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Research Article

CHANGES IN CHLOROPHYLL CONTENT AND STOMATAL PARAMETERS IN WILD PEAR SPECIES DURING SUMMER

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Summary: In this experiment, seasonal changes in chlorophyll concentration from July to late September with intervals of 20 days, and stomatal parameters in different wild species of pear were analyzed. Leaves were collected from the middle part of annual shoots with length 0.8-1.0 m. Our results showed that chlorophyll content and stomatal parameters in leaves were different during vegetation. The lowest chlorophyll content was found in *Pyrus korshinskyi*. The highest values were measured in *P. aromatica*, *P. nivalis*, and *P. pyrifolia*. Almost the same pattern was detected when calculating chlorophyll content either per fresh weight or per unit leaf area. The biggest stomatal area was observed in *P. bretschneideri*, *P. ussuriensis*, *P. pyrifolia*, *P. aromatica*, while in *P. betulifolia* the smallest stomatal area was measured. In the morning when plants were well water-saturated, the wild species *P. korshinskyi*, *P. aromatica*, *P. calleryana* and *P. bretschneideri* showed a larger stomatal area compared to afternoon measurement done at 3:00 p.m., whereas *P. betulifolia* and *P. ussuriensis* did not show significant variations. The drought tolerant species *P. korshinskyi* showed the highest density of stomata per leaf area (mm^2) as well as the largest covered area per leaf.

Keywords: Chlorophyll; wild pear species; stomatal density; leaf area.

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INTRODUCTION

The main organ of photosynthesis in higher plants is the leaf. Its specific structure allows providing autotrophic type of metabolism that is typical for plant organisms. Photosynthesis and respiration play a key role in plant productivity and recycling of nutrients (Kräutler et al., 1997; Rodoni et al., 1998; Rotondi and Predieri, 2002; Rüdiger and Grimm, 2006). Martin et al. (1997) reported that lower vigor in red-fruited trees strongly correlated with lower Rubisco activity and decreased photosynthetic rate, but more weakly correlated with lower chlorophyll content. Rotondi and Predieri (2002) found that the lower chlorophyll content in leaves contributed to the decrease of photosynthetic efficiency in pear trees.

Chlorophyll content and stomatal behavior depend on water content in plants and soil, light intensity, leaf age, habitat

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conditions (Trejo and Davies, 1991; Babani and Lichtenthaler, 1996; Rotondi and Predieri, 2002; Xu et al., 2008; Ghasemi et al., 2011; Gashi et al., 2013), soil fertilizing conditions (Blackmer and Shepers, 1995; Ghasemi et al., 2011) and plant hormones (Rodriquez and Davies, 1982; Gomes et al., 1988; Zhang and Davies, 1990; Hartung and Slovik, 1991; Trejo et al., 1993).

Data available in literature on chlorophyll concentration and stomatal characteristics in pear wild species are limited. According to Ma et al. (2005), the chlorophyll content of three wild pear rootstock species decreased markedly with a rise in soil pH to 8.2 causing severe leaf chlorosis, while at pH 5.5 chlorosis was not observed. However, the chlorophyll content values differed in the three pear rootstock. The same pattern was observed in the experiment of grafting with "Housui" pear cultivar, which showed different concentrations of chlorophyll depending on the rootstock. Many authors have reported that the success of commercial varieties grafted onto selected tolerant rootstocks is mainly determined by the rootstock genotype (Byrne et al., 1989; Alcantara et al., 2003; Ma et al., 2005).

Ghasemi et al. (2011) quantified the chlorophyll content in Asian pear (*Pyrus serotina* Rehd.) using a chlorophyll content meter (CCM-200) and identified a positive and linear correlation between the CCM-200 readings and the data for chlorophyll *a*, chlorophyll *b*, total chlorophyll, and total nitrogen content in the leaves. The authors concluded that CCM-200 can be used to predict both chlorophyll and nitrogen content in Asian pear leaves. However, this conclusion raised doubts as their experiment was carried out only in June and the CCM-200 values were measured and compared within a short period during vegetation.

