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Research paper

# Intraspecific plasticity in hydraulic and stomatal regulation under drought is linked to aridity at the seed source in a wild pear species

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**Adaptations of fruit trees to future climate are a current research priority due to the rapid increase in air temperature and changes in precipitation patterns. This is aimed at securing sustainable food production for our growing populations. Key physiological traits in trees conferring drought tolerance are resistance to embolism and stomatal control over water loss. Recently, we have shown in the field that a native wild pear species performs better under drought than two cultivated pear species. A comparative greenhouse study was conducted to investigate traits associated with drought tolerance in four ecotypes of a wild pear species (*Pyrus syriaca Boiss*), compared with a wild pear species (*Pyrus betulifolia Bunge*) commonly used as a pear rootstock. Seed sources were collected from semi-arid, sub-humid and humid sites across northern Israel. Measurements of water relations, leaf physiology, hydraulic conductivity and percent loss of hydraulic conductivity (PLC) were conducted under standard irrigation, drought and recovery from drought. The four *P. syriaca* ecotypes maintained significantly higher leaf gas exchange values and water-use efficiency and had lower PLC than the rootstock species under prolonged drought as well as during recovery. Across the four ecotypes, stomatal closure occurred at stem water potential (Ψ) around −3.5 MPa; however, Ψ at 50% PLC ranged from −4.1 MPa in the humid ecotype to −5.2 MPa in one of the semi-arid ecotypes, rendering the latter with a higher hydraulic safety margin (the Ψ difference between stomatal closure and 50% PLC). Divergence of the ecotypes in xylem vulnerability to embolism closely matched the mean annual precipitation at their seed sources. Thus, selection of pear ecotypes from populations in semi-arid sites may be better than the currently used plant material for preparing our cultivated species for hotter and drier future climate.**

***Keywords*:** fruit trees, local adaptation, phenotypic plasticity, recovery from drought, water stress.

## Introduction

Demand for food is intensifying with an increasing human population; at the same time, greenhouse gas emissions and land-use changes are altering our global climate (IPCC 2014). The altered climate trend shows increasing frequency of extreme temperature (Ganguly et al. 2009, Coumou and Robinson 2013, Stéfanon et al. 2014) and drought (Groisman and Knight 2008, Hansen et al. 2012), especially in drier regions. Under these conditions, physiological functions and hydraulic balance in trees could be weakened, which can lead to lower crop yield (Ciais et al. 2005) and increased mortality (Allen et al. 2014, McDowell and Allen 2015), further compromising food production. However, currently, commonly grown varieties are not suitable (Paudel et al. 2019), as most of the traditional breeding has focused on yield and productivity. Therefore, plants' physiological traits must be adjusted to the changing conditions to ensure agronomical productivity. Studies on mechanisms of growth and survival of relatives of cultivated species and their ecotypes, which are adapted to a hotter and drier climate, could potentially enable us to prepare our trees for hotter and drier future climate.

Wild relatives may provide valuable genetic resources to improve cultivated species while maintaining production. However, plant traits often vary across space, particularly when species encounter contrasting environmental conditions throughout their native range. Both genetic and environmental factors determine the variation of traits. One such example is ecotypes, which are genotypically adapted to specific environmental conditions. Species adaptations to local environments and genetic variation can create substantial phenotypic differentiation (Linnen and Hoekstra 2009). Determining the patterns and mechanisms of natural selection in the wild is of fundamental importance to understanding the differentiation of ecotypes. However, it is often unknown the extent to which adaptive genetic variation is distributed among ecotypes between distinct habitats. Intraspecific phenotypic variation can reflect micro-geographic adaptation and divergent selection (Patsiou et al. 2020, Voltas et al. 2018). Phenotypic plasticity can change traits rapidly within generations and may differ drastically across populations in dissimilar resource environments (West-Eberhard 1989). One such example can be found in ecotypes established across gradients of water availability, where selection may favor a phenotype that has enhanced drought tolerance in the driest sites (e.g., transpiration, growth; Crispo 2008, Klein et al. 2013, Voltas et al. 2018, Patsiou et al. 2020). Careful examination of an ecotype's environmental plasticity may reveal individuals that can increase productivity under optimal conditions and can sustain production under stressful climatic conditions (Nicotra et al. 2010, Valladares et al. 2007).

Ecotype trials are valuable for measuring the adaptive potential of a crop to a changing environment, which may be useful for the improvement of fruit trees (Dwivedi et al. 2008, Hajjar and Hodgkin 2007, Heywood et al. 2007). For instance, ecotypes of some species may contain specific adaptations to environmental factors experienced in natural conditions that are desirable for agriculture (Hajjar and Hodgkin 2007). Similarly, in ecotypes established across gradients of water availability, selection may favor traits that enhance drought tolerance in the driest sites (e.g., stomatal regulation, lower hydraulic conductance and lower embolism rate), resulting in the adaptive ecotype's differentiation, i.e., advantageous performance under drought compared with the cultivated variety (Matesanz et al. 2002).

Among the physiological mechanisms of drought stress and drought-induced tree mortality, a few key traits arise (Klein 2015). High performance under drought requires that a tree maintains its water transport capacity and carbon supply (Klein 2015, Klein et al. 2011, Nardini et al. 2012). The primary response to drought includes stomatal closure for maintaining water potential ($\Psi$) in the xylem to avoid embolism spread through air-seeding and to delay hydraulic failure (Brodribb and Feild 2000, Sperry 2000). Water potential when 50% of xylem vessels are embolized ($\Psi_{50}$) and when the stomata are fully closed ($\Psi g_{s(90)}$) are considered as important traits to screen for drought tolerance (Brodribb and Holbrook 2004, Martin-StPaul et al. 2017, Sperry 2004). The difference between $\Psi_{50}$ and $\Psi g_{s(90)}$ is the hydraulic safety margin (HSM; Klein et al. 2011). Intraspecific variation among the ecotypes in these traits has been observed in response to a wide range of environmental conditions (Klein et al. 2013; Beikircher et al. 2013; Reddy et al. 2019). In searching for new fruit tree varieties and species, it is therefore essential to test adaptive plasticity in those traits in different ecotypes to potential future climate conditions (Paudel et al. 2019). Greenhouse experiments were carried out to test the environmental variations of physiological traits within the tree species and study the relationships between trait variability and seed source environments (i.e., Sork et al. 2013).

Syrian pear (*Pyrus syriaca*) is a deciduous tree in the Rosaceae. It is the only pear species that grows in the wild in Lebanon, Turkey, Syria and Israel (Figure 1). The habitats of this species include open forests and some rocky mountains. This species has the potential to hybridize with *Pyrus communis* (Ercisli 2004). *Pyrus syriaca* has higher tolerance to drought and more grafting compatibility with *P. communis* than quince rootstocks (Al Maarri et al. 2007). *Pyrus syriaca* fruits are edible, but of lower quality than that of other pear varieties. On the other hand, using wild varieties as rootstocks could improve the drought resistance of the whole tree. In a recent study, we found a positive relationship between drought resistance and climate in the species native habitat. Wild pear and almond were more drought resistant than their respective cultivated relatives, but not wild plum, which was from wetter and colder sites (Paudel et al. 2019). In the present study, we expand on this aspect and test whether it acts at the intraspecific level as well, in *P. syriaca*.

A wild pear from a range of eastern Mediterranean regions was selected from its southern native range. We hypothesized that variance in hydraulic and stomatal regulation in response to environmental conditions, in wild pear ecotypes, is related to the native environmental conditions. We further hypothesized that ecotypes that are currently growing in hotter and drier conditions provide the best resources to improve the rootstocks and cultivars of commercially grown pears. Specific hypotheses were: (i) plasticity and ecotypic variation are expressed in percent loss of hydraulic conductivity (PLC) and growth, but not in leaf gas exchange variables or intrinsic water-use efficiency (WUEi; based on Klein et al. 2013); (ii) ecotypic traits match the climate at the geographic location of the seed source; and (iii) not all of the wild pear ecotypes tested are more drought tolerant than the cultivated rootstock.

