

Comparative study of newly-bred black locust clones with regard to photosynthetic rate and water use efficiency: early evaluation

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SUMMARY

Black locust (Robinia pseudoacacia L.) is one of the most important tree species in Hungary, due to its positive economic impacts. Research to increase its yield, improve its stem quality and enhance its drought tolerance has been ongoing since the 1960s. Of the current research works in this field, the clone trial of the Forest Research Institute, University of Sopron, established in 2020 in the Nyírség region, is worth highlighting. In this experiment 4 newly-bred clones and a state-approved black locust cultivar ('Üllői') are being tested. In the summer of 2022, 'on site' measurements of assimilation parameters – net assimilation (A_n), transpiration (Tr) – were carried out using the LI-6800 portable photosynthesis system. From the data obtained, the water use efficiency (WUE) was calculated. The results of the statistical analysis (Kruskal-Wallis H test) have shown significant differences ($p < 0.05$) between the clones for all three parameters (A_n , Tr , WUE) tested. The NK2 clone has performed the highest value for all the parameters studied. However, no significant differences were found between clones NK2 and PL040 for Tr or between NK2 and control ('Üllői') for WUE. Studies of this kind contribute to the improvement of black locust growing through the production and selection of cultivars, which are relatively resistant to the negative effects (drought) of climate change.

Keywords: photosynthesis; transpiration; water use efficiency; black locust clones

INTRODUCTION

Black locust (*Robinia pseudoacacia* L.) is a fast-growing, nitrogen-fixing, anisohydric (drought tolerant), broadleaved tree species, native originally to North America that has been introduced into several other countries around the world as well. Due to its valuable and resistant wood, also its excellent nectar production, it has a key role in the field of plantation forestry worldwide. *Robinia pseudoacacia* (hereinafter *R. p.*) is adaptable to many sites and climates, so it has great importance in facing the negative effects of the climate change (Huntley, 1990; Grünwald et al., 2009; Mantovani et al., 2014a; 2014b; Nicolescu et al., 2020; Ciuvăţ et al., 2022; Szyp-Borowska et al., 2022). However, it should be noted, that producing black locust timber with good quality is possible only on sites with adequate moisture and well-aerated and preferably light soils, rich in nutrients and humus (Rédei et al., 2008; Nicolescu et al., 2018). Although it has many positive economic impacts, it should be noted, that it is considered an invasive tree species in many countries. A site-specific approach, which combines both tolerance in some areas and strict eradication at valuable sites, seems to be the best option for achieving a sustainable coexistence of *R. p.* with humans and nature (Vítková et al., 2017; Sádlo et al., 2017; Puchałka et al., 2021).

In Hungary, *R. p.* is the most commonly planted tree species: it covers the 24% of the Hungarian stocked area. It provides 19% of the annual timber output of the country. However, 80% of the forest products are firewood and only 20% are industrial wood (sawlogs, props and poles), based on the data of the Hungarian National Land Centre (NLC, 2022).

Breeding of black locust to improve its stem quality, increase its yield and nectar production, and enhance its drought tolerance has been ongoing since the 1960s. These research works, led by Forest Research Institute (hereinafter FRI), have resulted in several state-approved (*R. p.* 'Üllői', *R. p.* 'Nyírségi', *R. p.* 'Jászakiséri' etc.) and candidate (*R. p.* 'Homoki', *R. p.* 'Bácska', *R. p.* 'Vacsi', etc.) cultivars in Hungary (Keresztesi, 1988; Rédei et al., 2017; Keserű et al., 2021; Ábri et al., 2021). Currently, FRI has some experimental plantations and clone trials in the country, where cultivars and new clones with fast juvenile growth are tested. One of these plantations is near the settlement of Napkor, where 4 new clones – *R. p.* 'Laposi' (breeding code: NK1), *R. p.* 'Napkori' (breeding code: NK2), *R. p.* 'Farkasszigeti' (breeding code: PL040), *R. p.* 'Püspökladányi' (breeding code: PL251) – and 'Üllői' state-approved cultivar are studied (Ábri et al., 2022).