Coskun et al. (2011), hypothesized that differences among chlorophyll content of different varieties may be due to different responses to high temperature stress. Additionally, chlorophyll concentration and photosynthesic rate were found to decrease at temperatures of 35-38°C (Berry and Bjorkman, 1980; O'Mahony et al., 2000).

Studies on chlorophyll concentration and stomatal parameters in *Purus* wild species during vegetation as well as the application of the data for breeding purposes are limited. Many researchers have focused only to certain commercial crops or special treatments. In this study, we present results on the seasonal changes of chlorophyll concentration and stomatal parameters in wild pear species.

MATERIALS AND METHODS

Plant materials

The experiment was carried out in the Pear Research Station, NIHHS, RDA (Republic of Korea) during the summer of 2013. The changes in chlorophyll concentration and stomatal parameters in pear leaves were monitored from July to late September with an interval of 20 days in the wild species *P. korshinskyi* Litv., *P. boissieriana* Buhse, *P. betulifolia* Bung, *P. pyrifolia* Nakai, *P. ussurienseis* Maxim, *P. nivalis* Jacq, *P. calleryana* Decne, *P. bretschneideri* Rehd and *P. aromatica*. Sampling of leaves for total chlorophyll measurements was done in the afternoon (at 3:00 p.m.) and for stomatal parameters

at 8:00 a.m. and 3:00 p.m. Leaves from the middle part of annual shoots with a length of 80-100 cm were used for the measurements.

Determination of chlorophyll content and stomatal parameters

Chlorophyll was extracted from leaves of annual shoots with 100% methanol and the absorbance was read at 651 and 664 nm using Eon Microplate Spectrophotometer USA. Chlorophyll content is expressed as mg/g FW; mg/cm^2 FW; mg/total leaf FW.

The stomatal area was determined under electron microscope AXIO (Carl Zeiss, Germany), magnification x50-400.

Leaf area (cm^2) was measured using a LI-3100 Area meter (USA).

RESULTS

Chlorophyll concentration in leaves during vegetation

Seasonal variations in chlorophyll content are presented in Figs. 1-3. Chlorophyll content of some wild species showed stable values during the vegetation period. However, in *P. aromatica, P. nivalis* Jacq and *P. pyrifolia* Nakai, a higher fluctuation rate was observed, with the highest values being recorded in mid-August, especially in *P. aromatica* showing a maximum value of 2.455 mg/g FW. The content of chlorophyll in *P. korshinskyi* Litv. was significantly lower than in the other species and in late July it fell to 0.225 mg/g FW.

