



## Impact of Drought Stress on Photosynthetic Response of Some Pear Species

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### ABSTRACT

To investigate photosynthetic response of some pear (*Pyrus* spp.) species to drought stress, a pot experiment was conducted using a factorial experiment based on completely randomized design (CRD) with three replication under greenhouse condition. The factors included five pear species including: *P. bioisieriana*, *P. communis*, *P. glabra*, *P. salicifolia* and *P. syriaca* and three levels of drought stress [(100%, 60% and 30% of field capacity (FC)]. According to the obtained results, different levels of drought stress significantly restricted morphological and physiological responses in all studied species. Increasing drought stress intensity reduced leaf relative water content (RWC), net photosynthetic rate, stomatal conductance, transpiration rate and intercellular carbon dioxide concentration when compared to their values in control plants. However, root/shoot dry weight ratio, specific leaf weight and stomatal density per unit of area were increased. In *P. glabra* exposed to severe stress (30% of FC), the values of root/shoot dry weight ratio (0.85 g), specific leaf weight (23 mg cm<sup>-2</sup>), stomatal density per unit of area, relative water content (73%) and net photosynthetic rate (3.9 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) were significantly higher than the other species. *P. syriaca*, *P. salicifolia*, *P. bioisieriana* and *P. communis* were placed in the next ranks, respectively based on their response to drought. In conclusion, *P. glabra* is reported as a more effective species in mitigating the adverse effects of drought by boosting its protective mechanisms than the other pear species.

### Introduction

Pear (*Pyrus* spp.) belongs to the Rosaceae family with about 50 species it has been identified worldwide; however, only three species are widely cultivated (Li et al., 2016). It has a

cultivated area of 3 million m<sup>2</sup> and an annual production of 2 million tons, after bananas, oranges, apples and grapes, is the fifth most important fruit in temperate regions (Food and Agriculture Organization of the United Nations, 2011). Considering that the use of wild plant materials is a useful and practical method to

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improve drought tolerance in breeding programs (Sisko et al., 2009; Ashraf, 2010), therefore, wild pear cultivars and genotypes in the Iranian plateau have long been the focus of attention due to their resistance to various biotic and abiotic stresses (Javadi et al., 2005). Water stress disrupts horticultural crop growth, development and finally results in low productivity particularly in arid and semi-arid parts of the world (Kumar et al., 2019). Under drought stress, some disorders generally occur in the vital physiological processes, such as in gas exchange responses (Hu et al., 2010; Hoshika et al., 2013), cell dehydration (Manes et al., 2006) and chlorophyll degradation (Liu et al., 2019). Plants use various resistance mechanisms such as escape, tolerance and avoidance to cope with stress. Some of the mechanisms are related to: reduction of water out flow from the plant by the reducing stomatal conductance (Romero and Botía, 2006; Brodribband McAdam, 2017), morphological modification and improving water uptake by developing efficient root systems (Kumar et al., 2019), and leaf anatomical modifications (Yadollahi et al., 2011). The evaluation of drought tolerance in *Pyrus betulaefolia* showed that with increasing dehydration, net photosynthesis, transpiration rate and stomatal conductance decreased significantly while the rate of ion leakage increased (Li et al., 2016). Furthermore, Liu et al (2019) showed that in *Pyrus betulaefolia* dehydration stress by stimulating synthesis of abscisic acid (ABA) from the roots and accumulation in the leaves, reduces the stomatal conductance. Evaluation of the responses of three wild *pyrus boissieriana* populations to water scarcity stress showed that dehydration reduces the leaf RWC, net photosynthesis, stomatal conductance, carbon

fixation, transpiration and xylem water potential.

Plant populations belonging to semi-arid regions are more tolerant to drought stress than the populations originated from semi-humid regions at higher altitudes (Zarafshar et al., 2014). Decreased photosynthesis and stomatal conductance under drought stress has been reported for many plant species, including pear (Rajametov, 2017; Li et al., 2019), grape (Ghaderi et al., 2011) and apple (Sircelj et al., 2007).

The aim of this study was to investigate the effect of drought stress on water relations and photosynthetic gas exchanges of five pear species with the aim of screening for drought tolerance.

## Materials and Methods

### *Plant materials and growth condition*

A pot experiment was conducted as factorial based on completely randomized design (CRD) with three replications in two experimental units in research greenhouse of West Azarbaijan Agricultural and Natural Resources Research Center from April to September 2019. Factors included three levels of drought stress: 100% of field capacity (FC) (control), 60% of FC (moderate stress) and 30% of FC (severe stress), and five pear species including: *P. boissieriana*, *P. communis*, *P. glabra*, *P. salicifolia* and *P. syriaca* were used. The one-years old pear seedlings were collected from different regions of Iran (Golestan, East and West Azarbaijan and Kurdistan provinces) and transferred to plastic pots (23×25 cm) containing 10 kg of soil. Each replication was composed of one plant per pot, in total 90 experimental parcels were used. The soil properties are presented in Table 1.

**Table 1.** Soil physical and chemical characteristics

Textural classification	Clay (%)	Silt (%)	Sand (%)	Organic matter (g/kg)	pH	N (g/kg)	P (mg/kg)	K <sup>+</sup> (mg/km)	CaCl <sub>2</sub>	Electrical conductivity (ds/m)
Sandy loam	18.31	55.53	55.27	0.871	7.7	2.10	51.00	442.05	15.20	2.8

60 days after transplanting (acclimatization period), drought treatments (100, 60 and 30% of FC) were applied. The field capacity of each pot was estimated by the gravimetric method (Green *et al.*, 2004). At first, a certain amount of soil was poured in to each pot based on weighting. Then 4 pots were randomly selected and saturated. Pot weights were re-calculated after 48 hour of drainage and the soil was dried for 24 hour at 105°C. According

$$\text{Field capacity(FC)} = \frac{\text{soil weight in field capacity(FCW)} - \text{weight of dry soil(DW)}}{\text{weight of dry soil(DW)}} \times 100 \quad (1)$$

### **Root/ shoot dry weight**

Roots were well washed using a micro-mesh sift and watered roots, shoots and main stem (without leaves) were oven-dried at 75 °C for 48 h and dry weight determined for 3 seedlings per treatment.

