Physiological, biochemical and molecular responses in four *Prunus* rootstocks submitted to drought stress

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Summary

The understanding of the mechanisms that determine plant response to reduced water availability is essential to improve water use efficiency of stone fruit crops. The

physiological, biochemical and molecular drought responses of four *Prunus* rootstocks (GF 677, Cadaman, ROOTPAC 20 and ROOTPAC® R) budded with 'Catherina' peach cultivar were studied. Trees were grown in 15 L containers and subjected to a progressive water stress during 26 days monitoring soil moisture content by TDR. Photosynthetic and gas exchange parameters were determined. Root and leaf soluble sugars and proline content were also measured. At the end of the experiment, stressed plants showed lower net photosynthesis rate, stomatal conductance and transpiration rate and higher intrinsic leaf water use efficiency (A_N/g_s). Soluble sugars and proline concentration changes were observed, in both root and leaf tissues, especially in an advanced state of stress. The accumulation of proline in roots and leaves with drought stress was related to the decrease in osmotic potential and increase of water use efficiency whereas the accumulation of sorbitol in leaves and raffinose in roots and proline in both tissues was only related with the increase in the water use efficiency. Due to the putative role of raffinose and proline as antioxidants and their low concentration they could be ameliorating deleterious effects of drought-induced oxidative stress by protecting membranes and enzymes rather than acting as active osmolytes. Higher expression of *P5SC* gene in roots was also consistent with proline accumulation in the tolerant genotype GF 677. These results indicate that accumulation of sorbitol, raffinose and proline in different tissues and/or the increase in *P5SC* expression could be used as markers of drought tolerance in peach cultivars grafted on *Prunus* rootstocks.

Introduction

Stone fruits include peach and nectarine, almond, apricot, plum prune and cherry plum and sweet and sour cherry. Stone fruits are the sixth group of crops produced in the world (41 million t) after banana and plantain fruits, citrus fruits, pomes, grapes and coconuts (FAOSTAT, 2011). Peach and nectarine are the most economically important plant species from the *Prunus* genus, the eleventh fruit crop in production (22 million t) in the world and the fourth in Europe (FAOSTAT, 2011). However, they are mostly cultivated in soils with water availability limitations, such as the Mediterranean area. The scarcity of water resources and high irradiance and temperature during summer are characteristics of this cultivation area (García et al. 2007, Flexas et al. 2010). In these conditions, drought is one of the most important environmental stresses in agriculture limiting crop production (Cattivelli et al. 2008).The need for water conservation and evaluation of the existing and/or newly developed germplasm of crop plants for their tolerance to drought has become urgent (Morison et al. 2008, Sivritepe et al. 2008).

Rootstocks are considered to have influence on the response of the grafted tree to water stress by altering stomata size and operation, transpiration and water potential and vegetative growth (Martínez-Ballesta et al. 2010, Schwarz et al. 2010, Hajagos and Végváry, 2013). The capacity of rootstocks to confer drought tolerance to the scion has also been shown in other woody plants, such as grapevine (Iacono et al. 1998) and apple (Atkinson et al. 2000). Because the responses to drought stress are different according to the plant genetic background (Rampino et al. 2006), one of the strategies to improve fruit tree response to water deficit conditions is the use of tolerant rootstock genotypes. In the Mediterranean area, the choice of proper rootstocks with multiple tolerances to the main abiotic stresses is crucial to prevent future problems in the orchard and to reduce management costs (Jiménez et al. 2008, Moreno et al. 2008). Thus identifying the physiological, biochemical and molecular mechanism and responses in peach trees

submitted to drought stress would provide understanding and facilitate the screening procedures for the selection of tolerant rootstocks.

In comparing the relative drought tolerance among tree genotypes, several traits have been associated with an improved water stress response and have been proposed as an effective selection criterion to identify plants with better performance. These include, among others, the induction of high osmotic adjustment, water use efficiency, chlorophyll content, antioxidant capacity and stronger protective mechanism, and low reductions in leaf relative water content growth capacity and photosynthetic capability (Cregg 2004, Cattivelli et al. 2008, Lovisolo et al. 2010, Liu et al. 2012). Water deficit can induce responses in plants at all levels of organization: cell, metabolism and molecular level (Krasensky and Jonak 2012). The primary effects of drought in trees are usually the reduction in plant stomatal conductance, water potential, osmotic potential, leaf elongation and leaf photosynthesis leading to a reduction of water losses but also of plant productivity (Jones 2007, Lovisolo et al. 2010). Stomatal closure is probably the most important factor controlling carbon metabolism under moderate drought stress (Chaves et al. 2009). Decline in intracellular CO_2 levels results in the over-reduction of components within the electron transport chain leading to generation of reactive oxygen species (Mahajan and Tuteja 2005). Plants accumulate osmolytes, such as the amino acid proline and the sugars raffinose and sorbitol, to prevent membrane disintegration and enzyme inactivation (Mahajan and Tuteja 2005, Chaves et al. 2009), to reestablish the cellular redox balance by removing the excess levels of ROS and/or to maintain cell turgor by osmotic adjustment (Krasensky and Jonak 2012). The capacity to accumulate proline has been correlated with tolerance to many stresses, including drought, high salinity and heavy metals (Krasensky and Jonak 2012). At the molecular level, genes involved in the synthesis of osmoprotectants are induced under stress (Krasensky and

Jonak 2012). The change in expression of genes of the biosynthetic pathway of the raffinose and sorbitol sugars has been studied in woody trees submitted to osmotic stress, such as mandarin and apple (Gimeno et al. 2009, Zhang et al. 2011). Another important plant adaptation under drought stress is the increase of water use efficiency (WUE). It is a component of drought tolerance in water limited environments that potentially affects yield (Bongi et al. 1994, Nicotra and Davidson 2010) that can be measured as the molar ratio between photosynthetic rate and leaf transpiration (Morison et al. 2008).

The aim of the present work was to evaluate the physiological and biochemical responses of four *Prunus* rootstocks (Cadaman, GF 677, ROOTPAC 20 and ROOTPAC® R) budded with 'Catherina' peach cultivar and submitted to drought stress under controlled conditions. The differences among genotypes and the relationship of the responses with growth induction were evaluated. The interaction between physiological and biochemical parameters was tested to identify drought tolerance markers that could be implemented in the peach rootstock breeding programs for marker assisted selection. The study was complemented at the molecular level with expression of key genes related to drought tolerance to know the control of these responses.

Material and methods

Plant material and experimental conditions

Micropropagated Cadaman [CD; *Prunus persica* (L.) Batsch × *P. davidiana* (Carr.) Franch], GF 677 (GF; *P. dulcis* Miller × *P. persica*), ROOTPAC 20 (R20; *P. besseyi* Bailey × *P. cerasifera* Ehrh.; formerly known as PAC 9801-02) and ROOTPAC® R (RR; *P. cerasifera* × *P. dulcis*) rootstock plants budded with var. 'Catherina' (*P. persica*) were obtained from Agromillora Iberia S.L. (Subirats, Barcelona, Spain).

Rootstocks were grown for 2 weeks in 300 cm^3 pots containing a peat substrate, then they were micrografted. Thirty plants per genotype were transferred to 15 L containers with a medium of 1:1 sand-peat substrate (TKS-1, Floragard, Oldenburg, Germany) and 2 g kg^{-1} osmocote 14-13-13 (The Scotts Company LLC, Ohio, US). Plants were grown in a greenhouse in Zaragoza, Spain (41º 43' N, 0º 48' W) under normal day light conditions during April and May 2011. During this period, the mean light-time was of 14 hours and 6 min. The mean average day- and night-time temperature and humidity were 23 and 18ºC, and 53 and 67 %, respectively. Plants were trained to a single shoot and watered to runoff every day during 21 days. On May 4 (day 0 of the experiment) plants of each genotype were randomly separated in two water treatments: well-irrigation and water-stress. Soil volumetric water content was monitored by TDR ("Time Domain Reflectometry") with 20 cm length probes vertically inserted into the containers. The probes were connected to a TDR100 cable tester (Campbell Scientific, Logan, UT, USA) by a 1.2-m-long coaxial cable (50 Ω impedance), and the TDR signals were transferred to a computer that calculates the volumetric water content using the software TDR-Lab V.1.0 (Moret-Fernández et al. 2010). The soil water retention curve of the experimental soil, needed to determine the water content of the soil field capacity (-33 kPa), was estimated using TDR-Cells as described in Moret-Fernández et al. (2012). This experiment also allowed obtaining the calibration function to estimate the soil water content by TDR. Control plants were watered daily and water status was maintained at full field capacity (the soil volumetric water content was of 29%). Water stressed plants were also irrigated daily but adding about 80% of the water evapotranspired the previous day (García et al. 2007), and subjected to progressive water stress during 26 days (Figure 1). Every morning, the soil volumetric water content of drought stressed plants was measured, then a target soil volumetric water content

corresponding with the recovering of about the 80% of the water evapotranspired the previous day (of the genotype of higher evapotranspiration) was established. Finally, pots received only the water needed to reach this value. It is found that dry-down responses are often confounded with plant size in studies using containers (Cregg 2004). Using this methodology, the variations in decline of the volumetric water content of pots among genotypes was minimized, regardless of their plant size.