Photosynthesis is the basis of plant productivity. The assimilation rate is determined by CO₂ fixation, which is affected by many environmental factors such as temperature, CO₂ concentration, light intensity, as well as the water and nutrient supply (Farquhar and Sharkey, 1982; Pethő, 1998; Meng et al., 2014). Due to the transpiration, water is transferred from the soil to the atmosphere through plants as an important part of the evaporation process and terrestrial water cycling (Jiao et al., 2019; Lyu et al., 2022). It is also affected by the environmental factors. The relationship between tree growth and physiological parameters has been proved in many studies (Shiple, 2006; Mantovani et al., 2014b; Jiao et al., 2019).

Water use efficiency (WUE) is defined as the amount of carbon assimilated as biomass produced per

unit of water used by the plant. It shows the relationship between plant productivity and water use. The resilience of plant material to drought stress is accompanied by greater WUE. Breeding plants and trees with both high carbon gain and WUE is a crucial aim for sustainable agriculture and forestry (Flexas et al., 2013; Hatfield and Dold, 2019).

The aim of this research was to study and compare black locust clones with regard to their plant physiology parameters such as net assimilation (A_n), transpiration (Tr) and WUE. In this paper, the recent results of the study of physiological traits of clones NK1, NK2, PL040, PL251 and state-approved cultivar ‘Üllői’ are presented.

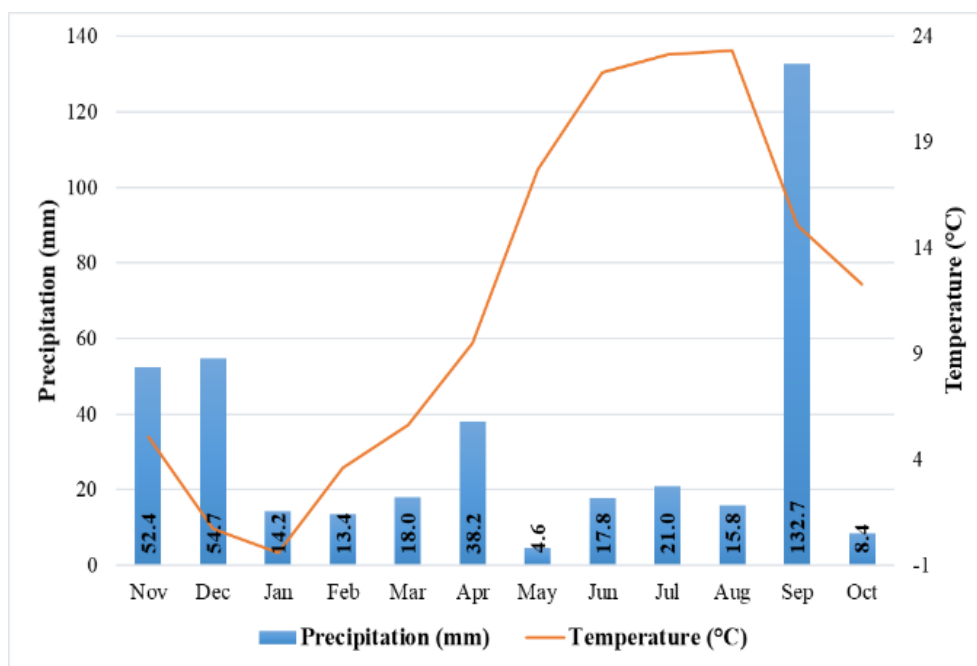
MATERIALS AND METHODS

The clone trial, near the settlement of Napkor (Nyírség region, Eastern Hungary), was established in 2020 on acid sandy soil with low humus content. The vegetatively propagated clones (NK1, NK2, PL040, PL251) and the ‘Üllői’ state-approved cultivar were planted in three different growing spaces per tree (6.25

m^2 , 9.0 m^2 and 16 m^2), but our investigation was carried out only in 6.25 m^2 growing space.

