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ORIGINAL ARTICLE

Physiological drought resistance mechanisms in wild species vs. rootstocks of almond and plum

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Abstract

Key message **Among wild relatives of cultivated almond and plum, a desert almond species, but not a montane plum species, showed higher drought resistance than the common rootstocks routinely used in orchards.**

Abstract Water shortage is a severe environmental factor causing growth disruption and yield-\loss in many agricultural plant species. As fruit trees are likely to suffer from the effects of severe drought in the future, wild relatives of cultivated crops can provide plant breeders a unique material to improve the drought resistance of modern crop varieties. We conducted a drought and rewatering greenhouse experiment along 51 days with young trees of almond (the desert wild species *Prunus ramonensis* vs. the commonly used rootstock hybrid *Prunus dulcis* × *Prunus persica*) and plum (the montane wild species *Prunus ursina* vs. the rootstock *Prunus cerasifera* × *Prunus persica*). To decipher the drought resistance mechanisms in these trees we monitored physiological responses. Expression dynamics of cellular water channels from the plasma intrinsic protein (PIP) aquaporin family were measured in the almond species. Our results indicate a higher drought resistance in wild almond compared to the rootstock, but not in the wild plum species. Under drought, *P. ramonensis* had ~ ninefold higher photosynthesis activity, ~ 50-fold higher water-use efficiency and lower vulnerability to embolism than the rootstock. In the almond species, PIP downregulation was linked with maintenance of hydraulic conductivity, and vice versa for upregulation. This study implies that there is a link between drought resistance in wild tree species and their native habitat conditions, with an advantage for the desert, but not the montane, species. Finally, our study highlights the need to protect and conserve wild relatives of fruit tree species, partly as potential plant materials to be used by breeders to improve the resilience of orchard tree species to drought.

Keywords Wild species · Cultivated species · Water relations · Drought tolerance · Drought · Photosynthesis

Introduction

Abiotic stresses limit plant growth and productivity (Madlung and Comai 2004; Pandey et al. 2017) and might lead to reduced fruit yield and even mortality, e.g. in the case of fruit trees under drought (Wagner et al. 2021). In the past decades, tree breeding programs were mainly focused on yield at the expense of other traits such as abiotic stress resistance (Feurtey et al. 2017; Dalal et al. 2017). Even in commercially grown species, abiotic stress can more aggressively restrict potential yield than biotic stress and can sometimes trigger the outbreak of the latter (Foyer et al. 2016; Hossain et al. 2018). The upsurge in the number of areas affected by droughts, increasing heatwave events, their duration, frequency and intensity due to climate change, turn the discovery of drought resistance traits into an urgent need (Stocker 2014; Dalal et al. 2017; Klein et al. 2019).

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Moreover, drought resistance needs to be seriously addressed when assessing the potential economic profit within a tree breeding program. Incorporating such functional traits in the next generation of tree varieties is seen as a vital step towards ensuring global food security in the background of the increasing global population and diminishing of growing regions (Abdolshahi et al. 2015; Reynolds et al. 2017). Future breeding programs will benefit from a better understanding of the intricate physiological and molecular mechanisms that may improve the drought resistance of trees.

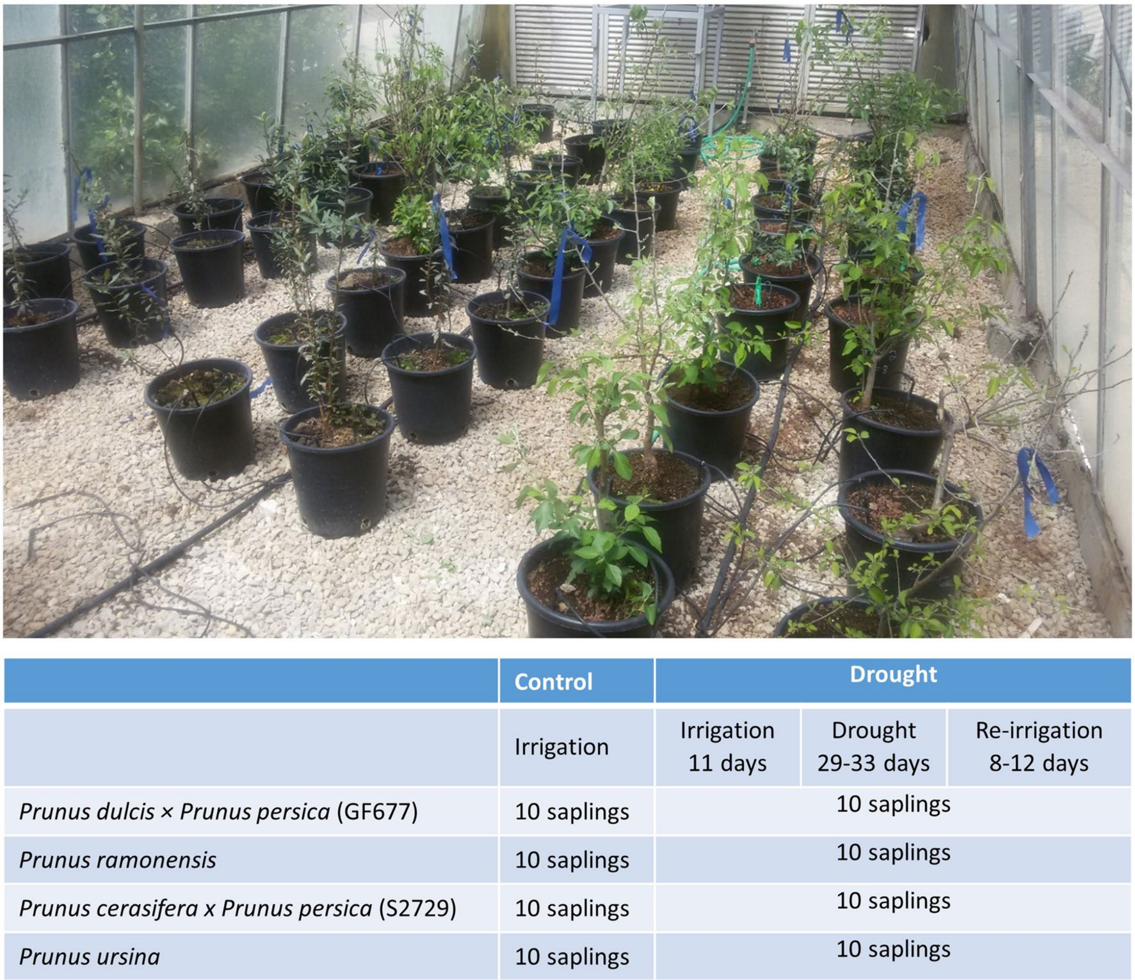
Trees' ability to cope with drought is broadly determined by their capacity to manage their carbon reserves and their hydraulic conductivity (McDowell et al. 2008; Sala et al. 2012; Choat 2013; Blackman et al. 2019). On the one hand, maintaining water status at relatively safe levels via strict stomatal control may lead to carbon reserve depletion. On the other hand, maintaining open stomata allows water potentials to drop close to critical thresholds of xylem embolism, which may lead to hydraulic failure. This tradeoff is the result of responses at physiological and molecular levels. In respect to carbon reserves, starch serves as a buffer for fluctuations in the assimilation rate during periods of insufficient carbon supply due to drought stress (Sala et al. 2012). Regarding the maintenance of hydraulic conductivity, its axial component is regulated by stomata, while its radial component is regulated by aquaporins, which are membrane water channels (AQPs). AQPs belong to a highly conserved group of membrane proteins called major intrinsic proteins that facilitate water transport across biological membranes. Plasma membrane intrinsic proteins (PIPs) are a subfamily of AQPs, taking part in the regulation of water balance and hydraulic repair under water stress, also implied to promote water flow into embolized conduits to restore their transport function (Almeida-Rodriguez and Hacke 2012; Zwieniecki and Secchi 2017). The hydraulic functions of PIPs have been described for leaves and roots, but few studies have focused on the stem (Cochard et al. 2007; Secchi and Zwieniecki 2014, Zwieniecki and Secchi 2017; Paudel et al. 2017). Studying the xylem-specific PIPs expression patterns is essential for understanding their role under drought. Furthermore, the relation between PIPs expression patterns and hydraulic traits should integrate into a more holistic view of tree's drought response.

