

Light response curve analysis of juvenile black locust clones: A case study from eastern Hungary

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Abstract: Assimilation (*A*) and photosystem II (*PSII*) efficiency value light response curves [*A/PPFD* and *PSII/PPFD* curves (*PPFD* – photosynthetic photon flux density)] of promising black locust clones (NK2 and PL251) and the registered Üllői clone were analysed to study the net assimilation rates and *PSII* efficiency within the function of *PPFD* levels. The natural logarithmic regression functions fitted well to the measured data points for *A/PPFD*, R^2 values varied between 0.9515–0.9884. For *PSII/PPFD* values, we used the exponential regression function with R^2 ranging from 0.9948 to 0.9989. Except for PL251, the *A/PPFD* curves of the tested clones increased steadily with increasing illumination levels but flattened at the 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPFD* level due to the effect of photorespiration on the assimilation rate. For PL251, the *A/PPFD* curve decreased at the 1 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPFD* level. Unlike *A/PPFD* results, the *PSII/PPFD* exponential curve decreased as the *PPFD* level increased. Europe is forecast to experience significant negative climate change factors, including increased drought, heat, and irregular precipitation. Under such conditions, relatively drought-tolerant tree species such as black locust will play a vital role in new afforestation and uninterrupted wood supply. Consequently, growing and improving newly bred black locust clones, including the ecophysiological studies of relatively drought-tolerant clones, is increasingly vital.

Keywords: assimilation; drought tolerance; ecophysiology; photosystem II

Black locust (*Robinia pseudoacacia* L.) is planted in Europe for its fast growth, excellent nectar production, relative drought tolerance, and site adaptability (Nicolescu et al. 2020). It is also an invasive tree, but strict adherence to its growing technology and optimal site selection can help reduce its invasiveness (Vítková et al. 2017).

In Hungary, black locust improvement started in the 1960s (Ábri et al. 2023a). The present study selected and propagated black locust clones with good stem quality and high yield. The Hun-

garian Forest Research Institute and Napkor Erdőgazdák Zrt. recently established an experimental plantation that included a clone trial involving four newly-bred clones and a state-approved cultivar – Üllői black locust (Rédei et al. 2020) as a control. The trial compared these new clones and the Üllői cultivar for growth, assimilation parameters and water use efficiency (Ábri et al. 2022, 2023b; Ábri, Csajbók 2023).

Many stress factors negatively affect plants today. Global climate change has increased the two

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most significant negative factors – drought and high temperatures. The two factors combined can severely damage trees and impair photosynthesis, which affects other physiological parameters (Flexas, Medrano 2002; Chaves et al. 2009; Farooq et al. 2009; Brito et al. 2019). Figure 1 illustrates possible mechanisms that lead to decreasing photosynthesis during water stress.

Many studies have examined black locust physiology and water stress response (Zheng et al. 2012; Mantovani et al. 2014; Meng et al. 2014; Küppers et al. 2018; Choi et al. 2021; Lange et al. 2022; Ábri et al. 2023b; Ábri, Csajbók 2023).

Zheng et al. (2012) demonstrated that black locust photosynthetic light response curves initially show a rapid increase in the net CO₂ assimilation rate (*A*) under photosynthetically active radiation (*PAR*) between 0 μmol·m⁻²·s⁻¹ and 200 μmol·m⁻²·s⁻¹. Then, *PAR* likely becomes a limiting factor in photosynthesis because net assimilation increases before slightly decreasing as *PAR* increases. Leaves cannot utilise high-intensity light due to enzyme system limitations (the light supersaturation phenomenon). Küppers et al. (2018) created a model to estimate the carbon flux in sun-exposed and shaded black locust leaves. The model showed that the maximum observed daily carbon gain in sun-ex-

posed leaves was 536.3 mmol CO₂·m⁻²·day⁻¹, while the annual carbon gain was 46 824 mol CO₂·m⁻².

The black locust (*Robinia pseudoacacia*) exhibits paraheliotropism to protect its photosynthetic system. Arena et al. (2008) examined leaves with restricted and unrestricted movement. Restricted leaves showed a significant decrease in net photosynthesis, stomatal conductance, effective quantum yield of photosystem II (*PSII*), and photochemical quenching, while intercellular/environmental CO₂ concentration and non-photochemical quenching significantly increased. Unrestricted leaves showed higher photosynthetic performance throughout the day. Under intense sunlight, unrestricted leaves recovered the maximum quantum yield of *PSII* photochemistry by the end of the day, while restricted leaves did not.