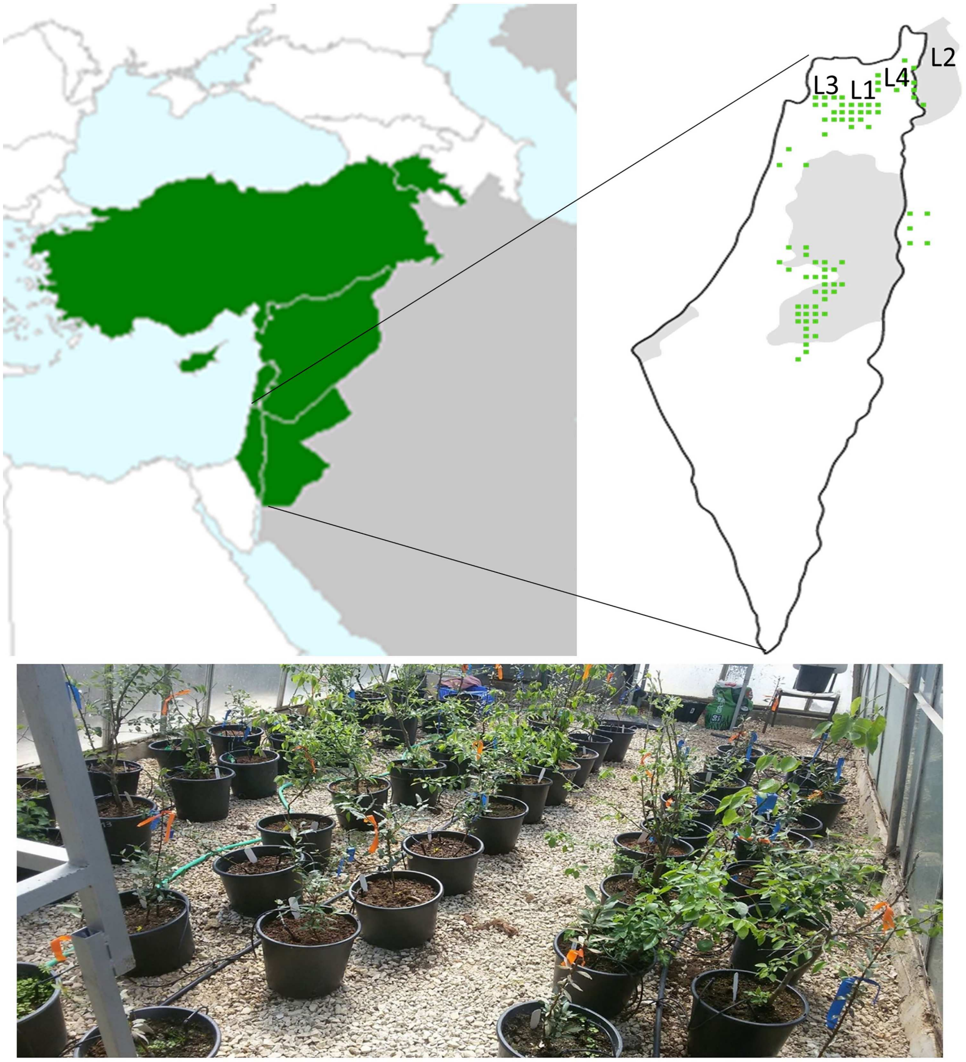

Figure 1. Distribution of the wild pear species *P. syriaca* (at national scale; green area) and specifically within Israel (green dots: top), experimental setup (bottom). Seed sources for the studied ecotypes L1–L4 are indicated (see Table 1 for details).

## Materials and methods

### Study species and ecotypes

To identify suitable future rootstocks for cultivated pear species using our wild relatives, intraspecific variation of *P. syriaca* was tested for its response to water deprivation in comparison with the most commonly used cultivated rootstock (*Pyrus betulifolia*). The four ecotypes of *P. syriaca* examined originated from distinct geographic regions in Northern Israel, with varying climatic histories. We used four contrasting ecotypes of *P. syriaca* from its fragmented distribution range in northern Israel (*Ps*L1–*Ps*L4; Figure 1). *Pyrus syriaca* is a relatively rare species across northern Israel, with small populations occurring in isolated sites within the Mediterranean forest covering mountain ranges in the Galilee and Golan Heights (Table 1). The dry season regularly occurs from April through November in all the habitats, but the annual precipitation amount and the prevailing temperatures diverge (Table 1). Ecotypes *Ps*L1 and *Ps*L4 are native to a dry and hot climate, while ecotypes *Ps*L2 and *Ps*L3 are native to a warm and humid habitat (Table 1). We compared the four *P. syriaca* ecotypes with *P. betulifolia*, originally selected from its wild habitat, which we will refer to as the cultivated species. Saplings were collected from a commercial nursery near The Galilee Research Institute (MIGAL, Kiryat Shmona, Israel). *Pyrus betulifolia* is a wild pear species native to China that was integrated into agriculture as a rootstock for pear (Jin et al. 2017, Li et al. 2016, Stern et al. 2013). *Pyrus betulifolia* is genetically compatible with *P. communis*, and scions grafted onto this rootstock gain more vigorous growth and drought resistance than *P. communis* (Li et al. 2016).

### Saplings and experimental setup

For each ecotype, seeds were collected from 6 to 10 trees and prepared for germination. A total of 200–300 seeds were collected from ~100 fruits from each site. Seeds were cleaned and washed in a 0.1% solution of the fungicide Captan (Merpan 50 pouder; Adama, Be'er Sheva, Israel) prior to sowing in trays.

Table 1. Climatic conditions at seed sources of the four *P. syriaca* ecotypes used in this study. Climate data are from the Israel meteorological service. The long-term annual mean potential evapotranspiration (PET) is ~1450 mm across the sites, producing different annual aridity index terms. S-Arid, semi-arid; S-Humid, sub-humid (United Nations Environment Programme Classifications). Elev., elevation; P, mean annual precipitation; T, mean diurnal temperature.

| Ecotype | Origin | Latitude | Longitude | Elev. (m) | P (mm) | P/PET (AI) | Aridity | T Jan (°C) | T Aug (°C) |
|---|---|---|---|---|---|---|---|---|---|
| *Ps*L1 | Meron | 32.999 | 35.4131 | 1,200 | 1,150 | 0.79 | Humid | 6 | 24 |
| *Ps*L2 | Merom Golan | 33.1288 | 35.7786 | 1,000 | 830 | 0.57 | S-Humid | 5.7 | 23.1 |
| *Ps*L3 | Kziv | 33.0443 | 35.2563 | 200 | 720 | 0.5 | S-Arid | 10.9 | 24.6 |
| *Ps*L4 | Yiftach | 33.1247 | 35.5568 | 420 | 570 | 0.39 | S-Arid | 10.3 | 25 |

For cold stratification, trays were sealed with black plastic bags and kept wet and dark at 4 °C for 30 days. Following this step, trays were moved to growth rooms with fluorescent light. When seedlings were 5–10 cm tall, they were transferred to the greenhouse. The *Ps*L1 was grown in Keren Kayemeth LeIsrael nursery, while others were grown in the greenhouse in MIGAL. The rootstock was grown in a commercial nursery and collected when seedlings were 1 year old.