Plant physiological measurements were made on well-watered and water-stressed plants the days 0, 7, 12, 16, 20, 23 and 26 after starting the experiment. Root and leaf tissue on well-watered and water-stressed plants were collected the days 16 and 26, except for roots of ROOTPAC 20 the last sampling (insufficient plant material). Plant material was rinsed in distilled water, immediately frozen in liquid nitrogen and stored at -80ºC until their use for the biochemical and molecular determinations.

Morphological parameters

Primary shoot axis growth (axis length) was measured daily for each genotype and treatment (n= 5) from the beginning (Day 0) to the end (Day 26) of the experiment. Fresh and dry weight of roots, leaves and stem were measured at day 26 for all genotypes except for ROOTPAC 20 due to insufficient plant material. Mean mature leaf area was estimated from the area of six expanded leaves per plant at day 26. Leaves were dried at 80ºC for 24 h to obtain the dry weight. Specific leaf area (SLA) was calculated as area divided by dry weight ($cm^2\ g^{-1}$).

Stem water potential, osmotic potential and RWC parameters

A single mature leaf (fifth expanded leaf) of each of six replicate plants was assayed for stem water potential (Ψ_s). Leaves were enclosed in aluminium foil-covered plastic

envelopes to stop transpiration and allow equilibrating with Ψ_s 30 min before measurement. Midday Ψ_s was measured using a Schölander-type pressure chamber (PMS instrument, Corvallis, OR, USA). After measurement, leaves were wrapped in aluminium foil, frozen in liquid nitrogen and stored in plastic bags at -20ºC (García-Sánchez et al. 2007). After thawing, osmotic potential (Ψ_π) was measured with a Psychrometer Tru PSi SC10X (Decagon devices, Pullman, WA, USA).

Leaf relative water content (RWC) was measured on a mature leaf (sixth expanded leaf) per plant. Leaves were immediately weighed to obtain a leaf fresh weight (FW) and petioles were submerged into water overnight in the dark at 5ºC to reduce respiration during night period and avoid dry weight losses. Fully hydrated leaves were reweighed to obtain turgid weight (TW) and dried at 80ºC for 24 h to obtain dry weight (DW). RWC was calculated as 100×(FW-DW)/(TW-DW) according to Morgan (1984).

Photosynthetic parameters measurement

Photosynthetic rate (A_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (E) was measured using a portable photosynthesis system (LI-6400XT, Licor Inc, Lincoln, Nebraska, USA). Measurements were conducted between 10:00 to 12:00 (GMT) in the same leaves used for Ψ_s determinations (n=6). Parameters were measured with saturating light (1400 µmol m^{-2} s^{-1} provided by an external light source), 400 µmol CO_2 mol^{-1} and 30.5ºC (average leaf temperature during measurements) of leaf block temperature. Leaf water use efficiency (WUE) was calculated as the ratio between A_N and g_s.

Chlorophyll concentration parameter

The chlorophyll (Chl) concentration per unit leaf area was estimated after 26 days of drought stress using a SPAD 502 meter (Minolta Co., Osaka, Japan). Six SPAD measurements were taken homogeneously distributed throughout the third expanded leaf of control and drought plants (n=6). After calibration by extraction of Chl from leaf disks (Abadía and Abadía 1993), SPAD measurements were converted into Chl concentration per unit leaf area (nmol Chl cm^{-2}).

Proline content parameter

After 16 and 26 days of the stress period, leaf and root proline were determined using the methodology described by Bates et al. (1973) and Ábrahám et al. (2010). Plant tissue (n=6) was ground to a fine powder in a pre-cooled mortar with liquid nitrogen. About 0.1 g fresh weight per sample was homogenized with sulfosalicylic acid 3% (Panreac Química S.A.) and supernatant was reacted with ninhydrine (Sigma-Aldrich). The absorbance was read at 520 nm and free proline concentration was calculated from a calibration curve using proline as a standard (Sigma-Aldrich). Free proline content was reported as mg g^{-1} DW.

Soluble sugar determination

After 16 and 26 days of the stress period, leaf and root soluble sugar content was determined by HPLC. Plant tissue (n=6) was ground to a fine powder in a pre-cooled mortar with liquid nitrogen. Polar compounds from about 0.1 g fresh weight were extracted into aqueous ethanol at 80°C, in three steps, each lasting 20 min (step 1: 0.75 ml 80% ethanol; steps 2 and 3: 0.75 ml 50% ethanol). The mixture of each step was centrifuged for 10 min at 4800 g and slurries were pooled (Moing et al. 2004). The ethanol was allowed to evaporate in a speed-vac and dry extracts were solubilised in 1

ml double-distilled water. Soluble sugars were purified using ion exchange resins (Bio-Rad AG 1-X4 Resin 200-400 chloride form, Bio-Rad AG 50W-X8 Resin 200-400 mesh hydrogen form). Samples were concentrated to 0.2 ml, filtered and 20 µl were injected and analysed by HPLC, using a Ca-column (Aminex HPX-87C 300 mm × 7.8 mm column Bio-Rad) flushed with 0.6 $ml \cdot min^{-1}$ double-distilled water at 85ºC with a refractive index detector (Waters 2410). Concentrations of the main sugars: fructose, glucose, raffinose, sorbitol, sucrose and xylose, were calculated for each sample using mannitol as internal standard. Sugar quantification was carried out with Empower Login software from Waters (Milford, Mass, US) using commercial standards (Panreac Química S.A.). Soluble sugar amount was reported as mg g^{-1} DW.

RNA isolation and reverse transcription

To evaluate the initial molecular response to reduced water availability, total RNA was isolated from Cadaman and GF 677 scion leaf and roots tissues of plants (n=4) submitted to control and drought stress during 16 days using the protocol of Meisel et al. (2005). Analysis were only done in Cadaman and GF 677 because they showed different responsiveness to drought. After DNase I treatment (Thermo Scientific, Waltham, MA, USA) to eliminate possible genomic DNA contamination, 2 µg of total RNA were reverse transcribed using an oligo $(dT)_{18}$ as a primer with RevertAid H Minus first strand cDNA synthesis system (Thermo Scientific).

Primer design and expression analysis by real-time PCR

Samples from cDNA synthesis were used to evaluate the expression of *raffinose synthase* (*SIP1*), *Δ-1-pyrroline-carboxylate synthase* (*P5SC*), leaf *sorbitol-6-phosphate dehydrogenase* (*S6PDH*) and root *sorbitol dehydrogenase* (*SDH*) genes. Gene

sequences were identified by Blastn against the "Peach Genome v1.0 predicted transcripts" database in GDR (http://www.rosaceae.org) with an E-value>$1e^{-05}$. Query sequences were *Arabidopsis SIP1* (AT5g40390) and *P5SC* (AT2g39800) (http://www.arabidopsis.org/), and *Malus domestica S6PDH* (D11080) (Kanayama et al. 1992) and *SDH2* (AF323505) (Park et al. 2002). Finally, gene-specific primers were designed using Primer3Plus (see Table S1 available as Supplementary Data at Tree Physiology Online) (Untergasser et al. 2007).

Real-time PCR was performed on a Applied Biosystem 7500 Real Time PCR (Life Technologies, Carlsbad, California, USA) using the Kappa SYBR Fast Maxter Mix (Kapa Biosystems, Cambridge , MA, USA). Two technical replications for each of the four biological replicates were performed. PCR was conducted with the following program: an initial DNA polymerase activation at 95°C for 180 s, then followed by 40 cycles of 95°C for 20 s, 60°C for 20 s, and 72°C for 30 s. Finally, a melting curve was performed, and the PCR products were checked with 2% agarose gel in 1× TAE with ethidium bromide. Fluorescence values were baseline-corrected and averaged efficiencies for each gene and Cq values were calculated using LinRegPCR program (Ruijter et al. 2009). Gene expression measurements were determined with the Gene Expression Cq Difference (GED) formula (Schefe et al. 2006). The gene expression levels were normalized to a peach *AGL-26-like*. This gene was chosen as internal reference among other tested genes (*actin 2*, *elongation factor 1α*, *ubiquitine 2*) based on the average expression stability, M, calculated with geNORM software (Vandesompele et al. 2002). Data were normalized relative to the values of the drought tolerant GF 677 rootstock (Alarcón et al. 2002) under control conditions. Normalized data in this manner allowed for the comparison of the magnitude of gene expression both across genotypes and treatments.

Statistical analysis

Data were evaluated by two-way variance (ANOVA) analysis with the programme SPSS 19.0.0 (SPSS, Inc, Chicago, USA). Previously, data was evaluated by Levene's homoscedasticity test and transformed if necessary. When treatment interaction terms were significant ($P < 0.05$), means were separated using Duncan's multiple range test at $P \leq 0.05$. Means of two samples were compared using a Student t-test. Regression analysis was carried out by Pearson's correlation. Gene expression differences were evaluated by the non-parametric Mann-Whitney U test ($P < 0.05$).

Results

Morphological determinations

After 26 days of growth under control conditions, Cadaman, GF 677 and ROOTPAC® R induced higher growth ($P < 0.001$) than ROOTPAC 20 (Figure 2a-d). Apical growth of GF 677, ROOTPAC 20 and ROOTPAC® R decreased significantly after 18, 22, and 14 days of being submitted to drought stress (t-test, $P < 0.05$). After 26 days of experiment, GF 677 (Figure 2b) plants showed the highest apical growth ($P \leq 0.001$) whereas ROOTPAC 20 (Figure 2c) the lowest, in both treatments. Cadaman, GF 677 and ROOTPAC® R showed lower shoot dry weight with drought stress (Table 1). Shoot to root dry weight ratio was lower in drought stressed plants and in the ROOTPAC® R rootstock (Table 1). However, water deficit did not reduce the specific leaf area (SLA) of leaves in all rootstocks studied (Table 1).