This paper analysed the photosynthesis parameters (net assimilation rate and *PSII*) of the newly bred PL251 and NK2 black locust clones and a state-approved Üllői cultivar in the function of photosynthetic photon flux density (*PPFD*) by recording light response curves. We have three hypotheses: (i) *A* of the clones PL251 and NK2 is higher than the Üllői at every *PPFD* level; (ii) the *A* increases with *PPFD*; (iii) the efficiency of *PSII* declines in higher *PPFD* levels.

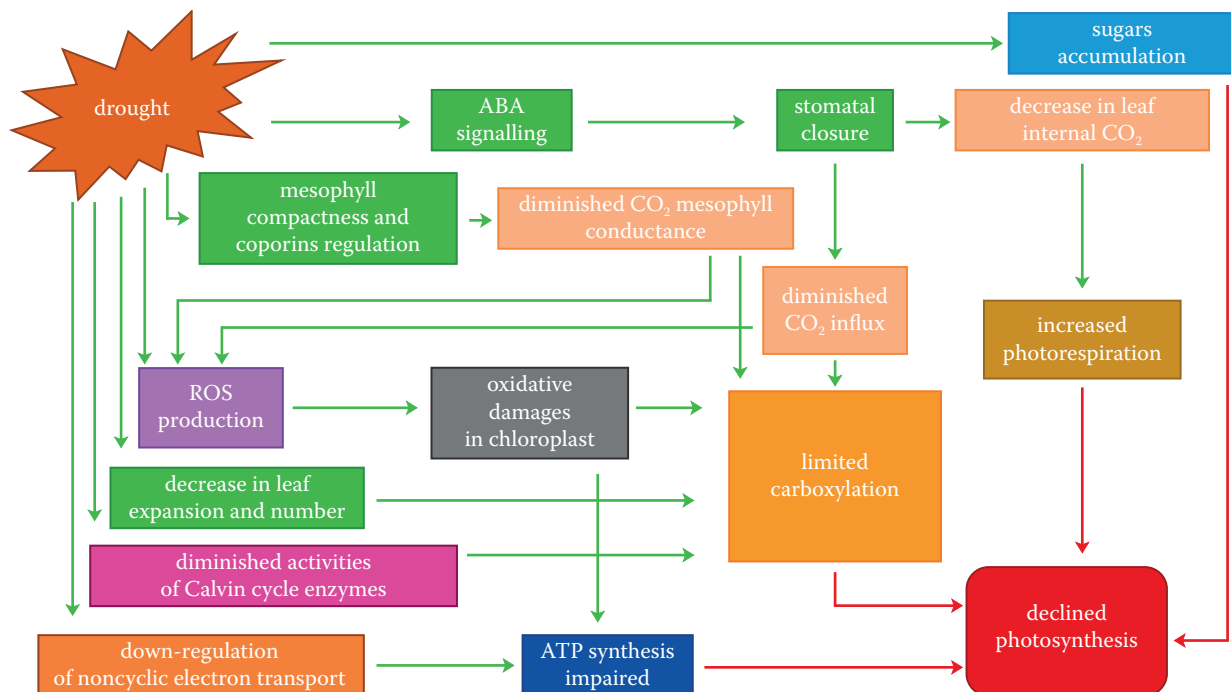


Figure 1. Possible mechanisms that lead to a decrease in photosynthesis during drought

ABA – abscisic acid; ATP – adenosine triphosphate (Farooq et al. 2012); ROS – reactive oxygen species