Based on 20 years (2002–2021) of meteorological data (HCSO, 2022), in Napkor, the annual average temperature is 10.9 °C, and the annual precipitation is 550.6 mm. 2022 (the period between January and October) was a year of extreme drought. The number of consecutive days without rainfall (0.1 mm >) was the greatest in June, August and October with 20 days, in each month. In Hungary, the main growing period of trees, when 80% of the organic material is produced, is from May to August (Führer, 2010; Mátyás et al., 2018). In this period the amount of the precipitation was only 59.2 mm, which is considerably below the 20-year average (253.3 mm). Furthermore, the monthly average temperature in June (22.3 °C), July (23.2 °C) and August (23.4 °C) was higher than the usual averages – 20.2, 21.7, 21.3 °C, respectively – in this period. In addition, it should be noted that the average temperature in June and July this year was 22.9 °C in the country, the hottest since 1901 (HMS, 2022). The monthly average temperature and the monthly precipitation from 2021 November to 2022 October are shown in Figure 1.

Figure 1: Monthly sums of the precipitation and averages of temperature (Napkor, 2021 November – 2022 October), based on meteorological (met) data of Napkor met station (HMS, 2022)



The field measurement was carried out on 29 June 2022 from 9:00 to 11:00 am. The physiological parameters (A_n and Tr) of the clones were measured with LI-6800 (LI-COR, Lincoln, Nebraska, USA) portable photosynthesis system (Figure 2). This system has two high-precision infrared gas analyzers to measure CO₂ and H₂O mole fraction in the air. The Li-6800-01A multiphase flash fluorometer head was used as a light source, the aperture was 2 cm². The CO₂ concentration was controlled in the chamber: 400 μ mol

mol⁻¹ using injector and CO₂ patrons. The ambient CO₂ level was 383 μ mol mol⁻¹. The air temperature ranged from 33 to 38 °C and the relative humidity varied between 34.60 and 50.45% in the chamber. We measured the leaves of clones six times per leaf on three trees per plot (5). Readings were logged when the measured parameters stabilized, but after a minimum of 2 min.



Figure 2: LI-6800 (LI-COR) portable photosynthesis system (Napkor, 2022)



From the data of A_n ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and Tr ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) measurements we could calculate the WUE (kg m^{-3}). We used the following formula (Tanner and Sinclair, 1983) for this:

$$\text{WUE} = (A_n * 44) / (Tr * 18), \quad (1)$$

Shapiro-Wilk test was used for testing normality. The data were nonparametric, thus, we used the Kruskal-Wallis H test to compare the A_n , Tr and WUE values of the clones. The relationship between A_n and Tr was also studied. For the pairwise comparison IBM SPSS 25.0 statistical software package was used and the natural logarithmic regression functions were calculated and plotted with MS Excel 2016.

RESULTS AND DISCUSSION

Assimilation and water management studies in field experiments in an ‘in situ’ way are crucial for understanding the photosynthetic process of *R. p.*. Measurements and studies regarding assimilation processes have been carried out by many scientists in the laboratory on different plants, but relatively few research works have been published on the results of ‘on-site’ measurements under field conditions (Mantovani et al., 2014a; Meng et al., 2014; Zhou et al., 2017; Zhu et al., 2017; Zheng et al., 2019; Lyu et al., 2022; Szyf-Borowska et al., 2022). We also studied these parameters of clones NK1, NK2, PL040 and PL251 in the summer of 2021 (Ábri et al., 2022).