Water potential and RWC

The stem water potential (Ψ_s) of control plants ranged between -1.11 and -0.50 MPa (Figure 3a-d). In stressed plants, Ψ_s decreased progressively during the experiment (Figure 3a-d) as a response to the reduction in soil water content (Figure 1). The Ψ_s of water stressed plants was significantly lower than control plants after 16 days for all genotypes. After 26 days of stress, Ψ_s was lower for the rootstocks GF 677 (-1.99 MPa) and ROOTPAC® R (-1.94 MPa) than for the other two rootstocks, Cadaman (-1.67 MPa) and ROOTPAC 20 (-1.64 MPa).

The leaf osmotic potential (Ψ_π) was significantly lower in drought stressed plants at 16 and 26 days of experiment (Table 2). This last day, GF 677 and ROOTPAC® R rootstocks showed larger decrease in Ψ_π with drought than the other rootstocks (GF 677 and ROOTPAC® R decreased Ψ_π more than 0.69 MPa whereas Cadaman and ROOTPAC 20 did it less than 0.32 MPa). The leaf relative water content was also significantly lower in drought stressed plants at 16 and 26 days of experiment, although no differences were found among genotypes (Table 2). If an estimate of the leaf osmotic potential at full turgor is obtained [using the following formula: $\Psi_\pi^{100} = \Psi_\pi$ x (RWC/100), the osmotic potential is estimated by the extrapolation of values at 100% RWC], and the osmotic adjustment is calculated (difference between the Ψ_π^{100} of control plants and that of the stressed plants), a higher osmotic adjustment can be found in the genotypes GF 677 and ROOTPAC® R despite of the decrease in RWC with drought stress (data not shown).

Photosynthetic, gas exchange parameters and chlorophyll content

The variables monitored in this study (A_N, g_s, Ci and E) showed a decline similar to the change of water potential with drought stress from day 0 to 26 (data no shown). After 16 days of water stress, drought plants showed lower net photosynthesis rate (except in

Cadaman), stomatal conductance, transpiration rate and intercellular CO_2 concentration, and higher leaf intrinsic water use efficiency (Figure 4a-e). Among genotypes, ROOTPAC 20 induced the lowest A_N, g_s and E, and the highest WUE (Figure 4a-c and e). At the end of the experiment (26 days), photosynthetic and gas exchange parameters were affected by drought in a similar way (Figure 5a-e). A significant interaction was found for A_N and WUE (Figure 5a and e). WUE was greater on drought stressed ROOTPAC® R and GF 677 rootstocks, the later being not significantly different from ROOTPAC 20.

Leaf chlorophyll concentration was not significantly affected by drought after 26 days of stress (Figure 5f). However, the ROOTPAC 20 rootstock showed lower leaf Chl concentration than the other rootstocks.

Soluble sugars and proline content

Main soluble sugar identified and quantified in peach leaves was sorbitol (between 68 and 123 mg g^{-1} DW), followed by sucrose (between 31 and 68 mg g^{-1} DW) (Tables 3 and 5). However, main soluble sugars in roots were sucrose (between 16 and 37 mg g^{-1} DW) and glucose (between 9 and 28 mg g^{-1} DW), followed by sorbitol (between 8 and 19 mg g^{-1} DW) (Tables 4 and 6). The less abundant soluble sugar was raffinose in leaves (between 0.1 and 0.5 mg g^{-1} DW) and xylose in roots (between 0.2 and 1.8 mg g^{-1} DW).

After 16 days of water stress, drought did not affect leaf and root soluble sugars concentration, except root fructose (Tables 3 and 4). Leaf and root proline concentration was also not affected by drought. However, significant differences among genotypes were evident for other compounds. On one hand, leaf fructose, raffinose and proline were significantly lower in ROOTPAC 20 genotype, whereas leaf proline was higher in

GF 677, followed by ROOTPAC® R plants (Table 3). On the other hand, root raffinose and sucrose were lower in GF 677 than in Cadaman and ROOTPAC® R (Table 4). However, no significant differences were found in root total soluble sugars and proline concentration among these three rootstocks. No significant correlations between physiological and biochemical parameters were found at this time point.

After 26 days of water stress, more significant differences were detected. Drought affected leaf and root soluble sugars and proline concentration, except leaf glucose and total sugars (Tables 5 and 6). Sorbitol concentration increased with water stress in leaves whereas decreased in roots. These changes were accompanied with the decrease of the other main soluble sugars (sucrose in leaves, glucose and sucrose in roots), causing no change of total sugars in leaves and a decrease in roots. However, drought induced the accumulation of proline in both tissues, leaves (1.7 fold) and roots (2 fold). Root proline accumulation was especially induced with water stress by ROOTPAC® R (Table 6).

Significant correlations between physiological and biochemical parameters were found after 26 days of water stress (Table 7). The Ψ_π was positively correlated with leaf fructose (r=0.51, $P<0.001$), leaf and root sucrose (r=0.56, $P<0.001$; r=0.53, $P<0.001$, respectively), root sorbitol (r=0.48, $P<0.01$) and root xylose (r=0.56, $P<0.001$) but negatively correlated with leaf sorbitol (r=-0.37, $P<0.05$) and leaf and root proline (r=-0.65, $P<0.001$; r=-0.44, $P<0.05$, respectively) (Table 7). The WUE was positively correlated with leaf sorbitol (r=0.36, $P<0.05$), leaf and root proline (r=0.65, $P<0.001$; r=0.55, $P<0.001$, respectively) and root raffinose (r=0.44, $P<0.05$), but negatively with leaf fructose (r=-0.35, $P<0.05$), leaf sucrose (r=-0.56, $P<0.001$) and root sorbitol (r=-0.58, $P<0.001$) (Table 7).

Gene expression of SIP1, P5SC, S6PDH and SDH

In order to evaluate if there is an initial molecular response to reduced water availability, expression of the genes involved in the synthesis of the main osmolytes accumulated under drought in scion and roots were evaluated after 16 days of stress. The study was conducted in two rootstocks (Cadaman and GF 677), budded with the peach cultivar 'Catherina', that showed different physiological and biochemical response to water stress.

Scion leaves on Cadaman and GF 677 showed significant up-regulation of *SIP1* under drought stress (Figure 6a). In roots, expression of *SIP1* also increased with drought in both rootstocks (Figure 6b), but differences were only significant for Cadaman rootstock. The expression of *P5SC* remained stable with stress in the scion leaves for both rootstocks (Figure 6c). However, drought induced up-regulation in the roots (Figure 6d), especially in the more tolerant rootstock GF 677 (2.3 fold). The expression of S*6PDH* remained stable with stress in the scion leaves for both rootstocks, but GF 677 showed enhanced expression in comparison to Cadaman rootstock (Figure 6e). The expression of *SDH* in roots decreased significantly with drought for Cadaman rootstock (Figure 6f), however the expression of other *SDH* isoforms significantly decreased with drought in both rootstocks (data not shown).

Discussion

The comprehensible study of the adaptive mechanisms and responses to water stress for the development of tolerant lines of deciduous trees is becoming increasingly important. The choice of proper rootstocks with tolerance to drought stress is crucial to prevent future problems in the orchard and to use water in a more sustainable way.

Several studies carried out with *Prunus* species submitted to water stress have shown a significant decrease in plant water status and gas exchange parameters (Escobar-Gutiérrez et al. 1998, Lo Bianco et al. 2000, Rieger et al. 2003, Mellisho et al. 2011). In this study, the Ψ_s, Ψ_π and RWC of the different *Prunus* rootstock combinations were generally diminished after 16 days of water stress. The RWC of other peach scion-rootstock combinations also decreased as found in our study as the soil water level stress increased (Kaynas and Atatürk 1997). Other authors found that Ψ_π also decreased in an initial maturing peach variety grafted onto GF 677 and subjected to low water availability during almost one month (Mellisho et al. 2011). However, the RWC and Ψ_π were not significantly different between control and stressed scion leaves of peach trees when drought was imposed in short term (eight days withholding water) (Escobar-Gutiérrez et al. 1998). As suggested by our study, *Prunus* trees showed adaptation to progressive drought stress probably because they have capacity to accumulate active solutes. Furthermore, drought monitored and imposed as in the present experiment, growing plants in pots, seems to mimic the field responses to drought stress of trees (Mellisho et al. 2011) and allowed the identification of drought responses induced by the rootstocks regardless of growth size induction.

We found that the most vigorous rootstocks GF 677 (*P. dulcis* Miller × *P. persica*) and ROOTPAC® R (*P. cerasifera* × *P. dulcis*) (Pinochet 2010) induced higher water use efficiency. This strategy could be explained by the genetic variation across *Prunus* species. The capacity of avoiding water loss via transpiration found in this study is related to the tolerance of the peach-almond hybrids GF 677 to drought (Alarcón et al. 2002). An evaluation of the capability of maintaining functional xylem conduits under extreme drought conditions of different *Prunus* species showed that *P. dulcis* and *P. cerasifera* species were more tolerant than *P. persica* (Cochard et al. 2008). Another

explanation could be related with the influence of the rootstock in growth vigor since it has been observed that scions grafted on dwarfing rootstocks showed more serious water stress symptoms (Hajagos and Végvári 2013). GF 677 and ROOTPAC® R rootstocks seem to have the strategy of tolerate lower water potentials and tissue water status whist still acquiring carbon but also still maintaining its photosynthetic capacity. However, a dwarfing rootstock such as ROOTPAC 20, presented lower tolerance capacity with an impaired photosynthetic capacity. Anatomical differences in stem induced by the different vigor of cherry rootstocks would support this idea (Hajagos and Végvári 2013).