Almond (*Prunus dulcis* (Mill.) D. A. Webb) and plum (*Prunus domestica* L.) belong to the important genus *Prunus* (family Rosaceae). These trees are adapted to temperate and Mediterranean regions, where they were cultivated in different countries, having a vast economic importance (Xuan et al. 2010; Secchi and Zwieniecki 2014). Productive stone fruit trees are usually grafted on a rootstock having a different genetic background providing an advantage for agronomic purposes (Reighard et al. 1996; Font I Forcada et al. 2009). *Prunus dulcis* × *Prunus persica* (GF677) and *Prunus cerasifera* × *Prunus persica* (S2729) are well-known interspecific hybrids that are used as rootstocks for almond and plum, respectively, and are of interest to breeders and horticulturists worldwide (Arab and Shekafandeh 2016). Northern Israel has a Mediterranean climate, in which summer months are characterized by lack of precipitation, elevated temperatures and high irradiance levels, subjecting native wild tree species to a continuous water deficit. On this basis, wild relatives of commercial species that grow in Israel might serve as a potential source for rootstock material to increase the quality of cultivated almond and plum production under stress conditions. Our recent studies on mature *Prunus* and *Pyrus* species in the field (pear, plum and almond) showed that under drought, both wild pear and wild almond species performed better than their cultivated relatives (Paudel et al. 2019, 2020). Nevertheless, our field comparison was incomplete for three reasons: (1) cultivated and wild trees were growing in separate sites, experiencing different conditions; (2) wild species were compared with their cultivated relatives, and not with the rootstocks which they should potentially replace; and (3) PIPs expression was measured in *Pyrus*, but not in *Prunus* species. Here, in a subsequent greenhouse experiment, we compare common almond and plum rootstocks with their wild relatives, making the first step in the evaluation of these wild species as potential rootstocks in the future. The main objective of this research was to investigate the mechanisms underlying drought resistance of cultivated *Prunus* rootstocks and their wild relatives. Specifically, we aim to (1) test whether wild relatives of cultivated fruit tree species have higher drought resistance than the common rootstocks. (2) Identify the underlying mechanisms of stomatal regulation and water transport under drought in the studied tree species, including interspecies differences. For the almond species, where we confirmed the hypothesis in goal (1), we also aim to (3) identify carbon storage response to drought and (4) quantify the expression profiles of PIP gene family members in the studied tree species. We hypothesized that the wild species have higher drought resistance than the rootstock of their cultivated relatives, facilitated by lower vulnerability to embolism.

Materials and methods

Experiment setup and plant material

The study was conducted on saplings of commercial almond and plum rootstocks and their locally available wild relatives during May–July 2018. These rootstocks (*Prunus dulcis* × *Prunus persica* (GF677) & *Prunus cerasifera* × *Prunus persica* (S2729)) were chosen because they are commonly used in agriculture. *Prunus ramonensis* (Danin) is an



	Control	Drought		
	Irrigation	Irrigation 11 days	Drought 29-33 days	Re-irrigation 8-12 days
Prunus dulcis × Prunus persica (GF677)	10 saplings	10 saplings		
Prunus ramonensis	10 saplings	10 saplings		
Prunus cerasifera x Prunus persica (S2729)	10 saplings	10 saplings		
Prunus ursina	10 saplings	10 saplings		

Fig. 1 *Prunus* saplings in the greenhouse experiment and a schematic representation of the experimental set up

endemic species to Israel found in the central Negev, characterized by its small glabrous fruit and apiculate leaves (Danin 1980; Browicz and Zohary 1996; Zohary and Hopf 2000; Das et al. 2011; Potter 2011). *Prunus ursina* (Kotschy) is native to the Levant and southeast Turkey and is characterized by short pedicels and relatively pubescent stem and leaves. GF677 is genetically compatible with both of its parents and scions grafted onto this rootstock gain a more vigorous growth and drought resistance (Rom 1987; Arab and Shekafandeh 2016). S2729 also contributes to a more vigorous growth (DeJong et al. 1994). To the best of our knowledge, studies regarding the drought resistance of this hybrid are currently lacking. As almond and plum are deciduous species, shedding their leaves during winter, we conducted the experiment in spring when leaves were fully developed. Two-year-old saplings (20 per species) were grown in a greenhouse at the Fiterman Plant Growth Center, Weizmann Institute of Science (Fig. 1). Saplings of rootstocks were purchased from a commercial nursery, where they were propagated from shoot cuttings, and saplings of wild species were collected from the Israel Forest Service (Jewish National Fund) nursery, where they were grown from seeds. Saplings were grown in silty-clay loam 10 L pots inside the ventilated greenhouse and under natural daylight (5–10% light extinction under the glass). Ventilation of the greenhouse helped reducing air temperature and humidity to levels close to ambient levels, 25–35 °C, 50–70% relative humidity, typical for summer in Israel. Saplings were approximately 1 m tall at the onset of the experiment. Mean stem diameter at 10 cm above ground was 8.0 ± 1.2 mm, with individual saplings ranging between 3.3 and 15.6 mm. Based on these dimensions, shoot biomass was estimated at 10 g, and assuming a root/shoot ratio of 0.5, root biomass was 5 g, and whole plant biomass was 15 g. According to Poorter et al. (2012), a plant biomass to pot volume ratio smaller than 2 g L^{-1} (1.5 g L^{-1} in our case) ensures that root binding by pot volume was highly unlikely. About half of the saplings from each species were kept as controls and the remaining half were subjected to drought treatment by withholding irrigation completely for 29 or 33 days (below). Trees were healthy with no visible signs of biotic or abiotic stress. The control group was drip-irrigated with 0.5 L per day which maintained soil moisture of about 20%. Soil water content on a volumetric basis (SWC, % v/v) was measured using a dielectric constant EC-10 soil moisture sensor (Decagon Devices Inc., Pullman, WA, USA). Measurements were performed twice before the dry-down cycle, six times during the dry-down, and twice following re-irrigation.

The duration of the drought treatment (29 and 33 days for *GF677* & *S2729* and *Prunus ramonensis* & *Prunus ursina,* respectively) was determined at the point when stem water potential was below − 6 MPa, a hydraulic damage threshold for many *Prunus* species (Cochard et al. 2008). Following the drought treatment, saplings were subjected to a recovery period (12 and 8 days for *GF677* & *S2729* and *Prunus ramonensis* & *Prunus ursina*, respectively) during which they were re-irrigated with 0.5 L per day. The duration of the recovery period was determined at the point when stem water potential increased back to above − 5 MPa. To study the tree's response to drought, samples were taken and measurements were performed on eleven campaign days at different time points along the experiment: twice before the onset of drought stress, six-seven times during the drought stress, and two–three times following the end of drought stress. In a few cases, instrument malfunction towards the end of the experiment prohibited measurement, and a single data point is reported for the recovery phase. The measurements were performed between 7:30 and 10:30 am. The measured parameters included stem water potential (WP), hydraulic conductivity (Kh) and percent loss of hydraulic conductivity (PLC), leaf gas exchange (rates of photosynthesis, transpiration, and stomatal conductance), starch concentration, and stem diameter at base and height, which were done on three trees per treatment and species. At the end of the experiment, saplings were not sacrificed, and hence biomass measurements were not performed.

