

## Comparative growth, hydraulic conductivity, and sap flow responses of seedlings of two pyrus species to drought stress

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**Abstract:** Drought is one of the major abiotic stresses limiting the advancement of the pear industry in the arid and semiarid regions of China. Currently, the production of pear seedlings mainly depends on the grafting propagation of rootstocks from *Pyrus betulaefolia* Bunge and *Pyrus calleryana* Decne. However, limited information is available on the drought stress potential of these rootstocks. Here, we conducted pot experiments with controlled watering conditions on both rootstocks' seedlings and compared their growth, plant hydraulic conductivity, and stem sap flow velocity. Both rootstocks' seedlings were challenged with three drought regimes i.e. light (LD), moderate (MD), and heavy (HD) drought stress. The results indicated that *P. betulaefolia* performed better in growth than *P. calleryana* under the same drought regimes. Under LD, MD, and HD the relative growth of the new shoots, leaf-specific canopy hydraulic conductivity, leaf, stem, and root hydraulic conductivity, and stem sap flow velocity of *P. betulaefolia* exhibited reduction as compared to control (CK). Similar reduction trends were noted for *P. calleryana*, with slight variations in the case of stem-specific canopy hydraulic conductivity.

**Key words:** Drought regimes, pear breeding, hydraulic resistance, stem sap flow velocity

### 1. Introduction

Grafting has been commonly practiced for many centuries in the cultivation of horticultural crops. The use of dwarfing rootstocks has enabled a high-density plantation to produce maximum yield (Hayat et al., 2020; Khan et al., 2020). Rootstock regulates scion phenotype, including precocity, fruit size, yield, quality characteristics, and tolerance to various environmental stresses (Hayat et al., 2021; Devin and Bujdoso, 2022). Pears rank third in the Chinese fruit industry after oranges and apples. With 18.0 million tons of fresh pear production in 2021/22, which makes 76.59% of the global production, China leads the world in pear production (USDA, 2022). In China, 13 *Pyrus* species have been described and hundreds of cultivars have been developed (Teng, 2011). Two frequently used rootstocks in China are *Pyrus betulaefolia* Bunge and *Pyrus calleryana* Decne, respectively (Dong et al., 2015). *P. betulaefolia* mainly grows in North China, Northwest China, and Southern Northeast China, whereas *P. calleryana* is distributed in East and South China. Most of the pear-growing areas in China are facing changes in water availability, precipitation, and drought spells. Particularly, Shanxi has significantly lower precipitation as

compared to other provinces in North China i.e. Beijing, Hebei, and Inner Mongolia. Similarly, other provinces in East China and South China have received reduced amounts of precipitation e.g., Guangxi had 1560 mm of rainfall in 2018, which reduced to 1383 mm in 2021. Thus, the uneven distribution, as well as reduced precipitation show, increased drought spells in these areas. Overall, soil water deficiency in China has been a major problem for agricultural production since ancient times (Gao, 2018). Additionally, climate change and human activities have resulted in a shortage of water resources for agriculture (An et al., 2011). The waste water discharged from human life and production, the transformation of natural water, and the implementation of engineering measures to reduce floods have all led to the shortage of water resources. These circumstances have led to an aggravation of aridity, which has direct effects on cultivation management, yield, and fruit quality of horticulture crops (Ercisli et al., 2005; Erturk et al., 2014; Li et al., 2020). Since pear trees have high plant height and deep root system, which make it a more water-intensive species than other crops (Zhou et al., 2019).

One can obtain drought resistance through hybridization and selection (e.g., breeding) of rootstock

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candidates and then using the drought resistant genotypes in grafting. Therefore, rootstock plays a decisive role in drought resistance (Ikinici et al., 2014; Wang et al., 2018b; Yildirim et al., 2021; Ugur 2022; Yildiz et al., 2022). Drought resistance varies significantly among different pear rootstocks (Cheng et al., 1996). Earlier reports on the relationship between rootstock tissue structure and drought resistance have shown that the drought stress resistance potential of rootstocks can help the plant to have higher stomatal density, bigger root hydraulic conductivity, and higher biomass accumulation (Fan and Li, 2008). Physiological (stomatal conductance, photosynthesis, transpiration, xylem water potential) and biochemical (leaf pigments, free proline, malondialdehyde, and hydrogen peroxide production) differences within the *Pyrus boissieriana* rootstocks have been associated with drought stress tolerance and recovery (Zarafshar et al., 2014). A recent study indicated that the type of rootstocks can affect the growth, water relations, gas exchange, and anatomy of Flemish Beauty pear under water stress (Sharma and Sharma, 2008). Apart from *Pyrus* species, work on other orchards e.g., *Prunus* species, have shown that some rootstocks can extract water from the soil more effectively as compared to others based on leaf area, dry mass, and root characteristics (Stott, 2017). Nevertheless, the majority of studies have focused on the effect of rootstock on growth and physiological and biochemical properties in different pear species (varieties). To our knowledge, the intrinsic mechanism behind the difference in drought resistance between the two common rootstocks, *P. betulaefolia*, and *P. calleryana* has not been studied.