Almost the same pattern was detected

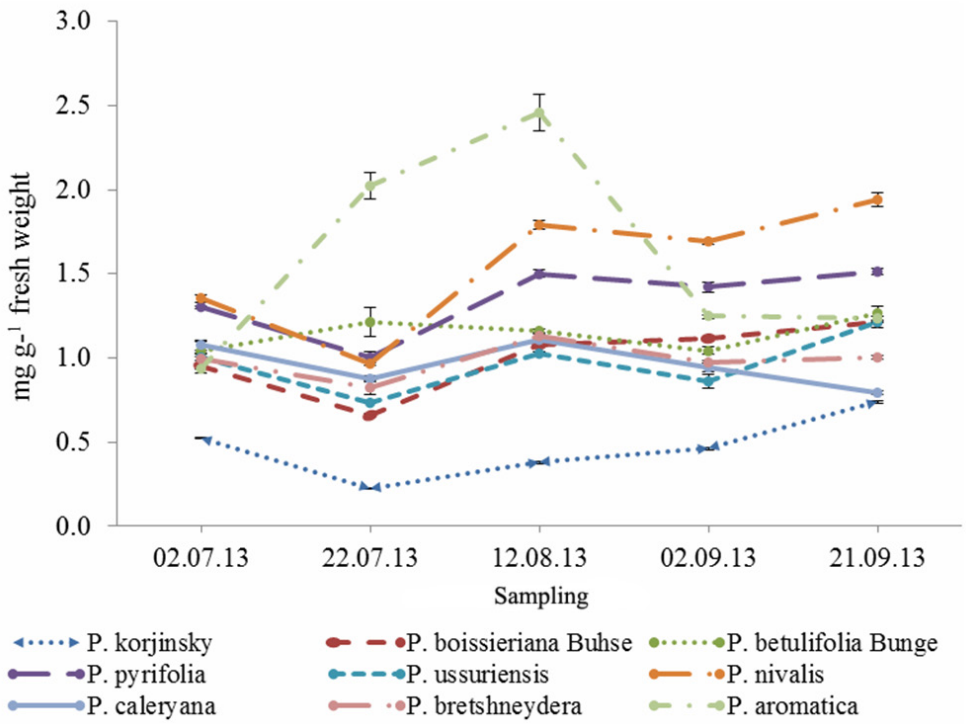

Figure 1. Changes in total chlorophyll content in leaves of wild pear species during vegetation. Data are means ± SD (n= 10).

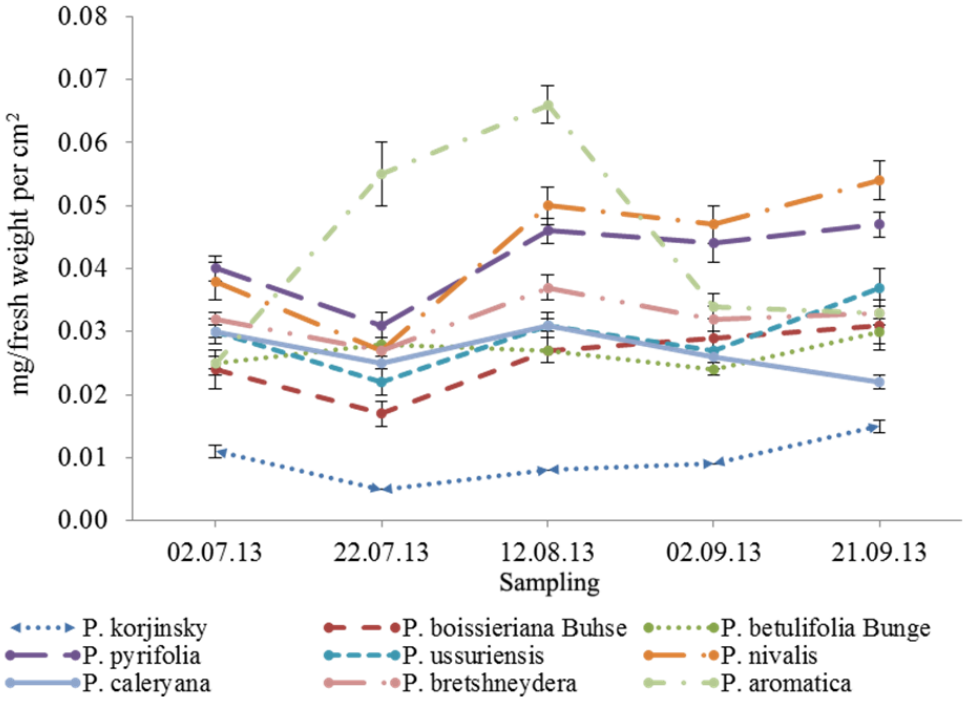

Figure 2. Concentration of chlorophyll per cm² leaf area during vegetation. Data are means ± SD (n= 10).

when calculating the chlorophyll content per fresh weight of cm² leaf area (Fig. 2). The lowest value for chlorophyll content (0.005 mg/cm² FW) was determined in *P. korshinskyi* Litvin. in late July, whereas *P. aromatica* showed the highest value (0.066 mg/cm² FW) in mid-August.

As well known, the wild species of pear have leaves with a different size and in comparison with the total leaf area, total chlorophyll content per fresh weight of leaf also varied (Fig. 3). The wild species *P. aromatica*, *P. pyrifolia*, *P. ussuriensis*, *P. calleryana*, and *P. bretschneideri* which had a greater leaf area showed also a higher chlorophyll concentration, whereas the species *P. nivalis* Jacq, *P. betulifolia* Bung, *P. boissieriana* Buhse which were identified with high concentration of chlorophyll per cm² area in this case showed the lowest values.