### **Stomata density per unit area**

For microscopic observation of stomata density, the epidermis was stripped from the lower part of leaf and from a similar place from the leaves of the same size as the plant. The number of stomata per unit of area (mm<sup>2</sup>) was counted and recorded using a fluorescence illumination microscope (NICON ECLIPSE Ts2R, Japan), magnification 40X (Miskin *et al.*, 1972).

### **Specific leaf weight (SLW)**

Specific leaf weight was calculated in four fully expanded young leaves from plants of each species under different drought stress treatments. Leaf area was determined with leaf area meter (AM-200 model) and then, the dry weight of these leaves were determined after oven drying for 24 h at 70 °C. Specific leaf weight was calculated as the ratio of leaf dry weight to leaf area.

### **Relative water content (RWC)**

The relative water content (RWC) of leaves was measured according to Turner (1981). Immediately after sampling, fresh leaves were weighted (FW) and then immersed in distilled water for 4 h at room temperature. The turgid

leaves were then blotted dry and weighed (TW) and then leaves were oven dried drying at 80 °C for 48 h and dry weight (DW) was measured. The leaf relative content was calculated using the following formula:

$$\text{RWC (\%)} = \frac{[(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100}$$

### **Net photosynthetic rate (Pn), stomatal conductance (g<sub>s</sub>), transpiration rate (E) and intercellular carbon dioxide concentration**

At the end of the experiment and in all treatments, upper fully expanded leaves were selected for assay. Net photosynthetic rate, transpiration rate, stomatal conductance and intercellular carbon dioxide concentration were carried out according to Jiang *et al.* (2020) and using a portable photosynthesis system HCM-1000 (WALZ, Germany) equipped with a leaf chamber fluorimeters with an 8 cm cuvette area. The standard conditions for leaf stabilization in the cuvette were: air flow rate of 800 ml min<sup>-1</sup>, reference CO<sub>2</sub> concentration was 350 ppm, chamber temperature was 20 °C and the light intensity was 1200 to 1400 μmol m<sup>-2</sup> s<sup>-1</sup>. Measurements were taken in the morning from 10:00 to 11:00.

### **Data analysis**

The data were analyzed by analysis of variance (ANOVA) with statistical analysis system (SAS) (Version 9.01, SAS Institute, Cary, NC, USA). Significant differences among values of all

parameters were determined at  $P < 0.01$  according to Duncan's Multiple Range Test. All experimental data were expressed as the means of least three independent biological repeat and the correlation curve between the parameters was drawn by Microsoft Excel software.

## Results

Analysis of variance revealed that the effects of

drought treatments, species and their interaction on some morphological traits, including root/shoot dry weight, specific leaf weight, stomata density per unit of area, relative water content, net photosynthetic rate, stomata conductance, transpiration rate and intercellular carbon dioxide concentration of pear species were significant ( $p < 0.01$ ) (Table 2).

**Table 2.** Analysis of variance for the effects of various level of drought, species and interaction of drought treatment and species on some morphological and physiological parameters in five pear species (*Pyrus* spp.)

Source of variation	df	Mean of square (MS)							
		Root/ Shoot dry weight	Specific leaf weight	Stomatal density per area unit	Relative water content	Net photosynthetic rate	Stomatal conductance	Transpiration rate	Intercellular carbon dioxide concentration
Replication	2	0.001 <sup>ns</sup>	0.484 <sup>ns</sup>	15.555 <sup>ns</sup>	0.497 <sup>ns</sup>	33.888 <sup>ns</sup>	0.837 <sup>ns</sup>	1.303 <sup>ns</sup>	14.520 <sup>ns</sup>
Drought	2	0.304 <sup>**</sup>	50.265 <sup>**</sup>	13.128 <sup>**</sup>	1544.447 <sup>**</sup>	6157.222 <sup>**</sup>	7856.171 <sup>**</sup>	134.198 <sup>**</sup>	2783.800 <sup>**</sup>
Species	4	0.057 <sup>**</sup>	9.580 <sup>**</sup>	936.66 <sup>**</sup>	56.307 <sup>**</sup>	1444.244 <sup>**</sup>	131.236 <sup>**</sup>	10.851 <sup>**</sup>	1869.319 <sup>**</sup>
Drought × Species	8	0.019 <sup>**</sup>	2.428 <sup>**</sup>	473.33 <sup>**</sup>	99.651 <sup>**</sup>	44.527 <sup>**</sup>	36.062 <sup>**</sup>	1.636 <sup>**</sup>	191.863 <sup>**</sup>
Experimental error	28	0.004	0.346	94.126	21.302	8.793	2.688	0.105	44.847
Cv (%)	-	13.395	4.461	6.747	6.361	6.725	4.181	5.877	5.766

Under each parameter, means of main effects followed by different letters in columns indicate significant differences at  $P \leq 0.05$  (Duncan's test). Main effects are pooled means for observations under each variable. For the analysis of variance, ns, \* and \*\* indicate non-significant ( $P > 0.05$ ) and significant at  $P \leq 0.05$  and  $P \leq 0.01$ , by F-test, respectively

### Root/shoot dry weight

The analysis of variance showed significant differences ( $p < 0.01$ ) in root/shoot dry weight among the drought stress treatments, species, and their interaction (Table 2). Based on the results of comparing means, in control treatment (100% of FC), the highest root/shoot dry weight was related to *P. syriaca*, while *P. bioessieriana* had the lowest root/shoot dry weight. Also, there was no significant difference in root/shoot dry weight between *P. glabra* and *P. communis* species. Drought stress caused a significant increase in root/shoot dry weight among studied species. In moderate stress (60% of FC), *P. glabra* and *P. Syriaca* with 0.114 g and 0.243 g increase compared to control (100% of FC) had the highest root/shoot dry weight and after that, *P. salicifolia*, *P. bioessieriana* and *P. communis* were in the next orders, respectively. Under

severe stress (30% of FC), highest root/shoot dry weight was observed in *P. glabra* with 0.66 g increase compared to the control treatment (100% of FC) and 0.334 g increase compared to the moderate stress (60% of FC) and after that, *P. syriaca*, *P. salicifolia*, *P. bioessieriana* and *P. communis* were placed in the next ranks (Fig. 1).