Plant responses to different water stress (drought) regimes include alterations in physiological and morphological processes. Additionally, the molecular and biochemical machinery is also affected under drought stress scenarios (Jiménez et al., 2013; Sabir, 2016). Studies have shown that drought can affect both the above-ground (stem and leaf) and below-ground organs (roots) (Robin et al., 2003; Singh et al., 2015; Umezawa et al., 2006). Among the above-ground organs, the changes in shoot length, new shoot growth, leaf area, and stem diameter are the major traits affected by drought (Shafqat et al., 2021). Whereas, root traits including root area, length, volume, and diameter can be significantly affected by drought (Serra et al., 2014; Yildirim et al., 2018). Previous studies have significantly improved our understanding of the variation patterns in water conductivity in annual woody plants upon stress conditions and explained the biological basis of water absorption and stress adaptation mechanisms in fruit trees (Han et al., 2015; LiandZhai, 2000; Zhang et al., 2013; Zhang and Shan, 2001). Many studies have demonstrated that as the main route of water transport, xylem conduits change and cavitation and

embolism are present under extreme conditions (Sperry et al., 1987), leading to variation in anatomical structure-dependent hydraulic characteristics (Li and Zhai, 2000), which directly affect water absorption and transportation (North and Noble, 1996). Water deficits will inevitably cause a reduction in water conductivity in the stem, root system, and leaf because of hydraulic resistance, decreasing the growth potential of the plant (Gonçalves et al., 2007; Tombesi et al., 2010). Changes in water conductivity at the tissue and organ level are requirements for plants to adapt to various adverse conditions. However, studies on water conductivity characteristics in various pear rootstocks and their changes in different organs of pear rootstocks under drought stress are very rare, particularly on the two rootstocks mentioned above.

Here, we performed drought simulation experiments using *P. betulaefolia* and *P. calleryana* and analyzed their drought stress responses and adaptation of the hydraulic structure. Our results about changes in growth potential, plant hydraulics, and stem sap flow velocity in response to drought provide data on the adaptability of two rootstocks for pear propagation under water deficit conditions.

## 2. Materials and methods

### 2.1. Plant material and growth conditions

Seeds of *P. betulaefolia* and *P. calleryana* were collected from Zhongtiao Mountain area, Jiangxian County, Shanxi Province, and Hubei Fruit Tea Research Institute, respectively. After sowing seeds, use potted seedlings as plant materials. In November 2016, the seeds were germinated and planted in trays with fine sand as the planting matrix after stratification. In May 2017, six-leaf seedlings were transferred into plastic pots with an upper diameter of 16.1 cm, lower diameter of 11 cm, and height of 14 cm. Potting soil was prepared using rotted pig manure, garden soil, and fine sand at a ratio of 1:2:3 (by volume), and seedlings were managed according to standard procedures. In February 2018, 150 seedlings of each of the two rootstocks with comparable sizes were transferred into pots with a height of 18 cm, upper diameter of 21 cm, and lower diameter of 12 cm. The soil was composed of rotted pig manure, garden soil, and fine sand at a ratio of 1:2:1 (by vol.). Each pot was filled with 2.90 kg of soil with a saturated soil water content of 41.3%. One plant was planted in each pot and was managed according to standard procedures. In April 2018, plants were moved to a rain shelter for testing, and their average height and average base diameter were approximately 55.0 cm and 0.8 cm, respectively. The photosynthetic active radiation was 23–29 mol·m<sup>-2</sup>·d<sup>-1</sup>.

### 2.2. Drought treatment

To prevent the effects of natural rain, the experiment was carried out inside an arch shed at the horticultural station of Shanxi Agricultural University. In early May

2018, seedlings showing robust growth and similar growth potential were selected for measuring related indicators. An artificial drought simulation experiment was performed with the following treatments: light drought-stress (LD): relative water content of the soil was  $60\% \pm 5\%$ ; moderate drought-stress (MD): relative water content of the soil was  $40\% \pm 5\%$ ; heavy drought-stress (HD): relative water content of the soil was  $20\% \pm 5\%$ ; and CK: relative water content of the soil was  $80\% \pm 5\%$ . The relative water content of the soil is presented as the percentage of soil water content relative to the saturated soil water content (FanandLi, 2008). Water was added to supplement for water evaporated at 6 pm every day. Leaf changes and damage levels were monitored during the experiment, and soil volumetric moisture was determined.

### 2.3. Investigation of growth potential in seedlings

At 9:30 am–11:30 am every 15 days, 6 healthy leaves were marked in the middle and upper part of each seedling as the leaves to be tested. Measures include net photosynthetic rate ( $P_n$ ,  $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) and transpiration rate ( $T_r$ ,  $\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), to calculate water use efficiency (RWUE,  $\mu\text{mol} \cdot \text{mmol}^{-1}$ ), the formula is  $\text{RWUE} = P_n/T_r$ . Growth potential was determined by measuring the relative growth of new shoots (cm), stem basal diameter (cm), total leaf area of the plant (TLAP,  $\text{m}^2$ ), and root parameters. The new shoots growth and stem basic diameter were measured with a ruler. The total leaf area of the plant is measured with a leaf area meter (Yaxin-1241, China). The root system was scanned with a scanner (Epson Perfection V800, Indonesia), and root length (m), average root diameter (mm), root surface area ( $\text{cm}^2$ ), and root volume ( $\text{cm}^3$ ) were determined using the software WinRhizo.

### 2.4. Determination of plant hydraulic characteristics

At 45 days after drought treatment, seedlings with similar growth status were selected for measuring the water conductivity of the plant canopy and stem and the hydraulic resistance of the root system using a high-pressure flow meter (HPFM, Dynamax hpfm-gen3, USA). Determination of water conductivity resistance was performed as described by (Tyree et al., 1998): the aboveground part of the plant was cut at the diameter measurement site and quickly connected to the HPFM to determine canopy hydraulic resistance ( $R_{\text{canopy}}$ ). The leaves were then removed to determine stem hydraulic resistance ( $R_{\text{stem}}$ ). Root hydraulic resistance ( $R_{\text{root}}$ ) was measured by connecting the cut root to the HPFM. Vector calculation was applied to calculate hydraulic resistance (Gascó et al., 2006). Absolute water conductivity ( $K_h$ ) and corresponding hydraulic resistance are reciprocal ( $K_h = 1/R$ ), and the water conductivity of the canopy and stem was obtained using this formula. The leaf-specific water conductivity ( $K_{\text{leaf}}$ ) of each organ was the ratio of its absolute water conductivity ( $K_h$ ) to the supported leaf area

( $S_{\text{leaf}}$ ). The stem-specific water conductivity ( $K_{\text{stem}}$ ) was the ratio of its absolute water conductivity ( $K_h$ ) to the area of the stem cross-section ( $S_{\text{stem}}$ ).

