Genetics and Plant Physiology – 2017, Volume 7(1–2), pp. 62–77

Research Article

PHYSOLOGICAL STATE OF DIFFERENT PEAR CULTIVARS DURING SUMMER

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Received: *07 December 2017* Accepted: *31 March 2017*

Summary: The present study was conducted to evaluate the dynamics of water regime together with physiological activity in different pear cultivars during the summer of 2013 based on the changes in water content in leaves (WCL) and annual shoots (WCS), water deficit in leaves (WDL) and annual shoots (WDS), electrolyte conductivity (EC), leaf anatomical structure, stomatal parameters and total chlorophyll content. A trend in water regime was observed showing that WCL, WCS in all cultivars decreased from early June to late August, regardless of the climatic condition. WDL and WDS differed significantly compared with WCL and WCS while WDL seasonally fluctuated in all cultivars except for Bartlett, which showed a high stable rate. An imbalance between WDL and WDS was observed. According to our results, there was no significant relationship between water regime and EC, stomatal parameters, anatomical structure and total chlorophyll content during the summer period when the air and soil were sufficiently moistened. Therefore, under the studied humid conditions, the pear cultivars studied were not able to show either their adaptability in terms of water regime and physiology or their real ability to cope with stress that could appear during the dry season or under controlled conditions.

Keywords: Water content; chlorophyll content; electrolyte leakage; epidermis; stomata; mesophyll.

Abbreviations: EC – electrolyte conductivity; RH – relative humidity; WCL – water content in leaves; WCS – water content in annual shoots; WDL – water deficit in leaves; WDS – water deficit in annual shoots.

Citation: Rajametov Sh., 2017. Physiological state of different pear cultivars during summer. *Genetics and Plant Physiology*, 7(1–2): 62–77.

INTRODUCTION

Water availability is one of the major factors affecting plant productivity. The necessity to regulate this factor, particularly through irrigation, is primarily concerned with the actual need of plants for water, and the characteristics of their water regime (Petinov, 1962; Kushnirenko, 1964). The water content in tissues of fruit plants depends on the growing conditions as well as on the age of organs and whole organisms. The shortage of water in plants significantly affects morpho-physiological characteristics of plant organs (Bahanova, 2003; Rajametov

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et al., 2010; Zayseva, 2011). Water regime of leaves is a significant factor underlying physiological state of trees. In drought years when the decreasing relative humidity reduces the activity of the root system, a marked inhibition in the growth of leaves and shoots was observed (Ulyanovskaya et al., 2005).

Plants usually respond to a changing environment in a complex, integrated way allowing them to adapt to the specific set of conditions and constraints present at a particular time. This involves an array of physiological and biochemical modifications including leaf wilting, reduction in leaf area and stomata, leaf abscission, stimulation of root growth, changes in relative water content, generation and accumulation of reactive oxygen species which disrupt cellular homeostasis by reacting with lipids, proteins, pigments and nucleic acids resulting in lipid peroxidation, membrane damage, inactivation of enzymes, thus affecting cell viability (Bajji, et al., 2001; Bahanova, 2003; Bartels and Sunkar, 2005). Molecular responses to abiotic stresses, on the other hand, include stress perception, signal transduction to cellular components, gene expression, and, finally, metabolic changes imparting stress tolerance (Agarwal et al., 2006; Lata and Prasad, 2011). Stress-induced genes function not only to protect cells from stress by production of important proteins, but they also regulate the expression of downstream genes for signal transduction (Ingram and Bartels, 1996; Bray, 1997; Shinozaki and Yamaguchi-Shinozaki, 1997, Nakashima et al., 2000; Bohnert et al., 2001).

Chlorophyll content and stomata are vital for gas exchange, photosynthesis and respiration (Trejo and Davies, 1991; Trejo et al., 1993; Rotondi and Predieri, 2002; Pruzinska et al., 2007). Generally, water balance and physiological properties of plants have been studied under the influence of different environmental factors, but the literature information about the changes in water potential and physiological activity of pear plants during summer is limited. Investigations have focused on short periods of treatment or special treatments. Therefore, the main purpose of this work was to study water regime in leaves and annual shoots together with changes in leaf anatomy, total chlorophyll content and electrolyte conductivity in different pear cultivars during the vegetation period under the natural conditions in Republic of Korea.

MATERIALS AND METHODS

Experiments were carried out in the Naju Pear research station (RDA) during the summer of 2013 using pear cultivars of different origins: Bartlett (USA), Nashvati iz Pishkarina (UZB), Niitaka (JAP) and Chuwhangbae (KOR). It should be noted that all cultivars originated from humid areas except for Nashvati iz Pishkarina whose genealogic was formatted in the dry area. All experiments were conducted under natural conditions.

Water regime in annual shoots and leaves was studied in the afternoon at 3:00 p.m. in a position between 30 and 70 cm, and each shoot was about 0.80 – 1.0 m in length and 0.7 – 1.0 cm in diameter.

Water content in annual shoots and leaves was estimated by the formula:

$$WC\% = (W_1 - W_2) \times 100/W_1$$

where WC% – water content; W_1 – initial mass of shoots or leaves; W_2 – dry mass of shoots or leaves.