Stem water potential and leaf gas exchange

Stem water potential (stem WP) was measured at 7:30 am, i.e. at a time when the plant is assumed to be in close-to-equilibrium with the soil water potential, on leaves sampled from three randomly selected trees per treatment. Stem WP was measured on the same branches used for gas exchange, hydraulics, starch content and transcription level of PIPs measurements. Stem WP was measured using a Scholander-type pressure chamber (PMS Instrument Company, Albany, OR, USA) within 30 min after cutting (branches were kept in a sealed bag until reaching the lab). Leaf CO_2 and H_2O gas exchange measurements were conducted simultaneously on young mature leaves using a Walz photosynthesis system (GFS-3000, Walz, Germany) equipped with a standard 2 × 4 cm leaf cuvette. Measurements were performed at ambient leaf temperature of 25 °C, 50% relative humidity, photosynthetically active radiation of 1200 µmol m^{-2} s^{-1}, 90% red light and 10% blue light, and 400 µmol CO_2 mol^{-1}. Three leaves were measured from each species. These measurements were performed in the morning, i.e. between 07:30 and 10:30, to avoid the midday depression of leaf gas exchange due to the high temperatures in summer noontime. Intrinsic water-use efficiency (WUEi) was calculated as the ratio between the rate of carbon assimilation in photosynthesis (P_n) and stomatal conductance (g_s) according to the following equation:

$$\mathrm{WUEi} = P_\mathrm{n}/g_\mathrm{s}. \tag{1}$$

To characterize the stomatal sensitivity to water potential, the dependence of g_s on stem WP was plotted for each of the species using JMP (Carey, NC, USA), where the 'Logistic 2P fit curve' function was applied. We registered stem WP as the regressor, g_s as the response, and grouped by species. Sigmoidal curves were produced according to the equation:

$$g_\mathrm{s} = \frac{1}{1 + \exp(a(WP - b))}, \tag{2}$$

where a and b are the empiric parameters for the curve's slope and inflection point, respectively.

Xylem hydraulic conductivity and xylem embolism

Three branch segments with lengths of ~ 15 cm each were submerged in water and cut from each tree using protocols adjusted from Sperry et al. (1988) and Wheeler et al. (2013). Branch cutting was done underwater, using a special bottle apparatus adjusted on the branch prior to cutting. Branch length was chosen according to the most common vessel length across the related *Prunus* species (Cochard et al. 2008; Scholz et al. 2013). Branches were placed underwater during transportation to the lab to allow relaxation of the xylem tension. Next, the segments were further cut while submerged in water in the lab on both edges into the final measured size of 10 cm. Xylem hydraulic conductivity was measured with a 70 cm column of water (7 kPa) to capture the native specific hydraulic conductivity (K_s-native), calculated by dividing the flow rate by the pressure gradient and the xylem cross-sectional area (calculated from the diameter of debarked shoots). The same segments were then flushed with 19 mM KCl aqueous solution at a High-Pressure Flow Meter (Dynamax Inc., Texas, USA) (Tyree et al. 1995) for 20 min at pressures of 0.05–0.1 MPa to facilitate the maximum hydraulic conductivity K_s-max. We then calculated the percentage loss of hydraulic conductivity (PLC = 100 × (K_s-max − K_s-native)/ K_s-max), as a measure of xylem transport failure caused by embolism. Our methodological approach yielded similar results with a micro-CT imaging technique, applied on branch segments from mature trees of the species studied here (Paudel et al. 2019). The xylem vulnerability to embolism was approximated using correlations of PLC by stem WP for each of the species to produce empiric curves. To generate these curves, data were uploaded to JMP (Carey, NC, USA), where the 'fit curve' function was applied. We assigned stem WP as the regressor, PLC as the response, and grouped by species. Sigmoidal curves were produced according to the equation:

$$\mathrm{PLC} = \frac{100}{1 + \exp(a(WP - b))}, \tag{3}$$

where a and b are the empiric parameters for the curve's slope and inflection point, respectively (Pammenter and Van der Willigen 1998).

Statistical analysis

All parameters were each analyzed using a repeated-measurements analysis of variance (ANOVA) with species and drought treatment as fixed factors, assuming the variance within subjects (i.e., the trees) was homogeneous. For all analyses, there were three to four replicates for each treatment and species. Effects of species and treatments and their interaction were tested statistically using two-way ANOVA. When the effects of treatments were statistically significant, differences among groups were post hoc tested with Tukey's HSD test. In figures showing tree responses along time, different letters indicate significant differences among sampling times and species. All analyses were performed in JMP (SAS, Cary, NC, USA).

Results

Soil moisture and stem water potential

Stem water potential values of GF677, *P. ramonensis,* S2729 and *P. ursina* were rather similar, ranging from − 1.2 to − 1.9 MPa at the beginning of the experiment (Fig. 2a–d). Stem WP of the trees under control treatment remained at the same range throughout the experiment. With drought onset, stem WP decreased gradually in GF677 and S2729, reaching a minimum value of − 7.3 MPa in both rootstocks after ~ 30 days without irrigation. In comparison, the *P. ramonensis* and *P. ursina* showed a slightly slower decrease and reaching − 6.6 and − 6.1 MPa at the end of drought; however, species differences were not significant (Table 1). Upon rewatering, stem WP of all species increased back close to the pre-treatment values. Stem WP during drought was significantly different from baseline and re-irrigation values in almond but not in plum, however, the effect of treatment on stem WP was significant across species. At the beginning of the experiment, soil water content (SWC) was not homogeneous among species, yet mostly equated before the drought (Fig. 2f) or on day 11 for control plums (Fig. 2g). Expectedly, following withholding irrigation, SWC rapidly decreased, from 14.5 to 26.3% (v/v) to a minimum of 0.0–1.4% (v/v) during drought (Fig. 2). A rapid recovery with resuming irrigation was observed in all species, and, moreover, in *P. ramonensis*. Overall, differences between species and treatments were significant (Table 1).

Xylem embolism

The four species showed a drought-induced increase in PLC, from 14.7 to 0% and 11.9% and 3.8% at the beginning of drought to 93.4% and 87.4% and 75.4% and 70.4% at the end of drought in *GF677* & *P. ramonensis* and *S2729* & *P. ursina,* respectively (Fig. 3b, d). *GF677* had the steepest increase in PLC while *P. ramonensis* stayed at a consistently low level (23.6–33.2%) during 26 days of drought, followed by a dramatic increase toward the end of drought (87.4%.). The *P. ramonensis* sample taken 12 days after re-irrigation was completely embolised, probably due to delayed drought effect (e.g. water demand exceeding the lowered hydraulic capacity). Both plum species exhibited a similar trend to *GF677*. Variations among trees were larger than in any other parameter, reflecting the complex distribution of embolism in xylem, moreover among different branches of a tree and among different trees. Still, across species, PLC at peak drought was significantly higher than at pre-drought (Fig. 3b, d). The effect of treatment on PLC was significant, although high levels of PLC were also measured in irrigated saplings (Fig. 3a, c), as a result of temperature spikes (up to 35 °C, increasing evaporative demand; see Methods), unmatched by irrigation amounts, which were constant. Notably, irrigated rootstocks had higher and earlier PLC peaks than the native species (Fig. 3a, c). Fitting the correlations between stem WP and PLC among the species displayed four species-specific sigmoidal curves of xylem vulnerability to embolism (Fig. 4). Although these are not proper vulnerability curves (constructed under controlled conditions), they provide a good proxy for the shape of the vulnerability curve for each of the four species. Xylem vulnerability to embolism was greater for the two rootstocks than for the two wild species across the part of the water potential range relevant to drought stress, i.e., between − 3 and − 8 MPa. Almond's