Saplings were transplanted into silty-clay loam soil in 10-l pots inside the greenhouse under 25 °C day/17 °C night and natural daylight. One-year-old saplings were grown in a greenhouse at the Fiterman Plant Growth Center, Weizmann Institute of Science (Rehovot, Israel), for 6 months (Figure S1 available as Supplementary data at *Tree Physiology* Online). At the beginning of the experiment, plants were selected for similar height (70 ± 14 cm) and diameter (6.5 ± 8 mm) and randomly assigned into three blocks; each consists of two treatments (i.e., control vs drought; Figure S1 available as Supplementary data at *Tree Physiology* Online). Saplings were healthy, with no visible signs of biotic or abiotic stress. In each block, two to three saplings of *Pb* and each of the *Ps* ecotypes were used in each treatment: control vs drought (drying and wetting conditions), which lasted for 60 days. In the drought treatment, standard irrigation was supplied for the first 12 days and then irrigation was stopped until maximum embolism was reached (after 32 days), and then re-irrigation was applied for another 14 days. The cultivated rootstock and *Ps*L3 had a total of 18 saplings each, while *Ps*L1, *Ps*L2 and *Ps*L4 had a total of 12 saplings each. Saplings that did not grow well were excluded from the experimental design; because of this we had only 12 saplings each of *Ps*L1, *Ps*L2 and *Ps*L4. In spring 2018 (1 May), when the leaves were fully expanded and mature, initiation of treatment began. For the first 2 weeks, standard irrigation (0.5 l per day) was applied to all of the saplings, and then irrigation was stopped for 30 days, with 0.25 l provided once after 2 weeks. After this, standard irrigation was resumed for another 2 weeks.

### *Xylem hydraulic conductivity and embolism*

Three branch sections ranging from 25 to 50 cm (70% of samples >40 cm) from each irrigation treatment (control and drought) were cut underwater from each sapling, according to protocols described by Wheeler et al. (2013). Branch length was selected according to the most abundant vessel length across the related *Prunus* species (Cochard et al. 2008, Paudel et al. unpublished data) and *Pyrus* species (Rodriguez-Zaccaro et al. 2019). Branches were dipped under degassed, distilled water until analysis in the lab (duration of about 1 h) to permit the relaxation of the xylem tension. Next, the segments were further cut underwater in the lab from two edges into the final measured size of 10 cm. Native xylem conductivity was measured with a 19-mM KCl aqueous solution in a 70-cm column of water (7 kPa) in order to capture the native hydraulic conductivity (Ks-native). The same segments were then flushed with a 19-mM KCl solution through a high-pressure flow meter (Dynamax Inc., Houston TX, USA; Tyree et al. 1995) for 15–20 min at pressures of 0.05–0.1 MPa to calculate the native percentage loss of hydraulic conductivity (PLC), as an indirect measurement of embolism.

Native PLC (%) and stomatal conductance (mmol $m^{-2}$ $s^{-1}$) were plotted against stem water potential ($\Psi$) and fitted with a sigmoidal curve for each sampling using SAS software (SAS Institute Inc., Cary, NC, USA), according to the following equation (Pammenter and Van der Willigen 1998):

$$Y = \frac{100}{1 + \exp\left(S\left(\Psi_x - \Psi_{50}\right)\right)}, \qquad (1)$$

where Y is either PLC or $g_s$, $\Psi$ (MPa) is stem water potential; $\Psi_{50}$ (MPa) is the xylem pressure inducing 50% loss of hydraulic conductivity and S (% $MPa^{-1}$) is the slope of the native vulnerability curve at the inflection point. The HSM was estimated as the difference between $\Psi g_{s(90)}$ and $\Psi_{50}$. To determine $\Psi g_{s(90)}$, all $g_s$ values measured during the six measurement days were plotted against $\Psi_s$ measured at the same time. Stem water potentials at 12 and 90% loss in stem hydraulic conductivity were considered as P12 and P50. This means that the point at which 12 and 90% reduction in $g_s$ was observed (compared with the highest value measured) was defined as $\Psi g_{s(12)}$ and $\Psi g_{s(90)}$, respectively. The time course of stem hydraulic conductivity and percent loss of hydraulic conductance (embolism formation) were measured weekly throughout the experimental period, following the above procedures.

### Soil water conditions and stem water potentials

Soil water content (SWC, % v/v) was measured weekly during standard irrigation, drought and recovery periods using a dielectric constant EC-10 soil moisture sensor (Decagon Devices Inc., Pullman, WA, USA). Measurements were made close to the roots at a 10-cm soil depth during each measurement day (Figure S2 available as Supplementary data at *Tree Physiology* Online). Correction for sensor bias was checked, according to Paudel et al. (2019). Stem water potential ($\Psi$) was measured weekly at midday on leaf cohorts sampled from four randomly selected saplings per treatment throughout the experimental period (Figure S2 available as Supplementary data at *Tree Physiology* Online). In each sapling, the fourth leaf from the tip of the branch was covered with aluminum foil at least 30 min before taking measurements, to allow equilibration between the leaf tissue and the stem xylem. The $\Psi$ was measured on the branches used for gas exchange and hydraulic measurements. Following cutting, $\Psi$ was measured immediately within 2 min of collection using a Scholander-type pressure chamber (PMS Instrument Company, Albany, OR, USA) in the greenhouse.

### Leaf physiology

Prior to stem water potential measurements, stomatal conductance, net $CO_2$ assimilation and transpiration measurements were done on fully grown young mature leaves using a Walz photosynthesis system (GFS-3000, Effeltrich Walz, Germany) fitted out with a 2 × 3 cm standard leaf cuvette. Measurements were completed at a leaf temperature of 25 °C, 50% relative humidity and a photosynthetically active light intensity of 1200 μmol $m^{-2}$ $s^{-1}$, 10% blue light and 400-μmol $CO_2$ $mol^{-1}$ in the cuvette. Leaves were measured from four saplings in each treatment. These measurements were performed periodically (weekly) throughout the experimental period around midday (i.e., between 10:00 and 14:00h). Intrinsic WUEi was calculated as the ratio between net $CO_2$ assimilation and stomatal conductance.

### Plant growth

Height and diameter were measured after transplanting (i.e., initial), biweekly and at the end of the experiment (final). During initial measurements, the sapling root collar was marked; therefore, the diameter was measured at the same location each time. Measurements were made on the same plants throughout the study period. The shoot height was measured from a set point 5 mm above the cotyledon to the tip of the shoot. The root collar diameter was determined by taking digital caliper measurements in perpendicular directions.

### Statistical analysis

Soil water content, stem water potential, leaf gas exchange, hydraulic conductivity and growth data were analyzed using a repeated-measurements analysis of variance (ANOVA) with species and drought treatments as fixed factors, with the assumption that the variance within subjects (i.e., the saplings) was homogeneous. For all analyses, there were at least three replicates of all treatments. The effects of ecotypes and drought treatments and their interaction were tested statistically at the drought period and after recovery time using two-way ANOVA. When effects of treatments were statistically significant, differences among groups were post hoc tested with Tukey's HSD test. Individual native vulnerability curves were generated for individual saplings (note—we used three saplings) and individual $\Psi_{12}$, $\Psi_{50}$, $g_s$ (max), $\Psi g_{s(90)}$ and $\Psi_{50}$ were generated and then ANOVAs were performed. These analyses were performed using JMP (SAS). For each species, the response of stomatal conductance to decreasing water potential was fitted with a logistic model using the NLIN procedure in SAS (SAS 9.4).