Water deficit in shoots and leaves was determined as a percentage of its total content at a state of complete saturation (shoots and leaves should be kept in water for 24 h) and expressed as:

$$WD\% = (WA \times 100)/W$$

where WD – water deficit; WA – water absorbed at saturation of the shoots and leaves, which is determined by the difference of mass of shoots and leaves before and after complete saturation; W – presence of water, the difference between the mass of shoots and leaves after complete saturation with water and dry mass of samples.

Electrical conductivity (EC) was measured with an Orion conductivity TDS meter model 124 conductimeter (Orion, Germany). In order to determine electrolyte leakage 3 leaves from each cultivar were collected, weighed and cut into segments (ca. 0.5 cm). Segments originating from the same shoot were put into 40 ml of distillated water in a test tube and allowed to stand for 15 h in the dark at 20°C. An initial electrical conductivity measurement (ECi) was done at the beginning of this rehydration period. All tubes were heated for 30 min in water at 95°C. Then, the tubes containing the segments were returned into the dark at 20°C and kept for 15 h. Following these readings, the total electrical conductivity (ECt) was measured. Electrolyte leakage (%) is expressed as: (ECi/ECt) × 100.

Stomatal area was determined in leaves from the middle part of annual shoots in the morning and afternoon using an electron microscope AXIO (Carl Zeiss, Germany, and magnification – x50 – 400). The leaf area (cm^2) was measured in mid-August using a LI-3100 Area meter (USA). For determination of leaf anatomical structure leaf samples were initially fixed in 2.5% glutaraldehyde for 90 min at 4°C and then rinsed four or five times with 0.1 M phosphate buffer (pH 7.2). The second fixing process was achieved using 1% osmium tetraoxide for 90 min and the samples were rinsed again with 0.1 M phosphate buffer five times. The fixed samples were dehydrated in alcohol series with increasing concentrations. Dehydrated samples were laid in a silicon mold with epon + D.M.P. 30 for 4 days at 60°C. After polymerization, the embedded samples were sectioned in 1 µm thickness using an ultramicrotome (Ultracut R. Leica Co., Austria) and observed under a light microscope AXIO (Carl Zeiss, Germany, and magnification – x200).

Total chlorophyll content was analyzed spectrophotometrically in the morning and afternoon using Eon Microplate Spectrophotometer (USA). Leaf disks each of 6.25 mm in diameter, were punched from the medium part of annual shoot leaves. The disks were placed immediately into 25 mL of 100% methanol, and pigments were allowed to be extracted in the dark at 4°C for 14 h. The absorbance was read at 651 and 664 nm. Chlorophyll content is expressed as (mg g^{-1} fresh weight).

RESULTS AND DISCUSSION

Normally, climate conditions during the summer in South Korea are usually characterized with warm, long sunny days and high level of precipitation. During the experiment, air temperature and humidity were recorded. Our data showed that the temperature increased from June to mid-August and reached a maximum of about 35°C in August (Fig. 1).

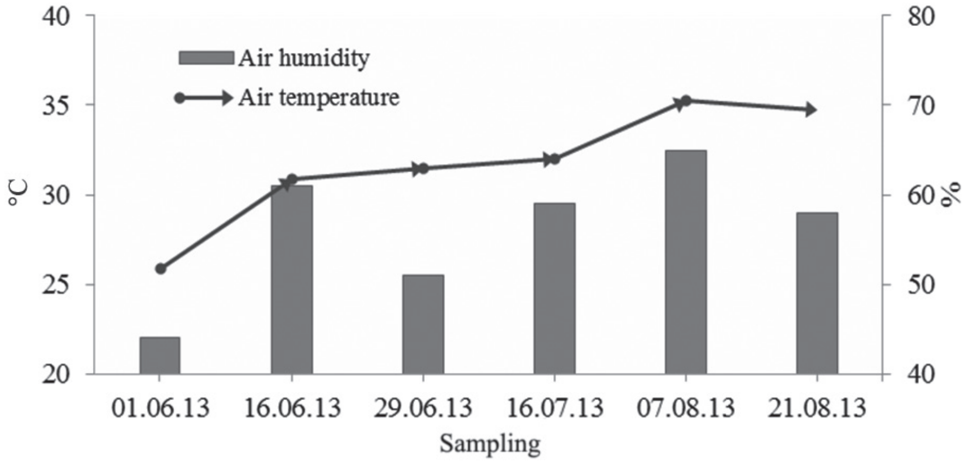

Figure 1. Climatic conditions during the investigation period of 2013, Naju.

Relative humidity (RH) was consistently high during the summer, especially at night when it reached 99%, but in the afternoon it was also relatively high (above 50%). Monthly mean rainfall was higher in July and in August and total rainfall was about 700 mm during the summer. Soil moisture content at a depth of 1.0 m exceeded 30-40% (data not presented); thus all cultivars were well supplied with water.

Our results showed that the water regime in leaves and annual shoots was unstable and ranged depending on period and cultivar. So, regardless of the climate condition WCL in all investigated cultivars decreased from early June to late August as was reported in fruit crops (Bahanova, 2003; Zakharchuk and Ryazanova, 2013). The same pattern was detected in WCS of pears, but the varietal differences in this parameter were more pronounced (Figs. 2, 3).