### Specific leaf weight (SLW)

Based on the analysis of variance in Table 2, the effects of drought stress treatments, species and their interaction on SLW were significant ( $P < 0.01$ ). The results of mean comparison revealed that with increasing levels of drought stress, the SLW were increased significantly. In control treatment (100% of FC) there was no significant difference in SLW of studied pear species. Both levels of drought stress led to a considerable increase in SLW of studied pear

seedlings. Under moderate stress (60% of FC), *P. syriaca* and *P. salicifolia* with 15.351 and 11.98 mg cm<sup>-2</sup> had more and less SLW, respectively. Nevertheless, there was no significant difference in SLW of *P. glabra* and *P. bioissierana* species. In severe stress (30% of FC), *P. glabra* and *P. syriaca* with 8.303 and

6.423 mg cm<sup>-2</sup> increase compare to moderate stress (60% of FC) and 13.848 and 13.657 mg cm<sup>-2</sup> increase compare to control stress (100% of FC) showed the highest SLW and after that, *P. salicifolia*, *P. bioissierana* and *P. communis* were placed in the next ranks, respectively (Fig. 2).

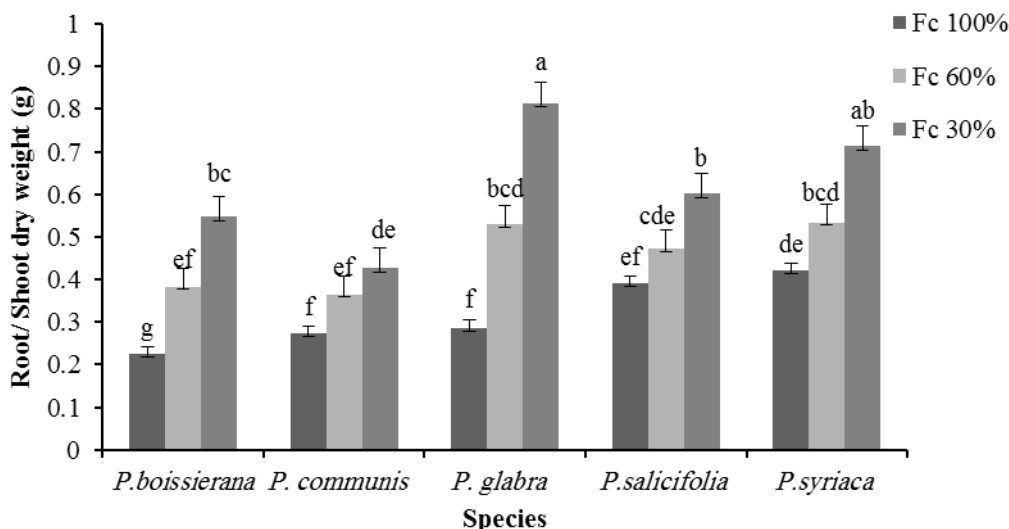


Fig. 1. Interaction of various levels of drought stress [% of field capacity (FC)] and pear species on root/ shoot dry weight. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n = 15$ ).

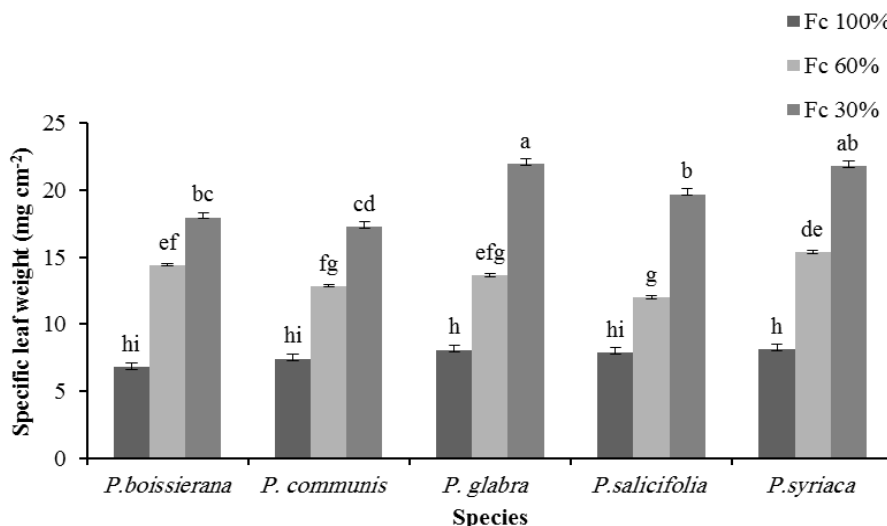


Fig. 2. Interaction of various levels of drought stress [% of field capacity (FC)] and species on specific leaf weight. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n = 15$ ).

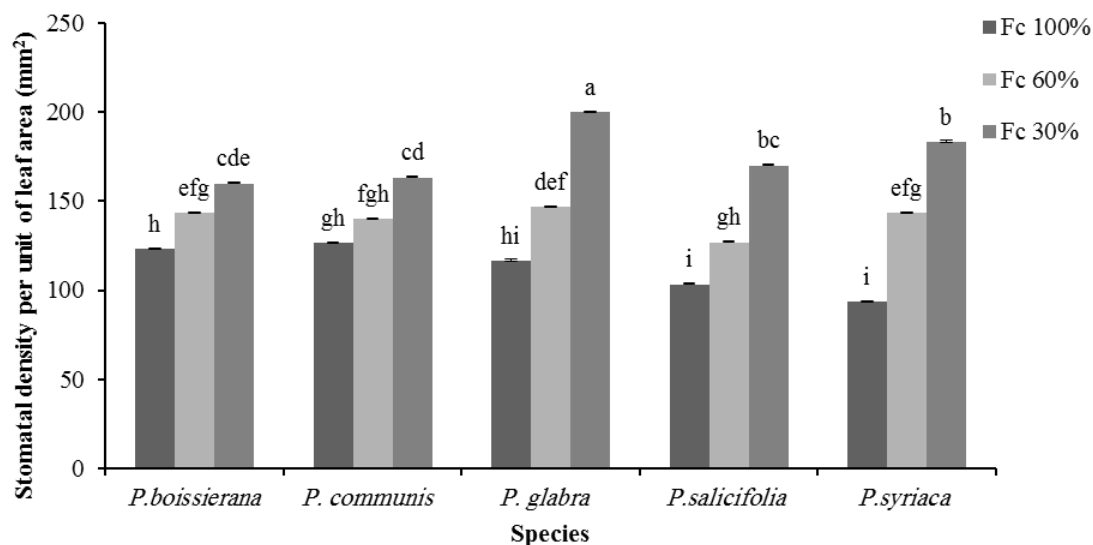
### Stomata density per unit of leaf area