Leaf size and stomatal parameters of wild pear species during vegetation

The biggest leaf area and fresh weight were detected in *P. ussuriensis* and *P. bretschneideri,* followed by *P. pyrifolia* and *P. calleryana* - higher than 40 cm² and 1.2 g, respectively (Table 1) while the smallest ones were detected in *P. korshinskyi* Litv, *P. boissieriana* Buhse and *P. nivalis* Jacq - below 13 cm² and 0.4 g, respectively. In the case of leaf weight per cm² leaf area some differences were identified. Besides *P. ussuriensis*, *P. bretschneideri* and *P. pyrifolia,* the species *P. nivalis* Jacq was also distinguished with high leaf weight per cm² - over 0.030 g.

Similar to chlorophyll concentration, stomatal parameters also varied among the studied species during the vegetation period. The stomatal area varied depending on the species and in some cases on the

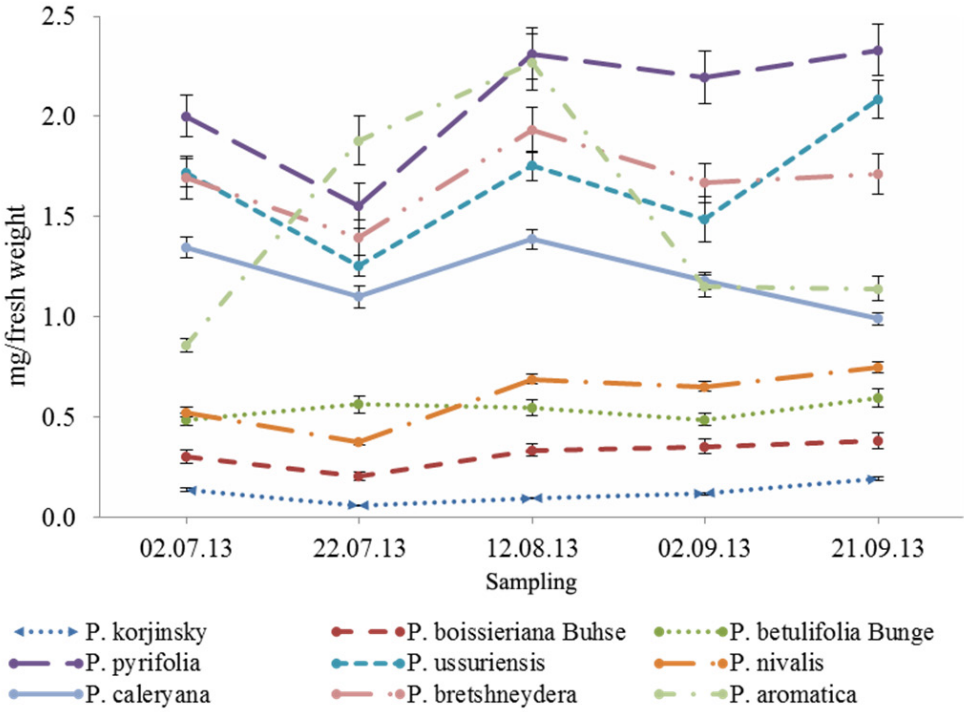

Figure 3. Changes in chlorophyll content per total leaf during vegetation. Data are means ± SD (n= 10).

Table 1. Leaf parameters in different wild pear species.

Species	Leaf weight [g]	Leaf area, [cm^2]	Leaf weight [g/cm^2]	Leaf	
				Length [cm]	Width [cm]
P. ussurienseis Maxim	1.712±0.060^{az}	55.2±1.5^{a}	0.031±0.002^{a}	12.1±0.2^{b}	7.0±0.1^{a}
P. bretschneideri Rehd	1.636±0.066^{a}	54.6±1.8^{a}	0.030±0.001^{a}	13.7±0.3^{a}	6.3±0.1^{b}
P. pyrifolia Nakai	1.489±0.052^{b}	49.5±1.3^{b}	0.030±0.001^{a}	10.5±0.2^{d}	7.2±0.1^{a}
P. calleryana Decne	1.243±0.031^{c}	44.1±0.9^{c}	0.028±0.001^{ab}	11.2±0.2^{c}	6.4±0.1^{b}
P. aromatica	0.962±0.034^{d}	34.5±1.0^{d}	0.028±0.001^{ab}	9.5±0.1^{e}	6.1±0.1^{b}
P. betulifolia Bung	0.459±0.017^{e}	18.9±0.6^{e}	0.025±0.001^{b}	7.1±0.2^{f}	4.4±0.1^{c}
P. nivalis Jacq	0.395±0.009^{ef}	13.0±0.4^{f}	0.031±0.001^{a}	6.0±0.1^{g}	3.2±0.1^{e}
P. boissieriana Buhse	0.300±0.021^{f}	12.5±0.7^{f}	0.026±0.003^{b}	4.8±0.2^{h}	3.6± 0.1^{d}
P. korshinskyi Litv.	0.294±0.015^{f}	12.1±0.5^{f}	0.025±0.002^{b}	4.1±0.1^{i}	3.7± 0.1^{d}

