedlings, ABA accumulation enhanced the root/shoot ratio (Sharp, 2002; Sharp et al., 2004; Spollen et al., 2000), while a reduction of ABA content in rice roots has been advocated as a mean to better exploit subsoil water under mild or transient water deficit (Siopongco et al., 2008; Siopongco et al., 2009). The actual ABA concentration seems to be the key. Studies by Sharp and co-workers showed that at low water potential, ABA accumulation is required to maintain maize root elongation, but excessive accumulation did not promote it further and even slightly inhibited it (Sharp, 2002; Sharp et al., 1994). The higher ABA accumulation observed in Flega, as compared to Patones, might reduce its root growth rate as observed here and in a previous work (Canales at al., 2019b). This, together with the overall higher root thickness would increase the resistance to water transport resulting in the more negative water potential observed. By contrast in Patones, the observed new root growth with higher conductivity (Steudle, 2000), might contribute to its better water status.

According to our data, the different hydraulic conductance observed in the two genotypes was associated with changes not only of the root density but also of root anatomy. Root cross sections of the two genotypes showed increased lignification, which acts as apoplastic barrier for water and ion flow (Stasovski and Peterson, 1991; Taleisnik et al., 1999). This drought-induced root lignification may contribute to minimize water losses to the dry soil when the potential gradient is high and in the wrong direction (Steudle, 2000). Interestingly, important root anatomical differences were observed between the resistant and susceptible genotypes during water stress. Upon gradual water depletion, the two genotypes reduced the number of cortical cell layers and the cortex, stele and xylem area. These changes were only detected at the most reduced sRWC in the susceptible Flega while they initiated at earlier stages in the resistant genotype. A reduced number of cortical cell layers is considered an adaptive advantage under water deficit conditions because it shorten the radial length of apoplast that water has to cross to reach the stele, and it increase root hydraulic properties (Vadez, 2014). In addition, it might contribute to reduce the metabolic cost of root system development (Chimungu et al., 2014; Lynch and Ho, 2005). Plant resource allocation to root growth typically increases under drought to enhance water acquisition. A reduction of the metabolic cost of root growth would facilitate the plant access to water and confers superior productivity as it increases the metabolic resources available for further resource acquisition, growth and reproduction (Chimungu et al., 2014), as observed in Patones. However, the smaller distance between soil and stele could also be a drawback considering the inverse flux of water under drought conditions. The formation of root cortical lacunae or root cortical

aerenchyma that interrupt the radial pathway for water movement from the stele to the soil is considered a strategy to overcome this problem. The formation of these root aerenchyma also contribute to reduce the metabolic cost by transforming living cortical cells in air volume (Chimungu et al., 2014; Zhu et al., 2010). These lacunae were observed in Flega and Patones under drought stress reducing the living cortex area. Interestingly, the reduced stele area observed in Patones could also be related with a reduction of root respiration costs and plant tolerance to drought (Jaramillo et al., 2013). In addition, Patones also significantly reduced its xylem area. This characteristic has also been reported in drought tolerant rice genotypes (Henry et al., 2012) and would be a mean to decrease the risk of xylem embolisms occurring during the most severe drought conditions (Scoffoni et al., 2017). During drought stress the negative sap pressure increases and if this exceeds the threshold value defined by the anatomical characteristics, cavitation occurs (Hacke et al., 2001; Sperry and Tyree, 1988). Xylem vessels with a large diameter are more susceptible to embolism than smaller vessels (Smith et al., 2013; Tyree and Sperry, 1989). Thus, reducing xylem area under conditions of constrained water uptake is an adaptive response that has been previously reported in drought resistant plants (Haworth et al., 2017). Under non-limiting water supply, larger xylem vessels permit movement of water with lower resistance, facilitating growth (Hacke et al., 2000; Villar-Salvador et al., 1997). Our data, showing a highly significant positive correlation between root length and anatomical phenes (i.e. xylem area, average diameter, or cortex area) in well-watered conditions but negative correlation during gradual water depletion, support the latter statement and highlight the importance of the modulation of the different anatomical phenes as adaptive drought response.