Based on the analysis of variance, the drought stress, species and their interaction showed significant effect on stomata density per unit of

leaf area ( $P < 0.01$ ). With increasing drought stress levels, the stomata density per unit of leaf area was increased significantly. In control treatment (100% of FC), *P. communis*, *P.*

*boissieriana*, *P. glabra*, *P. salicifolia* and *P. syriaca* showed the highest stomatal density per unit of leaf area, respectively. In moderate stress (60% of FC), *P. glabra* and *P. salicifolia* with 147 and 140 stomata had more and less stomatal density per unit of leaf area, respectively. Nevertheless, there was no significant difference in stomatal density per unit of leaf area of *P. boissieriana* and

*P. syriaca* species. Under severe stress treatment (30% of FC), the highest stomatal density per unit of leaf area was related to *P. glabra* with 54 and 84 stomata compared to moderate stress (60% of FC) and control (100% of FC), respectively. After that, *P. syriaca*, *P. salicifolia*, *P. communis* and *P. boissieriana* were in the next ranks, respectively (Fig. 3).



**Fig. 3.** Interaction of various levels of drought stress [% of field capacity (FC)] and species on stomatal density per area unit. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n=15$ ).

### Relative water content (RWC)

Based on the results of variance analysis in table 2, the effects of studied factors on leaf relative water content were significant ( $p < 0.01$ ). The results of comparing averages revealed drought stress significantly decreased the relative water content. In control treatment (100% of FC) there was no significant difference between the leaf relative water content of pear species. Drought stress caused effective changes in relative water content and with increasing drought stress level, leaf relative water content was significantly decreased in all pear species. Under moderate stress (60% of FC), *P. glabra* and *P. communis* with 9 and 21 % decrease

compared to control treatment (100% of FC) had the highest and lowest relative water content, respectively. Also, there was no significant difference in relative water content of *P. boissieriana* and *P. salicifolia* species. In severe stress (30% of FC), the leaf relative water content was significantly variable among species. *P. glabra* with a 18 % decrease compared to control treatment (100% of FC) had the highest leaf relative water content and after that, *P. syriaca*, *P. salicifolia*, *P. boissieriana* and *P. communis* with 18, 23, 24 and 31 % decrease in leaf relative water content compared to control treatment (100% of FC) were in the following ranks, respectively (Fig. 4).

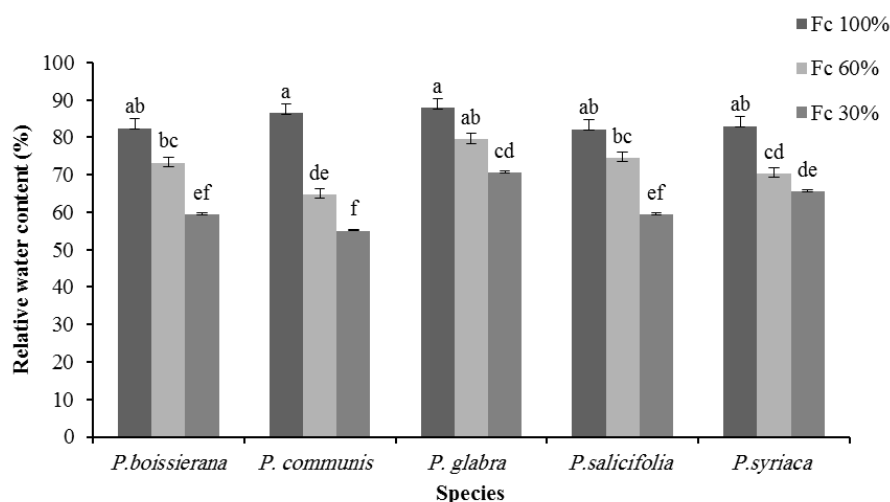


Fig. 4. Interaction of various levels of drought stress [% of field capacity (FC)] and species on relative water content. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n=15$ ).

#### **Net photosynthetic rate, stomatal conductance, transpiration rate and intercellular carbon dioxide concentration**

The analysis of variance showed significant differences ( $p < 0.01$ ) in net photosynthetic rate, stomatal conductance and transpiration rate among the drought stress treatments, species, and their interactions (Table 2). The results of mean comparison related to the interaction of drought and species on net photosynthesis showed that with increasing drought stress levels, the values of net photosynthetic rate, stomatal conductance and transpiration rate in all studied species

decreased significantly compared to the control treatment (100% of FC). In control treatment, the highest net photosynthetic rate was recorded in *P. glabra* and *P. syriaca*, *P. boissierana* and after that in *P. communis* and *P. salicifolia* species, respectively. In moderate stress (60% of FC), the highest net photosynthetic rate was found in *P. communis* and the lowest net photosynthetic rate was observed in *P. glabra* and *P. syriaca*. Under severe stress (30% of FC), *P. glabra* had the highest net photosynthetic rate and *P. syriaca*, *P. boissierana*, *P. salicifolia* and *P. communis* were in the next ranks, respectively (Fig. 5).