### 2.5. Determination of stem sap flow velocity

Stem sap flow velocity was monitored using a Flow 32 wrapped sap flow meter, and three replicates were performed for each treatment. Representative rootstocks were selected, and the measurement sites were located 10 cm above the soil surface. After installing a waterproof test probe with solar radiation prevention, the meter, which was powered by solar panels, was connected to the data acquisition unit to collect data every 15 min.

### 2.6. Data analysis

All the measurements were taken from triplicate samples. Excel 2013<sup>®</sup> was used for data statistics and drawing, SAS v9.0 (SAS Institute Inc., Cary NC, USA) software was used for data significance analysis ( $p < 0.05$ ) and marking.

## 3. Results

### 3.1. Changes in the growth of aboveground organs of pear rootstocks under drought stress

#### 3.1.1. Effect of drought stress on the relative growth of new shoots

Under normal water supply, the relative growth (RG) of the new shoots of *P. betulaefolia* was higher than that of *P. calleryana* ( $p < 0.05$ ), indicating that the former grew faster than the latter (e.g., Figure 1). We observed that with the increase in drought stress intensity (from LD to HD), the RG of new shoots of both examined rootstocks gradually decreased. Under LD, MD, and HD, the RG of new shoots of *P. betulaefolia* seedlings significantly decreased (Figure 1A). Similar trends were observed for RG of the new shoots in *P. calleryana* when challenged with LD, MD, and HD (e.g., Figure 1B). These observations indicate that the RG of both rootstocks is significantly affected by drought stress and there is a negative relation between the new shoot growth and drought intensity.

Considering the change in RG of new shoots in *P. betulaefolia* and *P. calleryana*, we observed that LD caused a 9.22% (nonsignificant) and 28.7% (significant) decrease from 15d–45d, respectively. Whereas under the influence of MD, the RG of new shoots decreased by 35.5% and 45.71% in *P. betulaefolia* and *P. calleryana*, respectively. Under HD, the RG of new shoots of both rootstocks was significantly lower than other treatments and CK. Taken together, it is stated that moderate and severe drought significantly affect the two *Pyrus* rootstocks, where *P. calleryana* was more sensitive to drought as compared to *P. betulaefolia*.

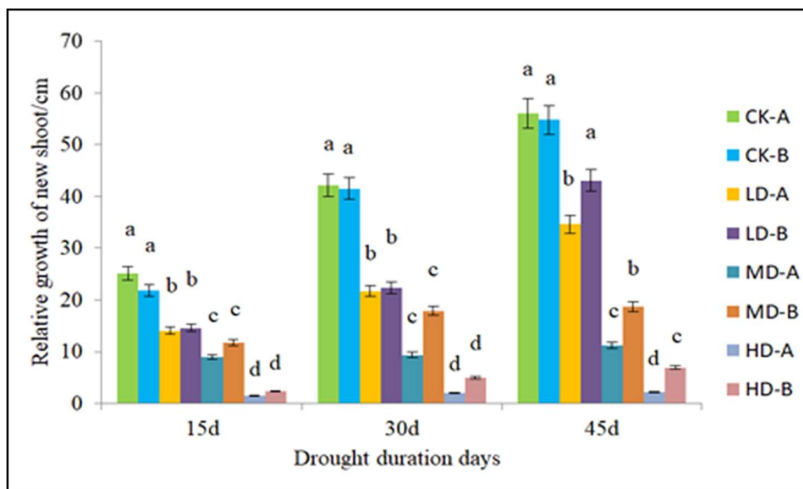
#### 3.1.2. Effect of drought stress on stem basal diameter and leaf area

Both *P. calleryana* and *P. betulaefolia* stems showed different stem thicknesses under drought stress. The overall stem thickness of *P. betulaefolia* was recorded as higher than

that of *P. calleryana*. In both tested rootstocks, the basal stem diameter decreased with an increase in drought stress intensity. Talking about *P. betulaefolia*, the reduction in basal stem diameter was more prominent under MD and HD as compared to LD. Whereas, in case of *P. calleryana*, LD actually resulted in slightly thicker stems (though nonsignificant), which then gradually decreased with the increase in drought stress intensity (e.g., Table). Overall, these results indicate that drought severity is linked with the reduction in the basal stem diameter.

The third above-ground organ i.e., the leaf was also affected by the imposed drought stress in both rootstocks. The TLAP of *P. betulaefolia* slightly increased in LD,

and then decreased in MD and HD as compared to CK. Whereas, in case of *P. calleryana*, the TLAP decreased in LD and MD, while it slightly increased in HD as compared to CK. However, the change in TLAP in both rootstocks under three different drought intensities was nonsignificant as compared to CK (e.g., Table). Overall, the TLAP of *P. betulaefolia* was higher than that of *P. calleryana*, though the changes were nonsignificant. These observations indicate that among the above-ground organs, the TLAP is not significantly affected by the changes in drought intensity. These results also imply that it is better to employ RG of new shoots and basal stem diameter as indicators of drought stress and not the TLAP.