## Results

### Hydraulic safety and stomatal regulation

Vulnerability to drought-induced embolism differed significantly between the species and among the four ecotypes, with *Ps*L4 being the most tolerant and *Ps*L3 the most vulnerable among the ecotypes. The $\Psi$ at the onset of cavitation ($\Psi_{12}$) ranged from −1.41 to −2.28 MPa and at 50% loss of native hydraulic conductivity ($\Psi_{50}$) from −4.2 to −5.1 MPa. In *P. betulifolia*, $\Psi_{12}$ was −1.25 and $\Psi_{50}$ was only −3.2 MPa (Figure 2). Maximum stomatal conductance (*gs*) was highest in *Ps*L3 (230 mmol $m^{-2}$ $s^{-1}$) and lowest in *Ps*L4 (161 mmol $m^{-2}$ $s^{-1}$; Table 2). We note that maximum *gs* can change with leaf area, but such effects are small considering that all saplings were of similar age and size (Figure 1; Materials and methods). Stomatal closure (negligible stomatal conductance) varied among species and ecotypes, with $\Psi g_{s(90)}$ of −2.6 MPa in *P. betulifolia* and −3.0 to −3.8 MPa in *P. syriaca* (Figure 2). Among the ecotypes, *Ps*L4 closed stomata at −3.7 PMa, followed by *Ps*L3 at −3.6 PMa. Differences in $\Psi_{50}$ between species greatly impacted the HSM values, with an HSM of 1.5 MPa in *Ps*L1 and 0.6 MPa for *P. betulifolia*, respectively. $\Psi_{50}$ and $\Psi g_{s(90)}$ were significantly correlated with mean annual precipitation and August temperatures in their native habitats (Figure 3).

### Time course of hydraulic conductivity and embolism formation

Ecotypes *Ps*L1 and *Ps*L2 had the highest Ks followed by *P. betulifolia*, while PLC was high only in *Ps*L4 during experimental period in control plants (Figure S3 available as Supplementary data at *Tree Physiology* Online). All ecotypes had significant reductions in Ks with the highest Ks in *P. betulifolia* and an increase in PLC with increasing water stress ($F_{1.23}$; $P < 0.05$; Table 3 and Figure 4). However, stressed *Ps*L4 had a less of reduction in Ks and increased PLC (by 58%), while the maximum reduction in Ks was in *P. betulifolia* (78%) and *Ps*L3

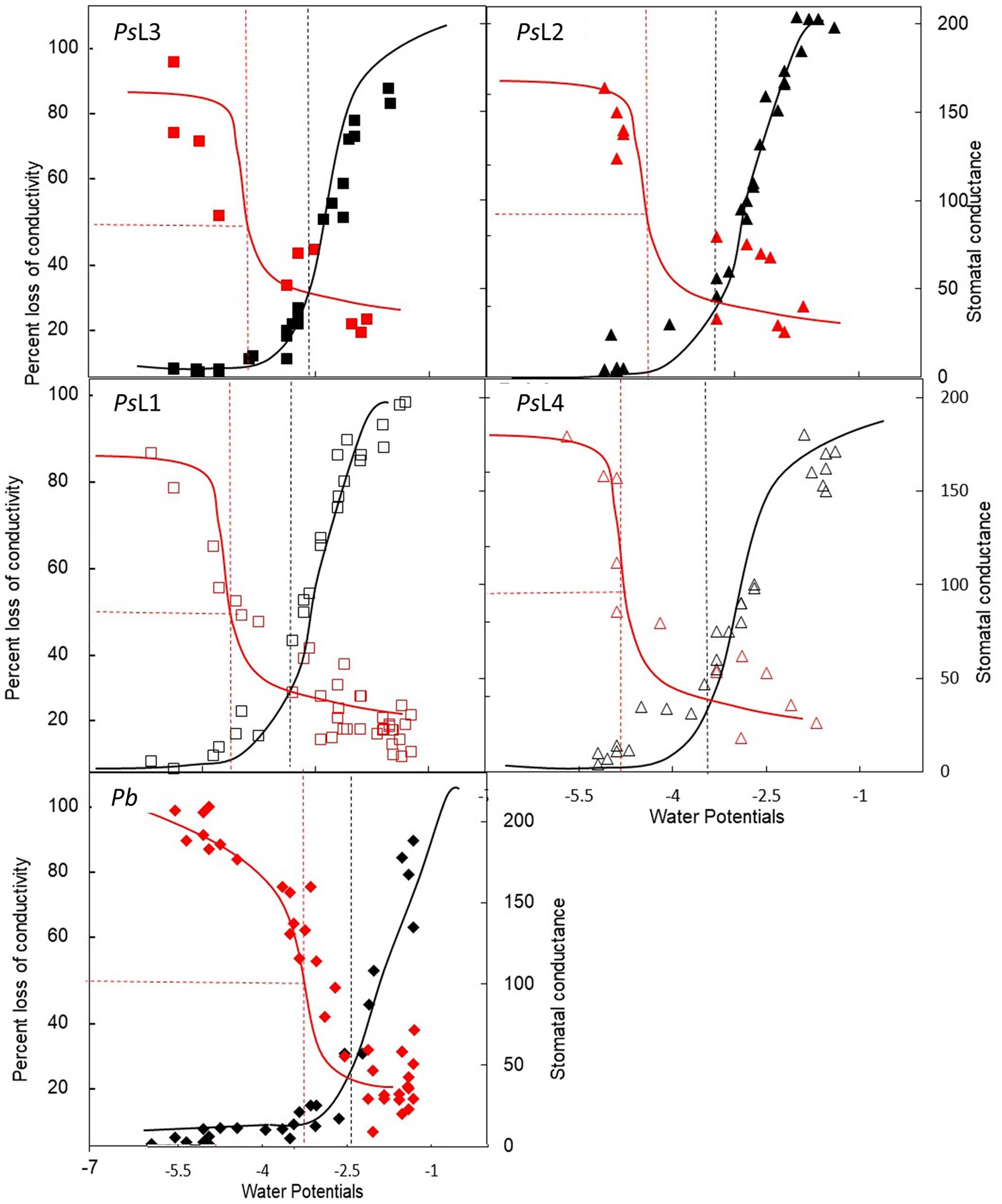

Figure 2. Changes in stomatal conductance (mmol m$^{-2}$ s$^{-1}$, black) and percent loss of native hydraulic conductivity (red) in response to stem water potential ($\Psi_s$, MPa) in four *P. syriaca* ecotypes (*Ps*) and in *P. betulifolia* (*Pb*). Vertical gray dashed lines represent $\Psi_s$ at stomatal closure. Red dashed lines represent 50, the water potential inducing 50% loss of conductivity (horizontal) and the $\Psi_s$ associated with it (vertical). See Table 2 for statistical analysis ($n = 3$).

(73%). During severe water stress, Ks was reduced by 81% in *P. betulifolia*, by 67% in *Ps*L1, by 67% in *Ps*L2, by 73% in *Ps*L3 and only by 54% in *Ps*L4 (Figure 4). Similarly, drought stress increased PLC from 19 to 82% in *P. betulifolia*, 15.0 to 69% in *Ps*L1, 15.9 to 69% in *Ps*L2, 17.5 to 86% in *Ps*L3 and 19.7 to 58. % in *Ps*L4 ($F_{2.6}$; $P < 0.05$; Figure 4). After recovery, *P. betulifolia* did not fully recover; however, Ks and PLC of the ecotypes recovered similar to those of plants receiving standard irrigation.