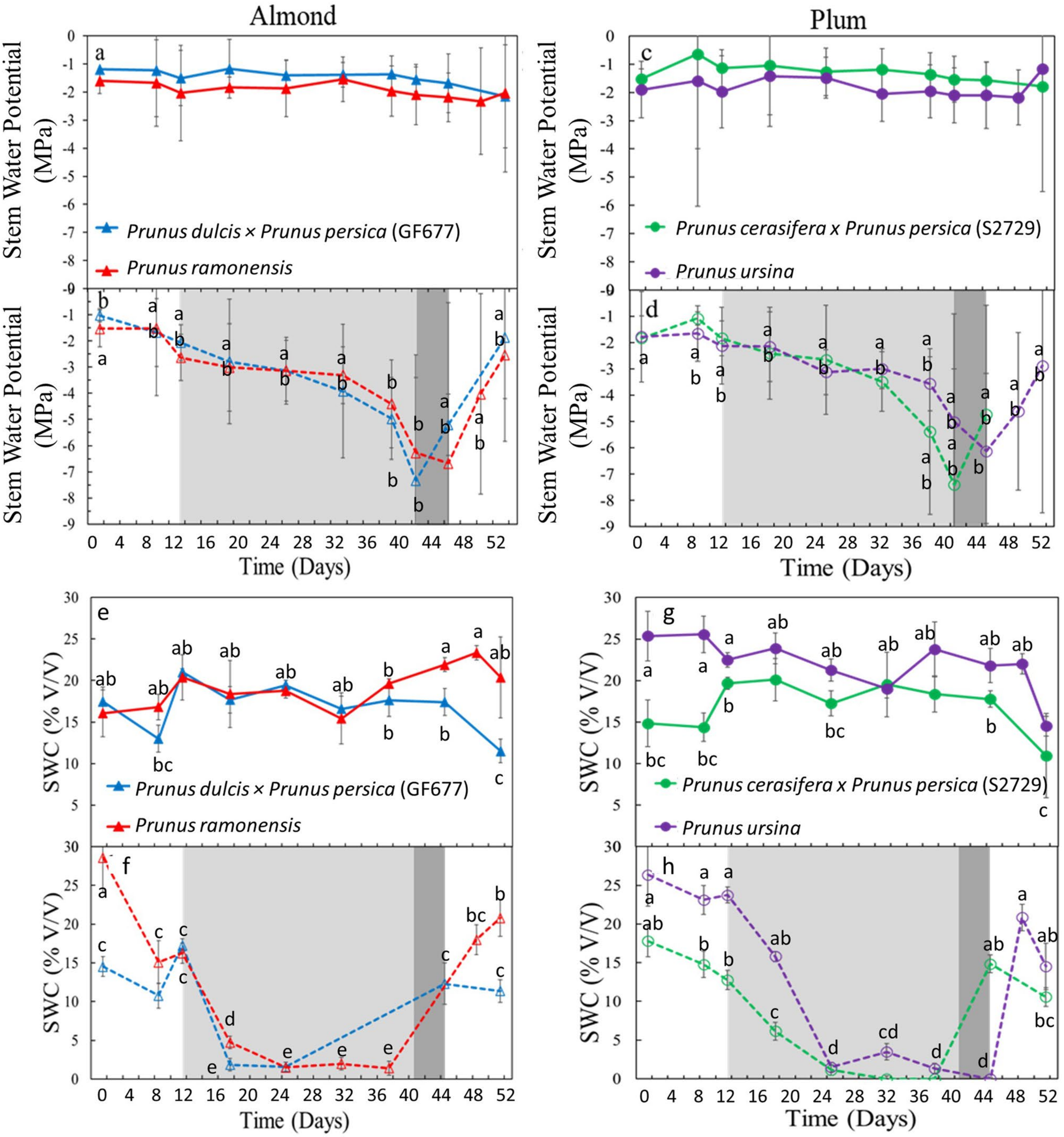


Fig. 2 Stem water potential (**a**–**d**) and soil water content in pots (**e**–**h**) of almond (left columns) and plum trees (right columns) in the greenhouse during 51 days of the experiment. Control group (solid line) and drought treatment (dashed line). Shaded areas, pale and dark denote the 29 and 33 days of drought imposed on drought treatment rootstock and wild species, respectively. Different letters indicate significant differences among sampling times and species. Error bars denote the standard error of the mean ($n = 3$). For stem water potential, instrument malfunction towards the end of the experiment prohibited measurement for drought-induced *Prunus cerasifera* × *persica*, and a single data point is reported for the recovery phase

rootstock and wild species had 50% loss of hydraulic conductivity around − 2.8 MPa and − 4.1 MPa, respectively, and plum's rootstock and wild species had 50% loss of hydraulic conductivity around − 3.3 MPa and − 4.7 MPa, respectively. We note, however, that the curve fitting was weaker for the two wild species than the rootstocks (Fig. S1).

Table 1 *P* values (probability > *F*, an effect is significant if $P < 0.05$, in bold) from ANOVA for species (rootstock vs. wild relative) and treatment (drought vs. control) in almond species (left) and plum (right), including the interaction between species and treatment

Parameters	Species	Treatment	Species × treatment	Species	Treatment	Species × treatment
	Almond			Plum		
Stem WP	0.2313	**< 0.0001**	0.1986	0.7349	**< 0.0001**	**0.0133**
Soil water content	**< 0.0001**	**< 0.0001**	0.1936	**< 0.0001**	**< 0.0001**	0.2978
K_s native	0.6984	**0.014**	0.6717	**0.0131**	0.0711	0.8633
PLC	0.1501	**< 0.0002**	0.5624	0.5619	**0.0029**	0.3177
Transpiration	**< 0.0001**	**< 0.0001**	0.1294	**0.0067**	**< 0.0001**	0.1953
Photosynthesis	0.2413	**< 0.0001**	0.3159	**< 0.0001**	**< 0.0001**	0.1969
Diameter	**< 0.0001**	0.4704	**0.0013**	**< 0.0001**	**0.0002**	**0.021**
Height	**< 0.0001**	**<0.0001**	**< 0.0001**	**< 0.0001**	**0.0019**	**0.0023**
Stem starch	**< 0.0002**	**< 0.0001**	**0.0063**			
PIP1:1	0.0688	0.8481	0.7921			
PIP1:2	0.1590	0.3439	0.3368			
PIP1:3	**0.0388**	0.9581	0.9083			
PIP1:4	**0.0035**	0.3950	0.4575			
PIP2:1	0.0596	0.6620	0.7727			
PIP2:2	0.1173	0.9814	0.9838			
PIP2:4	0.4677	0.5004	0.4677			

Photosynthesis and transpiration

Following irrigation cessation, a dramatic decrease in the photosynthetic activity was observed in *GF677* from 18.3 to 3.1 µmol m^{-2} s^{-1} (Fig. 5a–d). On the contrary, *P. ramonensis* maintained a remarkably steady photosynthetic activity (8.7–10.1 µmol m^{-2} s^{-1}) along with the experiment. In the middle of the drought period there was an increase in photosynthetic activity in the control *GF677* trees, which might be explained by the seasonal increase in solar radiation (Fig. S2). Similar to *GF677*, the photosynthetic activity of *S2729* decreased, yet gradually, with the onset of drought, starting from 22.5 µmol m^{-2} s^{-1} before the drought and reaching a minimum value of 0.3 µmol m^{-2} s^{-1} at the end of the drought. Moreover, the wild species *P. ursina* maintained a relatively stable, low, activity throughout the drought period of 3.8–6.7 µmol m^{-2} s^{-1}, reaching zero only at the end of the drought. Interestingly, *P. ursina* had a lower photosynthesis rate compared to the plum rootstock even under control conditions, and except for ~ 20 days into the drought (Fig. 5c, d). Upon rewatering, photosynthetic activity of S2729 slowly increased. Similar patterns were observed for transpiration (Fig. 5e–h). *GF677* and *P. ramonensis* showed a reduction in transpiration following drought. Under drought, transpiration values fluctuated mostly between 0.1 to 0.9 mmol m^{-2} s^{-1} and 0.3 to 2.3 mmol m^{-2} s^{-1} in *GF677 and P. ramonensis,* respectively. In addition to almonds, there was a reduction in transpiration following drought in *S2729*. In contrast, there was a gradual, not significant, increase in transpiration from 1.3 to 2.6 mmol m^{-2} s^{-1} in *P. ursina.* With resuming irrigation, transpiration increased in the wild species. In the end of the experiment, a decrease in transpiration was observed in the control trees. This coordinates well with the reduction in SWC at the same time point (Fig. 2e–h).

Intrinsic water-use efficiency was lower in *P. ramonensis* than in *GF677* among the control trees and at the beginning of drought, but became ~ 50 fold higher as a drought progressed (Fig. 6). This is probably a result of higher photosynthetic activity at a lower stomatal conductance of the wild species. Intrinsic water-use efficiency was lower in *P. ursina* compared to that of *S2729* in both control and drought treatments. Correlations between stomatal conductance and stem WP showed that *P. ramonensis* had higher stomatal conductance than the other three species and particularly than *GF677* (Fig. 7) at most of the range of stem WP. The opposite relationship was observed among the plum species. *S2729* had higher stomatal conductance than *P. ursina,* yet it was more sensitive to changes in WP than *P. ramonensis*, as well as the other two species.