**Figure 1.** Effects of drought stress on the relative growth of new shoot in two pear rootstock seedlings. The bars represent mean values of three replicates ± standard deviation. Where, A = *P. betulaefolia* Bunge and B = *P. calleryana* Decne. Different lower-case letters indicate significant differences at  $p < 0.05$ .

**Table.** The difference in growth parameters between *P. betulaefolia* and *P. calleryana* under the influence of low, moderate, and high drought stress.

Species	Treatment	Basal diameter/ cm	TLAP/ m <sup>2</sup>	Root length/ m	Root surface area/ cm <sup>2</sup>	Average diameter/ mm	Root volume/ cm <sup>3</sup>
<i>P. betulaefolia</i>	CK	0.847 ± 0.035 Aa	0.092 ± 0.003 Aa	44.71 ± 1.25 ab	654.62 ± 75.31 a	0.477 ± 0.031 a	8.143 ± 1.519 a
	LD	0.804 ± 0.006 ABab	0.102 ± 0.017 Aa	48.97 ± 3.71 a	642.40 ± 20.98 a	0.448 ± 0.025 a	7.101 ± 0.467 ab
	MD	0.717 ± 0.020 ABCbc	0.076 ± 0.001 Aa	54.94 ± 9.47 a	644.11 ± 103.46 a	0.406 ± 0.047 a	6.660 ± 1.552 ab
	HD	0.769 ± 0.035 ABabc	0.082 ± 0.039 Aa	26.26 ± 10.40 ab	309.45 ± 127.93 ab	0.409 ± 0.025 a	3.097 ± 1.358 ab
<i>P. calleryana</i>	CK	0.666 ± 0.010 BCcd	0.082 ± 0.001 Aa	38.36 ± 16.36 ab	500.38 ± 226.80 ab	0.459 ± 0.082 a	5.655 ± 2.733 ab
	LD	0.675 ± 0.077 BCcd	0.071 ± 0.008 Aa	31.63 ± 3.19 ab	399.39 ± 64.46 ab	0.395 ± 0.039 a	4.159 ± 1.012 ab
	MD	0.601 ± 0.027 Cd	0.065 ± 0.012 Aa	27.23 ± 8.92 ab	368.92 ± 162.14 ab	0.476 ± 0.026 a	4.187 ± 2.258 ab
	HD	0.580 ± 0.007 Cd	0.098 ± 0.031 Aa	16.41 ± 3.89 b	187.18 ± 42.29 b	0.39 ± 0.030 a	1.769 ± 0.405 b

### 3.2. Effect of drought stress on root characteristics of *P. betulaefolia* and *P. calleryana*

As shown in Figure 2, drought treatment caused dramatic differences in the morphological characteristics of *P. betulaefolia* and *P. calleryana*. There were no significant differences in all measured root parameters for *P. betulaefolia* among the various levels of drought treatment, but they shared a similar pattern of change (e.g., Table). Other than root length, which exhibited the order MD > LD > CK > HD, all other root parameters i.e. root surface area, average diameter, and root volume, decreased with an increase in drought intensity. Similarly, in *P. calleryana*, the root length and root surface area gradually decreased as drought conditions worsened. Whereas, the average root diameter in MD was the highest followed by CK, LD, and HD. While for the root volume, the reducing pattern was CK > MD > LD > HD. The root length, root surface area, and root volume were higher in *P. betulaefolia* as compared to *P. calleryana*, while there were minor or no differences for average root diameter (e.g., Table). Overall, the results indicate that under the influence of HD, the roots were severely affected.

### 3.3. Transpiration rate (*Tr*) of *P. betulaefolia* and *P. calleryana* under drought stress

No significant impact on the transpiration rate (*Tr*) of *P. betulaefolia* was noted under the influence of LD, while in

the case of *P. calleryana*, it increased and then decreased (e.g., Figure 3A). Under the influence of both MD and HD, a continuous significant reduction in *Tr* was recorded in both species. Under the influence of HD, the *Tr* of *P. betulaefolia* and *P. calleryana* were reduced by 88.6% and 92.5%, respectively (e.g., Figure 3A). These observations indicate that *P. calleryana* faces severe reductions in *Tr* as compared to *P. betulaefolia*.

### 3.4. Net photosynthetic rate and water use efficiency (RWUE) of *P. betulaefolia* and *P. calleryana* under drought stress

As shown in Figure 3B, the net photosynthetic rate (*Pn*) of the two rootstock seedlings decreased under drought stress compared with that under normal water supply, and there were significant differences between different drought treatments. Under LD treatment, the *Pn* values of the two seedlings were significantly higher than those of MD and HD. Under the condition of HD, the water use efficiency of *P. calleryana* was significantly higher than that of other treatments (e.g., Figure 3C).