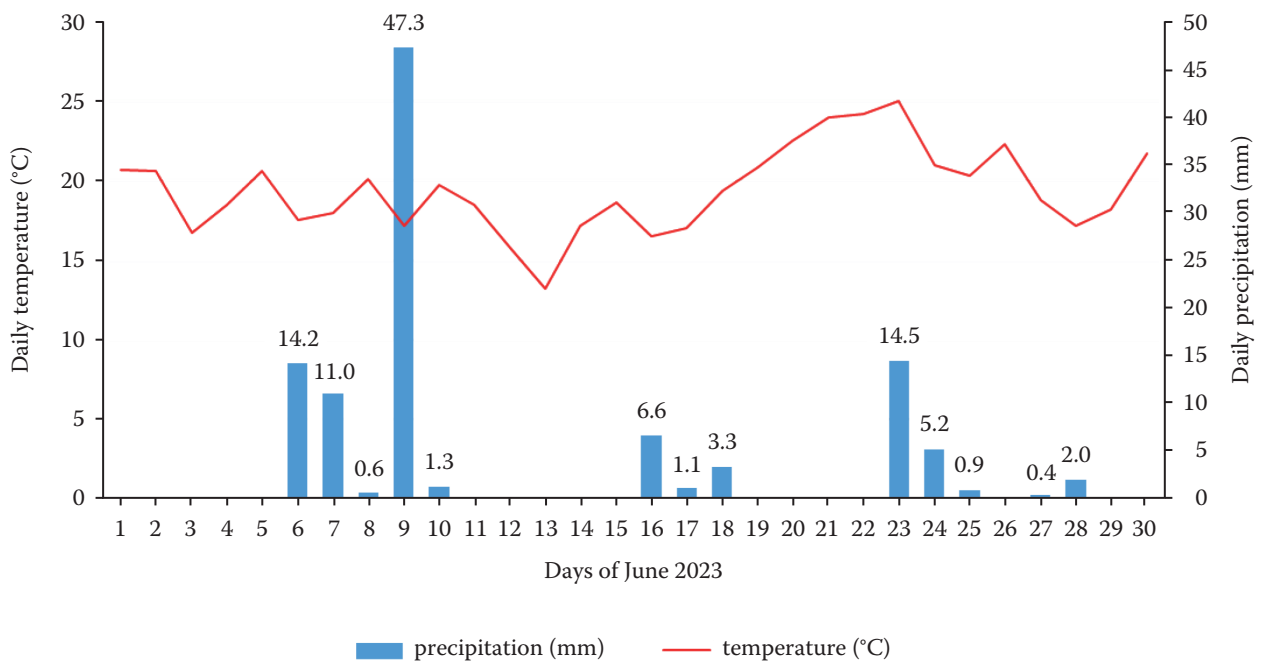


Figure 2. Daily temperature and precipitation data of the studied area in June 2023 (based on data from the Nyíregyháza-Napkor meteorological station of the Hungarian Meteorological Service)

MATERIAL AND METHODS

Study site. The experimental plantation was established in humic sandy soil in one of the main black locust growing areas in Hungary, the Nyírség region in the east (Ábri et al. 2022). Based on long-term (30-year average, 1991–2020) meteorological data provided by the meteorological station Nyíregyháza-Napkor (Hungarian Meteorological Service), the annual mean temperature was 10.6 °C and the annual mean precipitation was 537 mm in this region. The temperature was 28 °C during measurements. There was no rainfall before the clones were studied (Figure 2).

Measurements. Based on previous results from the Napkor clone trial (Ábri et al. 2022, 2023b), the study investigated the 4-year-old NK2 and PL251 clones and Üllői, which was used as a control, on June 30, 2023. LI-6800 (LI-COR, USA) portable photosynthesis system was used in the assimilation parameters measurements. The instrument recorded the net assimilation, transpiration, stomatal conductance, intercellular CO₂ concentration, PSII efficiency and other physiological parameters (Ábri et al. 2022, 2023b). The light was controlled in the sample chamber using 90% red (625 nm) and 10% blue (475 nm) light. We used decreasing photosynthetic photon flux density (PPFD)

in 9 steps (2 000; 1 500; 1 200; 900; 600; 300; 150; 50; 20 μmol·m⁻²·s⁻¹) for the light response curve. At each PPFD, *A* was recorded once the leaves had reached steady-state values, as described above. Leaf temperature at the time of all measurements varied from 26 °C to 29 °C.

The Li-6800-01A multiphase flash fluorometer head (LI-COR, USA) with a 2 cm² aperture served as a light source. The CO₂ concentration was controlled in the chamber: 400 μmol·mol⁻¹, using an injector and carbon dioxide cartridges. Light-adapted leaves were measured six times per leaf on three plants per plot. Readings were logged when the measured parameters stabilised but after a minimum of 120 s (Ábri et al. 2022).

