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UDC: 630*1(497.113)

Original scientific paper

Variation of Leaf Water Potential and Leaf Gas Exchange Parameters of Seven Silver Linden (*Tilia tomentosa* Moench) Genotypes in Urban Environment

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Received: 30 Mar 2020; Revised: 3 Apr 2020; Accepted: 27 May 2020

Abstract: Differences between genotypes are considered to be the most important requisite for a resilient urban forest. Analyses of physiological traits, such as leaf water potential and leaf gas exchange could provide useful insight into the capacity of different species and genotypes to grow in harsh urban environments. In the present study, a variation of midday (Ψ_{md}) and predawn (Ψ_{pd}) leaf water potential, net photosynthesis (A), rate of transpiration (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) of seven Silver linden genotypes (*Tilia tomentosa* Moench), planted in the urban environment in Novi Sad, were examined. Analysis of variance and LSD tests were used to show differences between studied silver linden genotypes. The results showed significant differences for all observed leaf gas exchange parameters (A , E , g_s , C_i , Ψ_{pd} and Ψ_{md}) between genotypes. The results indicate better physiological performances of genotypes T3, in comparison to other observed genotypes under the prevailing environmental condition of the studied site in the urban environment.

Keywords: leaf water potential, leaf gas exchange, Silver linden, genotypic variation.

1. Introduction

Silver linden (*Tilia tomentosa* Moench) is a highly valued ornamental species native to South-East Europe and South-West Asia (Rushforth, 1999). In Europe, four *Tilia* species are distributed naturally: Caucasian linden –*T. dasystyla* (Stev.), silver linden –*T. tomentosa* (Moench.), small-leaved linden –*T. cordata* (Mill.) and large-leaved linden –*T. platyphyllos* (Scop.) (Filiz et al. 2015). Apart from their natural abundance, especially *Tilia cordata* L. and *T. tomentosa* L. (Aničić et al. 2011), are commonly planted in parks and other categories of urban green spaces in the eastern Balkan region (Mauer and Tabel, 1995; Pawlikowski et al. 2010; Vaštag et al. 2018).

Differences between genotypes are essential for the ability of local populations to cope with varying environmental stresses, such as drought, heat wave, salinity stress, etc. (Possen et al. 2014). Plants were evidenced to endure these stress conditions with an array of morphological, physiological, anatomical adaptations (Vaštag et al. 2020). In that term, higher physiological

variation could lead to more exploitation of ecosystem niches and better productivity (Lindner et al., 2010). Indeed, high physiological variation among genotypes can enhance the resistance of stands to climate change (Roth et al. 2007). Having in mind the importance of physiological variation, a number of studies assessed between and within genotypic differences of leaf gas exchange parameters (Bhatt, 1990; Oren et al. 1996; Širčelj et al. 2007; Fini et al. 2009; Mozdzer and Zieman, 2010; Flood et al. 2011) and leaf water potential (Gaosegelwe and Kirkham, 1990; Bahrn et al. 2002; Klein, 2014; Reddy, 2019) of various species, including silver linden (Filiz et al. 2015).

The connection between physiological parameters has been a subject of many studies (Williams et al. 1994; Zufferey et al. 2000; Iandolino, 2004; Coupel-Ledru et al. 2014), highlighting the presence and the connection between them. Among all physiological parameters, the relationship between stomatal conductance (g_s) and leaf water potential (Ψ) was noted to have a key to the understanding of plants' function under the predicted climate changes (Silim et al. 2009; Jackson et al. 2015; Kostić et al. 2019). Namely, stomata were found to be highly sensitive to Ψ (Gaosegelwe and Kirkham, 1990). Indeed, Bahrn et al. (2002) evidenced that g_s can be reduced in the absence of a visible reduction of Ψ .