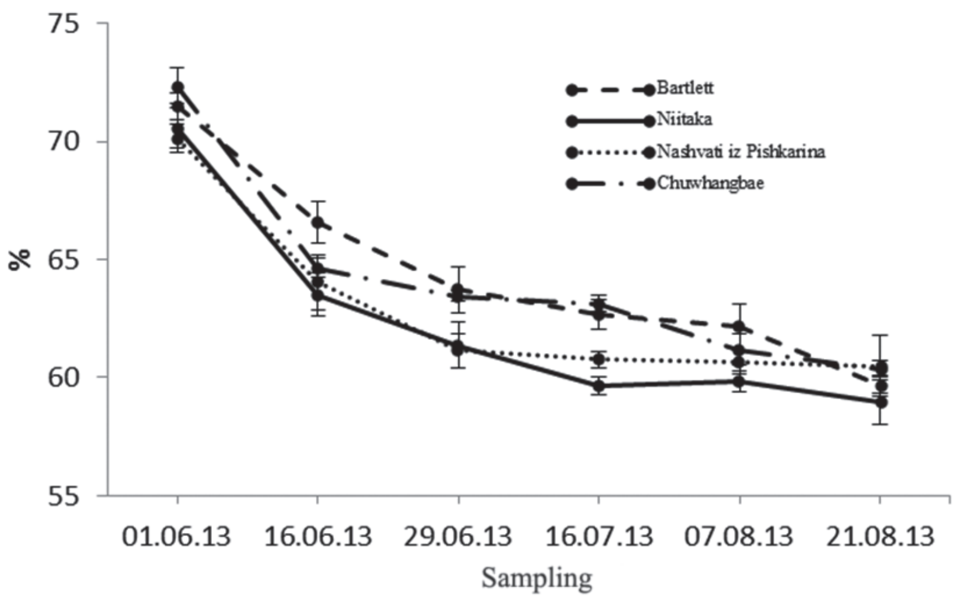

Figure 2. Changes in leaf water content in different pear cultivars.

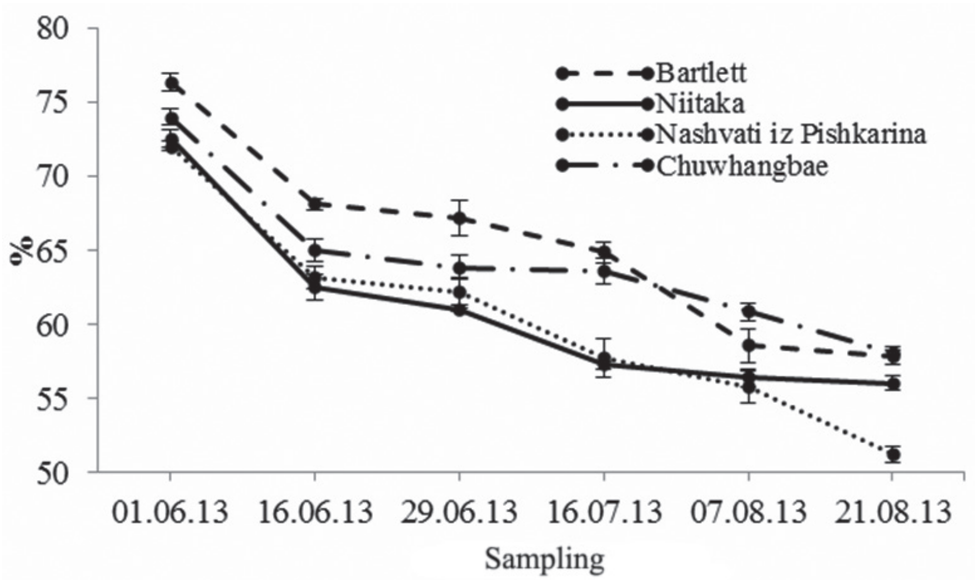

Figure 3. Changes in water content of annual shoots in different pear cultivars.

The cvrs. Niitaka and Nashvati iz Pishkarina had relatively low WCL and WCS in comparison with cvrs. Chuwhangbae and Bartlett.

High water concentrations in leaves and shoots are related to physiological activity of plant organs, the absolute maximal levels being observed in the beginning of blossom (Bahanova, 2003). Further on, the water content is reduced due to aging of the organs but it should be noted that the cultivars which show high WCL at the blossom stage do not show high stable values during the vegetation period. A gradual decline in water regime during the summer period is associated with the biological features of cultivars. Water content in plant organs is under the controll by some plant regulators, hormones and genes. Reduced water content leads to physiological and biochemical modifications in plants including leaf wilting, reduction in leaf area, leaf abscission; it induces leaf stomatal closure to reduce water loss through transpiration and decreases the photosynthetic rate in order to improve the water-use efficiency and root growth (Gomez et al., 1988; Agarwal et al., 2006; Bartels and Sunkar, 2005; Lata et al., 2011; Lata and Prasad, 2011). Thus, it can be assumed that the physiological and biochemical processes in plants depend on water regime and can vary in different cultivars.