The concomitant decrease on both photosynthesis and stomatal conductance, the lower values of intercellular CO_2 concentration and no presence of chlorophyll degradation could indicate that stomatal limitation was one of the main reasons for the declining in photosynthesis under drought stress, as it has been reported in citrus (García-Sánchez et al. 2007). No changes in chlorophyll concentration were previously found in 'Springcrest' peach cultivar grafted onto other *Prunus* rootstocks cultivated without irrigation (Bongi et al. 1994). Decline in intracellular CO_2 concentration may have resulted in generation of reactive oxygen species at the photosystem I (Mahajan and Tuteja 2005). Therefore, the presence of high content of osmolytes in the cells of stressed plants could have protected the photosynthetic apparatus (Krasensky and Jonak 2012). Probably, raffinose and proline could be involved in such tolerance, since their concentration was small to be osmotically effective.

Prunus trees showed a change in the soluble sugars composition with drought in both leaf and root tissues, especially in a late stress stage at 26 days of treatment (see the significances in the bottom part of tables 3 to 6). The decrease in fructose and sucrose concentration in both tissues, the increase in leaf sorbitol and decrease in root sorbitol

seems to be a common response to drought in the *Rosaceae* family (Lo Bianco et al. 2000, Rieger et al. 2003, Cui et al. 2004). It has been shown that sorbitol rather than sucrose is preferentially photosynthesized at the low photosynthetic rates of drought stressed peach leaves (Escobar-Gutiérrez et al. 1998). Moreover, sorbitol accumulation has been correlated with drought stress tolerance in several plant species (Krasensky and Jonak 2012). Given the high concentration found in our study, leaf sorbitol could behave as one of the major components involved in osmotic adjustment although we could not corroborate this possibility. The accumulation of other osmolytes such as raffinose and proline was also found in *Prunus* trees (Gholami et al. 2012), especially in roots. Raffinose was also accumulated in drought stressed plants of citrus (Gimeno et al. 2009) although the absolute concentration of this sugar in *Prunus* was low in comparison with sorbitol. Proline accumulation has been described as a tolerance mechanism used by plants to face drought stress and has been correlated with stress tolerance (García-Sánchez et al. 2007, Bandurska et al. 2009, Krasensky and Jonak 2012). Proline has been proposed to act as an osmolyte, a ROS scavenger and a molecular chaperone stabilizing proteins structure (Krasensky and Jonak 2012).

A different biochemical response to drought was also found in our study depending on the rootstock. The more vigorous and almond based rootstocks GF 677 and ROOTPAC® R showed higher accumulation of compatible solutes and, therefore, they seemed to induce a better drought tolerance response at both levels, physiological and biochemical. In fact, the physiological changes found have been correlated with the biochemical changes of the plant. On one hand, the decrease in osmotic potential has been related with the accumulation of leaf and root proline. On the other hand, the increase in WUE has been related with the accumulation of leaf sorbitol, root raffinose and leaf and root proline. Due to the putative role of sorbitol, raffinose and proline as

antioxidants (Ashraf et al. 2011, De Campos et al. 2011, Krasensky and Jonak 2012), they can be ameliorating deleterious effects of drought-induced oxidative stress by protecting membranes and enzymes. These osmoprotectants may confer to GF 677 and ROOTPAC® R genotypes a metabolic adaptation that could exert beneficial effects to drought at both root and peach scion. Whether they can also provide osmotic adjustment in peach leaves cannot be deduced from the analyses carried out in this study.

Finally, the increase in expression of *SIP1* and *P5SC*, genes that codify enzymes of the biosynthetic pathway of raffinose and proline respectively, were in general consistent with the accumulation of these osmolytes with drought. As in citrus (Gimeno et al. 2009), up-regulation of *SIP1* was translated into accumulation of raffinose in roots. Up-regulation of *P5SC* at an initial stage of drought stress was translated into accumulation of proline with time, especially in GF 677 roots (2.3 and 2.0 fold in expression and metabolite change after 26 days of stress, respectively). Higher expression of *P5SC* in correlation with proline accumulation was also found in safflower in a drought tolerant cultivar in comparison with a sensitive one (Thippeswamy et al. 2010). The expression of *S6PDH* in source leaves, gene that codify the enzyme involved in the biosynthesis of sorbitol as photoassimilate, was not affected by drought in an initial stage of stress. However, in apple this gene was induced by osmotic stress, especially with severe stress (Zhang et al. 2011). The change in transcript level has been associated with changes in S6PDH enzyme activity promoting sorbitol synthesis in peach leaves (Sakanishi et al. 1998). Several isoforms of *SDH*, genes that codify the enzyme that catalyzes the conversion of sorbitol to fructose in sink tissues, have been found expressed in roots of apple trees (Park et al. 2002). In *Prunus* roots, the expression of one or several *SDH* isoforms decreased at an initial stage of

drought stress, however, root sorbitol concentration seems to decrease with time, rather than the opposite.

In summary, the method used in this study mimic field conditions and appears to be suitable to test drought tolerance of peach rootstocks in controlled conditions. The biochemical responses to drought, mainly accumulation of sorbitol, raffinose and proline, were consistently related to the physiological responses to water stress that confer tolerance. Initial molecular responses were related with the biochemical responses observed. Therefore, we propose that the accumulation of leaf sorbitol, root raffinose and root and leaf proline could be implemented as drought tolerance markers for early selection of *Prunus* rootstocks for peach trees under controlled conditions. The differential expression of *PSC5* in roots could also be used as drought tolerance marker. The almond-based rootstocks GF 677 and ROOTPAC® R showed better performance to drought stress with both physiological and biochemical responses. The different rootstock performance could be related to their different genetic background and vigor. Further research will be needed to ascertain if these metabolic compounds participate in the osmotic adjustment of the plant and to disentangle the specific roles of proline and raffinose. This study would be the basis to proceed for future analysis at the whole-molecular level in order to disentangle the tolerance mechanisms to drought in *Prunus* rootstocks.

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Conflict of interest

None declared

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Table 1. Shoot dry weight, shoot to root ratio and specific leaf area (SLA) (n=5) in control and drought-stressed *Prunus* rootstocks (Cadaman, GF 677, ROOTPAC 20 and ROOTPAC® R) budded with var. 'Catherina', after 26 days of treatment.

		Shoot DW (g)	Shoot to root DW ratio	SLA (cm^2 g^{-1} DW)
Main factors				
Treatment	Control	11.3 b	3.2 b	155
	Drought	7.9 a	2.0 a	151
Genotype	CADAMAN	8.9	2.8 b	157
	GF 677	9.6	2.8 b	155
	ROOTPAC 20	N/D	N/D	158
	ROOTPAC® R	10.4	2.2 a	142
Interaction				
Control	CADAMAN	10.4	3.3	164 ab
	GF 677	11.9	3.4	164 ab
	ROOTPAC 20	N/D	N/D	143 ab
	ROOTPAC® R	11.7	2.8	147 ab
Drought	CADAMAN	7.3	2.3	150 ab
	GF 677	7.3	2.1	146 ab
	ROOTPAC 20	N/D	N/D	171 b
	ROOTPAC® R	9.1	1.6	137 a
Significance				
Treatment		*	***	ns
Genotype		ns	***	ns
Treatment × Genotype		ns	ns	**

Two-way ANOVA was performed for linear model, on raw data. Significance: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; ns, not significant. N/D, not determined. Comparison means by Duncan's test ($P < 0.05$) were shown for the significant interaction between treatment and genotype. Different letters indicate significant differences among data within the same factor or interaction.

Table 2. Scion leaf osmotic potential (Ψ_π) and relative water content (RWC) (n=6) in control and drought-stressed *Prunus* rootstocks (Cadaman, GF 677, ROOTPAC 20 and ROOTPAC® R) budded with var. 'Catherina', after 16 and 26 days of treatment.

		Ψ_π (MPa)		RWC (%)	
		Day 16	Day 26	Day 16	Day 26
Main factors					
Treatment	Control	-2.87 b	-2.69 b	86.7 b	88.3 b
	Drought	-3.12 a	-3.22 a	84.2 a	80.8 a
Genotype	CADAMAN	-2.85 b	-2.88	85.2	84.5
	GF 677	-2.89 b	-2.99	86.1	84.7
	ROOTPAC 20	-3.20 a	-3.05	86.0	84.8
	ROOTPAC® R	-3.05 ab	-2.94	84.6	83.9
Interaction					
Control	CADAMAN	-2.63	-2.72 de	87.9 b	88.5
	GF 677	-2.71	-2.64 e	86.4 b	88.7
	ROOTPAC 20	-3.20	-2.87 cd	85.8 b	87.4
	ROOTPAC® R	-2.95	-2.55 e	86.7 b	88.5
Drought	CADAMAN	-3.07	-3.04 bc	82.4 a	80.6
	GF 677	-3.08	-3.33 a	85.9 b	80.8
	ROOTPAC 20	-3.21	-3.19 ab	86.1 b	82.7
	ROOTPAC® R	-3.14	-3.33 a	82.5 a	79.4
Significance					
Treatment		***	***	***	***
Genotype		**	ns	ns	ns
Treatment × Genotype		ns	***	**	ns

Two-way ANOVA was performed for linear model, on raw data. Significance: ***, $P \leq 0.001$; **, $P \leq 0.01$; ns, not significant. Comparison means by Duncan's test ($P < 0.05$) were shown for the significant interaction between treatment and genotype. Different letters indicate significant differences among data within the same factor or interaction.