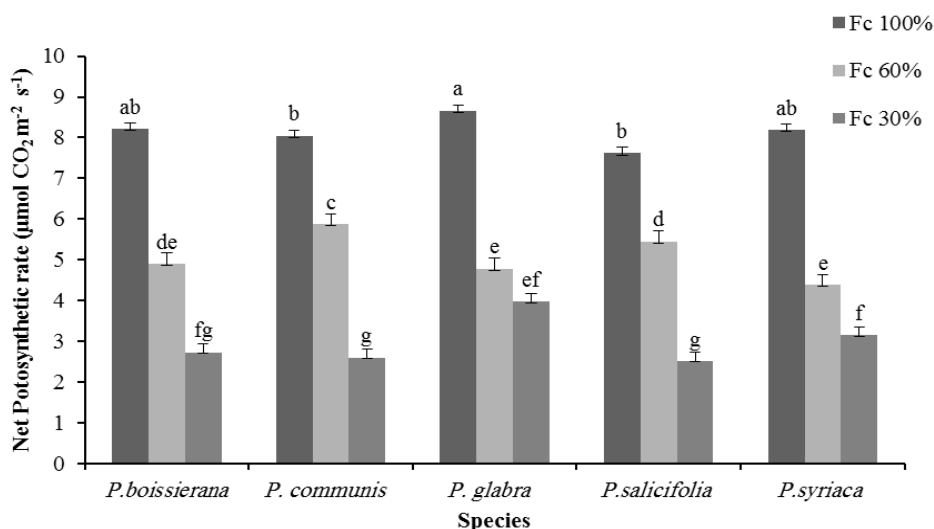
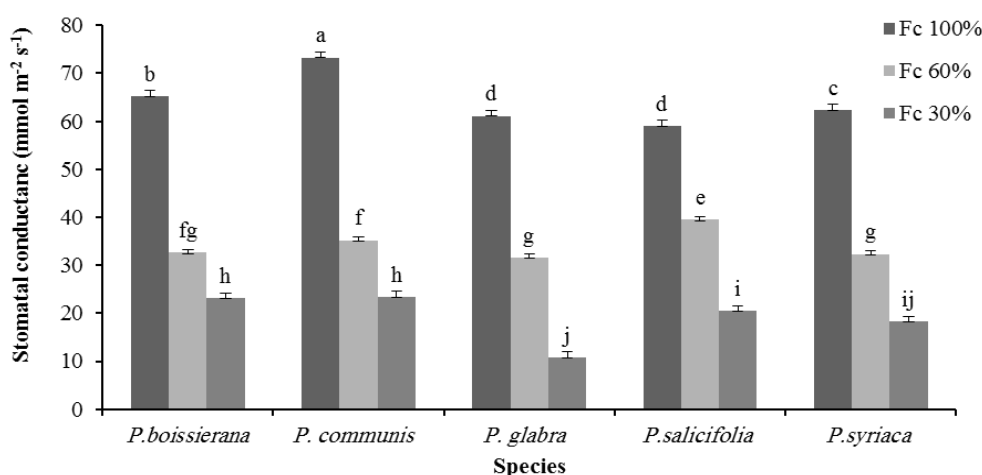


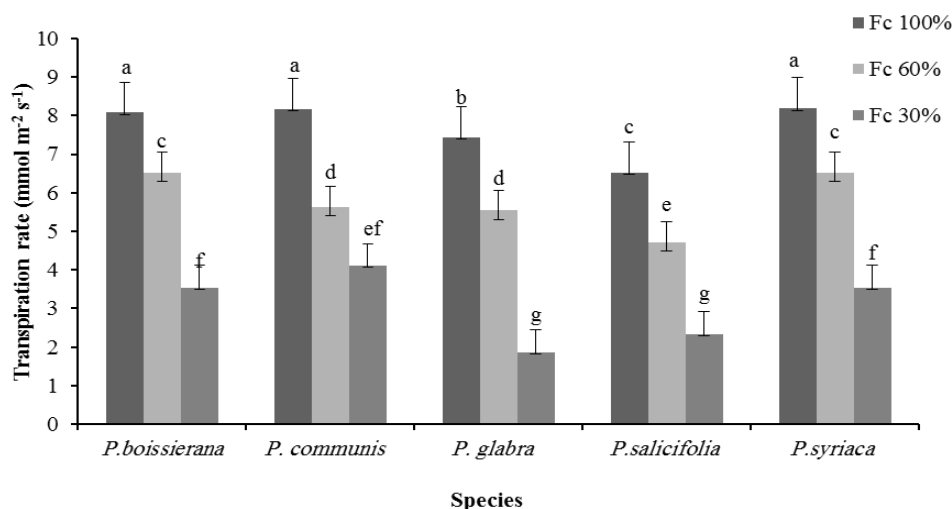
Fig. 5. Interaction of various levels of drought stress [% of field capacity (FC)] and species on net photosynthetic rate. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n=15$ ).

Also, in control treatment, *P. communis* had the highest stomatal conductance and later, *P. bioisieriana*, *P. syriaca*, *P. salicifolia* and *P. glabra* were in the next ranks. In moderate stress (60% of FC), the highest stomatal conductance was observed in *P. salicifolia* and the lowest stomatal conductance was observed in *P. glabra* and *P. syriaca*. Nevertheless, there was no significant difference in stomatal conductance of *P. glabra* and *P. syriaca* species. In severe stress (30% of FC), *P. communis* and *P. bioisieriana* had the maximum stomatal conductance and after them *P. salicifolia*, *P. syriaca* and *P. glabra* were in the next ranks, respectively (Fig. 6).

With increasing drought stress levels, the transpiration rate decreased significantly. In control treatment (100% of FC), *P. bioisieriana*, *P. communis* and *P. syriaca* showed the highest leaf transpiration rate, while *P. salicifolia* had the lowest transpiration rate. Under moderate stress treatment (60% of FC), *P. bioisieriana* and *P. syriaca* had more and *P. salicifolia* had less transpiration rate. Under severe stress (30% of FC), the maximum transpiration rate was in *P. communis* and minimum transpiration rate was belonged to *P. glabra* and *P. salicifolia* (Fig. 7).



**Fig. 6.** Interaction of various levels of drought stress [% of field capacity (FC)] and species on stomatal conductance. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n = 15$ ).



**Fig. 7.** Interaction of various levels of drought stress [% of field capacity (FC)] and species on transpiration rate. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n = 15$ ).



The results of mean comparison related to the interaction of drought and species on the concentration of intracellular carbon dioxide showed that with increasing the intensity of drought stress, the concentration of intracellular carbon dioxide decreased in all studied pear species. In control treatment (100% of FC), the highest concentration of intracellular carbon dioxide was found in *P. communis* and after that *P. salicifolia*, *P. bioessieriana*, *P. glabra* and *P. syriaca* were in the next ranks, respectively. In addition, under

moderate stress treatment (60% of FC), the highest concentration of intercellular carbon dioxide concentration was observed in *P. salicifolia* and the lowest intercellular carbon dioxide concentration was observed in *P. syriaca*. There was no significant difference in intercellular carbon dioxide concentration of *P. bioessieriana* and *P. glabra* species. In severe stress (30% of field capacity), *P. communis*, *P. bioessieriana* and *P. salicifolia* had the highest and *P. glabra* had the lowest concentration of intracellular carbon dioxide (Fig. 8).