Water shortage is an important and integral physiological index indicating the needs of plants for moisture. However, WDL and WDS differed significantly in comparison to WCL and WCS. So, WDL fluctuated in all cultivars except for cv. Bartlett (Fig. 4), which showed a high stable rate. Additionally, from early June to August, when the physiological activity and growth of plants are most intense (Bahanova, 2003; Gegechkori et al., 2013), all pear cultivars showed relatively high unstable values of WDL.

Regardless of the high temperatures in August, the values for WDL in cvrs. Niitaka, Chuwhangbae and Nashvati iz Pishkarina showed a decreasing trend.

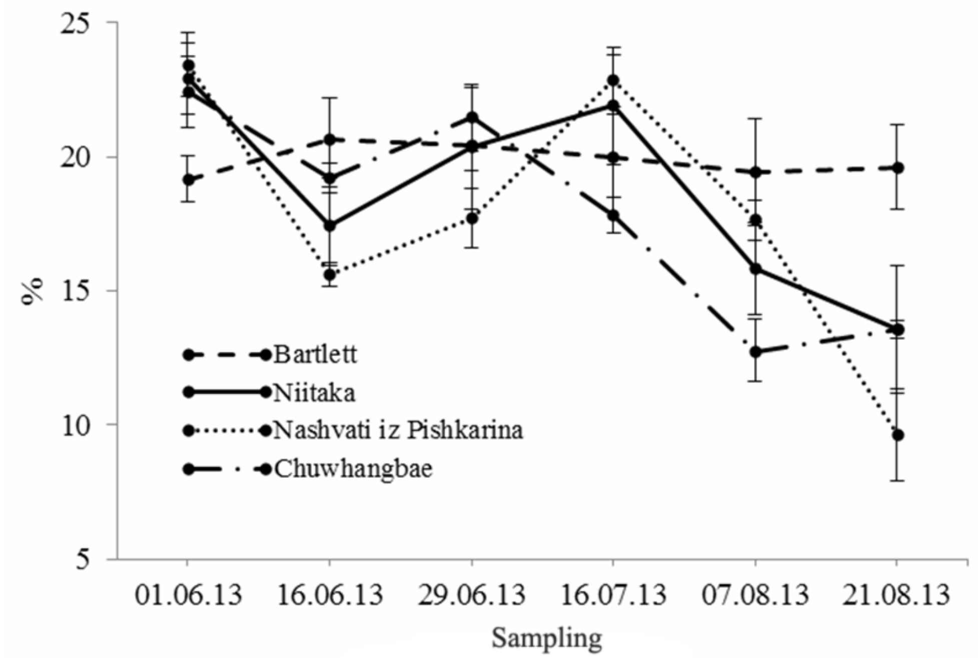

Figure 4. Changes in leaf water deficit in different pear cultivars.

Almost the same pattern of changes was observed in the experiments conducted under the conditions of Uzbekistan (Rajametov, 2008; Rajametov et al., 2010) where East and Central Asian pear cultivars showed low WDL values compared to the European cultivars. In comparison to Korean humid conditions where in June and July high (over 15%) and fluctuated values for WDL were measured, stable relatively low (about 10%) rates of WDL during the summer in Uzbekistan were

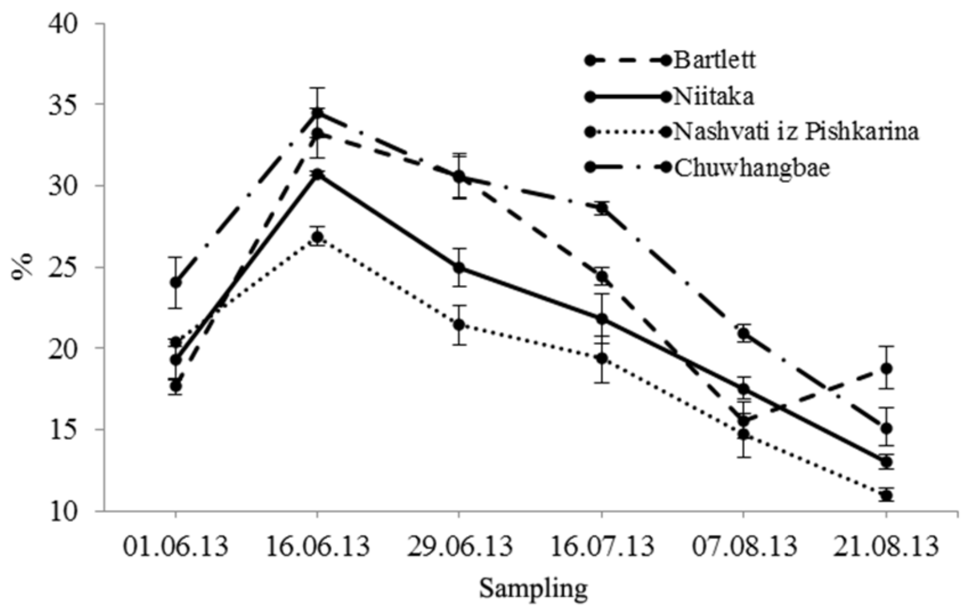

Figure 5. Changes in water deficit of annual shoots in different pear cultivars.

established. This might be attributed to the fact that the climate in Uzbekistan is dry without precipitation and and this activates plant's protective mechanisms to save water (Cruz et al., 2012).