Stem growth

Changes in stem diameter and height were variable among individual trees, yet several patterns emerged (Table 2). First, almonds increased in height but not in diameter, whereas plums increased in both. Second, growth was typically inhibited in trees under drought compared to control

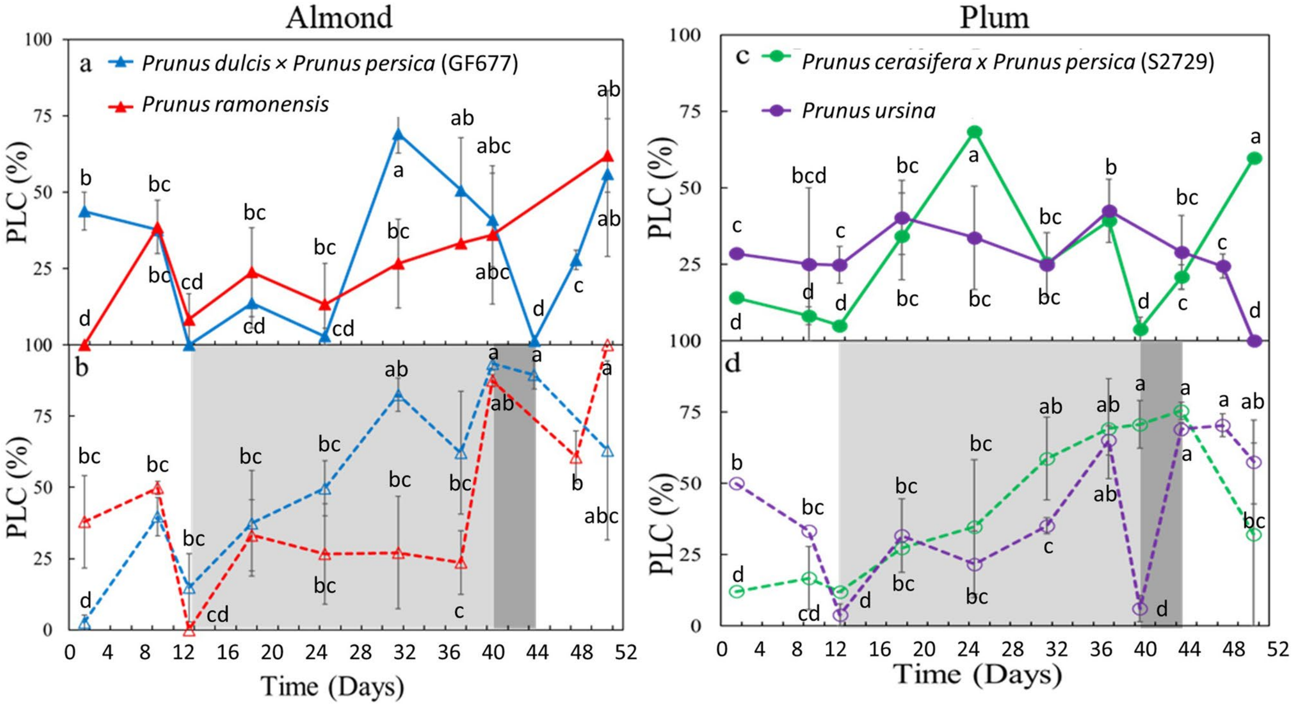


Fig. 3 Percent loss of hydraulic conductivity (PLC) in stems of almond (**a**, **b**) and plum trees (**c**, **d**) in the greenhouse during 51 days of the experiment. Control group (solid line) and drought treatment (dashed line). Shaded areas, pale and dark denote the 29 and 33 days of drought imposed on drought treatment rootstock and wild species, respectively. Different letters indicate significant differences among sampling times and species. Error bars denote the standard error of the mean ($n=3$)

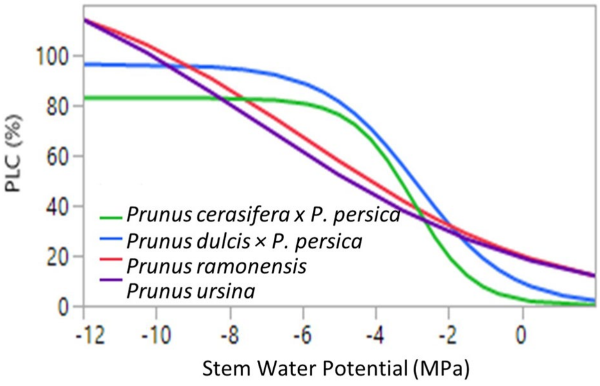


Fig. 4 Xylem vulnerability curves fitted for data points of each of the *Prunus* species. Estimates at PLC > 100 or WP > 0 MPa are due to the low number of measurements at WP < − 6 MPa or WP > − 1 MPa and are apparent only

trees. A single exception was *P. ursina*, where trees under drought maintained their height, while control trees had decreased height, and diameter growth was unaffected by drought. Third, the growth of the commercial hybrids was typically higher than that of the wild species, and more so under irrigation. A notable exception was *P. ramonensis* under drought, which grew in height during the late drought, when *GF677* trees were losing height.

Hydraulic conductivity

Stem-specific hydraulic conductivity (K_s-native) of the almond species decreased following drought. *GF677* showed a faster decrease reaching its minimum values 0.05 kg m^{-1} MPa^{-1} s^{-1} (Fig. 8) after 20 days of drought compared with *P. ramonensis*, which showed mild, and rather stable decrease reaching its minimum value 0.05 kg m^{-1} MPa^{-1} s^{-1} after 33 days of drought. K_s-native of the plum species was relatively stable and showed the same tendency as in the almond, of milder decrease among the wild species compared to the rootstock species. Stem hydraulic conductivity of all species barely recovered with resuming irrigation. In general, differences between species and treatment were significant (Table 1).

Discussion

We presented a study of drought resistance mechanisms in four *Prunus* tree taxa, from the whole-tree scale, through the tissue level. As far as we know, this study is the first study to compare drought resistance mechanisms among common almond and plum rootstocks, especially *Prunus cerasifera* × *Prunus persica*, with their wild relatives.