RESULTS

In 2023, the photosynthetic and PSII light response curves (*A*/PPFD curve and PSII/PPFD curve) of two clones (PL251 and NK2) with the best growth (Ábri et al. 2022, 2023b) and the Üllői cultivar were observed with decreasing PPFD (photosynthetic photon flux density) levels in 9 steps (2 000; 1 500; 1 200; 900; 600; 300; 150; 50; 20 μmol·m⁻²·s⁻¹). There were significant differences between the clones in both parameters at *P* < 0.0001 level. For *A*, the natural logarithmic regression functions

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fit well to the measured data points (R^2 values varied between 0.9515–0.9884), i.e. the curves of the functions showed the differences appropriately. When examining the assimilation rate of the two clones and Üllői as a function of *PPFD*, the present study found that PL251 was the weakest at the lowest *PPFD* level (0–20 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), with a value of $-7.04 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The difference between the Üllői ($-2.86 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and NK2 ($-3.03 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) was minimal. At the other *PPFD* levels, NK2 was the best, with *A* increasing steadily because of increasing *PPFD*, but the curve flattened at the $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPFD* level due to the effect of photorespiration on assimilation rates. The highest *A* value ($13.58 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) occurred at the $2\,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPFD* level. The Üllői cultivar and PL251 results varied and were characterised as follows. Üllői had a higher assimilation rate between 0 and $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPFD* levels; however, as light intensity increased, the PL251 clone proved superior. Although the Üllői curve ran lower than the PL251 curve at higher *PPFD* levels, its *A* value showed a steady increase, with the best value ($5.79 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at the highest *PPFD* level of $2\,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The assimilation rate of PL251 increased

to $1\,200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPFD* level, reaching the highest *A* value ($8.88 \mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at this level (Figure 3).

The exponential regression functions also fitted well to the measured data points for the *PSII* efficiency values (R^2 values are 0.9948 for NK2, 0.9964 for PL251, and 0.9989 for Üllői). Thus, the curves of the functions clearly exhibited the differences in this case as well, i.e. the curves of the functions showed the differences suitably. From the obtained results, the *PSII* efficiency of the tested black locust clones and the Üllői black locust decreased with increasing light intensity. At low *PPFD* levels (0– $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), the clone PL251 (0.70–0.61) produced the highest value, while at higher *PPFD* levels (600 – $2\,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), the NK2 clone had the best value (0.50–0.18). However, the differences between the clones tested and the Üllői black locust were minimal, especially between clone PL251 and Üllői, which both had similar *PSII* values at many of the same light intensities (on 900 ; $1\,200$; $1\,500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPFD* levels the *PSII* efficiency index was 0.37; 0.29; 0.22, respectively). NK2 at $900 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ *PPFD* level was visibly distinct from the other two (Figure 4).