The WDS levels in the studied cultivars showed a general decreasing trend from mid-June to August, as found for WCL and WCS, with the lowest WDS values being measured in cv. Nahsvati iz Pishkarina (Fig. 5).

In early June the values for WDS in all cultivars were low, then they increased, reaching a maximum in mid-June. The low WDS values in early June can be related to the high rate of water uptake by plant organs to ensure growth and well saturated with water leaves and shoots. With increasing temperature plant requirements for water are increased. The imbalance between WDL and WDS was probably due to the difference in water absorption, a property that is related to water demand of plant organs, age, leaf anatomical structure, xylem and root conductivity, transpiration rate (Trejo and Davies, 1991; Rotondi and Predieri, 2002; Kosma et al., 2009; Cruz et al., 2012; Aroca et al., 2012; Gegechkori et al., 2013). A strong shortage of water was observed in all cultivars in mid August and at the end of the month.

The electrolyte conductivity of leaves in the summer was characterized by a significantly different trend than that observed for water regime (Fig. 6). Many researchers have reported that the EC correlates with various physiological and biochemical parameters, characterizing plant response to environmental conditions such as spectral reflectance (Garty et al., 2000; Vainola and Repo, 2000), antioxidative enzyme synthesis (Liu and Huang, 2000; Sreenivasulu et al., 2000), membrane acyl lipid concentrations (Lauriano et al., 2000), water use efficiency (Franca et al., 2000; Saelim and Zwiazek, 2000; Bajji et al., 2001), transverse relaxation time of leaf water protons (Maheswary et al., 1999), stomatal

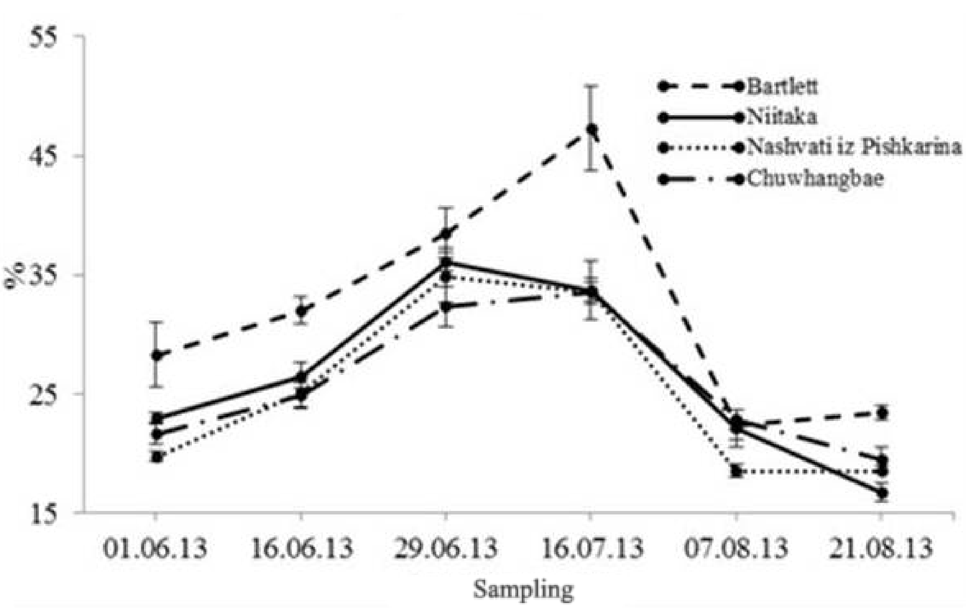

Figure 6. Changes in electrolyte conductivity of leaves during the summer of 2013.

resistance, osmotic potential and leaf rolling index (Premachandra et al., 1989). In the present study, EC rates showed a gradual increase from early June to mid-July, then with raising of the average daily temperature in early August, there was a sharp decline in the degree of cell membrane injury (below 25%), which was preserved until the end of August. Compared to the other varieties, cv. Bartlett had a relatively high level of electrolyte leakage from the cells, thus indicating a severe damage to cell membranes (Bandurska et al., 1997; Linden et al., 2000; Bajji et al., 2001). Thus, in our study, no relationship was found between EC, stomatal parameters and water regime in pear plants in the summer.

Analysis of leaf anatomical structure showed that cvrs. Niitaka and Chuwhangbae were characterized by higher values for leaf thickness, upper and lower epidermis layers, length of stomata slit between the guard cells (Table 1) as well as over 1.5 times bigger diameter of the main vascular bundles especially xylem (Fig. 7). However, they had lower density of upper and lower epidermis per 100 µm when compared with cvrs. Bartlett and Nashvati iz Pishkarina. When comparing the length and density of palisade mesophyll per 100 µm all cultivars showed a negligible difference except for cv. Bartlett. According to Bahanova (2003), the high leaf thickness, upper and lower epidermis layers and