Many environmental factors, such as salinity, high temperature, etc., can affect leaf gas exchange (Lahr et al. 2018; Lintunen et al. 2019; Vastag et al. 2019) and leaf water potential (Gillner et al. 2017; Sjöman, et al. 2018; Li et al. 2020) in urbanised area. From all environmental factors occurring in urban settlements, urban trees are highly sensitive to water deficit which plays the main role in their survival (Fini et al. 2009). This issue becomes even complicated as it was shown that environmental factors might affect gene expression of morphological and physiological traits (Day et al. 2002; De Souza et al. 2008). The presence of genetic variation in photosynthesis could indicate that natural selection had favoured different genotypes depending on the local environment (van Rooijen et al. 2015).

Physiological parameters are closely related to changes in environmental conditions (Williams and Araujo, 2002) and it could be used for urban forest management in the future (Filiz et al., 2015). Therefore, for this purpose Ψ_{md} , Ψ_{pd} , A, E, g_s , and Ci were used for research variation between silver linden genotypes. We studied leaf physiological variation of seven silver linden genotypes under the same urban environmental conditions. Our objectives were to: i) investigate variation in Ψ between genotypes and to assess the difference between midday (Ψ_{md}) and dawn (Ψ_{pd}); ii) investigate variation in leaf gas exchange parameters between seven genotypes; and iii) assess the relationship between physiological parameters.

2. Material and methods

Seven genotypes of *T. tomentosa* planted in the street of Antona Čehova (N 45.24°75'07'', E 19.83°11'38'', elevation 80 m a.s.l.) in Novi Sad, Serbia, were used for the present study. In order to estimate the variability in physiological traits of Silver linden, measurement of leaf gas exchange and Ψ were made on 30th of August 2019. For these measurements, fully expanded leaves were chosen from the same orientation.

2.1. Meteorological characteristics

The air temperature, daily precipitations and soil moisture of the 2019 growing season (from 1st of May until 4th of September 2019) were recorded automatically. The air temperature was measured with a MINIKIN RTHi (EMS, Czech Republic), while daily precipitation was assessed with an automatic precipitation collector (Pronamic, Denmark). Ψ_{soil} was measured with gypsum blocks (Delmhorst Inc., USA) at a soil depth of 50 cm, by automatically recording every 30 minutes (Figure 1). During the measurement, the air temperature was 24.8°C, soil moisture was 12.3% vol, and there was no precipitation.

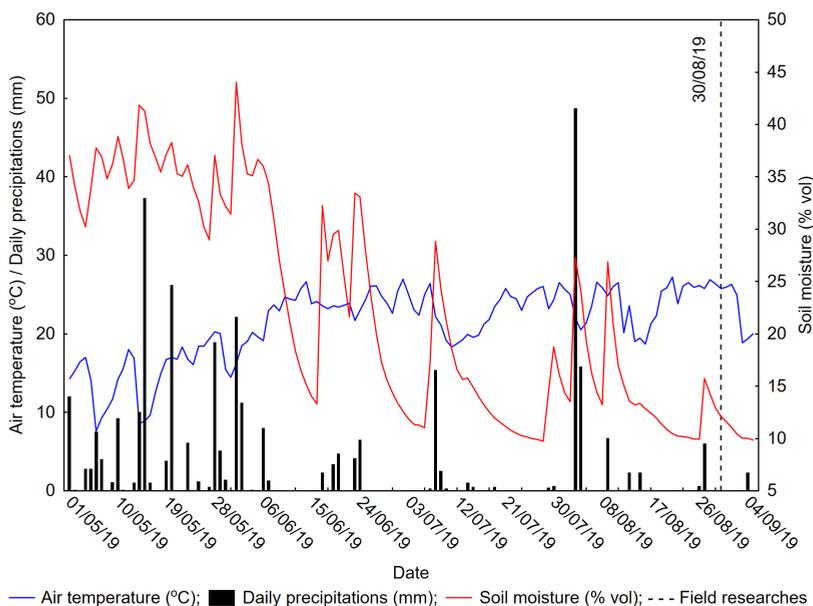


Figure 1. Air temperature (°C), daily precipitations (mm) and soil moisture (%vol) during growing season.