Assimilation parameters were measured in June (2022), when the rate of photosynthesis is the highest (Mátyás et al., 2018). There were significant differences ($p < 0.05$) found between the clones in all studied physiological traits (A_n , Tr , WUE). The highest assimilation rate ($20.25 \mu\text{mol m}^{-2} \text{ s}^{-1}$) was in the NK2, the lowest ($9.83 \mu\text{mol m}^{-2} \text{ s}^{-1}$) was found in the NK1 clone. In the pairwise comparison, the clone NK1 and the ‘Üllői’ state-approved cultivar ($11.30 \mu\text{mol m}^{-2} \text{ s}^{-1}$) did not differ significantly, nor did the clones PL040 ($15.02 \mu\text{mol m}^{-2} \text{ s}^{-1}$) and PL251 ($12.94 \mu\text{mol m}^{-2} \text{ s}^{-1}$). In addition to the highest photosynthetic rate, we have measured the greatest transpiration value ($10.08 \text{ mmol m}^{-2} \text{ s}^{-1}$) in the NK2 clone, but there was no significant difference observed between that and clone PL040 ($10.01 \text{ mmol m}^{-2} \text{ s}^{-1}$). The lowest transpiration rate was found in the ‘Üllői’ cultivar ($5.81 \text{ mmol m}^{-2} \text{ s}^{-1}$). Using the obtained data from the measurements of A_n and Tr , the WUE (kg CO_2 per $\text{m}^3 \text{ H}_2\text{O}$) was calculated for every clone. The best water use efficiency was observed in the clone NK2 and the cultivar ‘Üllői’ with values of 4.92 and 4.78 kg m^{-3} , respectively. The clone NK1 has the lowest WUE value (3.27 kg m^{-3}). There were no significant differences neither between NK2 and ‘Üllői’, nor between clones NK1, PL040 and PL251. The results with arithmetic means (\bar{x}), standard error (S.E.) and standard deviation (SD) are summarized in Table 1.

Table 1: Assimilation rate, transpiration and water use efficiency of the clones. The results of the pairwise comparison (Kruskal-Wallis H test)

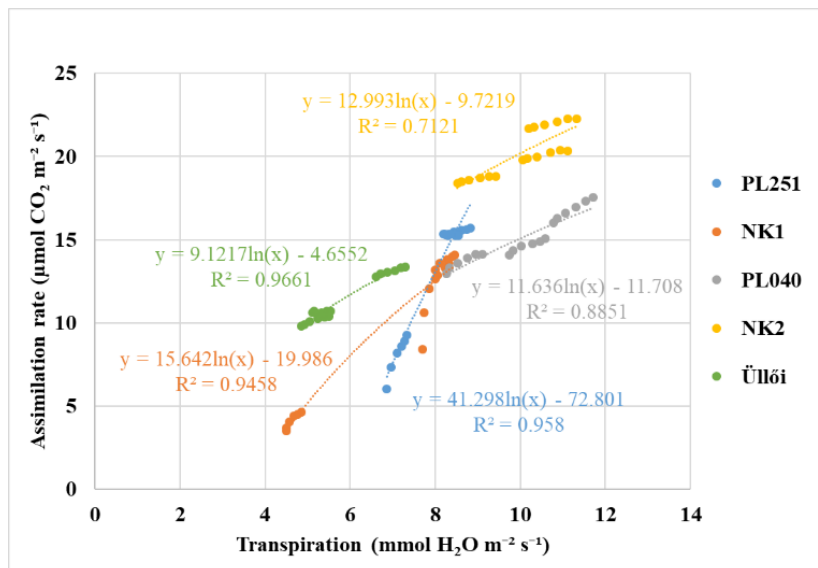
Clones	Assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)				Transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)				Water Use Efficiency (kg m^{-3})			
	n	\bar{x}	SD	S.E.	n	\bar{x}	SD	S.E.	n	\bar{x}	SD	S.E.
NK1	18	9.83 ^a	4.35998	0.478	18	6.95 ^{ab}	1.68781	0.212	18	3.27 ^a	0.87281	0.089
NK2	18	20.25^b	1.43250	0.478	18	10.08^c	0.91576	0.212	18	4.92^b	0.24054	0.089
PL040	18	15.02 ^c	1.41615	0.478	18	10.01^c	1.12657	0.212	18	3.68 ^a	0.14489	0.089
PL251	18	12.94 ^{bc}	3.61943	0.478	18	8.00 ^b	0.66774	0.212	18	3.89 ^a	0.84914	0.089
Üllői	18	11.30 ^a	1.34415	0.478	18	5.81 ^a	0.87269	0.212	18	4.78^b	0.18349	0.089

n = number of measurements; \bar{x} = arithmetic mean; SD = standard deviation; S.E. = standard error. The different letters mean significant difference at $p = 0.05$ among the clones.