Data are means ± SD (n= 20).
^{z}mean separation within columns by LSD test, P< 0.05.

time of the day. The biggest stomatal area was identified in *P. bretschneideri, P. ussuriensis, P. pyrifolia*, *P. aromatica* with values exceeding 700 μm^2 regardless of the investigation period (Table 2) whereas the smallest stomatal area was detected in *P. betulifolia* Bung (about 500 μm^2). Some species such as *P. bretschneideri*, *P. aromatica*, *P. korshinskyi* Litv. and *P. calleryana* were sensitive to the changing daily temperature and the area of their stomata was bigger in the morning (8:00 a.m.) compared to afternoon (3:00 p.m.). In *P. bretschneideri* and *P. aromatica* the afternoon decline was significant. However, the stomatal area in *P. betulifolia* and *P. ussuriensis* did not show significant changes during the day.

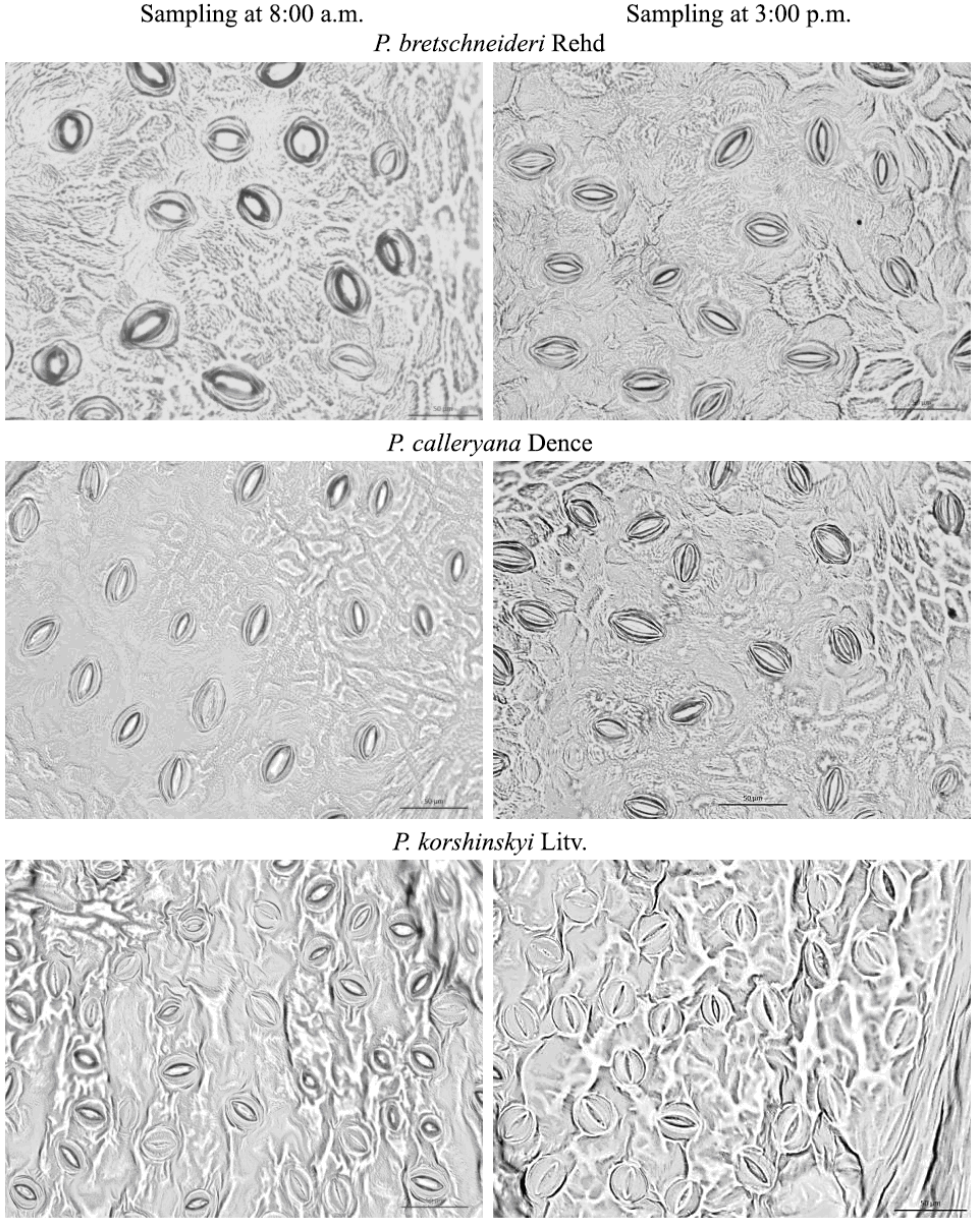

Fig. 4. Daily changes in stomata of some wild pear species.

Table 2. Stomatal parameters in wild pear cultivars.

Species	Stomatal parameters[y]									
	Area [µm²]		Stomatal pore length [µm²]		Stomatal pore width [µm²]		Density[x] [mm²]	Total density per leaf [thousand]	Covered area per cm² [%]	Total covered area per leaf [%]
	8:00 a.m.	3:00 p.m.	8:00 a.m.	3:00 p.m.	8:00 a.m.	3:00 p.m.				
P. bretschneideri Rehd	943.58±51.69[az]	856.22±24.97[a]	28.00±0.98[a]	31.89±0.62[a]	13.61±0.44[a]	10.82±0.27[b]	170.9±3.1[e]	9332.0	7.99±0.01	14.63±0.52