### *Time course of water relations and leaf physiology*

All ecotypes in the control treatment maintained their $\Psi$ (between −1.5 and −2.6 MPa) ($F_{5.005}$; $P < 0.0001$), whereas the drought-stressed plants had a significant reduction

Table 2. Hydraulic parameters of the reference rootstock (*P. betulifolia*) and the four ecotypes of *P. syriaca*. $\Psi_{12}$, $\Psi_{50}$, water potential inducing 12 and 50% loss of conductivity, respectively; *gs*, stomatal conductance; $\Psi g_s(90)$, water potential inducing 90% loss of stomatal conductance (i.e., stomatal closure).

| Species and ecotype | $\Psi_{12}$ | $\Psi_{50}$ | $g_s$ (max) | $\Psi g_s(90)$ | HSM |
|---|---|---|---|---|---|
| *P. betulifolia* | −1.25 ± 0.4 c | −3.30 ± 0.4 c | 188 ± 14 ab | −2.6 ± 0.40 | 0.60 |
| *P. syriaca* L1 | −1.85 ± 0.3 bc | −4.10 ± 0.4 bc | 183 ± 14 ab | −3.0 ± 0.25 | 1.10 |
| *P. syriaca* L2 | −1.75 ± 0.4 b | −4.60 ± 0.4 b | 204 ± 10 a | −3.4 ± 0.30 | 1.15 |
| *P. syriaca* L3 | −1.45 ± 0.3 c | −4.75 ± 0.3 ab | 197 ± 15 a | −3.7 ± 0.25 | 1.05 |
| *P. syriaca* L4 | −2.00 ± 0.5 a | −5.20 ± 0.4 a | 171 ± 11 b | −3.7 ± 0.30 | 1.50 |

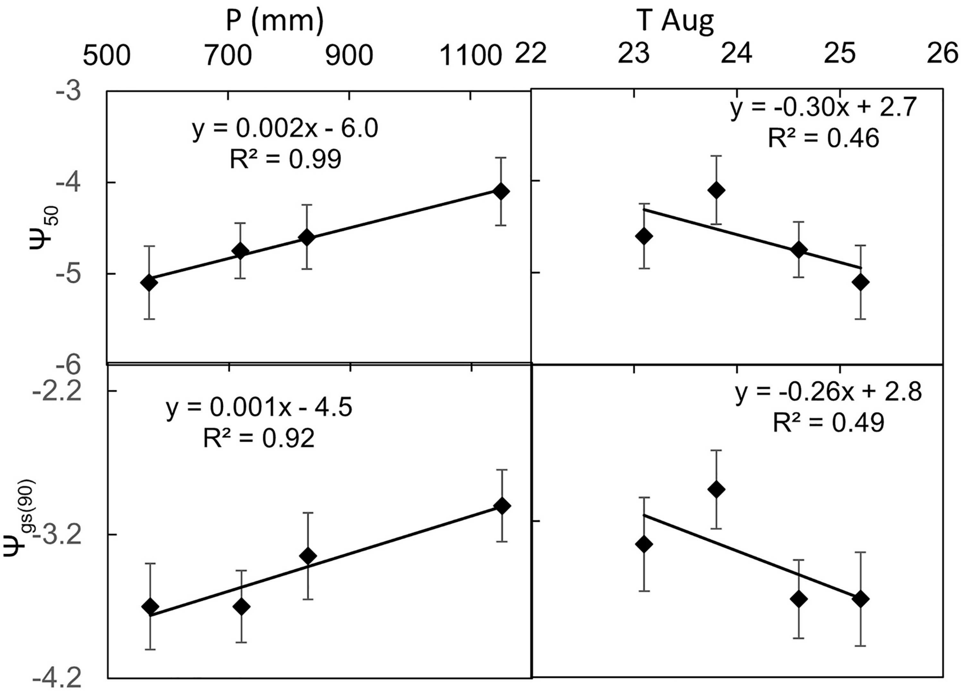

Figure 3. Correlations between water potential inducing 50% loss of hydraulic conductivity ($\Psi_{50}$) and water potential at stomatal closure ($\Psi g_s(90)$) with mean annual precipitation (P) and mean August temperature (T Aug). Each data-point represents one of the studied *P. syriaca* ecotypes.

in Ψ ($F_{2.5}$; $P < 0.05$; Table 3 and Figure S2 available as Supplementary data at *Tree Physiology* Online). Among the ecotypes, higher Ψ values were recorded in *Ps*L2 (−2.29 ± 0.15 MPa and − 5.23 ± 0.18 MPa at moderate and severe drought, respectively). However, a drought-induced reduction in Ψ was observed in all species and ecotypes; the maximum decrease in Ψ was observed mainly in *P. betulifolia*, *Ps*L3 and *Ps*L4 (Figure S2 available as Supplementary data at *Tree Physiology* Online). After 15 days of recovery, Ψ of all four ecotypes returned to values almost similar to that of plants receiving standard irrigation (Figure S2 available as Supplementary data at *Tree Physiology* Online); however, *P. betulifolia* did not fully recover. During recovery, there was a significant interaction between drought and species (Table 3 and Figure 2).

Expectedly, SWC was not consistent among the ecotypes at the control treatment but following withholding irrigation, SWC rapidly decreased from 17.5–20.3% (v/v) to a minimum of 0.5–2.4% (v/v) during drought (Figure S2 available as Supplementary data at *Tree Physiology* Online) and varied among the ecotypes ($F_{3.6}$; $P < 0.001$; Table 3). Rapid recovery after resuming irrigation was observed in both species and all ecotypes. Overall, differences between species and treatments were significant (Table 1). In moderate drought, SWC was 6–12% for *P. syriaca* and only 4% for *P. betulifolia* due to the vigorous growth and higher transpiration in the latter.

Under standard irrigation, net $CO_2$ assimilation was significantly different among ecotypes ($F_{2.64}$; $P < 0.05$; Figure 4, Figure S4 available as Supplementary data at *Tree Physiology* Online and Table 3). Compared with standard irrigation, net $CO_2$ assimilation was significantly reduced in the four ecotypes at moderate ($F_{3.5}$; $P < 0.05$) drought conditions. Maximum reduction of net $CO_2$ assimilation was observed in *P. betulifolia* (92 and 105%), and *Ps*L1 (only at severe drought (102%)), followed by *Ps*L3 (59 and 107%) and *Ps*L4 (57%). In comparison, a relatively lower reduction of net $CO_2$ assimilation was

Table 3. ANOVA results ($F$ values) for the effects of species, ecotype, drought and their interactions on sapling relative height (Ht) and trunk diameter (TD) increment, stem water potential ($\Psi_s$), pot soil water content (SWC), stomatal conductance ($g_s$), photosynthesis (Pn), transpiration (Tr), intrinsic WUE (WUEi), stem specific hydraulic conductivity (Kh) and percent loss of conductivity (PLC), at standard irrigation, 10 and 30 days of drought, and 14 days following re-irrigation. 'Species' indicates wild (mean response of the four ecotypes) or cultivated rootstock. Significant effects at the 0.05, 0.001 and 0.00001 are denoted by *, ** and ***, respectively