2.2. Assessment of leaf gas exchange and leaf water potential

Ψ was measured with a Portable Plant Water Status Console (SAPS II, model 3155; Soil Moisture Equipment Corp., Santa Barbara, USA) before dawn (Ψ_{pd} [MPa]) and at midday (Ψ_{md} [MPa]). For each tree, three leaves were sampled and measurements were made immediately after excision. Measurements were made on one fully expanded leaf.

Leaf gas exchange parameters (net photosynthesis (A [$\mu\text{mol m}^{-2} \text{s}^{-1}$]), rate of transpiration (E [$\text{mmol m}^{-2} \text{s}^{-1}$]), stomatal conductance (g_s [$\text{mmol m}^{-2} \text{s}^{-1}$]), and intercellular CO_2 concentration (C_i [$\mu\text{mol mol}^{-1}$]) were measured with a CIRAS-3 portable photosynthesis system (Amesbury, MA, USA) with red and blue LED light sources in a time scale between 9:00 AM and 11:00 AM. The measurements were recorded under photosynthetic active radiation (PAR) of $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$, while humidity, air temperature and the concentration of CO_2 were assessed during measurements. All measurements were made on one leaf per genotype and all parameters were recorded five times per leaf. All genotypes in this study had leaves fully developed, disease-free, and exposed to full sunlight in lower canopy (2 to 3 m above ground) (Herrick and Thomas, 1999; Tissue et al. 2001).

2.3. Statistical analysis

The statistical evaluation of the differences between the observed genotypes of Silver linden was made using ANOVA. The comparison of means was performed by applying the LSD test to determine the level of significance. Furthermore, the differences between the different genotypes were shown in the form of a diagram. All statistical analysis was performed in Statistica 13.3 (TIBCO Software, Inc.).

3. Results

Based on one-way ANOVA, there were significant differences in Ψ_{pd} and Ψ_{md} among Silver linden genotypes. The lowest mean value of Ψ_{pd} was observed for T4 (-0.9 ± 0.02 MPa), while T2 (-0.5 ± 0.48 MPa) had the highest mean value. Genotypes T1 (-0.7 ± 0.11 MPa), T3 (-0.72 ± 0.06 MPa), T5 (-0.67 ± 0.05 MPa), T6 (-0.58 ± 0.02 MPa), and T7 (-0.83 ± 0.09 MPa) were ranked in descending order into different homogenous groups. Genotypes T3 (-1.9 ± 0.05 MPa), T2 (-1.9 ± 0.01 MPa), and T6 (-1.85 ± 0.05 MPa) had the lowest mean value of Ψ_{md} , while genotype T5 (-1.65 ± 0.1 MPa) had the highest (Figure 2). Genotypes T1 (-1.7 ± 0.05 MPa), T4 (-1.8 ± 0.01 MPa), and T7 (-1.7 ± 0.1 MPa), were ranked in descending order into different homogenous groups. According to Mencuccini (2003) typical values of Ψ range between -1 and -2 MPa, down to -4 MPa in species occupying arid zones, and as low as -10 MPa in the most extreme cases (Tyree, 1997). Trees that have a low (more negative) Ψ tend to maintain leaf gas exchange at lower soil water potentials (Ψ_{soil}) and have an advantage where soil water deficits occur during the growth season (Mitchell et al. 2008; Blackman et al. 2010).