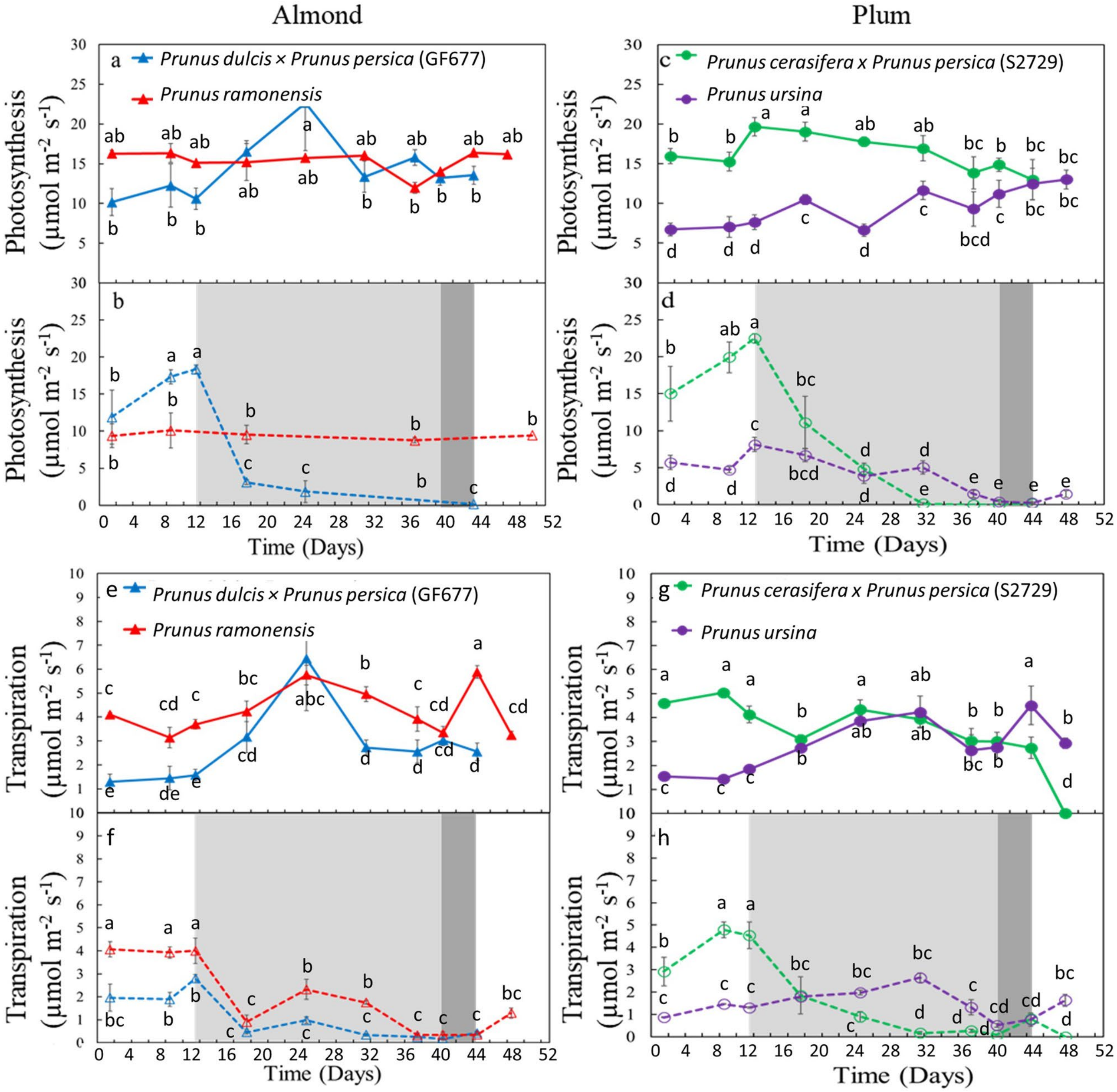


Fig. 5 Leaf photosynthesis (**a**–**d**) and transpiration (**e**–**h**) in almond (left columns) and plum trees (right columns) in the greenhouse during 51 days of the the experiment. Control group (solid line) and drought treatment (dashed line). Shaded areas, pale and dark denote the 29 and 33 days of drought imposed on drought treatment rootstock and wild species, respectively. Different letters indicate significant differences among sampling times and species. Error bars denote the standard error of the mean ($n=3$). Instrument malfunction towards the end of the experiment prohibited measurement for *Prunus dulcis* × *persica* and for *Prunus cerasifera* × *persica*, and a single data point is reported for the recovery phase

Our hypothesis of higher drought resistance in wild vs. cultivated species was confirmed for almonds, but not for plums. Among the almond species, our results showed a clear advantage of the wild species, *P. ramonensis,* over the rootstock *GF677*. This is due to (1) higher photosynthetic activity under drought than that of the rootstock (Fig. 5a–d); (2) higher WUEi under drought (Fig. 6); (3) higher stomatal conductance over a range of stem WP (Fig. 7); (4) higher embolism resistance of the wild species than in the rootstocks (Fig. 4); and (5) higher stem growth under drought than that of the rootstock (Table 2). Among the plum species, we could not obtain such an advantage of the wild species, *P. ursina,* over the rootstock *Prunus cerasifera* × *Prunus persica*. Although the wild plum species was less vulnerable

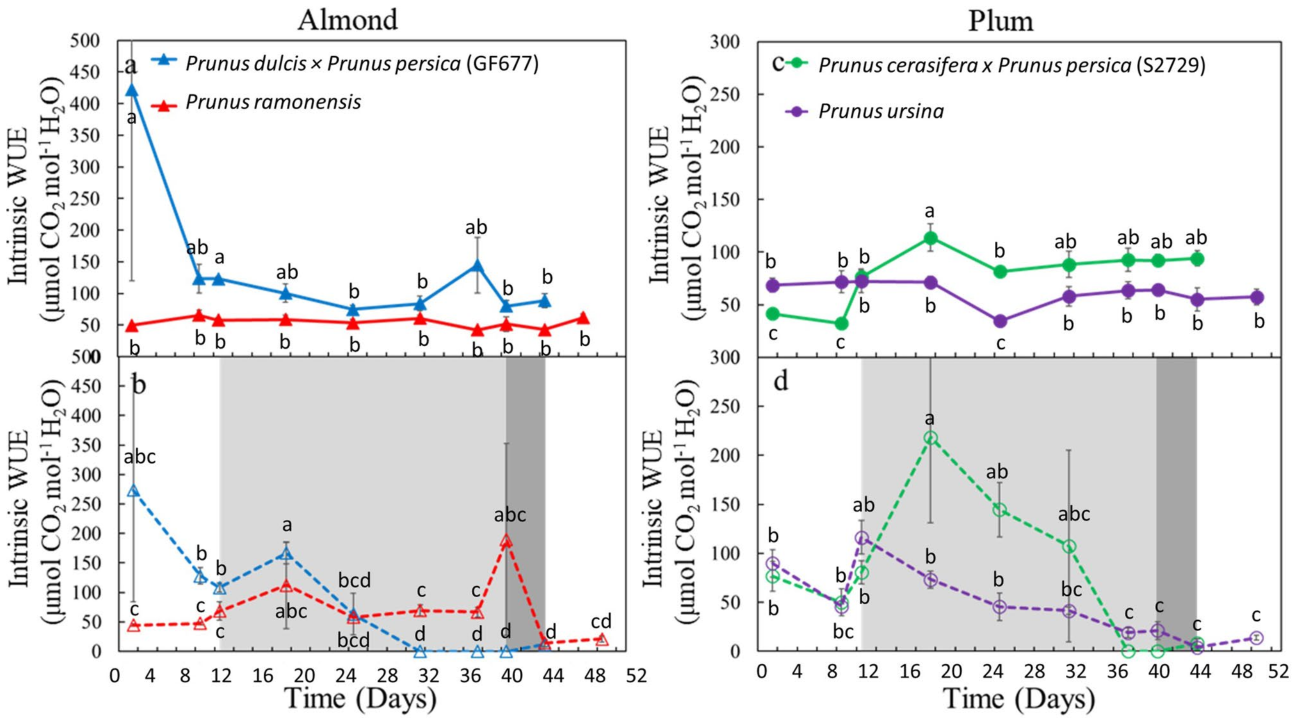


Fig. 6 Intrinsic water use efficiency of almond (**a**, **b**) and plum trees (**c**, **d**) in the greenhouse during 51 days of the experiment. Control group (solid line) and drought treatment (dashed line). Shaded areas, pale and dark denote the 29 and 33 days of drought imposed on drought treatment rootstock and wild species, respectively. Different letters indicate significant differences among sampling times and species. Error bars denote the standard error of the mean ($n=3$). Instrument malfunction towards the end of the experiment prohibited measurement for *Prunus dulcis* × *persica* and for *Prunus cerasifera* × *persica*, and a single data point is reported for the recovery phase

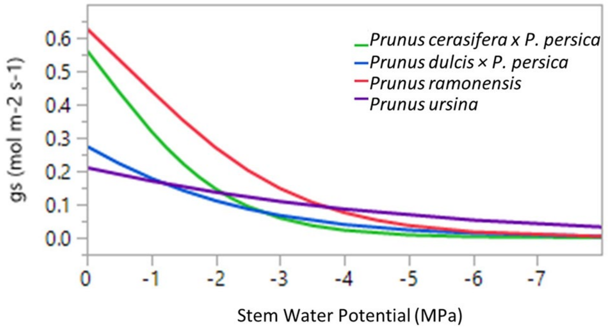


Fig. 7 Stomatal conductance (g_s) as a function of stem water potential (Ψl) for each of the prunus species

to embolism than the rootstock (Fig. 5), it experienced more negative WP (Fig. 7) and higher levels of embolism (Fig. 3), however, its K_s was less affected by drought (Fig. 8). Furthermore, *P. ursina* had lower WUEi under drought compared to the plum rootstock (Fig. 6). Finally, the rootstock had higher stomatal conductance than the wild species over a range of stem WP (Fig. 7).