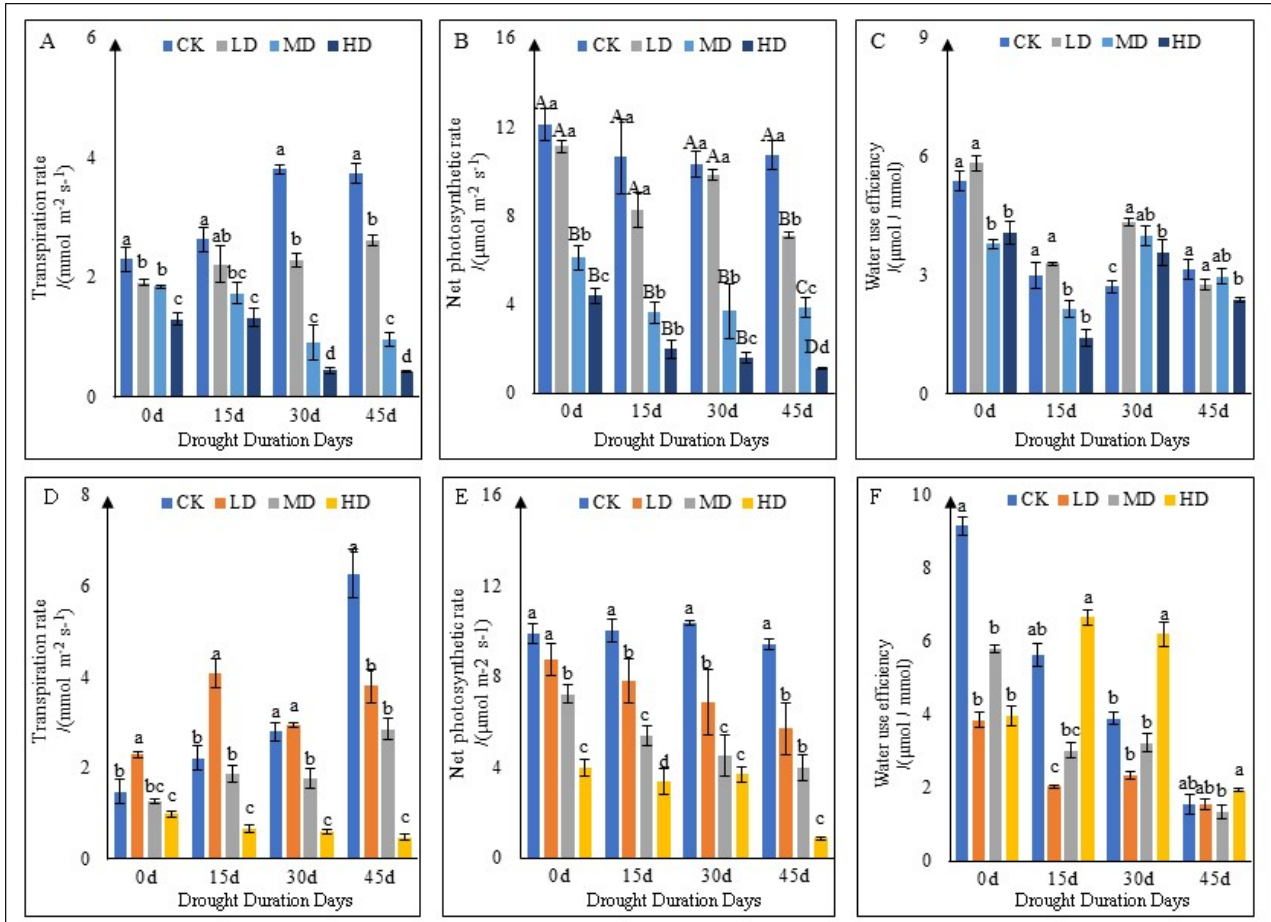
### 3.5. Effects of drought regimes on the hydraulic conductivity of *P. betulaefolia* and *P. calleryana*

#### 3.5.1. Changes in water conductivity of the two pear rootstocks under drought stress

The leaf specific  $K_{canopy}$  under LD treatment reduced to 86.6% and 74.9% in *P. betulaefolia* and *P. calleryana*,



**Figure 2.** Morphological differences in root systems between the two pear rootstocks under drought stress treatment. A1: *P. betulaefolia*, CK; A2: *P. betulaefolia*, LD; A3: *P. betulaefolia*, MD; A4: *P. betulaefolia*, HD. B1: *P. calleryana* a, CK; B2: *P. calleryana* a, LD; B3: *P. calleryana* a, MD; B4: *P. calleryana* a, HD.



**Figure 3.** Effect of drought stress on A, D) transpiration rate, B, E) net photosynthetic rate, and C, F) water use efficiency of *P. betulaefolia* (A, B, and C) and *P. calleryana* (D, E, and F). The bars represent mean values of three replicates  $\pm$  standard deviation. Different letters indicate significant differences at  $p < 0.05$ .

respectively, when compared to CK. With the increase in drought stress intensity i.e. MD and HD, the leaf specific  $K_{\text{canopy}}$  showed significant reduction i.e. it reduced to 74% and 59.7% in *P. betulaefolia* and 56.3% and 45.6% in *P. calleryana*, respectively. These results imply that the more severe drought intensity becomes, the higher it affects the leaf specific  $K_{\text{canopy}}$ . Overall, the leaf specific  $K_{\text{canopy}}$  was higher in *P. betulaefolia* as compared to *P. calleryana* (e.g., Figure 4A). Contrarily, LD (increased by 1.9%) did not significantly affect the stem specific  $K_{\text{canopy}}$  in *P. betulaefolia*, whereas MD (reduced to 77.86%) and HD (reduced to 46.26%) caused a significant reduction. In contrast, *P. calleryana* stem specific  $K_{\text{canopy}}$  was significantly affected, where it reduced to 84.4%, 66.0%, and 40.4% under the influence of LD, MD and HD, respectively. This suggests a reducing trend in stem specific  $K_{\text{canopy}}$  was noted for *P. calleryana* when subjected to LD, MD, and HD. Whereas, *P. betulaefolia* showed a slight increase in stem  $K_{\text{canopy}}$  under the influence of LD, which then decreased on MD

and HD (Figure 4B). Overall, stem  $K_{\text{canopy}}$  was higher in *P. calleryana* than *P. betulaefolia*. These observations suggest that *P. calleryana* is relatively more drought susceptible than *P. betulaefolia*.