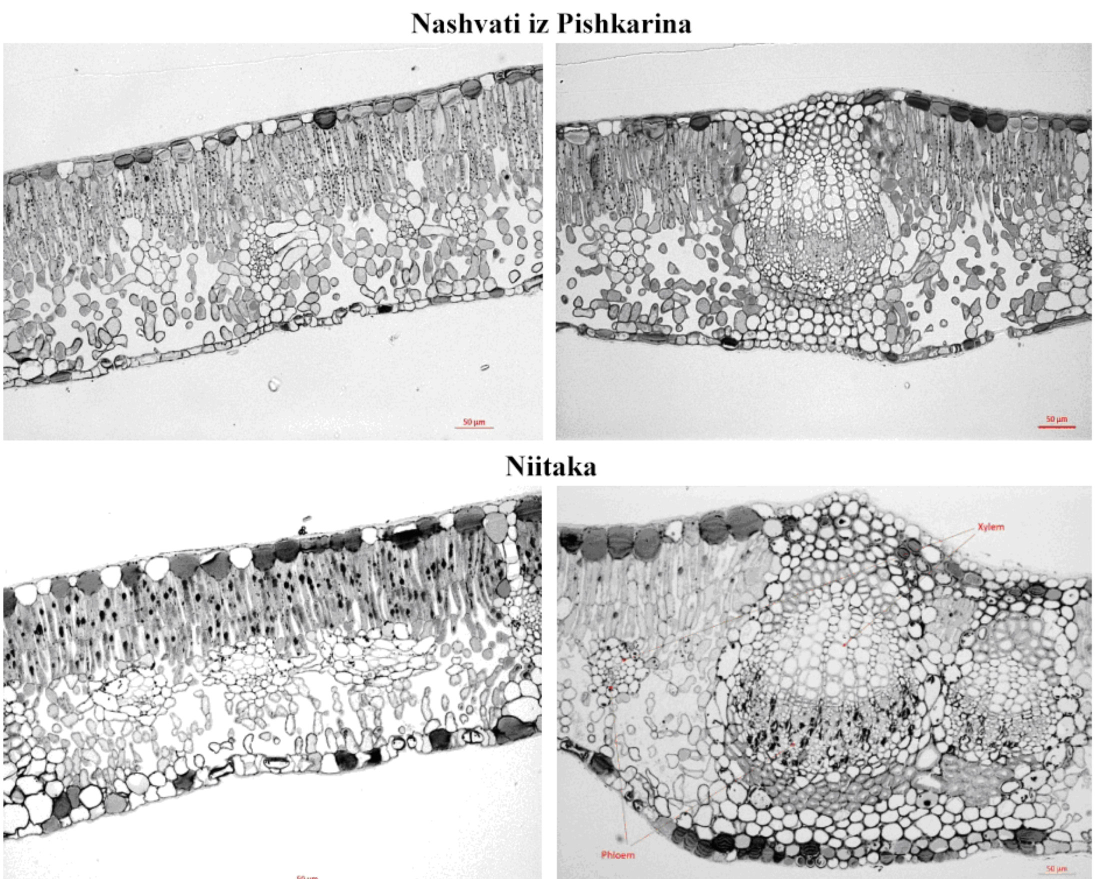

Figure 7. Leaf anatomical structure in pear cultivars originating from different habitats.

Table 1. Leaf anatomical parameters in different pear cultivars.

Cultivars	Thickness^{y}			Length of palisade mesophyll [µm]^{y}	Width of stomata slit between guard cells [µm]^{y}	Density^{y}			The main vascular bundles^{x}	
	Leaf [µm]	Upper epidermis [µm]	Lower epidermis [µm]			Upper epidermis per100 µm	Lower epidermis per100 µm	Palisade mesophyll per 100 µm	Diameter [µm]	Area [µm^2]
Nashvati	258.7±2.24^{bz}	17.24±1.04^{b}	8.57±0.32^{b}	115.7±3.30^{b}	25.32±0.98^{c}	4.9±0.13^{a}	6.9±0.36^{a}	11.0±0.28^{ab}	181.0±2.18^{c}	25750±622.2^{c}
Bartlett	263.0±3.78^{b}	17.34±0.77^{b}	9.30±0.31^{b}	128.3±2.11^{a}	26.41±1.38^{bc}	4.7±0.16^{a}	6.3±0.29^{a}	11.6±0.24^{a}	162.2±3.07^{d}	21250±714.6^{d}
Niitaka	299.3±3.49^{a}	30.07±1.38^{a}	20.47±1.15^{a}	117.8±4.30^{b}	29.30±1.11^{ab}	3.8±0.18^{b}	4.7±0.27^{b}	10.9±0.19^{b}	308.2±3.04^{a}	74690±1466.9^{a}
Chuwhang	295.8±4.88^{a}	26.55±1.65^{a}	20.77±1.06^{a}	111.4±4.06^{b}	30.77±1.19^{a}	3.7±0.16^{b}	4.5±0.20^{b}	10.9±0.26^{ab}	296.4±4.30^{b}	69120±1990.6^{b}

^{yx}Data represent the mean ± SD (n = 20 and 10).

^{z}Mean separation within columns by LSD test, $P \leq 0.05$.

density of stomata per mm^2 observed in apple varieties in a dry region contributed to the decrease in leaf transpiration rate and low WDL values, but this pattern was not observed in pears under the humid conditions of Republic of Korea.