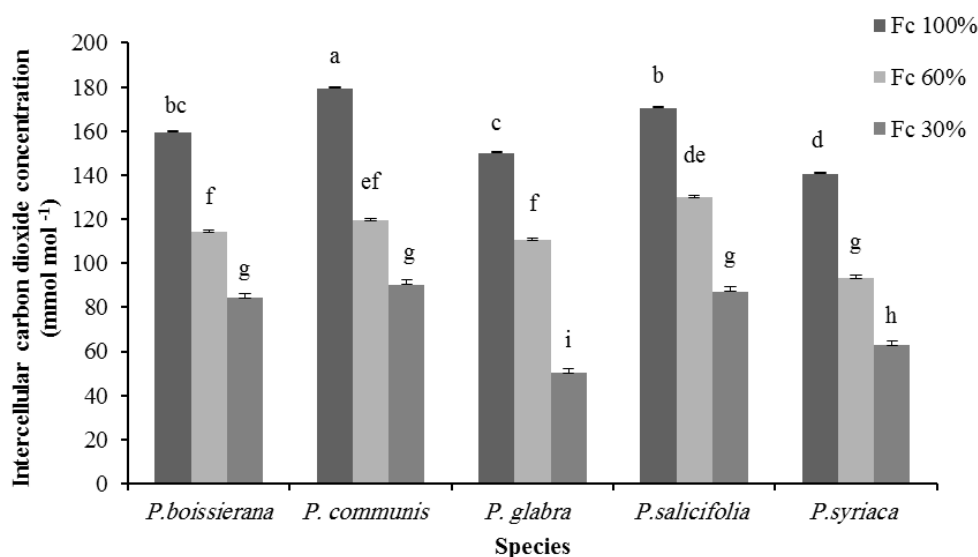


Fig. 8. Interaction of various levels of drought stress [% of field capacity (FC)] and species on intercellular carbon dioxide concentration. Similar letter/s shows not significant differences ( $P < 0.01$ ). Error bars show mean  $\pm$  SE ( $n=15$ ).

## Discussion

### Root/shoot dry weight

During drought stress, roots as one of the main organs of the plant, is strongly influenced by moisture deficit (Sangakkara *et al.*, 2010). When water availability is limited, the synthesis of ABA is increased by the roots. This hormone has growth inhibitory properties for the shoots, while promotes the growth of roots and increases the length of the roots (Xu *et al.*, 2000). The effects of drought stress on shoot growth are generally greater than root growth. It seems this low root sensitivity is due to the ability of roots to rapidly modulate osmosis in response to reduced soil water potential. This

condition allows continued water uptake and is also due to the increased flexibility of the root cell wall (Sharp *et al.*, 2004). In this regard, we showed that with increasing drought stress intensity, root and branch dry weight increased in all species. Under severe stress (30% of FC), the highest and lowest root/shoot dry weight were observed in *P. glabra* and *P. communis*, respectively (Fig. 1). These results are consistent with reports on *opuntia* (Snyman, 2004), pepper (Kulkarni and Phalke, 2009), oleander (Niu *et al.*, 2008), *Catharanthus roseus* (Jaleel *et al.*, 2008), rose (Niu and Rodriguez, 2009), almond (Yadollahi *et al.*, 2011) and grape (abbaspour and babae, 2017).

### ***Specific leaf weight (SLW)***

In this study, the trend of changes in SLW under different drought stress levels was similar in all species and with increasing drought stress intensity, SLW increased significantly. Under severe (30% of FC) stress, *P. syriaca* and *P. glabra* showed the highest SLW and *P. communis* had a minimum SLW (Fig. 2). SLW is one of the important traits in plants and is used as a reliable morpho-physiological marker to study drought stress tolerance in many plants (Ali et al., 2011). Many studies have shown that with increasing severity of dehydration stress, the amount of SLW increased (Rieger et al., 2003; Bacelar et al., 2006; Martinez et al., 2010). This trait depends on the leaf thickness, dry matter of leaves per unit of leaf area and the density of the leaf tissue (Xu and Zhou, 2005). The higher SLW of drought tolerant cultivars may be related to their higher carbon uptake potential (Ghaderi et al., 2014). Furthermore, Xu and Zhou (2005) stated that changes in SLW under water stress might be due to changes in the amount of carbohydrates, including starch. Also, the leaf relative water content is important in determining specific leaf weight and since under deficit stress, the number of hairs and the length of the pores decreases while the thickness of the cuticle, epidermis, hypodermis and the number of pores increases (Hameed et al., 2002), Thus cultivars that have a thicker cuticle wax layer, under dehydration conditions, retain more relative water content in their leaves, have a higher leaf specific gravity and are more tolerant of drought.

### ***Stomata density per unit of leaf area***

One of the first defense mechanisms of the plant against dehydration stress is the limitation of leaf area. With increasing drought stress, leaf area decreases sharply as a result of hormonal imbalance due to increased ABA and decreased levels of indole acetic acid (IAA) in stressed plants, reduced cell division and cell elongation

(Blum, 2005) and as leaf area decreases, stomata density and number of stomata per unit of leaf area increases (Sing and Usha., 2003; Najafian et al., 2009). Under water stress, the fluffs on the epidermal layer of leaves grow less. The spongy tissue in the middle of the leaf develops less and stronger mechanical tissues emerge. Morphological changes caused by drought are called Xeromorphs, which includes increasing the thickness of cuticle and cell membrane, reducing cell growth and leaf area and increasing the stomatal density per unit of leaf area (Jalili Marandi et al., 2011; Aliniaiefard et al., 2014; Aliniaiefard and van Meeteren, 2013, 2014, 2016; van Meeteren and Aliniaiefard, 2016). Drought stress disrupts mitotic divisions, cell elongation and expansion led to reduced growth and production traits (Heckenberger et al., 1998). Leaf surface development depends on ambient temperature, photosynthesis and the amount of nutrients availability. Decreased leaf area stimulated by drought is attributed to the stomatal close and inhibited photosynthesis (Rucker et al., 1995). As shown in Fig. 3, the stomata density per unit of leaf area in studied species subjected to drought stress treatment was significantly more than in the leaves of the well-watered plants, indicating that pear plants respond to drought by reducing cell division and elongation, reducing leaf area and thus increasing the number of stomata per unit of leaf area. Under severe stress treatment (30% of FC), the highest stomata density was related to *P. glabra* with 2 and 1.4- fold increase compared to control treatment (100% of FC) and moderate stress (60% of FC) and after that, *P. syriaca*, *P. salicifolia*, *P. communis* and *P. bioisieriana* were in the following ranks, respectively. There are many reports that showed that by reducing the number of stomata per unit of leaf area and by reducing the length of the stomata, the plant's tolerance to dehydration increases (Seif et al., 2021), which is consistent with our results in this research.