P. ramonensis had higher photosynthetic activity that seemingly was not affected by drought and was well-maintained (~ 10 µmol m^{-2} s^{-1}) under drought compared to that of *GF677* (Fig. 5a–d). This observation could be partly explained by its smaller leaves, at otherwise similar leaf number and thickness of the almond rootstock hybrid. As *P. ramonensis* is endemic to the hyper-arid Negev desert, having small leaves is an advantage in hot and dry environments with high intensities of solar radiation (Farris 1984; Steppe et al. 2011). This means a greater photosynthetic "apparatus" per unit leaf area, giving rise to higher photosynthetic rate on a leaf area basis. In addition, smaller leaves have a lower surface area, and hence lower evaporative losses. Indeed, our wild almond species had high water use efficiency (Fig. 6). A leaf energy balance model shows that maximizing P_n under high light and temperature requires the smallest leaf size possible (Okajima et al. 2012). This is also in good agreement with Rieger and Duemmel (1992) who showed lower specific leaf area and a higher rate of carbon assimilation in the xeric species, *P. andersonii*, compared to other *Prunus* species from mesic habitats. These values of photosynthesis in *P. ramonensis* under drought were higher with respect to those reported by Wang et al. (2015) for *Prunus mongolica*. Even in the greenhouse we did not obtain better performance of *GF677* compared to the wild species.

Table 2 Stem diameter (mm) and height (cm) increments (mean ± SD; relative to day 0) of almond and plum species during pre-drought (day 8); early drought (day 17); late drought (day 37); and recovery (day 51)

	Stem diameter increment (mm)				Stem height increment (cm)			
	Day 8	Day 17	Day 37	Day 51	Day 8	Day 17	Day 37	Day 51
Almond								
GF677 C	1.0 ± 1.3	1.4 ± 0.8	3.6 ± 1.2	4.2 ± 1.4	11.1 ± 8.0	20.3 ± 9.1	11.8 ± 6.1	11.2 ± 8.0
GF677 D	0.0 ± 0.5	0.0 ± 0.6	0.1 ± 0.5	− 0.1 ± 0.5	− − 7.7 ± 2.5	− 5.4 ± 2.9	− 2.4 ± 4.2	− 3.9 ± 4.6
P. ramonensis C	0.0 ± 0.8	0.4 ± 0.9	0.5 ± 0.8	0.9 ± 0.8	− 0.6 ± 8.0	− 3.3 ± 7.0	9.1 ± 15.1	9.8 ± 15.8
P. ramonensis D	0.7 ± 1.0	0.1 ± 1.6	− 0.5 ± 0.5	0.0 ± 1.3	1.7 ± 8.4	0.0 ± 7.9	5.0 ± 7.3	5.0 ± 7.5
Plum								
S2729 C	13.1 ± 0.2	15.2 ± 1.9	14.1 ± 0.8	13.8 ± 0.4	19.7 ± 11.9	24.7 ± 11.7	25.9 ± 10.8	30.7 ± 9.1
S2729 D	12.2 ± 1.8	12.7 ± 5.4	11.4 ± 5.4	11.1 ± 5.1	− 0.4 ± 13.0	4.3 ± 10.7	5.6 ± 11.9	4.8 ± 12.6
P. ursina C	5.2 ± 0.4	5.9 ± 0.3	5.4 ± 0.6	5.6 ± 0.4	− 1.7 ± 13.2	4.7 ± 9.6	4.7 ± 9.6	− 5.7 ± 3.2
P. ursina D	4.8 ± 0.5	5.4 ± 1.2	5.4 ± 1.0	5.1 ± 1.0	− 3.5 ± 8.6	− 0.5 ± 10.6	0.2 ± 10.9	0.2 ± 7.8

Drought treatment (D) and control (C). Negative values denote a decrease in stem diameter or height (e.g. as a result of apical meristem die-off) $n = 3$

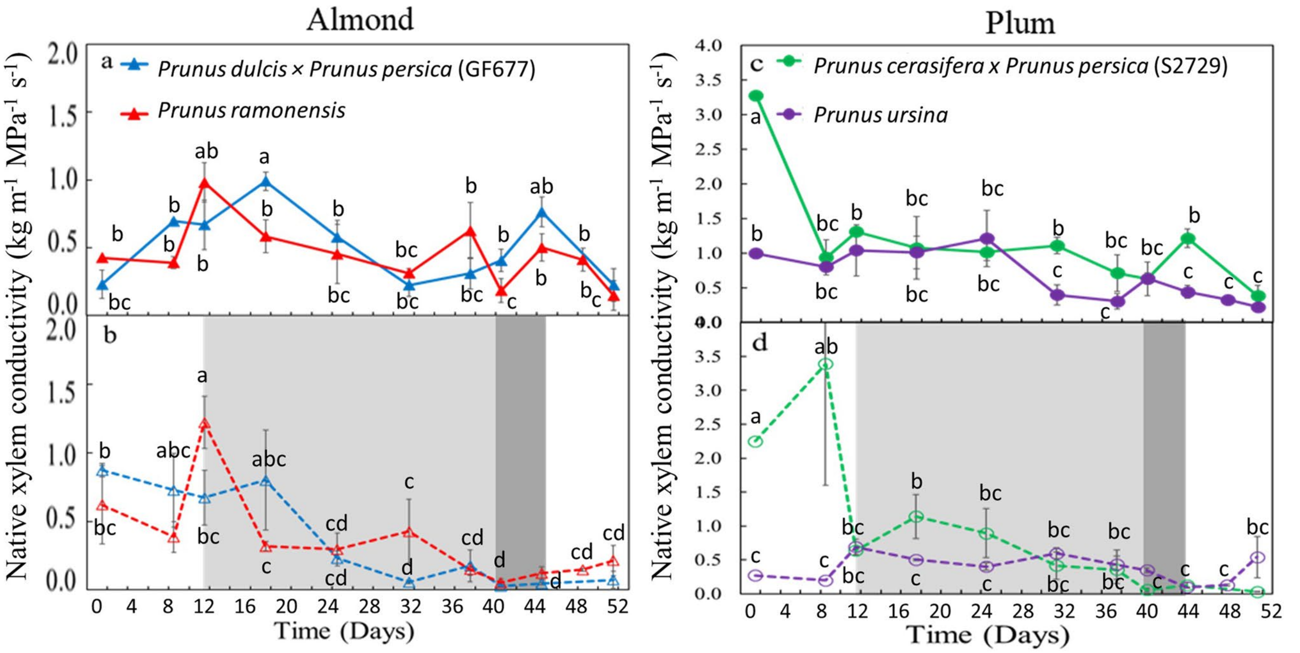


Fig. 8 Native specific hydraulic conductivity (K_s) in stems of almond (**a**, **b**) and plum trees (**c**, **d**) in the greenhouse during 51 days of the experiment. Note the different *y*-axis scale for almond and plum. Control group (solid line) and drought treatment (dashed line). Shaded areas, pale and dark denote the 29 and 33 days of drought imposed on drought treatment rootstock and wild species, respectively. Different letters indicate significant differences among sampling times and species. Error bars denote the standard error of the mean ($n = 3$)

Alternatively, derived from the high temperatures and excessive radiance which *P. ramonensis* has been adapted to in its natural habitat, there is a good probability that the conditions in our greenhouse experiment were favorable and allowed the high photosynthetic activity. Photosynthesis involves physiological and metabolic processes which decrease at high temperatures (Kurek et al. 2007; Sage and Kubien 2007). Desert plants have also adapted strategies to deal with the impacts of heat and stress, including the expression of heat shock proteins (HSPs) and heat-stable enzymes (such as Rubisco and Rubisco activase isoforms), and rapid induction of non-photochemical quenching (NPQ) of excess excitation energy (Brestic et al. 1995; Scharf et al. 2012). Future research on the desert almond should consider these, and other, adaptations. Notably, the photosynthetic rates measured here were higher than those measured in the field (Paudel et al. 2019). A possible explanation can be related to the young age of trees. Photosynthesis is highest in the youngest trees and declines precipitously from the seedling stage through the first decades of growth (Steppe et al. 2011). Similar, yet moderate, trend of photosynthetic activity was observed among the plum species. The wild species *P. ursina* had an advantage over the rootstock *S2729* in the more advanced stage of drought compared to wild almond which had an advantage over the rootstock immediately after drought began. Photosynthetic activity of *P. ursina* under drought was ~ 5 μmol m^{-2} s^{-1} (Fig. 5a–d) and ~ 10–15 μmol m^{-2} s^{-1} in the control group. The values are barely distinguishable from those of *P. ursina* in the field (Paudel et al. 2019). As discussed previously in our earlier study (Paudel et al. 2019), many *Prunus* species share the same Eastern Mediterranean origin where ecosystems are hot and dry, though differ in their biogeography. *P. ursina* distribution limit is to the north of that of *P. ramonensis*' and thus, it is adapted to lower temperatures. It is hence possible that the potential for superior drought resistance in wild tree species, relative to their cultivated relatives, is limited to wild species from dry and hot habitats.