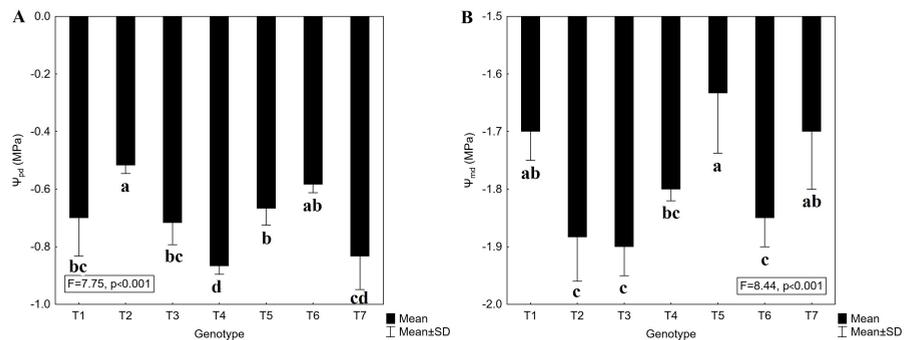


Figure 2. Leaf water potential for the observed silver linden genotypes. A) Pre-down leaf water potential (Ψ_{pd} [MPa]), B) Midday leaf water potential (Ψ_{md} [MPa]).

Regarding leaf gas exchange parameters, significant genotype differences were detected for all observed parameters (A , E , g_s , and C_i). The highest A values were detected for genotype T3 ($5.78 \pm 0.38 \mu\text{mol m}^{-2} \text{s}^{-1}$), while genotype T1 was observed to have the lowest ($3.78 \pm 0.08 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Figure 3.A). There were no statistically significant differences between genotypes T2 ($5.37 \pm 0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$) and T5 ($5.46 \pm 0.38 \mu\text{mol m}^{-2} \text{s}^{-1}$) as well as between genotypes T1 ($3.78 \pm 0.08 \mu\text{mol m}^{-2} \text{s}^{-1}$) and T6 ($3.58 \pm 0.36 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Figure 3a). The highest E value was recorded for genotype T3 ($4.09 \pm 0.01 \text{ mmol m}^{-2} \text{s}^{-1}$), while the lowest for T2 ($1.58 \pm 0.25 \text{ mmol m}^{-2} \text{s}^{-1}$). Genotypes T5 ($3.05 \pm 0.05 \text{ mmol m}^{-2} \text{s}^{-1}$), T6 ($2.47 \pm 0.04 \text{ mmol m}^{-2} \text{s}^{-1}$), T4 ($2.12 \pm 0.01 \text{ mmol m}^{-2} \text{s}^{-1}$), T1 ($2.03 \pm 0.05 \text{ mmol m}^{-2} \text{s}^{-1}$), and T7 ($1.87 \pm 0.04 \text{ mmol m}^{-2} \text{s}^{-1}$) were ranked in descending order into different homogenous groups (Figure 3b). The highest value of g_s was observed for genotype T3 ($158.6 \pm 0.55 \text{ mmol m}^{-2} \text{s}^{-1}$), while the lowest was recorded for genotype T2 ($40.4 \pm 0.89 \text{ mmol m}^{-2} \text{s}^{-1}$). T1 ($63.8 \pm 1.79 \text{ mmol m}^{-2} \text{s}^{-1}$) and T4 ($63.8 \pm 0.45 \text{ mmol m}^{-2} \text{s}^{-1}$) genotypes did not differ in terms of g_s (Figure 3c). C_i had the highest values in genotype T3 ($158.6 \pm 2.99 \mu\text{mol mol}^{-1}$), while T2 ($46.5 \pm 2.31 \mu\text{mol mol}^{-1}$) genotype had the lowest value. Genotypes T1 ($352 \pm 0.89 \mu\text{mol mol}^{-1}$), T4 ($327 \pm 2.38 \mu\text{mol mol}^{-1}$), T5 ($365 \pm 1.26 \mu\text{mol mol}^{-1}$), T6 ($374 \pm 2.08 \mu\text{mol mol}^{-1}$), and T7 ($295 \pm 3.56 \mu\text{mol mol}^{-1}$) were ranked in descending order into different homogenous groups (Figure 3d).

