UNIVERSITY of SOPRON

FACULTY OF WOOD ENGINEERING AND CREATIVE INDUSTRIES

10th HARDWOOD Conference Proceedings

12–14 October 2022 Sopron

Editors: Róbert Németh, Christian Hansmann, Peter Rademacher, Miklós Bak, Mátyás Báder

WOOD KPLUS

Mendel University in Brno

10^{TH} HARDWOOD CONFERENCE PROCEEDINGS

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UNIVERSITY OF SOPRON PRESS

SOPRON, 2022

10TH HARDWOOD CONFERENCE PROCEEDINGS
Sopron, Hungary, 12-14 October 2022

ISBN 978-963-334-446-0 (pdf)
DOI https://doi.org/10.35511/978-963-334-446-0
ISSN 2631-004X (Hardwood Conference Proceedings)

Constant Serial Editors: Róbert Németh, Miklós Bak

Cover image based on the beech specimens of Radim Rousek and Mátyás Báder by Miklós Bak, 2021

The manuscripts have been peer-reviewed by the editors and have not been subjected to linguistic revision.

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University of Sopron Press, 2022
Responsible for publication: Prof. Dr. Attila Fábián, rector of the University of Sopron

Growing technology and genetic testing of newly-bred black locust cultivar candidates in Hungary: A review

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Keywords: black locust, cultivar candidates, genetic evaluation, breeding, industrial tree plantation

ABSTRACT

Black locust is one of the most important tree species in Hungary, and forestry research to increase its yield, improve its stem quality and enhance its abiotic (drought) stress tolerance has been ongoing for decades. Among the current research projects focusing on black locust breeding and growing technology, the joint project of the University of Sopron, Forest Research Institute and Napkori Erdőgazdák Zrt. (Napkor Foresters Private Limited Company) is worth mentioning. This project aims to develop a propagation method for newly selected black locust specimens with excellent properties to produce homogeneous, uniform propagating material under operational conditions. In 2020, an experimental black locust industrial tree plantation was successfully established in the Nyírség region. Four newly selected clones (‘PL251’, ‘PL040’, ‘NK1’, ‘NK2’) and the ‘Üllői’ cultivar are being tested there in three different plant spacings. Our conference paper presents the results of the 2.5 m × 2.5 m plant spacing. In addition to the traditional measurements (height and diameter at the base), we also investigated plant physiological parameters (photosynthetic intensity, transpiration, water use efficiency) in a less common *in-situ* way. Studying the water use efficiency (WUE) of tree species is of great importance for sustainable plantation forestry. In the early evaluation of our research results, significant differences were found between the clones and the ‘Üllői’ cultivar in all the parameters studied. Based on these results, the clone ‘NK2’ seems to be the most promising in terms of growth and drought tolerance (WUE). In parallel, DNA-based studies have also been launched. Next-generation sequencing (NGS) was performed to explore the genetic background of specific stem features and wood properties, and simple sequence repeat markers (SSRs) were used for DNA fingerprinting of the selected clones as part of a broad-scale population genetic comparative study. The latter genetic experiment aims to evaluate the genetic diversity of Hungarian black locust in comparison with the genetic pattern of the original American populations, with a focus on the outstanding stem forms of some of the Hungarian cultivars.

INTRODUCTION

Black locust (*Robinia pseudoacacia* L.) is one of the most commonly planted exotic tree species globally. Despite its well-known ecological risks, black locust is an important economic tree species in countries such as Hungary, Romania, Ukraine, Poland, Germany, Italy and Greece (Rédei et al. 2011, Vítková et al. 2017, Nicolescu et al. 2020, Puchałka et al. 2021). It is a fast-growing, drought-tolerant tree species with very hard, durable wood. It is also adaptable to many sites and climates; thus, it plays a key role in current and future forest management by mitigating the negative effects of local and global climate change. Its excellent nectar production also makes it an important tree species for beekeeping (Keresztesi 1988, DeGomez and Wagner 2001, Nicolescu et al. 2018).