| Parameter | Ht | TD | $\Psi_s$ | SWC | *gs* | Pn | Tr | WUE$_i$ | Kh | PLC |
|---|---|---|---|---|---|---|---|---|---|---|
| Standard irrigation | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* | *F* |
| Species | 0.34 | 3.5* | 20.34*** | 2.56 | 8.47*** | 3.35 | 2.62 | 13.35 | 27.23** | 5.1* |
| Ecotype | 1.5 | 1.8 | 5.005*** | 1.76 | 2.8 | 2.64* | 4.05** | 2.64* | 1 | 0.54 |
| Drought | 2.66 | 0.24 | 0.4 | 2.46 | 2.9 | 0.003 | 0.48 | 0.023 | 0.013 | 0.79 |
| Drought*Species | 4.5 | 3 | 1.2 | 3.1 | 5.7 | 0.005 | 0.67 | 0.005 | 0.14 | 0.3 |
| Drought*Ecotype | 2 | 1.9 | 2 | 4.3 | 3.1 | 0.3 | 0.6 | 0.3 | 0.8 | 0.57 |
| 10 days of drought | | | | | | | | | | |
| Species | – | – | 0.12 | 11 | 3.5 | 0 | 3.6 | 0 | 33.23* | 7.1*** |
| Ecotype | – | – | 0.98 | 0.3 | 3.5* | 1.48 | 2.9* | 11.48* | – | – |
| Drought | – | – | 21.75* | 27.7*** | 2.5 | 3.7* | 2.55 | 3.7 | 0.013* | 4* |
| Drought*Species | – | – | 0.35 | 0.3 | 0.11 | 0.55 | 0.007 | 0.55 | 0.07 | 0.3* |
| Drought*Ecotype | – | – | 0.44 | 0.11 | 0.73 | 0.36 | 0.64 | 0.36 | – | – |
| 32 days of drought | | | | | | | | | | |
| Species | 0.93 | 21.93 *** | 0.04 | 0.68 | 10.2 | 0.55 | 6.25 | 22.55 | 5.29 | 7.1* |
| Ecotype | 0.85 | 11.95*** | 0.27 | 0.55 | 0.56 | 0.55 | 0.34 | 0.55 | 0.4 | 0.85 |
| Drought | 17.47* | 1.777* | 462.1** | 125.6** | 15.3*** | 18.46*** | 17.37*** | 18.46*** | 9.6* | 19.3*** |
| Drought*Species | 0.31 | 1.87 | 4.5* | 10.78** | 7*** | 1.6 | 2.3 | 1.6* | 0.013 | 6.6* |
| Drought*Ecotype | 1.2 | 0.34 | 2.5* | 2.56** | 3.6** | 2.12 | 2.9* | 2.12* | 1.23* | 2.6* |
| 14 days of recovery | | | | | | | | | | |
| Species | 6.5 | 21.7*** | 6.6 | 8.1 | 12.82 | 0.07 | 7 | 10.07 | 1.34 | 0.47 |
| Ecotype | 0.48 | 5.5** | 0.1 | 0.55 | 1.8 | 0.38 | 1.95 | 10.38 | 0.9 | 0.6 |
| Drought | 12.47* | 1.7 | 52*** | 26 | 27*** | 97*** | 23*** | 97*** | 5.12* | 13.64** |
| Drought*Species | 0.31 | 2.7 | 3.5* | 4.78 | 5.6* | 1.6 | 2.8 | 1.6* | 2.3 | 16.6* |
| Drought*Ecotype | 1.26 | 0.4 | 2.5 | 2.56 | 2.6* | 2.9 | 2.8 | 2.9* | 0.8 | 0.7 |

recorded in *Ps*L2 (48 and 90%) during water stress conditions (Figure 5). Under standard conditions, transpiration (Tr) was lowest in *Ps*L2 and *Ps*L4 ($F_{4.05}$; $P < 0.001$: Figure S4 available as Supplementary data at *Tree Physiology* Online). In the drought treatment, differences were significant for *P. betulifolia*, *Ps*L1 and *Ps*L3 in moderate drought, and across all the species and ecotypes under severe water stress ($F_{2.9}$; $P < 0.05$; Table 3).

Under the standard irrigation, *gs* differences were only significant for species and values were lowest in *Ps*L4 (Figure S4 available as Supplementary data at *Tree Physiology* Online). At moderate drought stress, *gs* significantly decreased in *P. betulifolia* (by 70%), *Ps*L1 (by 55%) and *Ps*L4 (by 63%; $F_{3.5}$; $P < 0.05$). At severe drought, *gs* significantly decreased compared with control in all species and ecotypes ($F_{2.56}$; $P < 0.001$). Maximum reduction in *gs* was observed in *P. betulifolia* (by 95%), *Ps*L3 (by 92%) and *Ps*L4 (by 91%), and a minimum reduction was found in *Ps*L1 (by 67%) and *Ps*L2 (by 70%). Water-use efficiency (WUE$_i$: $P_n/g_s$) differences were significant among ecotypes at standard irrigation. The WUE$_i$ significantly decreased in both species and all ecotypes, with the maximum reduction of WUE$_i$ in *P. betulifolia* (92 and 105% at moderate and severe drought) and *Ps*L1 (only at severe drought 102%), followed by *Ps*L3 (59 and 107% at moderate and severe drought) and *Ps*L4 (57%). In comparison, a relatively lower reduction of WUE$_i$ was recorded in *Ps*L2 (48 and 90%) during water stress conditions (Figure 5).

### Plant growth

Growth traits were significantly different among species ($F_{5.1}$; $P < 0.05$) in control conditions (Figure S5 available as Supplementary data at *Tree Physiology* Online and Table 3). After 32 days of droughts, differences in diameter increment were significant to species and ecotypes ($F_{11.95}$; $P < 0.0001$; Figure 6 and Table 3). *Pyrus syriaca* ecotypes were growing at a low, steady rate throughout the drought period (lower in *Ps*L4), whereas *P. betulifolia* grew faster and more vigorously, but height increase was dropped sharply in mid-drought. However, growth traits did not show a similar pattern among ecotypes, suggesting complex effects driven by prevailing local conditions at the seed sources (Table 3).

## Discussion

The current study was aimed at quantifying intraspecific plasticity in hydraulic and stomatal regulation under drought in a wild pear species. In turn, this was helpful for screening and selecting wild pear ecotypes that performed better than

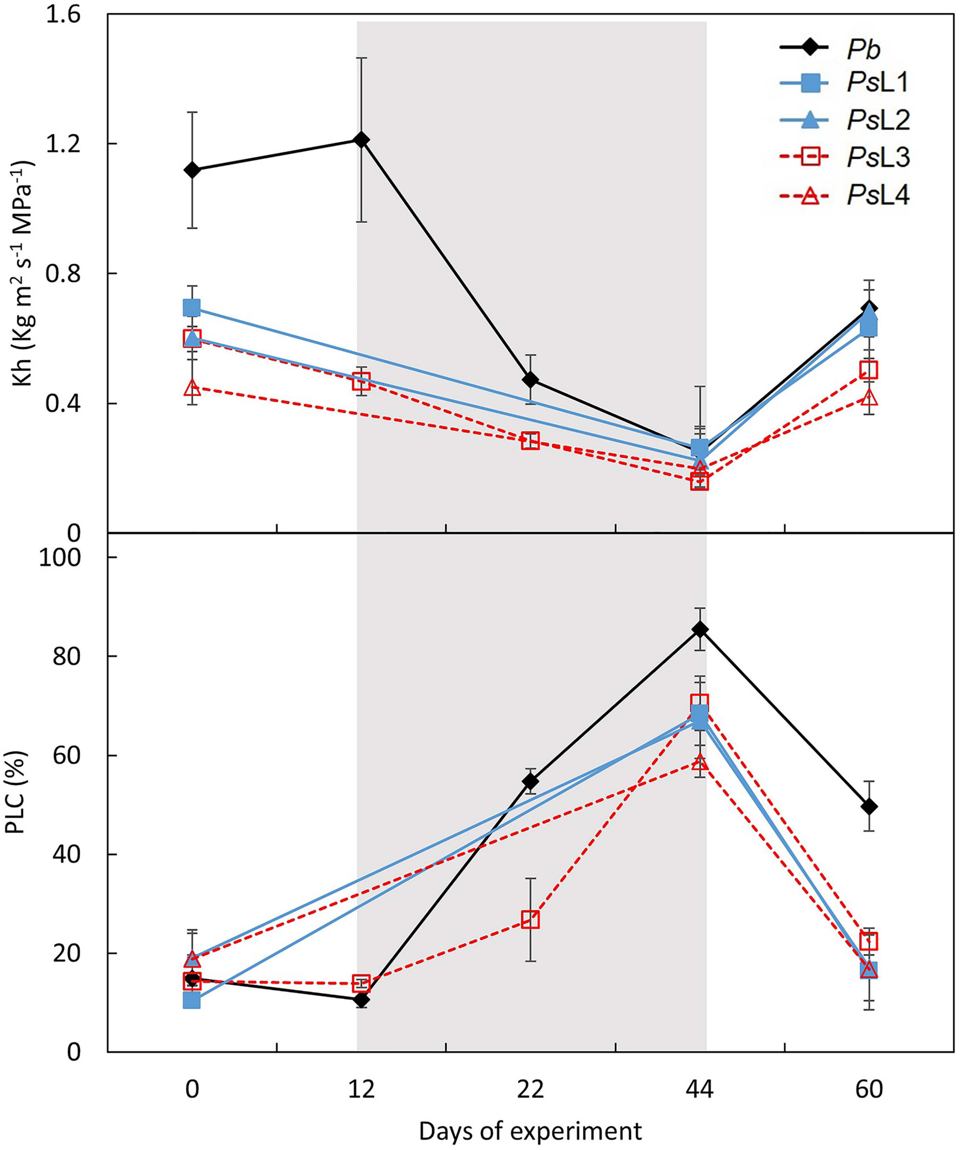

Figure 4. Native specific hydraulic conductivity (Kh) and PLC in course of the experimental period in *P. betulifolia* and *P. syriaca* L1–L4 (*Ps*L1-–*Ps*L4). The gray background denotes the imposed drought period. Error bars denote the standard error of the mean ($n$ = 3–10; see Table 3 for statistical analysis).