Analysis of stomatal parameters showed variability depending on species and times of the day (Table 2). In the morning (8:00 AM) in comparison to the afternoon (3:00 PM) stomatal area in cvrs. Niitaka and Bartlett expanded significantly from 773.0 to 835.1 and from 703.5 to 724.3 µm^2, respectively. In cvrs. Nashvati iz Pishkarina the values were reduced while in cv. Chuwhangbae the change was quite negligible. Stomatal response might be associated with temperature and RH, xylem conductivity, needs of plant organs to uptake water, involvement of ABA in the regulation of stomatal behavior (Rodriguez and Davies, 1982; Zhang and Davies, 1990; Hartung and Slovik, 1991; Gollan et al., 1992; Trejo et al., 1993).

The stomatal density per mm^2 in the studied cultivars varied from 164.8 to 214.3 (Table 2). In cvrs. Niitaka and Chuwhangbae stomatal density was high (exceeding 16-18%,) when expressed per total leaf area whereas in cvrs. Nashvati iz Pishkarina it was about 12%. We did not find a significant relationship between water regime and leaf anatomical structure during the summer when air and soil were sufficiently moist.

Total chlorophyll content varied in the studied cultivars, but regardless of the conditions the minimal values were noted in cv. Niitaka (Figs. 8, 9). According to Rotondi and Predieri (2002), the leaves of the pear cvrs. Abbé Fétel and Passe Crassane had high chlorophyll content and exhibited also high photosynthetic activity. Some researchers (Kushnirenko and Medvedev, 1969; Bahanova, 2003) have reported that increasing the chlorophyll content of leaves under water shortage is a protective response of plants and such cultivars are more drought resistant. In the present study, the highest total chlorophyll content during the summer was measured in cvrs. Nashvati iz Pishkarina (Figs. 8, 9).

This could suggest high photosynthesic activity in these varieties. The concentration of chlorophyll in all cultivars was slightly higher in the afternoon (3:00 p.m.) than in the morning. The values reached a maximum in mid-August and decreased thereafter. In our previous seasonal studies both negative and positive correlations were found between total chlorophyll and total nitrogen content in the same varieties, and the data fluctuated regardless of the time of study (data not presented). Park et al. (2007) found that chlorophyll content in apple leaves, measured by a portable chlorophyll meter (SPAD-502), increased from June to August but the correlation coefficient between SPAD reading values and nitrogen content in the leaves tended to gradually decrease with the progress of growth. According to Ghasemi et al. (2011) there was a positive and linear correlation between chlorophyll content and total nitrogen content in Asian pear leaves, however, the analysis was only made in June. The discrepancy between these results, as well as in results reported by other authors (Blackmer and Shepers, 1995; Wu et al., 1998; Richardson et al., 2002; Rotondi and Predieri, 2002; Kowalczyk-Jusko and Koscik, 2002), may be due to differences in genotype, experimental period and environmental factors.

Table 2. Stomatal parameters in different pear cultivars.

Cultivars	Stomatal parameters								
	Density of stomata^{y}		Total covered area in leaves [%]	Changes of stomatal area depending on time^{x} [µm^{2}]		Length of stomatal pore^{x} [µm^{2}]		Width of stomatal pore^{x} [µm^{2}]	
	per mm^{2}	per leaf (thousand)		08-00 AM	03-00 PM	08-00 AM	03-00 PM	08-00 AM	03-00 PM
Niitaka	214.3±6.7^{az}	14637.8	16.6	773.0±26.1^{b}	835.1±23.2^{b}	30.14±0.64^{a}	26.70±0.66^{b}	10.86±0.23^{bc}	11.42±0.41^{b}
Chuwhangbae	183.4±4.6^{b}	12972.8	17.6	961.0±42.0^{a}	968.5±32.2^{a}	31.70± 0.79^{a}	28.78±0.72^{a}	12.27±0.33^{a}	11.04±0.28^{b}
Nashvati	164.8±3.8^{c}	3064.3	11.9	761.7±43.5^{b}	720.5±35.0^{c}	23.82±0.77^{b}	25.47±0.79^{bc}	9.93±0.28^{c}	9.56±0.29^{c}
Bartlett	203.7±5.6^{a}	3523.0	14.3	703.5±28.5^{b}	724.3±29.1^{c}	25.51±0.77^{b}	24.19±0.62^{c}	11.52±0.56^{ab}	14.85±0.51^{a}

^{yx}Data represent the mean ± SD (n = 12 and = 30).

^{z}Mean separation within columns by LSD test, $P \leq 0.05$.