### **Relative water content (RWC)**

In this regard, the studied species of *P. glabra* with 18 % and 9 % decrease compared to control treatment (100% of FC) and moderate stress (60% of FC), respectively had better water status than other studied pear species. This is very important for physiological performance and survival under water stress (Fig. 4). Leaf RWC, leaf water potential, stomatal resistance, transpiration ratio, leaf temperature and plant canopy temperature are the most important characteristics effecting plant water relations. The leaf relative water content is an indicator to reflect the balance between leaf tissue water supply and transpiration rate (Lugojan and Ciulca, 2011) and assessing the water status of the plant and the metabolic activities of the tissues and is a suitable tool in the diagnosis of drought resistance. The relative content of leaf water in the early stages of growth and in young leaves is higher than mature and developed leaves. Cuticle thickness is one of the important factors in preserving the relative water of the leaf and Many different studies have shown that under drought conditions, tolerant cultivars have more leaf cuticle thickness and able to retain more water content in their cells (Siddique et al., 2001; Yadollahi et al., 2011; Ghaderi et al., 2011; Tani et al., 2019).

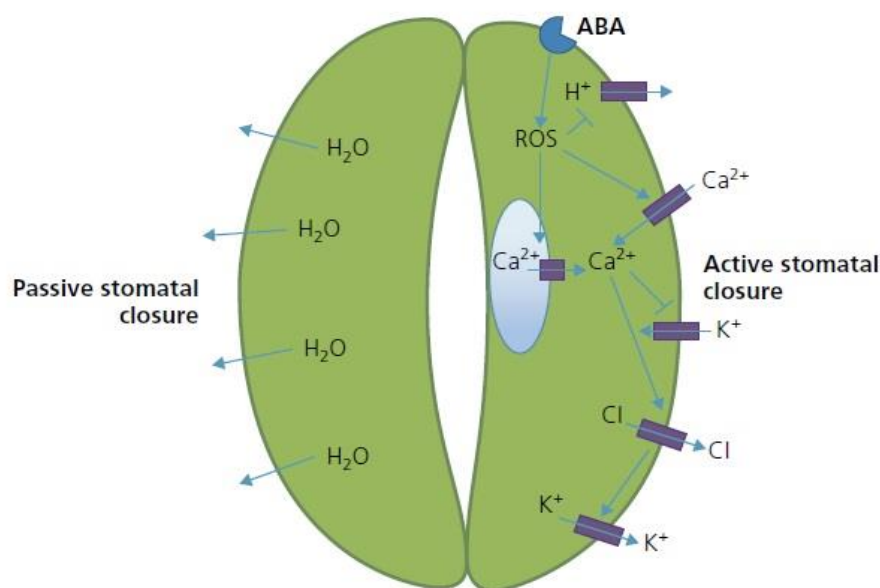
### **Net photosynthetic rate, stomatal conductance, transpiration rate and intercellular carbon dioxide concentration**

With increasing drought stress levels, in all studied species, net photosynthetic rate, stomatal conductance, transpiration rate and intercellular carbon dioxide concentration decreased significantly. Under severe stress (30% of FC) conditions, *P. glabra* and *P. communis* had the highest and lowest amount of net photosynthetic rate, respectively (Fig. 5). However, *P. communis* and *P. biossieri* had the maximum stomatal conductance and after that *P. salicifolia*, *P. syriaca* and *P. glabra* were in the next ranks, respectively (Fig. 6). Lack of

water is sensed by the roots and causes physiological and morphological changes in the plant by sending signals to branches through xylem. ABA synthesis, as one of the first root responses to water stress, stimulates the expression of genes that respond to drought stress (Carolina et al., 2015), Increases flow of  $K^+$  ions from the guard cells and loss of turgor pressure and closing of the pores perturbation (Guerrero and Mullet, 1986), reduce water loss and transpiration, and ultimately limit cell growth (Carolina et al., 2015). Environmental stresses, especially drought stress, have direct effects on the photosynthetic process and cause the destruction of key photosynthetic compounds, including electron transfer from the thylakoids, carbon reduction cycle and stomatal control of carbon dioxide entry and finally carbohydrate deposition, lipid peroxidation and disrupts the plant's water balance (Allen and Ort, 2001). Overall, the reasons limiting photosynthesis in water stress conditions are divided into two types of stomatal or non-stomatal limiting factors that they are due to the direct effect of water deficiency on plant biochemical processes. The stomata are highly sensitive to dehydration and closure of the stomata is one of the plant's first reactions to drought stress, which leads to a reduction in photosynthesis. Stomatal restriction reduces mesophilic access to carbon dioxide and reduces photosynthetic carbon assimilation during the light-breathing process. On the other hand, non-stomatal mechanisms include reduced synthesis of chlorophyll pigments, reduced yield and degradation of chloroplast structure, and damage to the processes of accumulation, transport, and distribution of photosynthetic products (Samarah et al., 2009). The stomata are a collection of stoma, guard cells and subsidiary cells (Berry et al., 2010). The abundance of stomata is mostly in the lower surface of the leaf (Pirasteh-Anosheh et al., 2016). Stomata generation occurs continuously and parallel to the leaf growth (Zhao et al., 2015). Stomata

morphological characteristics such as size, density and distribution significantly affect gas exchanges and their relationships with environmental factors such as soil water status (Anjum *et al.*, 2011). The stomata are much more sensitive to soil water scarcity than any perceive drought stress signals from the leaves such as reduction in leaf mesophyll turgor pressure (Hoshika *et al.*, 2013). The earliest leaf defense against desiccation is stomatal closure to avoid excessive dehydration (Lehmann and Or, 2015; van Meeteren and Aliniaefard, 2016), since it is much faster than changes in the leaf area, root system and chloroplast. During this process, ABA reaches the leaves through the transpiration stream for drought-induced root-to-leaf signaling, which induces the stomatal closure (Sikder *et al.*, 2016). Stomata also control transpiration, which can decrease leaf temperature by conducting transpiration (Ishida *et al.*, 2014). During dehydration, the amount of CO<sub>2</sub> uptake gradually decreases due to the closure of the stomata, which in turn reduces the content and activity of enzymes involved in the photosynthetic carbon reduction cycle, including its major enzyme, ribulose- 1, 5-phosphate carboxylase/oxygenase (RuBisCO)