Overall, this study showed that adaptation to gradual water depletion is based on many functional and morpho-anatomical phenes expressed in different organs at different levels (model presented in Fig 11). Since they are not mutually exclusive, different phene combination might lead to different adaptive strategies. The mechanims triggered by the two oat genotypes studied were not different in absolute terms although their fine-tuning differed widely. One of the key factors for drought tolerance in oat was the maintenance of root growth. Two interpretations can arise from the differential root growth observed in the two genotypes studied. On one hand, the limited capacity of Flega to promote root growth, would reduce water and nutrient availability in this genotype. Consequently, it would suffer more severely than Patones, leading to a reduction of stomatal conductance, transpiration and hydraulic conductivity. On the other hand, a dramatic and fast ABA response in Flega following drought sensing might contribute to the early reduction of stomata conductance hence hindering assimilation and root growth which could lead to a vicious circle aggravating and fastening its drought symptoms. From our time-course data we can infer that drought sensing occurs very early in both genotypes since they reduced shoot growth as early as 6 daww altering root/shoot ratio under drought. This early drought sensing triggered a dramatic ABA response in Flega as compared to that observed in Patones. The theory of an uncontrolled ABA response in Flega would be supported by the almost 2 fold ABA concentration detected in Flega by the end of the time-course, while the moderate concentration detected in

Patones was sufficient to almost completely close stomata. The possible role/side effect of this additional ABA production in Flega remains unknown as discussed above, although it could be related to its effect on auxin transport regulation. It has been recently reported that root auxin transport *via* PIN1 is limited in an ABA-regulated manner, with high ABA levels decreasing auxin concentrations, meristem size and root growth (Rowe et al., 2016). In agreement with this, ABA response in Flega, was observed at 6 daww, before root growth reduction. The two interpretations thus, are not incompatible but complementary. The root length increase in Patones, observed from 12 daww, may have contributed to a higher water availability, which contributed to maintain higher conductance, transpiration and water potential. Consequently, it may have had a positive feedback promoting further root growth. Our data suggest, nevertheless, that this would not have been possible if the initial and further ABA response in Patones had been similar to that observed in Flega.

Considering the early ABA increase in Flega, prior to any observed root growth decrease, we might say that Flega showed a quite conservative response. The early reduction of stomata and root conductance would primarily impact the water potential gradients along the soil-root-shoot continuum inducing water-saving reaction in the leaves (Maurel et al. 2010). The down regulation of the root conductance might also be considered as a protective reaction to restrict a possible backflow of water from the plant into the soil. However, this strategy resulted in earlier drought symptoms and lower yield and biomass under gradual water deficit as observed under controlled conditions and in the field. By contrast, the resistant Patones followed a more opportunistic and/or risky strategy since the high conductivity might facilitate a backward flow of water from the plant into the drying soil, even taking into account that its higher root growth increases water availability. The mechanism driving water flux (cohesion-tension) during transpiration would also place xylem under tension, making it vulnerable to cavitation-induced embolism. However, other mechanisms including the changes in root anatomy were orchestrated in order to minimize these risks and the overall result was a better leaf water status maintaining transpiration longer, which resulted in prolonged photosynthetic activity and higher yield and biomass in the field (Sánchez-Martín et al. 2015; Sánchez-Martín et al. 2014).

Our data show the importance of the orchestration of interconnected mechanisms to cope with gradual water depletion. Thus, selecting for increased transpiration without considering the increased conductivity necessary to maintain water status and/or the anatomical root changes that promote root growth and limit the risk of embolisms would not be successful. The strategies observed in the two genotypes cannot be labelled as beneficial or harmful in absolute terms as it would depend on the stress intensity or dynamics of the seasonal water deficit. Our data showed that under the gradual water depletion, the ‘water spending’ model observed in Patones has advantages over the most conservative ‘water saving’ model observed in Flega, which could be more appropriate in the harshest environments. The data presented showed a comprehensive

understanding of the successful sequence of responses, which can facilitate oat improvement for water stress in Mediterranean environments.