We also studied the lower leaf and lower stem conductivities in both genotypes under the influence of LD, MD, and HD. As far as the lower leaf conductivity is concerned, *P. betulaefolia* exhibited a reducing trend with the intensification of drought stress i.e. CK > LD > MD > HD. However, *P. calleryana* showed variations in lower leaf conductivity such that it reduced in LD, slightly increased in MD (though nonsignificant), and then a significantly increased in HD (e.g., Figure 4C). These changes show that *P. betulaefolia* lower leaf conductivity reduces consistently with the intensity of drought but *P. calleryana*'s lower leaf conductivity increases if drought is intensified. This was also confirmed by the lower stem conductivity measurements, where both rootstocks exhibited similar trends (e.g., Figure 4D).

**3.5.2. Changes in root system hydraulic resistance of *P. betulaefolia* and *P. calleryana* under drought stress**

Under normal watering conditions i.e. CK, the differences in  $R_{root}$  of both species were statistically nonsignificant, though *P. calleryana* had a slightly higher value. However, under LD, MD, and HD, we observed 1.12, 2.58, and 2.86-fold increases in  $R_{root}$ , as compared to CK, in *P. betulaefolia*. A similar increasing trend was noticed in *P. calleryana* under LD and MD. However, we observed a 36.6% decrease in  $R_{root}$  as compared to MD (e.g., Figure 5). Overall, we observed an increasing trend in  $R_{root}$  in both species with slight variation in *P. calleryana*.

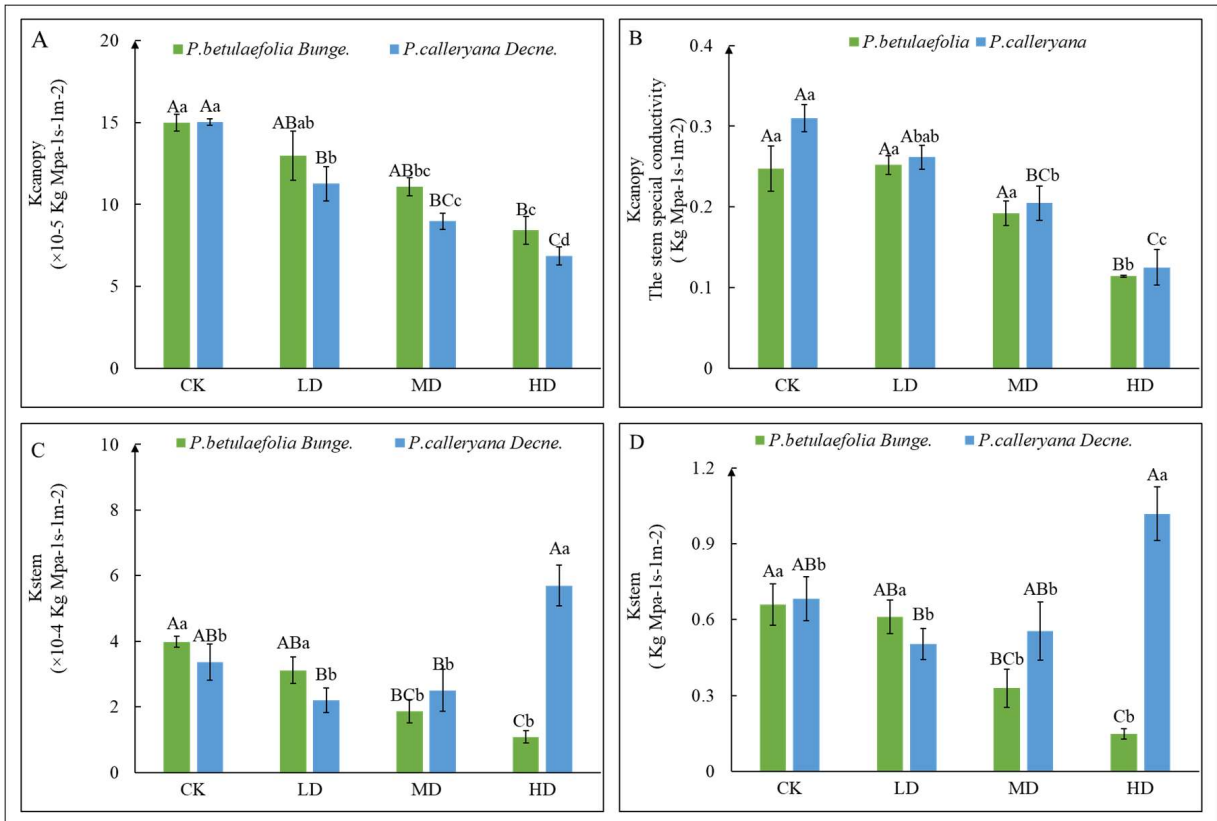
**3.6. Variation in stem sap flow velocity in *P. betulaefolia* and *P. calleryana* in response to drought stress**

Under the influence of LD, the sap flow velocity in *P. betulaefolia* was significantly higher than CK. It reached a peak of 18.29 g/h at 14:55; ~1.64-fold higher than CK. However, with an intensification of drought, the stem sap flow velocity significantly decreased to 72.6% and 42.2% in MD and HD, respectively, as compared to CK. Similarly, LD, MD, and HD also affected the stem sap flow velocity of *P. calleryana*, where it gradually decreased to