Table 3. Scion leaf soluble sugar and proline (mg g^{-1} DW) concentration (n=6) in control and drought-stressed *Prunus* rootstocks (Cadaman, GF 677, ROOTPAC 20 and ROOTPAC® R) budded with var. 'Catherina', after 16 days of treatment.

		Fructose	Glucose	Raffinose	Sorbitol	Sucrose	Xylose	Total sugars	Proline
Main factors									
Treatment	Control	13.2	25.8	0.35	107	57.0	1.21	205	1.2
	Drought	12.7	24.2	0.31	117	52.3	1.25	207	0.9
Genotype	CADAMAN	13.1 b	27.1	0.33 b	115	48.8	1.29	205	1.0 b
	GF 677	14.2 b	25.6	0.44 b	110	57.2	1.43	208	1.5 c
	ROOTPAC 20	10.5 a	22.6	0.20 a	105	53.3	1.01	193	0.6 a
	ROOTPAC® R	13.9 b	24.6	0.33 b	118	59.5	1.18	217	1.2 bc
Interaction									
Control	CADAMAN	13.0	27.3	0.21 ab	113	52.2	0.87 ab	206	1.0 bc
	GF 677	14.4	28.9	0.44 c	105	58.3	1.35 bcd	209	1.8 d
	ROOTPAC 20	10.3	20.8	0.30 bc	93	51.2	1.11 abc	176	0.5 a
	ROOTPAC® R	15.3	26.5	0.48 c	121	68.1	1.58 cd	233	1.5 d
Drought	CADAMAN	13.2	26.9	0.46 c	117	45.3	1.70 c	205	1.0 bc
	GF 677	13.9	22.3	0.44 c	114	56.1	1.51 cd	208	1.2 cd
	ROOTPAC 20	10.8	24.7	0.08 a	120	55.9	0.88 ab	213	0.6 ab
	ROOTPAC® R	12.7	23.0	0.20 ab	116	52.4	0.86 a	205	0.9 bc
Significance									
Treatment		ns	ns	ns	ns	ns	ns	ns	ns
Genotype		**	ns	**	ns	ns	ns	ns	***
Treatment × genotype		ns	ns	*	ns	ns	***	ns	*

Two-way ANOVA analysis was performed for linear model, on raw data. Significance: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; ns, not significant. Comparison means by Duncan's test ($P < 0.05$) were shown for the significant interaction between treatment and genotype. Different letters indicate significant differences among data within the same factor or interaction.

Table 4. Root soluble sugar and proline (mg g^{-1} DW) concentration (n=6) in control and drought-stressed *Prunus* rootstocks (Cadaman, GF 677, ROOTPAC 20 and ROOTPAC® R) budded with var. 'Catherina', after 16 days of treatment.

		Fructose	Glucose	Raffinose	Sorbitol	Sucrose	Xylose	Total sugars	Proline
Main factors									
Treatment	Control	7.6 b	23.3	2.0	13.2	27.8	0.7	74.6	0.7
	Drought	5.4 a	25.1	2.6	15.1	23.5	0.6	72.3	0.8
Genotype	CADAMAN	4.8 a	26.2	3.8 b	13.1	27.2 b	0.2 a	75.2	0.8
	GF 677	6.6 ab	26.9	1.3 a	14.8	17.8 a	0.9 b	68.4	0.8
	ROOTPAC 20	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	ROOTPAC® R	8.0 b	19.7	2.1 a	14.4	32.0 b	0.8 b	77.0	0.7
Interaction									
Control	CADAMAN	4.9	24.9	3.2	11.3	26.7	0.3	71.3	0.7
	GF 677	8.8	25.8	1.0	13.8	20.2	1.1	70.7	0.7
	ROOTPAC 20	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	ROOTPAC® R	8.9	19.8	1.9	14.2	35.1	0.7	80.6	0.7
Drought	CADAMAN	4.7	27.4	4.3	14.9	27.7	0.2	79.1	0.8
	GF 677	4.7	27.9	1.5	15.6	15.9	0.8	66.4	0.9
	ROOTPAC 20	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	ROOTPAC® R	7.0	19.5	2.4	14.7	28.4	0.8	72.7	0.7
Significance									
Treatment		**	ns	ns	ns	ns	ns	ns	ns
Genotype		**	ns	***	ns	*	*	ns	ns
Treatment × genotype		ns	ns	ns	ns	ns	ns	ns	ns

Two-way ANOVA was performed for linear model, on raw data. Significance: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; ns, not significant. N/D, not determined. Comparison means by Duncan's test ($P < 0.05$) were shown for the significant interaction between treatment and genotype. Different letters indicate significant differences among data within the same factor or interaction.

Table 5. Scion leaf soluble sugar and proline (mg g^{-1} DW) concentration (n=6) in control and drought-stressed *Prunus* rootstocks (Cadaman, GF 677, ROOTPAC 20 and ROOTPAC® R) budded with var. 'Catherina', after 26 days of treatment.

		Fructose	Glucose	Raffinose	Sorbitol	Sucrose	Xylose	Total sugars	Proline
Main factors									
Treatment	Control	10.5 b	21.1	0.2 a	88 a	44.8 b	0.8 a	165	0.9 a
	Drought	7.9 a	19.1	0.3 b	104 b	32.6 a	1.0 b	165	1.5 b
Genotype	CADAMAN	11.0 c	24.5 c	0.2 a	110 b	40.6	1.0 b	187 c	0.9 a
	GF 677	11.6 c	24.8 c	0.5 b	97 b	35.6	1.4 c	171 bc	1.3 ab
	ROOTPAC 20	5.7 a	12.6 a	0.3 a	72 a	43.2	0.5 a	134 a	1.2 ab
	ROOTPAC® R	8.1 b	17.2 b	0.1 a	99 b	35.8	0.7 a	161 b	1.5 b
Interaction									
Control	CADAMAN	11.3 d	23.5 c	0.2	96	44.5	0.8	176	0.7
	GF 677	14.8 e	29.8 d	0.5	91	44.6	1.5	183	1.2
	ROOTPAC 20	6.5 ab	11.0 a	0.1	75	49.1	0.4	142	0.8
	ROOTPAC® R	9.5 cd	19.8 bc	0.1	88	41.2	0.6	159	1.0
Drought	CADAMAN	10.7 d	25.6 cd	0.3	123	36.8	1.3	198	1.1
	GF 677	8.4 bc	19.7 bc	0.5	102	26.7	1.3	159	1.5
	ROOTPAC 20	4.6 a	14.6 ab	0.5	68	35.8	0.5	124	1.4
	ROOTPAC® R	6.9 b	15.0 ab	0.2	108	31.3	0.8	163	2.1
Significance									
Treatment		***	ns	**	**	**	*	ns	***
Genotype		***	***	***	***	ns	***	***	*
Treatment × genotype		*	*	ns	ns	ns	ns	ns	ns

Two-way ANOVA was performed for linear model, on raw data. Significance: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; ns, not significant. Comparison means by Duncan's test ($P < 0.05$) were shown for the significant interaction between treatment and genotype. Different letters indicate significant differences among data within the same factor or interaction.

Table 6. Root soluble sugar and proline (mg g^{-1} DW) concentration (n=6) in control and drought-stressed *Prunus* rootstocks (Cadaman, GF 677, ROOTPAC 20 and ROOTPAC® R) budded with var. 'Catherina', after 26 days of treatment.

		Fructose	Glucose	Raffinose	Sorbitol	Sucrose	Xylose	Total sugars	Proline
Main factors									
Treatment	Control	9.7 b	24.6 b	2.5 a	15.4 b	28.2 b	1.3 b	81.8 b	1.0 a
	Drought	4.8 a	17.0 a	3.8 b	9.4 a	17.9 a	0.7 a	53.6 a	2.0 b
Genotype	CADAMAN	4.7 a	20.1 b	4.6 b	15.5 b	20.0	0.9	65.7	1.1 a
	GF 677	8.9 b	25.0 b	2.0 a	10.4 a	23.2	0.9	70.4	1.1 a
	ROOTPAC 20	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	ROOTPAC® R	8.6 ab	16.1 a	2.9 a	10.8 a	27.2	1.2	66.7	2.5 b
Interaction									
Control	CADAMAN	4.6 a	22.5 bc	4.1	19.1	21.9	1.0	73.2	1.0 a
	GF 677	12.6 b	28.5 c	1.5	12.9	29.0	1.2	85.7	0.7 a
	ROOTPAC 20	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	ROOTPAC® R	13.6 b	23.0 bc	1.6	13.1	36.7	1.8	89.8	1.4 a
Drought	CADAMAN	4.7 a	17.2 b	5.2	11.1	17.7	0.8	56.8	1.2 a
	GF 677	5.8 a	22.2 bc	2.4	8.4	18.3	0.6	57.6	1.5 a
	ROOTPAC 20	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D
	ROOTPAC® R	3.5 a	9.1 a	4.1	8.6	17.7	0.6	43.5	3.6 b
Significance									
Treatment		***	***	*	***	**	**	***	**
Genotype		*	*	**	*	ns	ns	ns	***
Treatment × genotype		**	**	ns	ns	ns	ns	ns	*

Two-way ANOVA was performed for linear model, on raw data. Significance: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; ns, not significant. N/D, not determined. Comparison means by Duncan's test ($P < 0.05$) were shown for the significant interaction between treatment and genotype. Different letters indicate significant differences among data within the same factor or interaction.