P. ussuriensis Maxim	852.99±36.37[a]	857.15±40.82[a]	29.80±0.69[a]	31.14±0.85[a]	9.70±0.26[a]	11.18±0.51[a]	170.3±2.9[e]	9402.4	8.06±0.01	14.60±0.83
P. aromatica	807.97±30.52[a]	718.74±22.08[a]	29.39±0.64[a]	31.89±0.67[a]	11.12±0.38[a]	10.91±0.39[a]	177.1±6.3[de]	6109.3	4.39±0.00	12.73±0.47
P. pyrifolia Nakai	774.37±40.94[a]	850.46±32.49[a]	24.08±0.60[b]	28.28±0.65[a]	8.17±0.31[b]	10.73±0.40[a]	196.0±8.3[cd]	9702.0	8.25±0.01	16.67±0.66
P. calleryana Decne	711.66±23.48[a]	686.03±25.15[a]	28.17±0.65[a]	28.77±0.66[a]	9.75±0.28[b]	12.08±0.21[a]	210.5±8.0[c]	9283.0	6.06±0.01	13.74±0.60
P. korshinskyi Litv.	687.03±29.95[a]	637.22±20.55[a]	23.95±0.73[a]	23.02±0.48[a]	8.52±0.31[a]	8.29±0.27[a]	370.8±10.5[a]	4487.1	2.86±0.00	23.63±0.41
P. boissieriana Buhse	631.10±20.24[a]	672.40±36.24[a]	23.09±0.78[b]	27.14±0.85[a]	10.23±0.29[a]	13.02±0.54[a]	190.4±7.3[cde]	2380.2	1.60±0.01	12.80±0.73
P. nivalis Jacq	569.44±17.37[b]	790.54±35.73[a]	22.41±0.67[a]	27.41±0.77[a]	9.13±0.22[b]	11.96±0.45[a]	205.0±8.7[c]	2665.0	2.11±0.00	16.21±0.74
P. betulifolia Bung	515.30±16.92[a]	519.45±17.05[a]	20.66±0.59[b]	23.68±0.58[a]	8.02±0.21[a]	10.28±0.34[a]	263.3±11.6[b]	4977.0	2.54±0.00	13.42±0.34

[z]mean separation within columns by LSD test, $P \leq 0.05$.
[y]mean ± SD (n = 30).
[x]mean ± SD (n = 12).