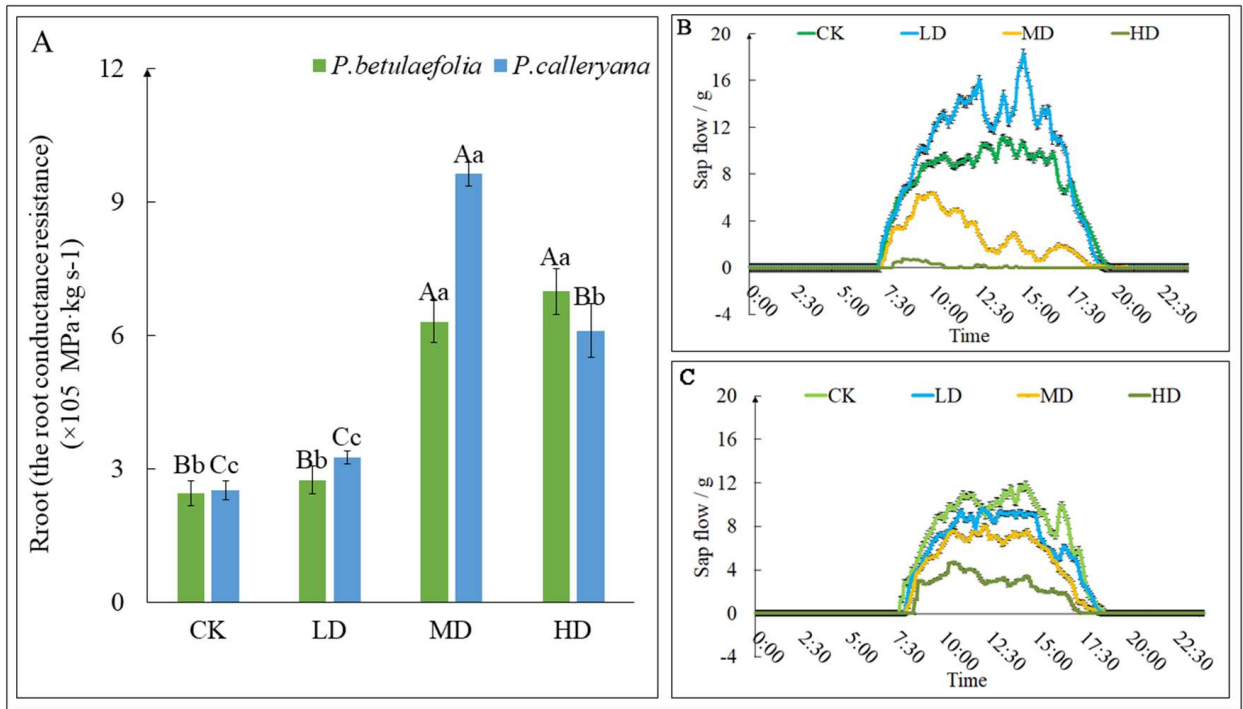
80.67%, 53.52%, and 6.63%, respectively, as compared to CK. Overall, *P. betulaefolia* initiated sap flow earlier than *P. calleryana*. With an increase in drought intensity, the reduction in stem sap flow velocity of *P. calleryana* was significantly greater than that of *P. betulaefolia* under the same drought conditions.

**4. Discussion**

Drought stress can significantly affect plant growth, development, and overall yield. It triggers multiple responses including limiting shoot growth, diameter, leaf growth, and emergence and growth of new shoots. Additionally, drought can significantly affect root traits, water use efficiency, and transpiration (Takahashi et al., 2020 and references therein). Under climate change and changing precipitation scenario in China, drought stress threatens pear yields (Wang et al., 2018). Among the strategies to mitigate drought stress in pears, the use of drought-tolerant rootstocks can be productive (Asayesh et al., 2023). A similar strategy has been reported in multiple fruit plant/tree species e.g., grapevine ones are frequently used (Fort et al., 2017), tetraploid Rangpur lime (Allario



**Figure 4.** Hydraulic characteristics of *P. betulaefolia* and *P. calleryana* under the influence of drought stress. A) leaf Kcanopy, B) stem Kcanopy, C) Kstem, and D) Kstem. The bars represent mean values of three replicates  $\pm$  standard deviation. Different lower-case letters indicate significant differences at  $p < 0.05$ .



**Figure 5.** A) Effects of 45-day drought stress on the hydraulic conductance resistance of roots of two types of rootstocks. The bars represent mean values of three replicates  $\pm$  standard deviation. Different letters indicate significant differences at  $p < 0.05$ . B) Changes in stem sap flow velocity in *P. betulaefolia*. C) Changes in stem sap flow velocity in *P. calleryana*.

et al., 2013), apple, and citrus (Hussain et al., 2018), etc. The two *Pyrus* species examined here for their drought stress resp in China (especially in Northern China) (Dong et al., 2015). Their uses (particularly of *P. betulaefolia*) as rootstocks have also been reported in North America and Europe. Therefore, the results obtained here are of greater importance.

Our results on the effect of three drought stress regimes on RG of new shoots, Tr, net photosynthetic efficiency, and  $R_{WUE}$  in both *Pyrus* species indicate that on 45d, the drought stress responses are more prominent as compared to CK (e.g., Figures 1–3). These observations are consistent with the recent results in *Glycyrrhiza uralensis* Fisch (Song et al., 2022). Considering this, and the fact that seedlings are relatively more sensitive/vulnerable to changes in water availability (Kupers et al., 2019; Maryam et al., 2020), we continued our experiments with the three drought regimes for a period of 45d. Based on earlier work on *Nerium oleander* L. (Niu et al., 2008), we know that higher RG of new shoots is related to drought tolerance, therefore, the slightly higher RG of new shoots in *P. calleryana* may hint that it is relatively drought tolerant, which is consistent with a previous study (Li et al., 2017). However, considering that the differences were nonsignificant for this trait and TLAP, it is not reasonable to judge their drought tolerance based on these characteristics. Drought

can significantly decrease the stem diameter in different crops e.g., soybean (Ohashi et al., 2009) and maize (Aslam et al., 2015), therefore, the reduction in stem diameter in both *Pyrus* species is understandable. Furthermore, the results that caused the most reduction in stem diameter are consistent with the results (Aslam et al., 2015).