Since the introduction of black locust into Hungary in the 18th century, the tree species has been closely associated with agriculture because its wood can be utilized for many agricultural and domestic purposes. In the 1960s, it was necessary to improve the quality of end products from black locust forests to meet consumer expectations and demands. Therefore, a national selection breeding programme was launched to introduce new cultivars into practical forestry use. During this period of black locust breeding, Béla Keresztesi and his co-workers selected several plus trees and tree groups of the so-called 'ship mast' stem form in local stands. As a result, more than 20 state-approved cultivars with names referring to their place of selection appeared (e.g. 'Üllői', 'Nyírségi', 'Kiskunsági', etc.). However, this initial Hungarian black locust cultivar assortment is no longer complete, and the availability and accessibility of different genotypes are rather limited (Keresztesi 1988, Rédei et al. 2011). Renewing the breeding programme became a seriously pressing issue. The FRI recognized the need for new cultivars in the 1990s when Károly Rédei continued Keresztesi's selection work. During this period, attention was focused not only on good stem form but also on fast juvenile growth and drought tolerance. This work resulted in five new candidate cultivars: 'Vacsi', 'Oszlopos', 'Homoki', 'Bácska', and 'Szálas'. Based on the first long-term field test results, two clones – 'Homoki' and 'Bácska' – seem the most promising for plantation forestry purposes (Rédei et al. 2013, Keserű et al. 2021). A yield table for selected black locusts was also developed (Rédei et al. 2021). The research results of black locust improvement received to date conclude that a higher value can be obtained in stem quality even if there is no significant difference in yields between the cultivars and seed-originated black locust stands (Keresztesi 1988, Rédei et al. 2017, 2020).

A joint selection project of the University of Sopron (Forest Research Institute) and Napkori Erdőgazdák Zrt. (Napkor Foresters Private Limited Company) has recently highlighted four brand-new, high-performance candidates. The new clones 'NK1', 'NK2', 'PL251' and 'PL040' have been selected for high-yield industrial timber production. Field trials with various experimental plots have been established in three locations (near Napkor, Nyírbogdány and Debrecen) with the selected genotypes to improve the growing technology of new industrial tree plantations with a special focus on one trial, near Napkor. In Napkor, the common clonal inventories are complemented by plant physiology studies measuring net assimilation, transpiration, and through these parameters, water use efficiency (Ábri et al. 2022). Water use efficiency (WUE) is defined as the amount of carbon assimilated as biomass produced per unit of water used by the plant (tree). It shows the relationship between plant productivity and water use. The resilience of genetic material to abiotic stress (temperature or water) is accompanied by greater WUE (Briggs and Schantz 1918, Hatfield and Dold 2019). This is crucial for forestry nowadays due to the negative effects of climate change.

This paper presents the results of the initial growth and plant physiology studies of the mentioned four newly selected black locust clones. We aim to assess the suitability of the new candidates to establish fast-growing tree plantations to produce high-quality timber in light of local and global climate change. Furthermore, we would like to demonstrate how new techniques and approaches can support conventional tree breeding activity. In addition to the tree physiology studies, genetic profiling of the Hungarian black locust resources has also been launched. Its concepts and methods will also be discussed.

MATERIAL AND METHODS

Field trial

The industrial tree plantation and comparative clone trial (UTM 563210 E; 5307770 N) near Napkor was established in 2020. The 2.66 ha site is in the north-eastern part of the Hungarian Great Plain (Nyírség). Based on meteorological data for the last 35 years (1985–2020), the average annual temperature is 10.4 °C, and the average annual precipitation is 527.4 mm (HMS, 2022). The soil in the site is humus sandy soil (Arenosol) (IUSS Working Group WRB, 2015) with low humus content and acidic effect. In the experiment, the timber production of four new black locust clones (candidate cultivars: 'PL251', 'PL040', 'NK1' and 'NK2') and the 'Üllői' state-approved cultivar as control are being tested in three different planting spaces (2.5 × 2.5 m; 3 × 3 m; 4 × 4 m). This paper presents the results of the clone test in 2.5 × 2.5 m planting space.

The vegetatively propagated seedlings were planted with a planting auger in the spring of 2020. After planting, the necessary nursing (cultivation of row and spacing, pruning) was performed.

Tree physiology measurements

Full inventories of the plantation were completed in the spring (May) and autumn (November) of 2021. The diameter at the base (mm) was measured with a Powerfix digital calliper and height (cm) with a levelling staff. We measured 216–224 trees per clone ‘PL251’, 204-201 per clone ‘NK1’, 178–200 per ‘PL040’, 124–120 per ‘NK2’ and 87–81 individuals of the control ‘Üllői’ during the full inventories.

We measured the assimilation parameters with an LI-6800 (LI-COR, Lincoln, Nebraska, USA) portable photosynthesis system. The instrument recorded the net assimilation, transpiration, stomatal conductance, intercellular CO_2 concentration and other physiological parameters (LI-COR, Inc. 2017). The light was controlled in the sample chamber using a 1500 μmol photon m^{-2} s^{-1} PAR, with 90% red (625 nm) and 10% blue (475 nm) light.