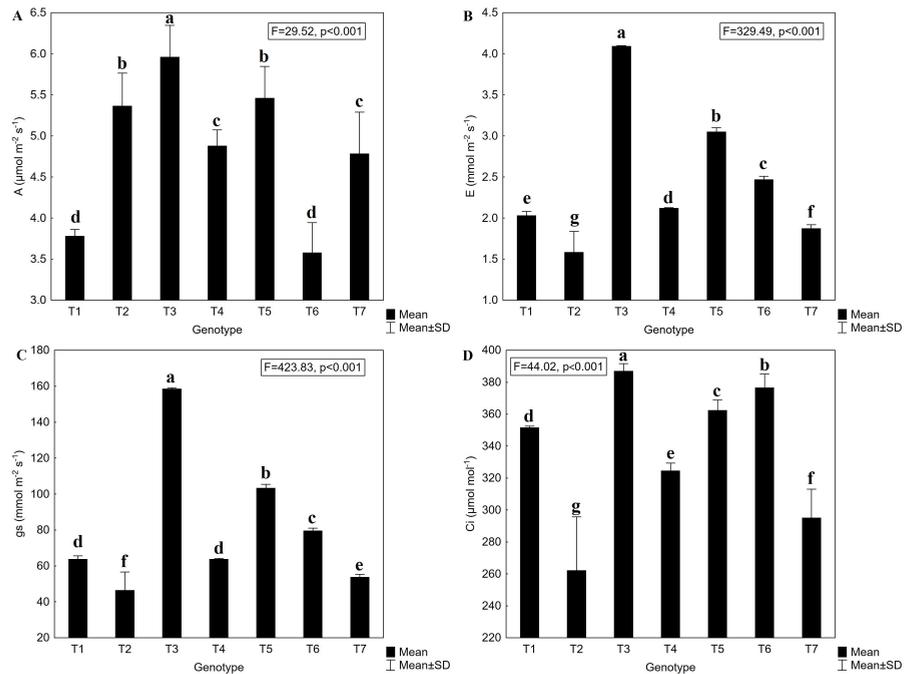


Figure 1. Leaf gas exchange parameters of seven silver linden (*Tilia tomentosa* Moench) genotypes. Net photosynthesis rate (A [$\mu\text{mol m}^{-2}\text{s}^{-1}$]); rate of transpiration (E [$\text{mmol m}^{-2}\text{s}^{-1}$]); stomatal conductance (g_s [$\text{mmol m}^{-2}\text{s}^{-1}$]); internal CO_2 concentration (C_i [$\mu\text{mol mol}^{-1}$]).

According to the correlation coefficients among the investigated parameters, a statistically significant positive correlation was observed between g_s and E ($r=1.00$, $p>0.05$). Furthermore, a statistically significant negative correlation was detected between A and Ψ_{md} ($r=-0.78$; $p>0.05$).

Table 1. Correlation coefficients between physiological parameters.

	g_s	A	E	Ψ_{md}
A	0.51	-		
E	1.00	0.49	-	
Ψ_{md}	-0.04	-0.78	-0.04	-
C _i	-0.04	-0.13	0.49	0.46

Legend: Net photosynthesis rate (A [$\mu\text{mol m}^{-2}\text{s}^{-1}$]); rate of transpiration (E [$\text{mmol m}^{-2}\text{s}^{-1}$]); stomatal conductance (g_s [$\text{mmol m}^{-2}\text{s}^{-1}$]); intercellular CO_2 concentration (C_i [$\mu\text{mol mol}^{-1}$]); midday leaf water potential (Ψ_{md} [MPa]). Bolded numbers show correlation coefficients which are significant ($p>0.05$).

4. Discussion

Urban trees are affected by climatic change over time (Sjöman et al., 2018). In order to become more resilient to threats from pathogens, damages of insects and the effects of changing climate, it is essential for urban forests to possess high diversity – at different levels (species,

genus, and family level) (Sjöman et al. 2018). At the species level, the most important task is to address the most successful genotype for urban environments. Regarding the suitability of species for urban settlements, lindens were chosen to possess many advantages such as: good shade casting capacity, flowers with sensational smell and high ornamental values, which are ideally suited to cityscapes (Pawlikowski et al. 2010).