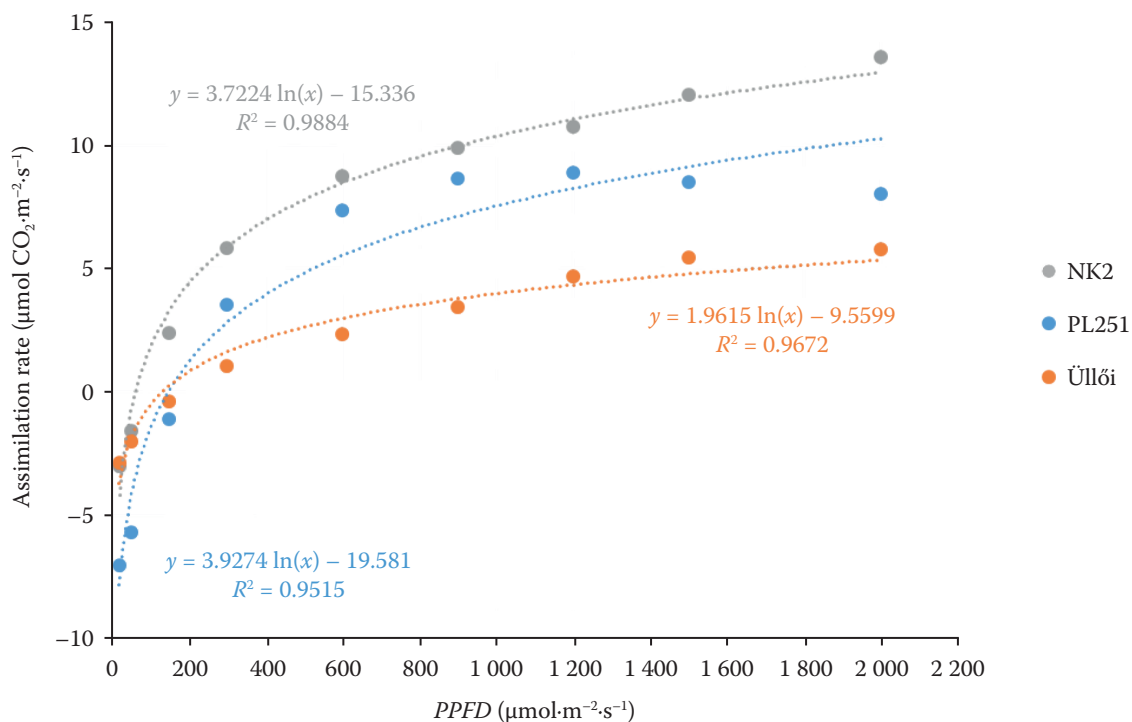


Figure 3. Assimilation rate light response curve of black locust clones PL251, NK2, and Üllői in Napkor (June 29, 2023) *PPFD* – photosynthetic photon flux density

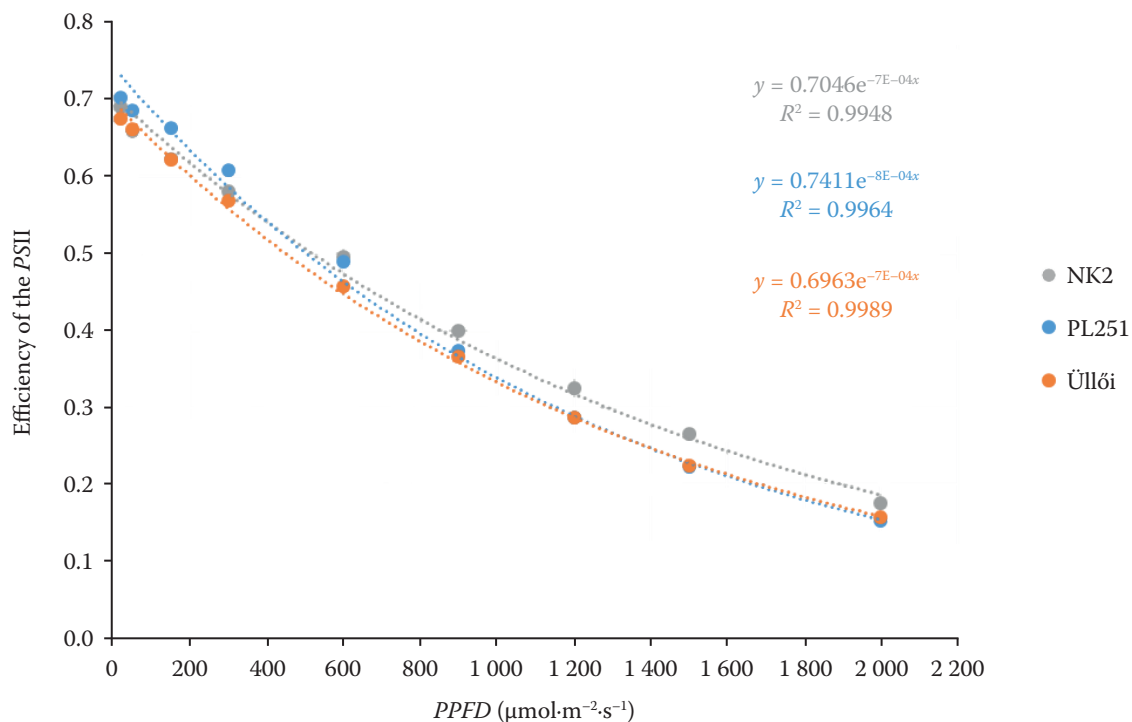
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Figure 4. Photosystem II (PSII) light response curve of black locust clones PL251, NK2, and Üllői in Napkor (June 30, 2023) PPFD – photosynthetic photon flux density

CONCLUSION

Black locust is planted in Europe for its ecological adaptability on degraded lands, fast growth, high biomass yields, and economic benefits. Ecophysiological studies are vital in this climate change era. The light response curves supply an opportunity for insights into black locust clone shade tolerance. NK2 clone rose above the other tested clones for shade tolerance and other studied parameters. The clone seems relatively drought tolerant, with a high assimilation rate and high PSII efficiency.

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