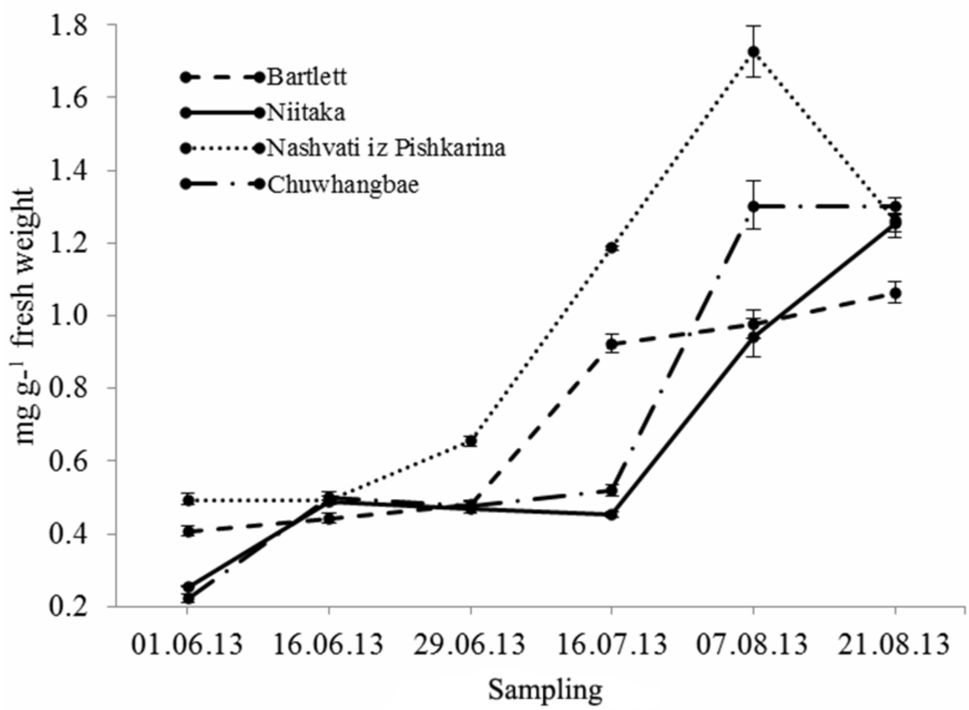

Figure 8. Total chlorophyll content in leaves of different pear cultivars measured at 8:00 a.m. during the summer of 2013.

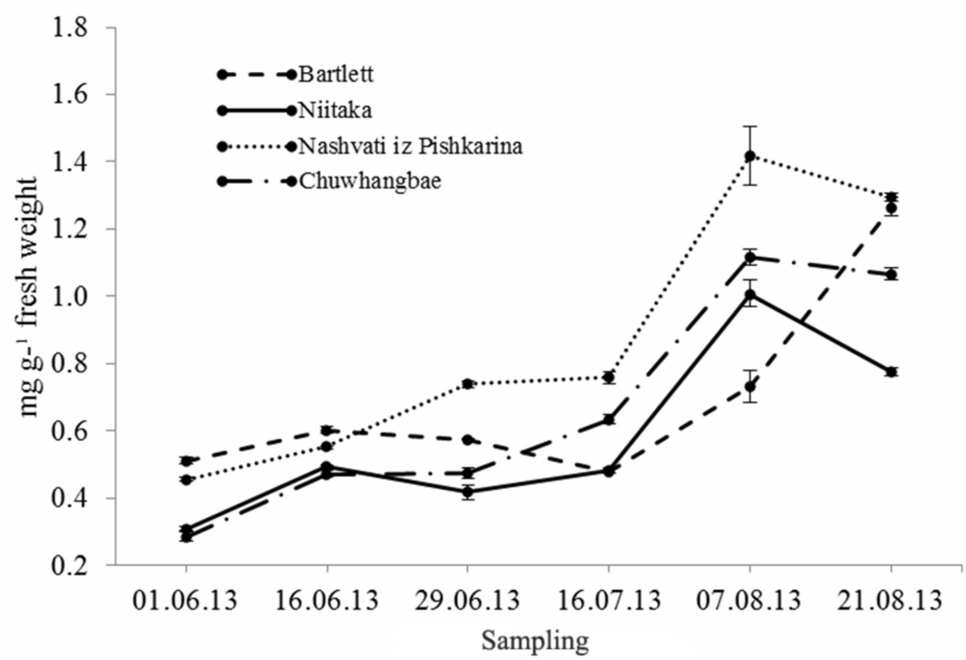

Figure 9. Total chlorophyll content in leaves of different pear cultivars measured at 3:00 a.m. during the summer of 2013.

In conclusion, our results demonstrated that the water regime, electrolyte conductivity, stomatal size and density as well as total chlorophyll content in the studied pear varieties were unstable and varied depending on investigation time, variety and climatic conditions. However, in humid summer conditions in South Korea, pear cultivars can not reveal their real ability in terms of water regime and physiological characteristics to resist stress factors.

The variety-environment interactions are very important in crop breeding in order to develop a specific variety suitable for a given region (Becker et al., 1999). Based on the presence or absence of an interaction effect, breeders may have to change the target area for cultivation or the selection scheme.

As for cultivation of a given cultivar in a different habitat, breeders in the future should pay attention to the development of new cultivars based on parental lines originating from external habitats. Thus, the same type of study should be conducted in semiarid areas where practically there is no rain in the summer and the humidity is relatively low compared to humid and rainy weather conditions. In such cases, other patterns of physiological responses can be observed and some of the newly developed cultivars may possess higher resistance than cultivars growing in a wetland.

ACKNOWLEDGEMENTS

This work was supported by funds of Pear research station, NIHHS, RDA, Republic of Korea. The author thanks Dr. Kang, Sam-Seok and technical staff for the assisstance to conduct this experiment.

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