According to the results of leaf gas exchange parameters, it is possible to identify a genotype that has the best physiological performance and which is assumed to perform better under the predicted climate changes in comparison to other genotypes. However, in the case of Ψ (Ψ_{pd} and Ψ_{md}) we found statistically significant differences among the observed genotypes. According to Sjöman et al. (2018), species with Ψ around -2.0 MPa respectively are considered to be highly sensitive to drought. Bearing in mind the result of Ψ_{pd} and Ψ_{md} (0.9-1.9 MPa) of the present study, we can conclude that the observed genotypes belong to the above-mentioned group. Similarly to this finding, Zhang et al. (2019) noted that *T. cordata Greenspire* cultivar appeared to be susceptible to drought in the urban environment. Moreover, the poor growth of Tilia-road side trees in urban area of Poland were attributed to the lesser ability of this genus to cope with side environments (Swoczyna et al. 2014)

Assessment of Ψ_{pd} and Ψ_{md} can provide valuable information that resolves which genotype and species can maintain physiological function more effectively in stress conditions (Lenz et al. 2006), and have the capacity to adapt to environmental change (Sjöman et al. 2018). Observing the values of Ψ_{pd} and Ψ_{md} of the present study, the highest values were detected for T3 genotype in comparison to the other six studied genotypes, suggesting its better performance. This was further supported as the same genotype exhibited the highest values of all leaf gas exchange parameters, namely, A, E, g_s , and Ci.

The present study evidenced high genotypic variation in all investigated leaf gas exchange parameters. Variation in A can lead to variation in growth rate and productivity, which are important factors in terms of species competition and yield (Flood et al. 2011). Genotypic variation in the A response could result from the differences in stomatal responses or the activation of the carboxylation process (Soleh et al. 2016). Coupel-Ledru et al. (2014) found that E was not only controlled by water deficit but is also under the influence of genetic. Furthermore, environmental factors, such as, temperature, wind, light, soil water, and relative humidity can also affect the process of transpiration (Taiz and Zeiger, 2002). Variation in g_s can result from different levels of water deficit, high temperature (Damour et al. 2010), and high urban CO₂ concentrations (Ziska et al. 2004), and can influence the A. Higher CO₂ concentrations might have relieved higher rates of A by urban trees, which would reduce water loss via stomatal conductance (Lahr et al. 2018).

Stomatal conductance (g_s) is influenced by the morphological and structural traits and the opening of stomata (Galmes et al. 2013), as well. Our results confirmed the relationship between g_s and E (Table 1), and this is not surprising given that g_s directly controls the transpirational water flow from leaf to the ambient air. The slow decrease of g_s will not affect E, but if g_s decrease strongly then it would be beneficial for increasing E (Ouyang et al. 2017). Ψ_{md} was negatively correlated to A (Table 1). Similarly to our findings, Giorio et al. (1999) and Zufferey et al. (2000) reported that low Ψ_{md} was coupled with low A values.

5. Conclusion

The results of the present study evidenced significant genotypic variation regarding all observed parameters of leaf gas exchange, namely A, E, g_s , Ci, Ψ_{pd} , and Ψ_{md} . The results of one-way analysis of variance noted high detrimental values of g_s , A, E, and Ci for T3 genotype compared to other observed genotypes. However, in terms of Ψ_{md} and Ψ_{pd} statistically significant genotypic variation was found in T5 and T3 genotypes. This study shows that physiological performance can be used in the selection of Silver linden genotypes. Namely,

under the predicted climate changes the selection of tolerant genotypes is of crucial importance in order to enhance the resilience of urban forests. Evaluation of the leaf water potential and leaf gas exchange parameters gives strong evidence for a genotype variation under urban environment.

Acknowledgments

This study was financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No: 451-03-68/2020-14/200197).

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