We also studied the relationship between the A_n and Tr of the clones. The results of logarithmic regression analysis (Figure 3) have shown strong relationship between the observed parameters. The highest R^2 value (0.9661) was in cultivar ‘Üllői’, while the lowest in clone NK2 (0.7121). The slope of the fitted function is

steepest for clone PL251, with the rate of assimilation increasing intensively with increasing transpiration. The assimilation rate of clone NK2 varied less with increasing transpiration, but was above the other values.

Figure 3: Logarithmic relationships between the assimilation and the transpiration values of the clones



The negative effects of climate change, such as rising temperature and CO_2 concentration, less and uneven rainfall, growing frequency of extreme droughts, pose a threat to the ecosystem, especially forests and tree plantations. These factors can affect the growth, the vitality and the assimilation process of trees (Hlásny et al., 2014; Keenan, 2015; McDowell and Allen, 2015; Mátyás et al., 2018).

Forestry is one of the fields most affected by climate change. Thus, forestry breeding, increasing the drought stress tolerance will be a key feature in maintaining functional forest cover in the face of climate change. For drought resistance, morphological traits, growth, yield, survival rate, and physiological traits, including WUE, stomatal conductance, intercellular CO_2 concentration, photosynthetic ability, can be used as target traits for selection (Szyp-Borowska, 2022).

In water stress, the rate of A_n and Tr , and thus the tree productivity decrease (Jiao et al., 2019). According to many studies (Xu et al., 2010; Mantovani et al., 2014a, 2014b; Lyu et al., 2022), *R. p.*, as an anisohydric tree species, can adapt to extreme – prolonged – drought conditions by improving its water use efficiency. Black locust can reduce its water loss through both reduced Tr and leaf size. However, under well-watered conditions it does not regulate its Tr , thus it cannot be considered a water-saving tree species (Mantovani et al., 2014a).

Based on our previous (Ábri et al., 2022) and current studies, we assume that clone NK2 has great drought resistance ability, due to the fact that it had the highest assimilation rate between the tested clones. This clone has the best performance also in the

parameter of WUE. These results have great relevance in extreme weather conditions prolonged drought and uneven rainfall – such as the ones in 2022.

CONCLUSIONS

Black locust is one of the most commonly planted exotic tree species in many countries. In Hungary, it plays a key role in the forest management of the country, due to its positive features, such as valuable, durable and resistant wood, excellent nectar production and its adaptability on degraded lands. To improve its stem quality, increase its yield and drought stress tolerance, research works have been on-going for more than 60 years. Recently, we have been studying newly-bred *R. p.* clones in a clone trial, near the settlement of Napkor. Besides the morphological and growth studies, we also investigate the physiological traits, such as A_n , Tr and WUE. Significant differences were observed between the clones in all studied parameters. The results show that the clone NK2 has the best performance in A_n and WUE with the values $20.25 \mu mol CO_2 m^{-2} s^{-1}$ and $4.92 kg m^{-3}$.

R. p. cultivars will have great importance in wide-spaced, short-rotation industrial tree plantations, which are primarily aimed at meeting the growing demand for quality wood. Furthermore, these plantations, established with fast growing tree species and cultivars offer ecosystem services such as CO_2 sequestration and landscape reclamation. Moreover, these plantations also play a great role in forest conservation, due to the mitigation of the environmental pressure by reducing the number of harvestings in the forests.

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