the common pear rootstock based on water relations, physiology and hydraulic traits in responses to drought stress followed by re-watering. Our main finding was that the ecotypes diverged in their xylem vulnerability to embolism. Their hierarchy matches the precipitation gradient among their geographical seed sources very well. The two wetter seed sources have cooler winters than the warmer sources, but the four seeds have similar summer temperatures. Based on (i) higher $g_s$ over a range of $\Psi$ (Figure 2), (ii) higher embolism resistance (Figure 2), (iii) higher HSMs (Table 2), (iv) higher photosynthetic activity under drought (Figure 5) and (v) higher WUEi under drought (Figure 5), the four wild pear ecotypes can be characterized into most tolerant (*Ps*L4), moderately tolerant (*Ps*L3) and less tolerant (*Ps*L1 and *Ps*L2). However, all ecotypes performed better during drought compared with the rootstock (*P. betulifolia*).

A tree's drought tolerance is determined by several factors, one of which is hydraulic safety. Between the two species and four ecotypes studied, hydraulic safety was highest in *Ps*L4. Compared with the most vulnerable ecotype, *Ps*L3, $\Psi$ at 50% loss of hydraulic conductivity ($\Psi_{50}$) of *Ps*L4 was ≥0.9 MPa lower (Table 2, Figure 2). This confirms our second hypothesis that the ecotype from the driest habitat is the most drought tolerant. The first hypothesis regarding the plasticity of traits was also confirmed: growth and vulnerability to embolism had higher plasticity than leaf gas exchange and WUEi. The third hypothesis was rejected, as all four ecotypes were more drought tolerant than the cultivated rootstock. There are only a few

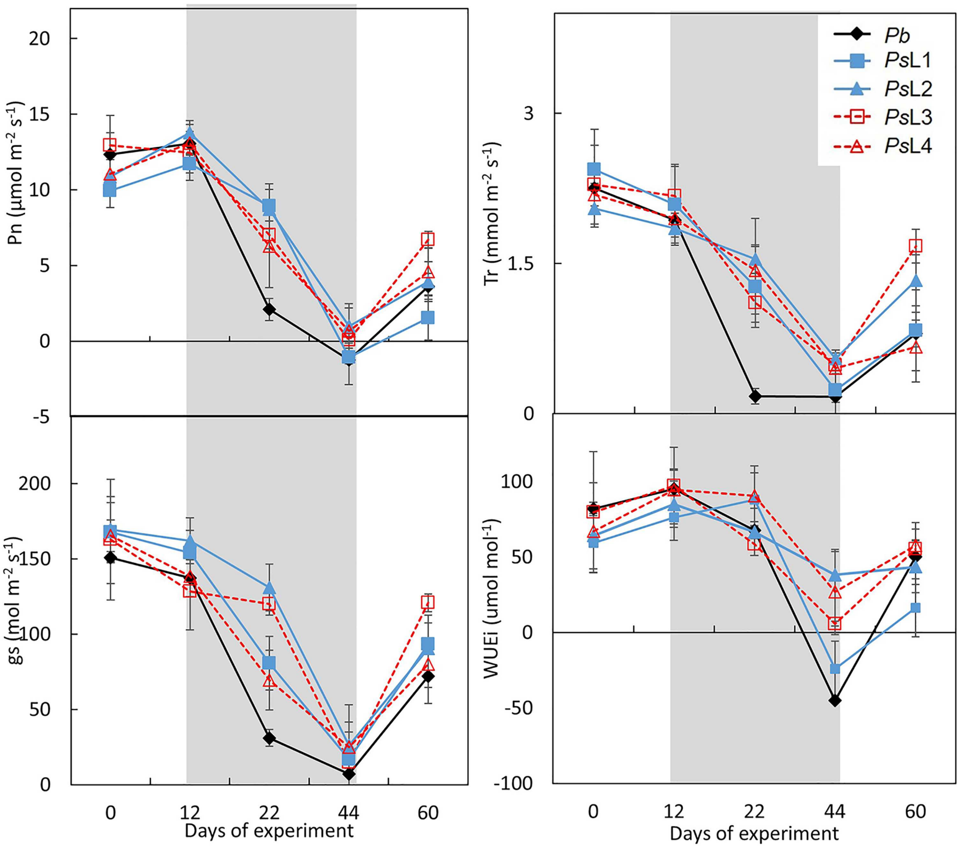

Figure 5. Changes in leaf gas exchange parameters in course of the experimental period in *P. betulifolia* and *P. syriaca* L1–L4 (*Ps*L1–*Ps*L4). Pn, net photosynthesis; *gs*, stomatal conductance; E, transpiration; WUEi, intrinsic WUE. The negative values in Pn, due to respiration, carried over to negative values in WUEi. The gray background denotes the imposed drought period. Error bars denote the standard error of the mean ($n = 3$–6; see Table 3 for statistical analysis).

other studies reporting vulnerability thresholds for *Pyrus*, but these point to relatively high variability in hydraulic safety within species. Paudel et al. (2019) found a range in cavitation onset from −1.2 to −1.7 MPa for cultivated pears and −1.6 to −2.0 for a wild relative, which is similar to *Ps*L4. Interestingly, the $\Psi_{50}$ levels estimated for cultivated and wild trees in the present study, −3.3 and −4.1 to −5.2 MPa, respectively, were well within the range reported for *Prunus* species (Cochard et al. 2008), and we can now confirm this range. The difference between the values measured by Paudel et al. (2019) and those measured in the current study could be related to genetic variation within *P. syriaca* ecotypes because of local adaptations, and effects of rootstock and grafting that influence tree vigor, anatomy and thus hydraulics in *P. betulifolia* (see Atkinson et al. 2005, Cohen et al. 2007, Bauerle et al. 2011). It is probably also a consequence of sapling age because we were using very young saplings (1.5 year old) and the water-saving strategy in small saplings increases the water economy compared with larger trees (Baquedano and Castillo 2006). Still, our results should be applicable for implementation at the nursery stage and, moreover, for establishment in the field. Previous studies found that there is a link between drought tolerance capacity and in situ drought occurrence (Klein et al. 2011, 2013, Pšidová et al. 2015, Voltas et al. 2018), At the same time, there are some notable examples showing lack of variation in $\Psi_{50}$ for conifer species (Klein et al. 2013, Maherali and DeLucia 2000) and some showing limited variation within angiosperm species that is correlated with climate (Herbette et al. 2011, Schuldt et al. 2016). It is possible that the potential for superior drought tolerance in ecotypes is limited to dry and hot habitats.