(Heldt, 1997). To maintain the carboxylation function of RuBisCO, photorespiration is increased and the end result of using additional reducing equations in chloroplasts is to increase the production of oxygen free radicals that leads to oxidative stress in chloroplasts, degradation of photosynthetic pigments (Lisar *et al.*, 2012), protein degradation, lipid peroxidation, DNA disorder and ultimately cell death (Farooq *et al.*, 2009). Due to the fact that the content of ABA is closely related to the rate of pore opening, plants can strongly regulate the concentration of ABA in stress conditions such as drought, cold and salt (Hu and Xiong, 2014). 9-cis epoxycarotenoid dioxygenase (NCED) is the main enzyme in ABA synthesis under drought stress. The ABA signaling pathway is triggered by drought stress, leading to enhance synthesis of NCED, which in turn promotes ABA, ascorbic acid (AsA) and peroxidase synthesis and ROS scavenging, allowing the drought-associated damage to be alleviated by ROS homeostasis and stomatal aperture adjustment (Xian *et al.*, 2014). Overall, two mechanisms are involved in closing the stomata under drought stress: Hydropassive and hydroactive (Fig. 9).



**Fig. 9.** Two mechanisms of stomatal closure; hydroactive (right) and hydropassive (left) pathways (adapted from Arve *et al.*, 2011).

In hydropassive mechanism, the synthesized ABA from roots and leaves is transported to the guard cells through ATP-binding cassette (ABC) transporters located in plasma membranes and activates the signaling pathways of closing the stomata (Mutava *et al.*, 2015). ABA stimulates the production of ROS, which act as a potent stimulus for the production of nitric oxide (NO) (van Meeteren *et al.*, 2020), the influx of  $\text{Ca}^{2+}$  through vacuole and plasma membranes, and the inhibition of membrane proton pumps. On the other hand,  $\text{H}^+$ -ATPases activity, which strongly polarizes the plasma membrane, is inhibited to induce stomata closure. As  $\text{Ca}^{2+}$  levels increase, anion channels are activated, causing generating an anion efflux from cells that depolarizes the membrane and causes  $\text{K}^+$  efflux through  $\text{K}^+$  out channels across both plasma and vacuole membranes. At the same time,  $\text{Ca}^{2+}$  inhibits  $\text{K}^+$  ions in the channels (Arve *et al.*, 2013). While the removal of  $\text{K}^+$  and  $\text{Cl}^-$  is well described in the literature, the role of sucrose and malate during stomatal closure is unclear. Sucrose can be cleaved by cytosolic invertase (cINV), and the resulting hexoses can be imported into the chloroplast in the form of Glc-6-P (Glc6P). Glc6P is used subsequently for starch biosynthesis. Malate can be removed from the cell via decarboxylation to pyruvate by malic enzyme (ME) and the subsequent complete oxidation in the cycle of mitochondrial tricarboxylic acid (CAC). Alternatively, malate can be converted to Phosphoenolpyruvate (PEP) via PEP carboxykinase and nicotinamide adenine dinucleotide-dependent malate dehydrogenase. Nevertheless, the plasma membrane is depolarized, the turgor pressure and cell volume decreased and the stomatal closure occurs (Santelia and Lowson, 2016). Hydroactive mechanism of stomatal closure increased ABA content in the leaves induce and regulate stomatal closure, at the same level in the roots, it increases the hydraulic conductivity and facilitates water absorption

and transpiration (Arve *et al.*, 2013). In this regard, under moderate stress treatment (60% of FC), *P. bioisieriana* and *P. syriaca* had more and *P. salicifolia* had less transpiration rate. Under severe stress (30% of FC), the maximum transpiration rate was in *p. communis* and minimum transpiration rate was belonged to *P. glabra* and *P. salicifolia* (Fig. 7). There are many studies showing that with increasing intensity and duration of drought stress, net photosynthesis, stomatal conductance, transpiration rate and intracellular carbon dioxide concentration decreases (Ghaderi *et al.*, 2011; Flexas *et al.* 2014; Cotrozzi *et al.*, 2016; Ghanbary *et al.*, 2017), which are in consistence with our results in this study. Also, the results of research on *Pyrus betulaefolia* L. seedlings under drought stress showed that the net photosynthesis and transpiration rate decreased significantly while stomatal resistance increased (Li *et al.*, 2016). Overall, the results showed that under drought stress, the rate of net photosynthetic reactions in the studied pear species decreased and the *P. glabra* species had a higher tolerance than others, which is consistent with research on grapes (De Lorenzi and Rana, 2001); almond (García-Tejero *et al.*, 2011; Zokaee-Khosroshahi *et al.*, 2014), onion (Pejic *et al.*, 2014), thyme (Ashrafi *et al.*, 2018), rosemary (Delphin *et al.*, 2005), and medicago (Tani *et al.*, 2019).

## Conclusion

Results of evaluation of morphological responses to drought stress of five pear species showed that *P. glabra* was more effective in mitigating the destructive effects of drought stress. This was occurred by reduction in reducing intercellular carbon dioxide concentration and gas exchange of their leaves. Relative leaf water content was reduced by drought exposure and *P. syriaca*, *P. salicifolia*, *P. bioisieriana* and *P. communis* were in the next rank of keeping higher RWC, respectively. It can be suggested that the

studied species would be tested outdoors and other physiological and biochemical parameters such as leaf water potential, leaf parenchyma thickness (fence and sponge mesophyll), etc. would be further examined.

### Conflict of interest

The authors indicate no conflict of interest for this work

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