Stem hydraulic conductivity of the almond species decreased following drought, with a faster decrease in *GF677* compared to that of *P. ramonensis* (Fig. 8), also shown by the embolism dynamics in these species (Fig. 3). Because *P. ramonensis* exhibited a constant, low, level of embolism (23–33%) during most days of drought and a steep increase only toward the end of drought (87%), it would appear to indicate an embolism avoidance strategy as part of its drought resistance. Extrapolating the curve based on our PLC measurements indicated a higher embolism resistance of the two wild species than in the rootstocks (Fig. 4). This is represented by the curve's shape which showed slower development of embolism in wild species. In addition, *P. ramonensis* further showed an embolism tolerance behavior as it performed well (Fig. 5a–d) under high levels of embolism (Fig. 3) and survived the drought treatment despite these levels, i.e. with a very low rate of functional vessels and low hydraulic conductivity (none of the trees died at the end of the experiment).

In the current study, we found much higher WP values among the species (Fig. 2a-d) with respect to our recent field study in which the most negative WP values were − 3.0 and − 3.8 MPa in *P. dulcis* and *P. ursina,* respectively (Paudel et al. 2019). Slightly more negative values of around − 4.0 MPa were measured in *Pyrus syriaca* (Paudel et al. 2020). Our greenhouse experiment imposed severe water stress on the trees, a stress they would not necessarily experience in their natural habitat and/or in the orchard. We obtained minimum WP values which ranged from − 6.1 to − 7.4 MPa. This concurs well with Cochard et al. (2008) who measured mean xylem pressures causing 50 percent loss of conductivity ranging from − 5 to − 7 MPa in *P. persica*, *P. dulcis* and *P. cerasifera,* using the cavitron technique.

PIP mRNA expression patterns in the wood of almond species

In addition to the above physiological analysis, the Supplementary Information presents the detailed dynamics of PIPs expression differences in sapwood following drought and rehydration. The findings support the idea that downregulation of PIPs under drought stress (relative to irrigated trees) is the mechanism which plants use to reduce membrane water permeability, and by that prevent water outflow from the tree. Nevertheless, we observed an upregulation of most PIP members in *GF677* following drought (Fig. S3). On the other hand, most PIP genes in *Prunus ramonensis* were downregulated during drought (Fig. S3). These results are consistent with our previous study in pear (Paudel et al. 2020), which showed high transcript levels of a few PIP members and a reduction in the expression of most of them in response to water stress. Indeed, a growing body of literature has investigated PIP expression under different environmental conditions, which seem to follow species-specific patterns and diverge between plant parts (i.e. root, stem, leaf). Under drought conditions, AQP transcription is either upregulated (Jang et al. 2004; Li et al. 2016) or downregulated (Smart et al. 2001; Jang et al. 2004; Secchi and Zwieniecki 2014).

There was a tradeoff between PIP expression, presumably connected to radial flow, and hydraulic conductivity, representing axial flow. *GF677* upregulated 6 of 7 PIPs (Fig. S3) and lost hydraulic conductivity (Fig. 8), whereas *Prunus ramonensis* kept downregulating PIPs and maintained hydraulic conductivity longer, but ultimately also failed. Furthermore, in all trees, PIP upregulation was linked with subsequent increase of embolism to > 80% (Fig. 3), at thirteen and twenty-six days of drought for *GF677* and *Prunus ramonensis* respectively. Many studies reported a correlation between PIPs expression level and hydraulic efficiency (North et al. 2004; Cochard et al. 2007; Perrone et al. 2012; Paudel et al. 2017; Pawlowicz and Masajada 2019). Alexandersson et al. (2005) have shown that PIP transcripts were down-regulated following drought stress in leaves. Furthermore, Jang et al. (2004) have shown that under drought stress the transcripts level of several PIP genes were rapidly decreased down to one-tenth. In another study (Jang et al. 2007), under dehydration stress transgenic Arabidopsis and tobacco plants overexpressing PIP1:4 or PIP2:5 displayed a rapid water loss. The general upregulation after thirteen days of drought in *GF677* might show the loss of hydraulic control in species that were cultivated toward high yield and not high drought resistance. At the end, all the trees survived, so it seems that they sacrificed their xylem transport in favor of other functions, perhaps radial water movement to living cells such as the phloem or the cambium.

Starch content in the wild *P. ramonensis* fluctuated between 0 and 6% d.w (Fig. S4) whereas in our previous field study (Paudel et al. 2019) we measured higher starch levels of ~ 6–9% d.w. The differences in starch concentration may be due to the tree's age (Steppe et al. 2011; Liu et al. 2018): two vs. twenty years old in this and the previous study, respectively. Starch content in *GF677* (considering both treatments) fluctuated more, between 0 and 12%. This is in line with our previous studies in cultivated pear and almond (Paudel et al. 2020, b) in which starch levels were ~ 8–11 and ~ 6–9% d.w., respectively. As previously proposed by Paudel et al. (2020), stems in tree species of the Rosaceae family might have higher starch content compared to stems in other deciduous trees, due to the high carbon investment in a large number and size of fruits. Changes in starch concentrations under drought were rather similar among almond species, with drought-induced degradation (Fig. S4) to produce soluble sugars, possibly for osmotic protection (Tsamir-Rimon et al. 2021). The increase in starch levels in spring among the control group probably reflected phenological changes. In this point, when leaves are fully developed, starch is being synthesized.

We are aware that our research has technical limitations, such as the lack of data in some of the measuring dates. It is plausible that these limitations may have influenced the significance of the statistical analysis. Further data collection would be needed to determine the differences' significance. Moreover, the lack of humidity control in the greenhouse may have led to undesired responses. Finally, because we conducted the experiment during the end of spring/beginning of summer with the typical phenological changes occurring at this time of the year we cannot be sure if the results are transferable to other seasons. Still, the observations presented here have potential implications for agricultural purposes. The choice of proper rootstocks with drought resistance is important to use water more sustainably and crucial to prevent yield losses in the orchard. Characterization of the various drought resistance mechanisms in *Prunus* may reveal whether drought resistance can be increased using rootstocks with drought-resistant characteristics. Furthermore, as many *Prunus* species are graft-compatible and can be interbred, the development of a single tolerant genotype may impact the cultivation of several species. In this regard, further experimental tests on scion-rootstock interactions need to be carried out to establish whether the wild relative can be used as a rootstock material. Further work on tissue localization and structural differences among the *Prunus* PIPs, including root PIPs, would help us to better understand their role and function under drought. In addition, we hope to combine aquaporins expression analysis together with microCT scans to support our finding on PIPs expression pattern together with previous findings in the literature regarding xylem refiling.

Author contribution statement HG performed all measurements and analyses under the supervision of TK. The study was designed by TK and IP. SBD consulted the bioinformatic analysis. AZ and GS were involved in the project regarding the plum species. HG and TK wrote the paper jointly, with contributions from the other authors.

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Data availability statement All data obtained in this study are reported in its text and figures. Information about primers used for *Prunus* Pdu-PIP aquaporin reference gene qPCR analyses is included in the Supplementary Information.

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