Table 7. Pearson's correlation coefficients for physiological and biochemical parameters at the end of the experimental period (26 days).

		Ψ_π	WUE
Leaf	Fructose	0.51 ***	-0.35 *
	Sucrose	0.56 ***	-0.56 ***
	Sorbitol	-0.37 *	0.36 *
	Proline	-0.65 ***	0.65 ***
Root	Raffinose	ns	0.44 *
	Sucrose	0.53 ***	ns
	Sorbitol	0.48 **	-0.58 ***
	Xylose	0.56 ***	ns
	Proline	-0.44 *	0.55 ***

Significance: ***, $P \leq 0.001$; **, $P \leq 0.01$; *, $P \leq 0.05$; ns, not significant.

Supplemental Table S1. Putative name, genome database for Rosaceae (GDR) identification codes and primer sequences used in real-time PCR of the genes assayed.

Putative gene function	GDR ID	Forward (F) and reverse (R) primer sequences	Amplicon size (bp)
AGL-26 like	ppa010708m	F 5'-TGCAACAGTGAAACATTTGG-3' R 5'-CATACAAACGAATGCCAACA-3'	103
Raffinose synthase (*SIP1*)	ppa001744m	F 5'-GGTGCCATCCAGTCCTTTGT-3' R 5'-TGCCCTCAATCCTGCAACTT-3'	121
Δ-1-pyrroline-carboxylate synthase (*P5SC*)	ppa002098m	F 5'-CGAATTGCTGTGGATGCAAAAGT-3' R 5'-GCGAAGGTCAACCACAAGATCA-3'	121
Sorbitol 6-phosphate dehydrogenase (*S6PDH*)	ppa009007m	F 5'-ACATGGCACGACATGGAAAAGAC-3' R 5'-AATTGGCTCACTTGAGGCTTGAT-3'	128
Sorbitol dehydrogenase (*SDH*)	ppa007458m	F 5'-CGAAGTTGGTAGCTTGGTGAAGA-3' R 5'-CTTGCACTGCTCACATCTCCA-3'	91

Figure legends

Figure 1. Daily soil volumetric water content of control and drought stressed pots containing *Prunus* rootstocks (Cadaman, CD; GF 677, GF; ROOTPAC 20, R20; and ROOTPAC® R, RR) budded with var. 'Catherina'. Each data point is the average of at least 6 pots.

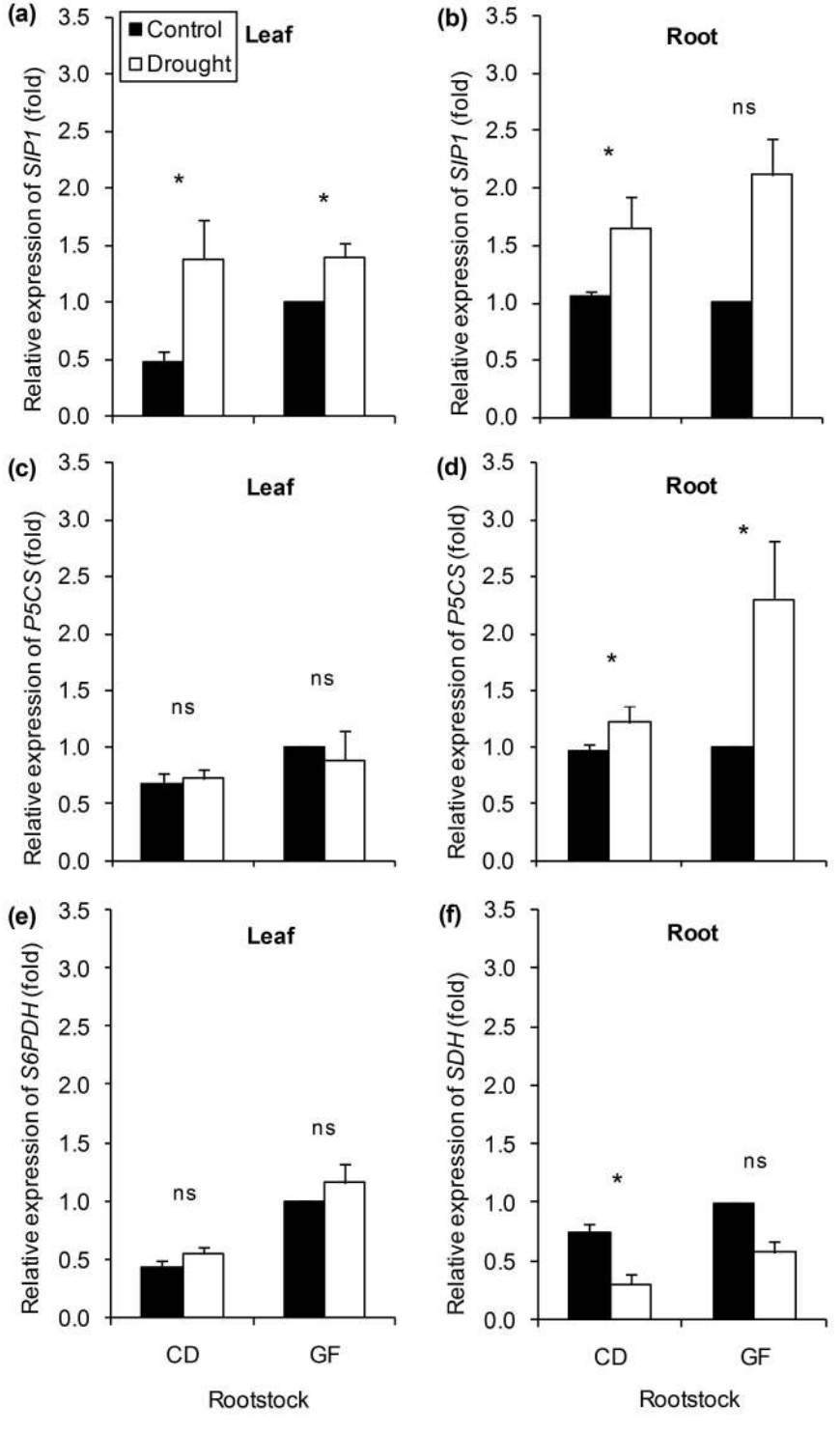
Figure 2. Daily scion apical growth of control and drought-stressed *Prunus* rootstocks Cadaman (a), GF 677 (b), ROOTPAC 20 (c) and ROOTPAC® R (d) budded with var. 'Catherina'. Vertical bars indicate the SE (n=5). Significant growth decrease (*, $P < 0.05$) in the drought vs. control treatment was indicated by an arrow (t-test).

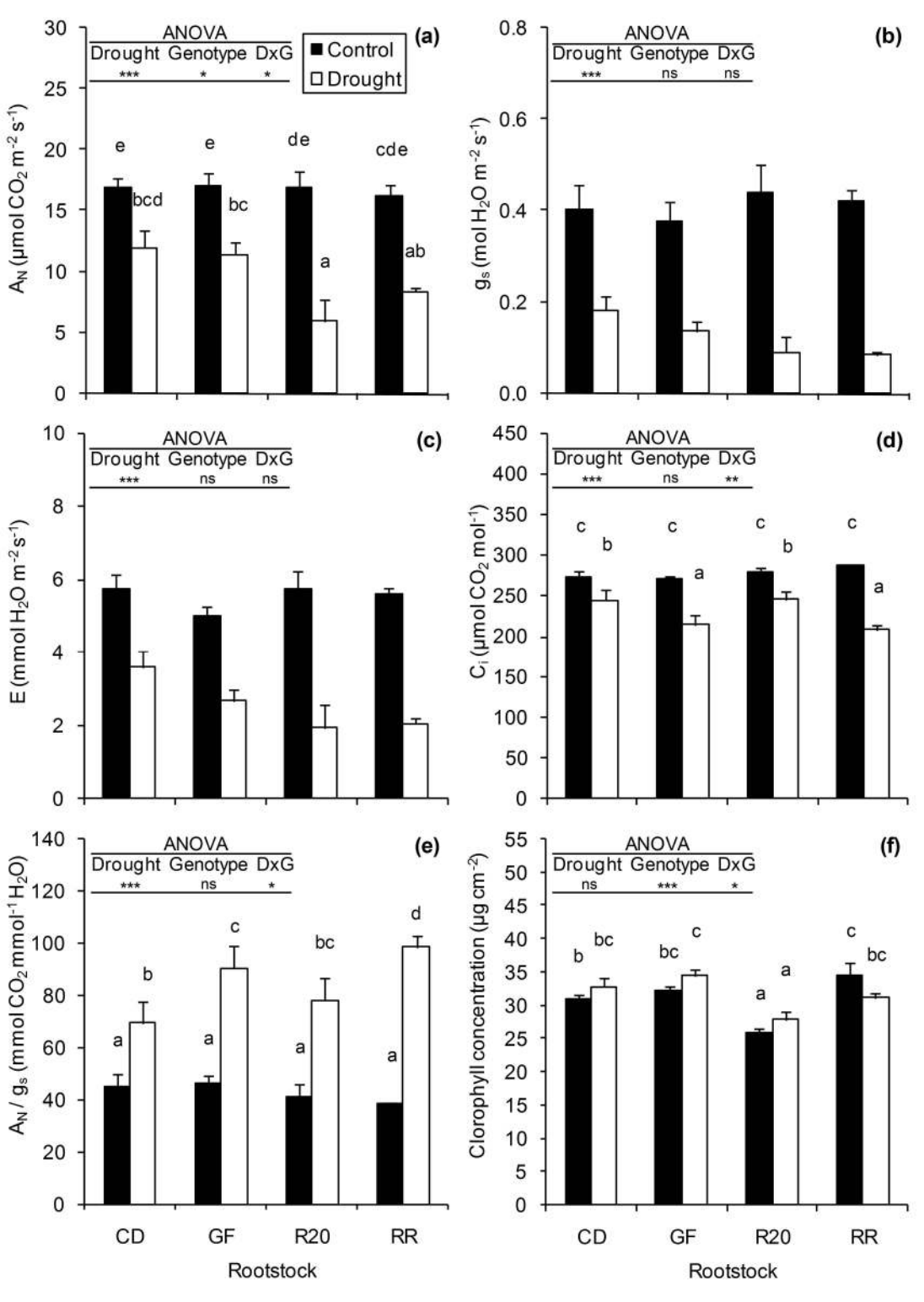
Figure 3. Midday stem water potential (Ψ_s) in scion leaves of control and drought-stressed *Prunus* rootstocks Cadaman (a), GF 677 (b), ROOTPAC 20 (c) and ROOTPAC® R (d) budded with var. 'Catherina'. Vertical bars indicate the SE (n=6). Different letters indicate significant differences among genotypes for drought treatment (Duncan's test $P < 0.05$).