No correlation was found between the afternoon reduction of the stomatal area and the somatal pore length and width in *P. bretschneideri*, *P. aromatica*, *P. korshinskyi* Litv., and *P. calleryana.* In contrast, the stomata in leaves of *P. boissieriana* Buhse, *P. pyrifolia*, *P. nivalis* Jacq which were compact and did not open fully in the morning, increased in size in the afternoon. Additionally, the density (number) of stomata also varied. The calculations of stomata per mm^2 leaf area showed that *P. korshinskyi* Litv. plants had a maximal value - 370, whereas *P. bretschneideri* and *P. ussuriensis* showed a minimal density of about 170 pieces. Regardless of the low density per mm^2, the number of stomata per leaf in *P. bretschneideri, P. ussuriensis, P. pyrifolia* and *P. calleryana* was higher compared with the other studied cultivars, reaching values of over 9000**.** The covered leaf area also differed among pear species with the maximum value being found in *P. korshinskyi* Litv. - a species distinguished with the highest stomatal density per mm^2, in which the stomata covered about 23.6% of the total leaf area. The lowest value (12.7%) was determined in *P. aromatica* plants.

DISCUSSION

In the present study, chlorophyll content in different wild pear species was monitored during summer. Our results showed differential responses which were species-specific. The maximal value for chlorophyll content was detected in *P. aromatica* in mid-August, followed by a rapid decrease till late September, whereas *P. korshinskyi* Litv. showed a low chlorophyll content during the vegetation period. It should be noted that *P. korshinskyi* Litv. plants grow under semi-arid conditions in Central Asia and this wild pear species is considered as drought tolerant (Likhonos et al., 1983; Rajametov et al., 2010), whereas *P. aromatica* plants grow under the humid conditions of East–Asian area (Rubtsov, 1944; Kajiura et al., 1983).

Plants of *P. nivalis* Jacq which had a high content of chlorophyll per g fresh weight showed low levels of chlorophyll amount per total leaf fresh weight. The same pattern was found in *P. betulifolia* and *P. boissieriana* Buhse. In contrast, *P. pyrifolia, P. bretschneideri* and *P. ussuriensis* which had the greatest leaf area were identified with high level of chlorophyll per total leaf fresh weight.

Similar to chlorophyll concentration, stomatal parameters showed also variability among species during the vegetation period. The biggest stomatal area was observed in *P. bretschneideri, P. ussuriensis*, *P. pyrifolia*, *P. aromatica,* while the smallest was detected in *P. betulifolia* plants. It should be noted that in the morning (8:00 a.m.) when plants were well saturated with water, *P. korshinskyi*, *P. aromatica*, *P. calleryana* and *P. bretschneideri* showed the biggest stomatal area. On the other hand, in the afternoon (3:00 p.m.) when temperature significantly increased while air humidity decreased, a decreasing trend in stomatal area was established, thus reducing transpiration rate from leaves. However, in some species, such as *P. betulifolia* and *P. ussuriensis* stomatal area did not significantly change in dependence of environmental conditions. A major role of abscisic acid (ABA) in the physiological processes leading to stomatal closure has

been reported (Davies, 1978; Trejo and Davies, 1991; Trejo et al., 1993). However, while many dehydration-responsive genes can be induced by ABA, some are ABA-independent (Shinozaki and Yamaguchi-Shinozaki, 1997).

The highest density of stomata per certain leaf unit (mm^2) and total leaf area was measured in *P. korshinskyi*. As said above, this species has originated under dry area, and this might be the reason for the high stomatal density measured.

In conclusion, the concentration of chlorophyll and stomatal density in leaves of wild pear species varied during vegetation. More detailed studies are needed to give a complex assessment of traits for future breeding programs.

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