Furthermore, the root morphology traits are considered functional traits that determine the adaptability of woody species e.g., *Pyrus pyraeaster* L. and *Sorbus domestica* L., to drought stress (Paganová et al., 2019). The relative decrease in root surface area, average diameter, and volume in *P. betulaefolia* and root length and root surface area in *P. calleryana* indicate that drought significantly hampers their growth similar to other plant species (Sánchez-Blanco et al., 2014). However, considering the decrease in root traits in *P. betulaefolia* as compared to *P. calleryana* suggests that the latter is relatively more tolerant to drought (e.g., Table). Similar results have been described in wheat (Tomar et al., 2016), rapeseed (Dai et al., 2020), and soybean (Hund et al., 2009), where tolerant genotypes tend to have better root trait performance under drought stress. Considering the results that drought intensification caused a concomitant decrease in overall root length, volume, area, and average diameter, it can be concluded that drought intensity is directly linked with the observed root characteristics. This is also consistent with earlier studies that root volume,



length, and surface area are the traits that enable a plant to maintain its productivity under drought, and the severer the water stress is, the more prominent the changes are in root architecture reviewed in Comas (Comas et al., 2013).

Plants adjust their hydraulic architecture by altering the growth patterns in both the above and below ground organs. It is hydraulic conductivity that determines drought responses in higher plants (Brodribb and Holbrook, 2006). Water conductivity can reflect the relationship between plant and soil moisture. Under drought conditions, leaf specific water conductivity is reduced with the decrease of soil water content in woody plants (Xu, 2013). A study by Zhang (2012) on the water conductivity of roots and different aboveground organs of *Malus hupehensis* revealed that stem embolism occurs upon water deficit, but leaf specific water conductivity of roots and stems under HD conditions is slightly higher than that under MD due to a robust conducting system and thick ducts. Strong drought resistance is related to such a characteristic, which is consistent with our findings here (e.g., Figure 4). In addition, we also showed that under normal water conditions, the water conductivity of *P. calleryana* was slightly better than that of *P. betulaefolia*. However, *P. betulaefolia* exhibited better adaptability, therefore, the reduction in leaf specific  $K_{\text{canopy}}$  was less significant, and hydraulic resistance maintained a slow elevation, resulting in a higher Tr. Moreover, under normal water conditions, the daily variation of stem sap flow velocity exhibited a “broad peak” curve in both *P. betulaefolia* and *P. calleryana*, which is consistent with a previous study by Du et al. (2017). *P. betulaefolia* started sap flow activity earlier but its peak velocity was marginally lower than that of *P. calleryana*. With a reduction in soil water content, the decrease in stem sap flow velocity was more substantial in *P. calleryana* compared to *P. betulaefolia*, upon the same severity of drought stress. Our data suggest that with the progression of drought stress, leaves of pear rootstock gradually dehydrate and wither, even turning yellow and dried up, leaf area decreases, transpiration is gradually reduced, and trunk thickening and growth slows down. The root system absorbs water passively to maintain the physiological and ecological water requirement of the plant. However, it is difficult for the root system to absorb water and sap flow activity is weakened due to increasing hydraulic resistance of the root system. The difference between *P. betulaefolia* and *P. calleryana* in response to water deficiency may be related to the relatively complex and highly strict regulatory mechanism of the genetic factors such as transcription factors and aquaporins in the root system similar to *Morus* spp (Reddy et al., 2017), however, this assumption should be further investigated through the application of omics techniques e.g., transcriptome, metabolome, and/or proteome analyses.

The comparative growth, hydraulic conductivity, and sap flow analyses of the two commonly used pear rootstocks i.e. *P. betulaefolia* and *P. calleryana* revealed that prolongation of drought causes severe damages. Both rootstocks exhibited decreasing growth trends in terms of above and below ground characteristics when drought stress was intensified. Based on the morphological changes in stem, leaf, and root, leaf and stem specific  $K_{\text{canopy}}$  changes,  $K_{\text{leaf}}$  and  $K_{\text{stem}}$ , and stem sap flow velocity, we can conclude that *P. calleryana* growth is seriously inhibited. Whereas, *P. betulaefolia* showcased a relatively better drought stress tolerance. Therefore, when considering these two rootstocks, *P. betulaefolia* should be preferred over *P. calleryana*.

## 5. Conclusions

Under normal water supply conditions, the diurnal variation of the liquid flow rate of *P. betulaefolia* and *P. calleryana* is a “broad peak” curve, and *P. betulaefolia* can start liquid flow activity earlier. Under mild, moderate, and severe drought stress, the leaf  $K_{\text{canopy}}$  hydraulic conductivity of *P. betulaefolia* and *P. calleryana* decreased significantly. After drought stress, the increase of root hydraulic resistance of *P. calleryana* was significantly greater than that of *P. betulaefolia*, which showed that the growth of *P. calleryana* was severely inhibited. After comprehensive analysis of the above physiological indicators, it was found that *P. betulaefolia* has a strong ability to resist drought stress.

## Author Contributions

Sheng Yang: Methodology, investigation, validation, data curation. Bin Xie: Methodology, investigation, validation, data curation. Li Liulin: Conceptualization, supervision, writing-review & editing.

## Conflict of interest

The authors declare no competing financial interests.

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#### Data availability statement

Not applicable.

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