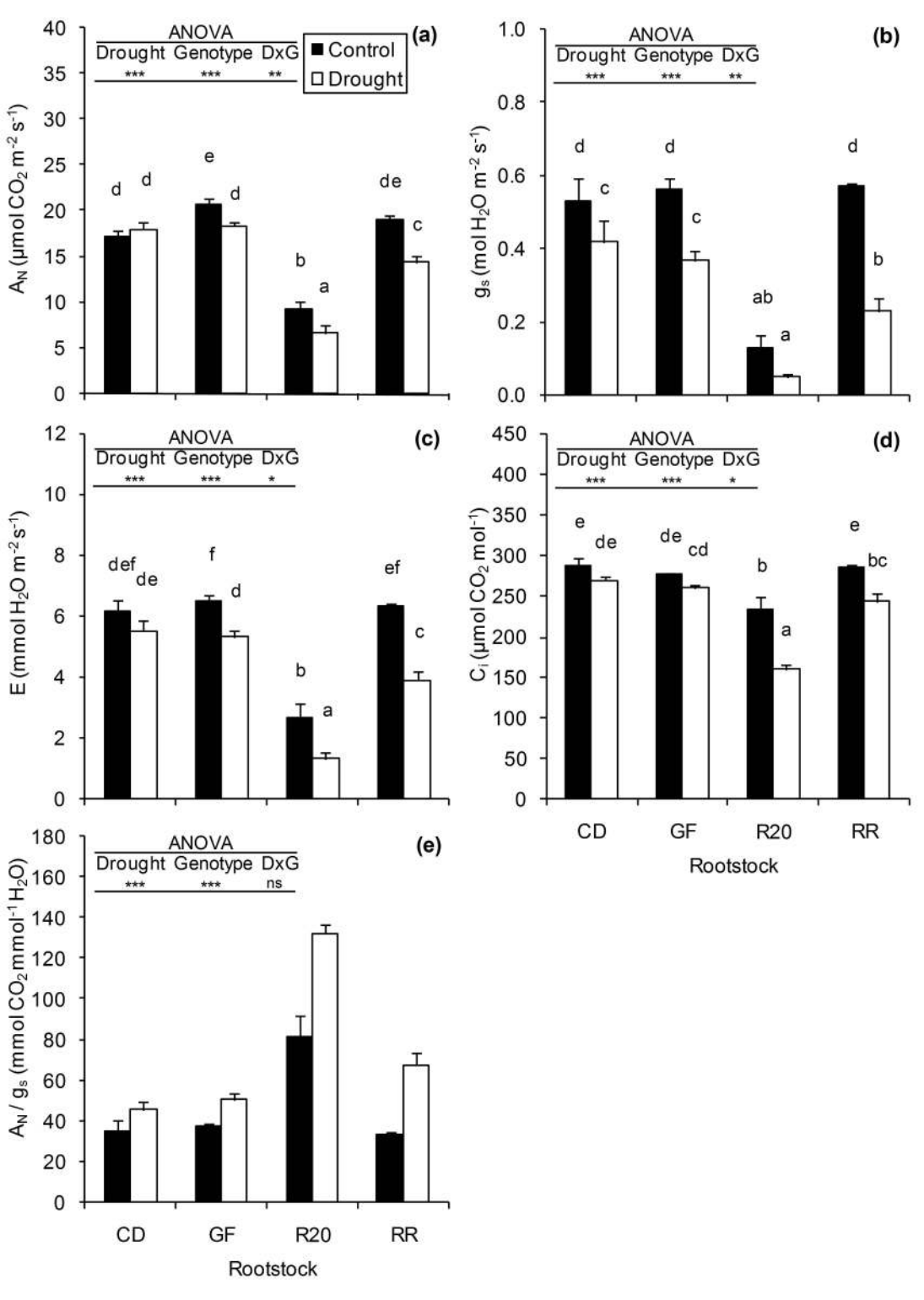
Figure 4. Photosynthesis rate (A_N) (a), stomatal conductance (g_s) (b), transpiration rate (E) (c) intercellular CO_2 concentration (C_i) (d) and water use efficiency (A_N/g_s) (e) in control and drought-stressed *Prunus* rootstocks (Cadaman, CD; GF 677, GF; ROOTPAC 20, R20; and ROOTPAC® R, RR) budded with var. 'Catherina' after 16 days of treatments. Vertical bars indicate the SE (n=6). Comparison means by Duncan's test ($P < 0.05$) were shown for the significant interaction between drought (D) and genotype (G). Different letters indicate significant differences.

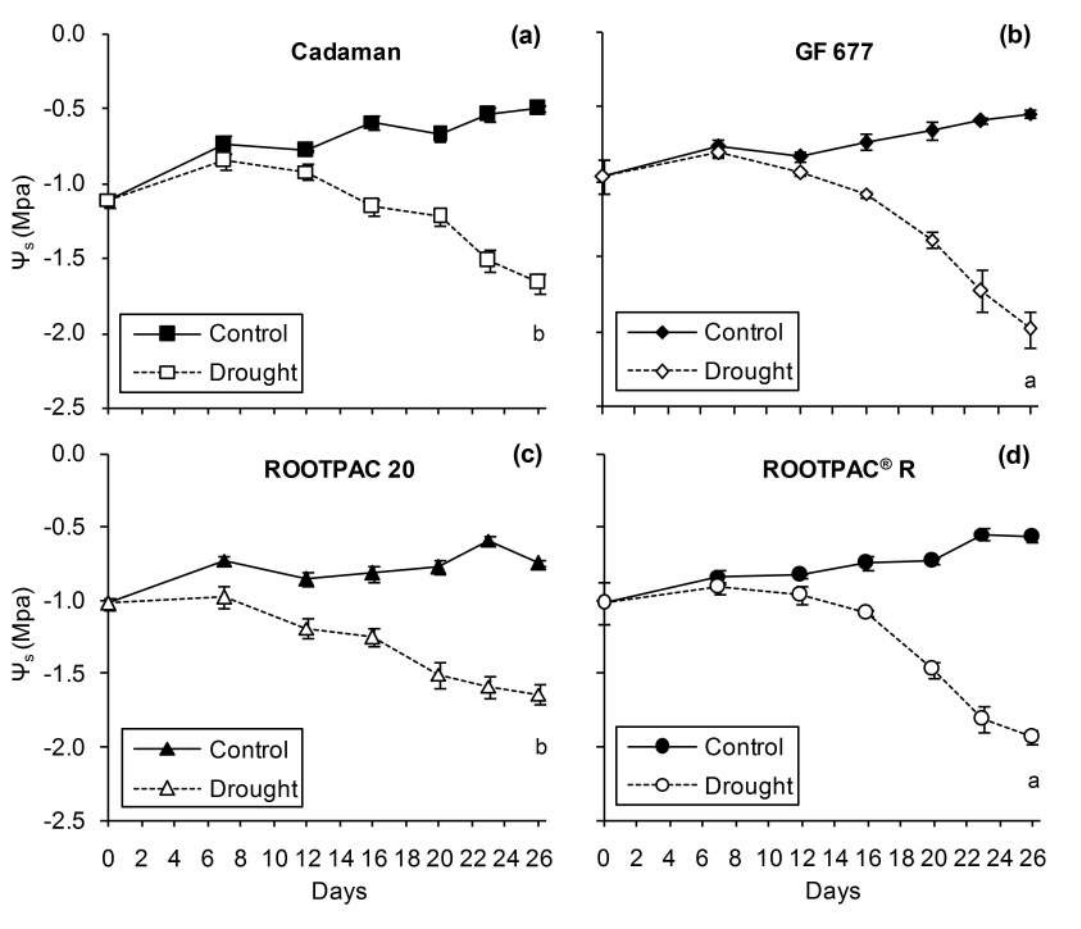
Figure 5. Photosynthesis rate (A_N) (a), stomatal conductance (g_s) (b), transpiration rate (E) (c) intercellular CO_2 concentration (C_i) (d), water use efficiency (A_N/g_s) (e) and chlorophyll concentration (f) in control and drought-stressed *Prunus* rootstocks (Cadaman, CD; GF 677, GF; ROOTPAC 20, R20; and ROOTPAC® R, RR) budded with var. 'Catherina' after 26 days of treatments. Vertical bars indicate the SE (n=6). Comparison means by Duncan's test ($P < 0.05$) were shown for the significant interaction between drought (D) and genotype (G). Different letters indicate significant differences.