CONCLUSION

In this study, we dissected two different strategies to cope with water deficit: the most conservative "water saving" strategy vs the more opportunistic and/or risky "water spending" strategy in oats. A dramatic and early ABA accumulation in the susceptible genotype resulted in a tight and rapid reduction of stomatal conductance. Despite of this, leaf water potential decreased concomitantly due to a decrease in root hydraulic conductivity. By contrast, the resistant genotype, showed a mild and slow ABA accumulation that allowed a longer maintenance of transpiration. This response was associated with an increase in root hydraulic conductance and conductivity through the promotion of total root length and the length of the thinnest roots. This was also coupled with anatomical changes reducing metabolic cost. These changes allowed the resistant genotype to maintain higher water potential, reducing drought symptoms and promoting growth under water deficit conditions. In summary, this work advances our knowledge about the resistance mechanisms engaged at root and whole plant levels to cope with drought in oats. It reveals the importance of the fine-tuning of interconnected mechanisms at biochemical, physiological, morphological and anatomical level as part of a complex strategy to cope with gradual water depletion. In addition, we identified several phene aggregates that could contribute to improve drought tolerance in oats.

AUTHOR CONTRIBUTION

FJC conducted most of the experimental work and data analysis. VA supervised the abscisic acid quantification and their results. APL supervised the root anatomy assessment and their results. OGT supervised the leaf water potential and root hydraulic conductivity as well as their results and contributed to the interpretation of results. NR and EP steered the research, designed experiments, and contributed to the interpretation of results and writing of the manuscript. All authors also contributed to critical reading and writing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Tables

Table 1. Pearson correlations between plant root conductivity and several physiological and morphological parameters. *, ** and *** indicates significant correlations at P<0.05, P<0,001 and P<0.001 respectively.

Transpiration	0.5929***		
Area	0.3726*	0.6820***	
Root length	0.4286**	0.6807***	0.7534***
	Root_Cond	Transpiration	Area

Figure Legends

Fig. 1. Stomatal conductance of oat plants during a drought time course. Stomatal conductance was measured in leaves of Flega (triangles) and Patones (circles), well watered (open symbols) and droughted (solid symbols) plants during a time course of gradual water depletion. Data are means of ten replicates ± standard error. ≠G, ≠T, ≠sRWC and GxT indicate overall statistical significance between genotypes (G), treatment (T), soil relative water content (sRWC) and their interaction, respectively. Different letters indicate significant differences (P<0.05) between means for a given time point. Measurements were taken at midday.

Fig. 2. Transpiration per unit leaf area and time in oat plants during a drought time course. Transpiration was recorded for each sampling time during 8 hours in the central part of the light photoperiod in susceptible Flega (triangles) and tolerant Patones (circles) well-watered (open symbols) and droughted (solid symbols) plants during a time course of gradual water depletion. Data are mean of five replicates ± standard error. ≠G, ≠T, ≠sRWC and GxT indicate overall statistical significance between genotypes (G), treatment (T), soil relative water content (sRWC) and their interaction, respectively. Different letters indicate significant differences (P<0.05) between means for a given time point.

Fig. 3. Abscisic acid content of oat plants during a drought time course. Abscisic acid was quantified in susceptible Flega (triangles) and tolerant Patones (circles) well-watered (open symbols) and droughted (solid symbols) leaves (a) and roots (b) during a time course of gradual water depletion. Abscisic acid content data are mean of three replicates ± standard error. Data corresponding to 6 days after withholding water in a and b panels are represented in a magnified scale in the right inset. (c) Response of transpiration to increasing abscisic acid concentration as soil became drier during a drought time course treatment. ≠G, ≠T, ≠sRWC and GxT indicate overall statistical significance between genotypes (G), treatment (T), soil relative water content (sRWC) and their interaction, respectively. Different letters indicate significant differences (P<0.05) between means for a given time point.