Interestingly, the point of stomatal closure was rather similar among the ecotypes, and hence, their safety margins were driven primarily by $\Psi_{50}$. Th is means that tree populations at the driest and warmest part of natural distributions can offer two distinct hydraulic advantages over other ecotypes: lower embolism vulnerability and higher HSMs. In contrast to conifers and ferns, narrow or negative safety margins are not uncommon in angiosperms and represent a strategy for optimizing carbon gain (Bréda et al. 2006, Brodribb and Holbrook 2004, Choat et al. 2012, Scoffoni et al. 2012). Indeed, Urli et al. (2013) and Choat et al. (2012) found that the xylem embolism threshold for the irreversible hydraulic failure of several angiosperm species was correlated more closely with $\Psi_{88}$ than with $\Psi_{50}$. Besides late stomatal closure, *gs* values also indicate that the rootstock

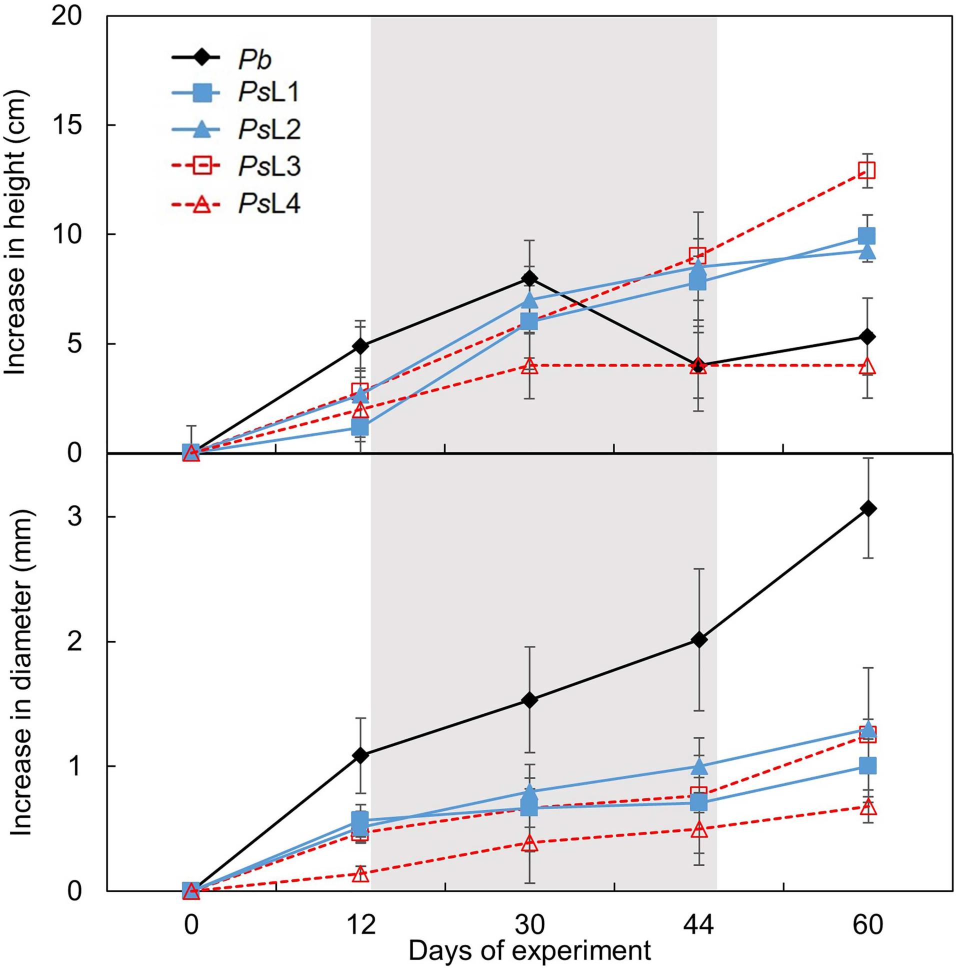

Figure 6. Stem height and diameter growth in course of the experimental period in *P. betulifolia* and *P. syriaca* L1–L4 (*Ps*L1–*Ps*L4). The gray background denotes the imposed drought period. Error bars denote the standard error of the mean ($n = 3$–10; see Table 3 for statistical analysis).

and four ecotypes in our study are all programmed to maximize carbon gain. Maximum *gs* values ranged from 210 (*Ps*L3) to 171 mmol m$^{-2}$ s$^{-1}$ (*Ps*L1; Table 3), higher than those reported for temperate angiosperms (e.g., Aasamaa et al. 2001, Eensalu et al. 2008). Stem Ψ in this range would already have induced considerable embolism in branches due to dehydration (Table 2, Figure 4). However, those previously reported measurements of $g_s$ were carried out on transpiring leaves, and it is known that Ψ of leaf can differ significantly from stem Ψ and xylem pressure (see Meinzer et al. 2001). The relatively high maximum *gs* values can be explained by stomatal features such as larger stomata and higher stomatal density (Sack and Holbrook 2006, Sellin et al. 2010).

Plant water status is a critical parameter that acts as a significant regulatory switch for both physiological and hydraulic responses during water stress. Water stress induces hydraulic signals from roots to leaves via the xylem, to exhibit the symptoms of drought to the shoot and leaf level (Sengupta et al. 2013). In pears, soil water stress caused a significant reduction in Ψ in all species and ecotypes, wherein *Ps*L2 and *Ps*L4 were able to maintain relatively higher and stable Ψ. At the same time, *Ps*L4 had the widest HSM, but *Ps*L3 had higher $g_s$ in mid-drought, and much higher growth when compared with the other ecotypes and species (Figure 6), supporting our hypothesis. Inconsistent with the above results, drought induced a significant reduction in net $CO_2$ assimilation and $WUE_i$ (Figure 5). The *Ps*L2 and *Ps*L4 plants exhibited higher net $CO_2$ assimilation and $WUE_i$ during water stress compared with others that are comparable with their water potentials (Reddy et al. 2019). A recent report has shown that similar behavior is observed in other plants; specifically, a slower reduction in the photosynthetic rate in unfavorable conditions indicates higher tolerance (Taïbi et al. 2017, Zhang et al. 2017). Notably, the photosynthetic rates measured here were slightly higher than those measured in the field (Paudel et al. 2019). A potential 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).

*Pyrus syriaca* had higher intrinsic WUEi than the rootstock, which had values of −45 to –28 mmol $CO_2$ $mol^{-1}$ $H_2O$, similar to values measured in apple (Regnard et al. 2010). The values of 68–89 mmol $CO_2$ $mol^{-1}$ $H_2O$ measured in the wild pear were closer to those measured in a broadleaf forest tree such as oak (Klein et al. 2013), and it was closer to our previous study (Paudel et al. 2019). In addition to this, this ecotypes' differentiation was significant at non-drought and intermediate drought conditions but not at prolonged drought, nor during recovery. Conversely, in those later times, there was differentiation among ecotypes in radial stem growth. But the ecotypes' distinction in growth had the opposite effect: the drier ecotype was growing less than the wetter ecotypes. This might hint to a tradeoff between growth and drought resistance (Paudel et al. 2018).

Based on our results, we conclude that *Ps*L3 and then *Ps*L4 ecotypes are more drought-tolerant under drought conditions as compared with the two other ecotypes and the rootstock. Late stomatal closure may, at least partially, explain the relatively high hydraulic safety found in our wild ecotypes, as this is the first prerequisite for maintaining carbon gain under decreasing $\Psi$. As expected, we found differences in hydraulic safety among the ecotypes, with *Ps*L4 being the most drought-tolerant, and providing an evidence for local adaptation to drier habitats. Selection of ecotypes growing at the driest sites for new rootstocks could provide more drought-tolerant fruit trees for the future climate.

## Authors' contributions

I.P. coordinated the measurements, performed most of the analysis and prepared the manuscript; T.K. initiated the project, designed the experiment and revised the manuscript; H.G. helped in the measurements; and A.Z. and G.S. helped in coordinating the ecotypes.

## Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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## Conflict of interest

The authors declare no conflict of interest.

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