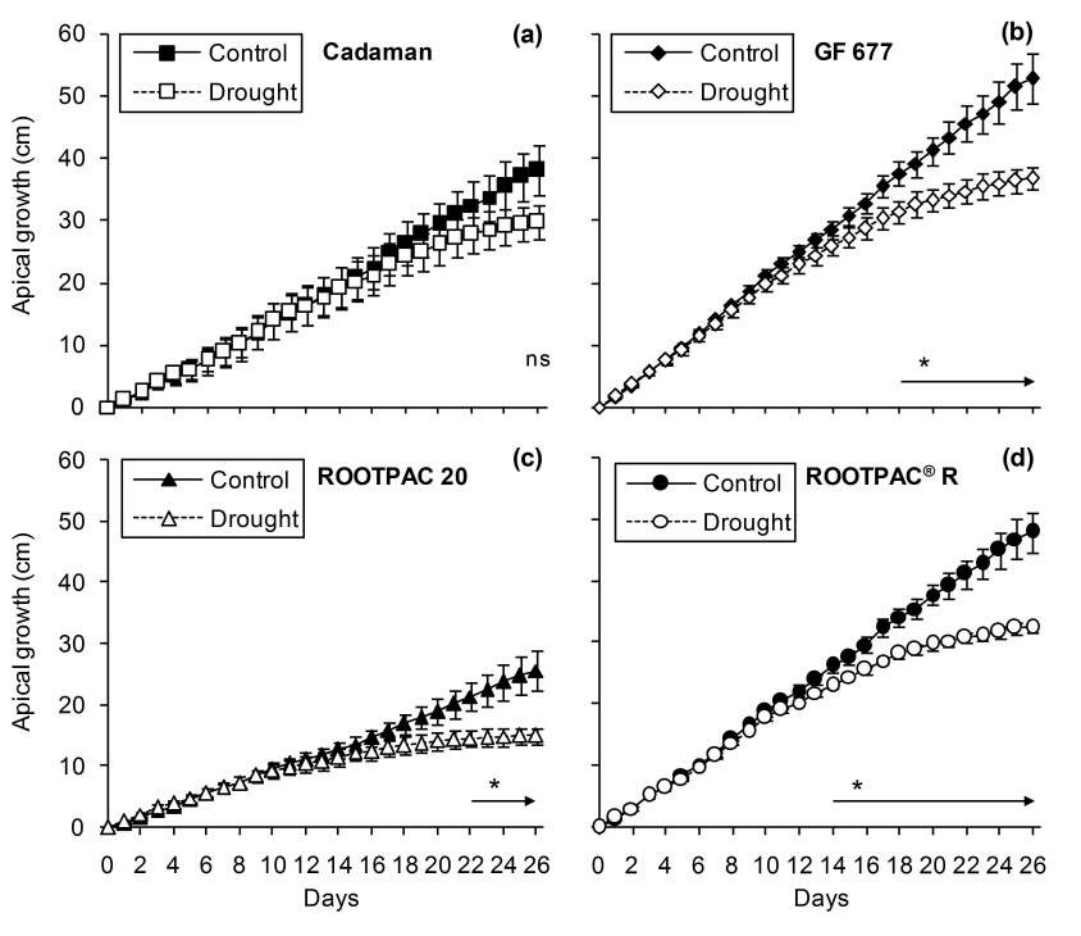
Figure 6. Expression profiles of *raffinose synthase* (*SIP1*) in scion leaves (a) and roots (b), *Δ-1-pyrroline-carboxylate synthase* (*P5SC*) genes in scion leaves (c) and roots (d), *sorbitol 6-phosphate dehydrogenase* (*S6PDH*) gene in scion leaves (e) and *sorbitol dehydrogenase* (*SDH*) gene in roots (f) of Cadaman (CD) and GF 677 (GF) rootstocks budded with var. 'Catherina' and submitted to control and drought treatments during 16 days. Gene expression is shown relative to control plants budded on GF 677. Error bars indicate the standard error (n=4). Asterisks indicate significance of difference between control and drought treatments: ns, not significant; *, $P < 0.05$.

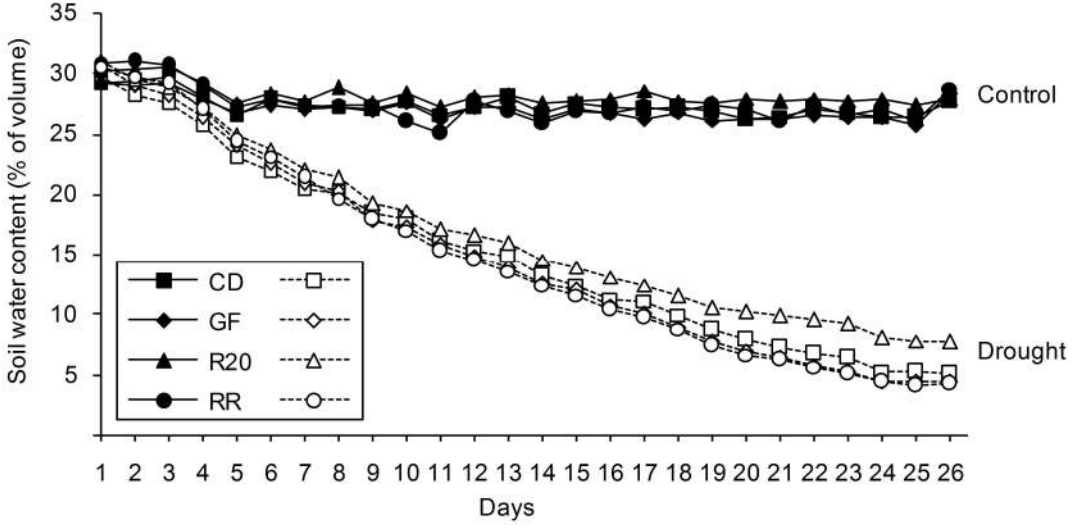

(a)
Control
Drought
Leaf
Relative expression of SlP1 (fold)
(b)
Root
Relative expression of SlP1 (fold)
ns
(c)
Leaf
Relative expression of P5CS (fold)
ns
(d)
Root
Relative expression of P5CS (fold)
(e)
Leaf
Relative expression of S6PDH (fold)
ns
(f)
Root
Relative expression of SDH (fold)
ns
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
CD
GF
Rootstock


(a)
ANOVA
Drought Genotype DxG
*** * *
Control
Drought
A_N (µmol CO_2 m^{-2} s^{-1})
e e de cde
bcd bc a ab
(b)
ANOVA
Drought Genotype DxG
*** ns ns
g_s (mol H_2O m^{-2} s^{-1})
(c)
ANOVA
Drought Genotype DxG
*** ns ns
E (mmol H_2O m^{-2} s^{-1})
(d)
ANOVA
Drought Genotype DxG
*** ns **
C_i (µmol CO_2 mol^{-1})
c b c a c b c a
(e)
ANOVA
Drought Genotype DxG
*** ns *
A_N / g_s (mmol CO_2 $mmol^{-1}$ H_2O)
a b a c a bc a d
(f)
ANOVA
Drought Genotype DxG
ns *** *
Clorophyll concentration (µg cm^{-2})
b bc bc c a a c bc
CD GF R20 RR
Rootstock
CD GF R20 RR
Rootstock


(a)
ANOVA
Drought Genotype DxG
*** *** **
Control
Drought
A_N (µmol CO_2 m^{-2} s^{-1})
(b)
ANOVA
Drought Genotype DxG
*** *** **
g_s (mol H_2O m^{-2} s^{-1})
(c)
ANOVA
Drought Genotype DxG
*** *** *
E (mmol H_2O m^{-2} s^{-1})
(d)
ANOVA
Drought Genotype DxG
*** *** *
C_i (µmol CO_2 mol^{-1})
CD
GF
R20
RR
Rootstock
(e)
ANOVA
Drought Genotype DxG
*** *** ns
A_N / g_s (mmol CO_2 $mmol^{-1}$ H_2O)
CD
GF
R20
RR
Rootstock


Cadaman
(a)
GF 677
(b)
ROOTPAC 20
(c)
ROOTPAC® R
(d)
Ψs (Mpa)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Control
Drought
b
a
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Days


(a)
Cadaman
Control
Drought
ns
(b)
GF 677
Control
Drought
*
(c)
ROOTPAC 20
Control
Drought
*
(d)
ROOTPAC® R
Control
Drought
*
Apical growth (cm)
Apical growth (cm)
Days
Days
0 2 4 6 8 10 12 14 16 18 20 22 24 26
0 10 20 30 40 50 60


Soil water content (% of volume)
35
30
25
20
15
10
5
CD
GF
R20
RR
Control
Drought
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Days