Figure 4. Root/shoot dry mass ratio of oat plants during a drought time course. Root/shoot dry mass ratio was measured in Flega and Patones, well watered (open symbols) and droughted (solid symbols) plants during a time course of gradual water depletion. Data are mean of five replicates ± standard error. ≠G, ≠sRWC and GxsRWC indicate overall statistical significance between genotypes (G), soil relative water content (sRWC) and their interaction, respectively. * and *** indicate significant differences between genotypes at P<0.05 and P<0.001, respectively, for a given time point.

Fig. 5. Leaf water potential in oat plants during a drought time course. Midday and pre-dawn leaf water potential was measured, during the steady period, in susceptible Flega (triangles) and tolerant Patones (circles) well-watered (open symbols) and droughted (solid symbols) plants during a time course of gradual water depletion . Data are means of four replicates ± standard error. ≠G, ≠T, ≠sRWC and GxT indicate overall statistical significance between genotypes (G), treatment (T), soil relative water content (sRWC) and their interaction, respectively. Different letters indicate significant differences (P<0.05) between means for a given time point.

Fig. 6. Root hydraulic conductivity in oat plants during a drought time course. Total root hydraulic conductivity (a) and root hydraulic conductivity per root unit (b) was measured in susceptible Flega (triangles) and tolerant Patones (circles) well-watered (open symbols) and droughted (solid symbols) plants during a time course of gradual water depletion. Data are means of four replicates ± standard error. ≠G, ≠T, ≠sRWC and GxT indicate overall statistical significance between genotypes (G), treatment (T), soil relative water content (sRWC) and their interaction, respectively. Different letters indicate significant differences (P<0.05) between means for a given time point.

Fig. 7. Root morphological related traits of oat plants during a water deficit time course. Root parameters were measured in susceptible Flega (triangles) and tolerant Patones (circles) well-watered (open symbols) and droughted (solid symbols) plants during a time course of gradual water depletion. Data are means of four replicates ± standard error. ≠G, ≠T, ≠sRWC and GxT indicate overall statistical significance between genotypes (G), treatment (T), soil relative water content (sRWC) and their interaction, respectively. * and ** indicate significant differences between genotypes at P<0.05 and P<0.01, respectively, for a given time point.

Fig. 8. Root cross sections of oat plants showing root anatomical features such as cortex (cor), xilem (xi) and stele (st). Pictures corresponded to third order roots of susceptible Flega (a, c) and tolerant Patones (b, d) well-watered (a and b) or droughted (c and d) plants.

Fig. 9. Root anatomical traits of oat plants during a drought time course. Number of cortex layer and vessels and area of stele, total cortex, living cortex and xylem were measured in susceptible Flega (triangles) and tolerant Patones (circles) well-watered (open symbols) and droughted (solid symbols) plants during a time course of gradual water. Data are means of four replicates ± standard error. ≠G, ≠T, ≠sRWC and GxT indicate overall statistical significance between genotypes (G), treatment (T), soil relative water content (sRWC) and their interaction, respectively. Different letters indicate significant differences (P<0.05) between means for a given time point.

Fig. 10. Scheme of Pearson correlations between plant root conductivity and several morphological and anatomical root traits in well-watered (W) and droughted (D) oat plants. The

color assigned indicates the strength of a particular correlation between two traits with red-related and blue-related colors for positive and negative correlations, respectively, as depicted in the color key. *, ** and *** indicates significant correlations at P<0.05, P<0,001 and P<0.001 respectively.

Fig. 11. Integrated model of 'water spending' strategy of the drought tolerance cultivar Patones as compared with the 'water saving strategy' of the susceptible cultivar Flega. The schematic brings together the biochemical, physiological, morphological and anatomical observations described in this paper. In Patones, the moderate accumulation of abscisic acid in roots and leaves allows fine modulation of stomatal closure maintaining transpiration for longer. Morphological and anatomical changes in roots favour hydraulic root conductance and conductivity improving water status and avoiding cavitation under the transpirative demand and reduce metabolic cost promoting root growth. By contrast, in Flega a dramatic increase of abscisic acid in roots and leaves lead to a rapid and tight stomatal closure. Reduced transpiration slowed root growth but it does not improve plant water status as root hydraulic conductance was also reduced. This reduction is due, at least partly, to the lack of adaptation through morphological and anatomical root changes.