The LI-6800-01A multiphase flash fluorometer head was used as a light source; the aperture was 2 cm^2. The CO_2 concentration was controlled in the chamber: 400 μmol mol^{-1} using injector and carbon dioxide patrons. Light-adapted leaves were measured six times per leaf on three plants per plot. Readings were logged when the measured parameters stabilized but after a minimum of two minutes.

To calculate water use efficiency data measured on the leaves, we applied the formula proposed by Tanner and Sinclair (1983) (Eq. 1).

$$WUE = (Ass*44)/(Emm*18) \tag{1}$$

where, WUE – water use efficiency (kg m^{-3}), Emm – transpirated H_2O (mmol m^2 s^{-1}) and Ass – assimilated CO_2 (μmol m^2 s^{-1}).

Genetic fingerprinting

The genetic evaluation of Hungarian black locust genetic resources has been initiated with the use of highly variable nuclear microsatellite (or Simple Sequence Repeat, SSR) DNA markers providing a unique fingerprint for the selected genetic material. Twenty SSR loci have already been tested from various sources (nuclear and EST-derived) following the original PCR protocols: Rops02, Rops04, Rops05, Rops08, Rops10 (Lian and Hogetsu 2002), Rops16, Rops18 (Lian et al. 2004), Rops109, Rops150, Rops200, Rops206 (Mishima et al. 2009), Rp-01, Rp-04, Rp-06, Rp-09, Rp-11, Rp-15, Rp-26, Rp-29, Rp-43 (Guo et al. 2017). Besides the four new candidates, six other candidate clones (PL035, Vacsi, Szálas, Homoki, Bácska, Oszlopos) and nine old cultivars from the available former black locust assortment were included in the study. Additionally, 16 tree samples from the original USA territory were also included. Data analysis was conducted using GenAlEx 6.5, which checked the uniqueness of each genotype and calculated the genetic distance matrix among samples (Peakall and Smouse 2006, 2012). To interpret the genetic relatedness of the analysed genotypes, a PCoA plot was generated based on the genetic distance matrix by GenAlEx.

RESULTS

Fig. 1 and Fig. 2 summarize the results of full inventories (May 2021, November 2021) and the data on height and diameter at the base. Fig. 1 also shows the linear relationship between the height and the diameter at the base of the studied candidate cultivars and the ‘Üllői’ black locust. Based on this, the results are as follows: PL251 – R^2 = 0.7982; NK1 – R^2 = 0.7777; PL040 – R^2 = 0.7391; NK2 – R^2 = 0.7008; ‘Üllői’ – R^2 = 0.7773. These results reveal a strong correlation between the studied parameters.

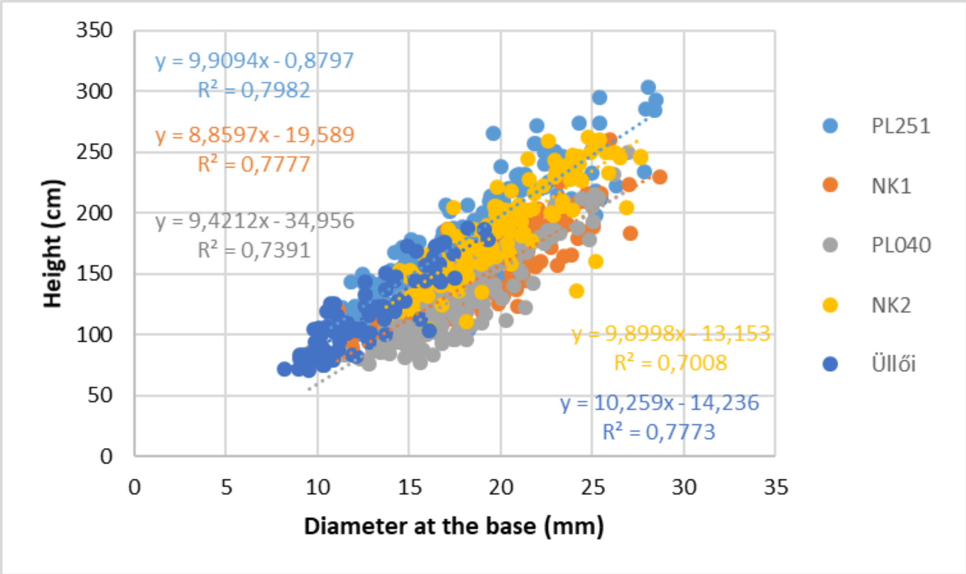

Figure 1: Results of full inventory (2021 May) and the relationship between the diameter at the base and the height

In the evaluation of the full inventory results, significant differences ($p = 0.05$) were observed between the candidate cultivars and the 'Üllői' state-approved cultivar tested for both height and diameter at the base (Fig. 2). The results of full inventories (May and November 2021) are almost similar.

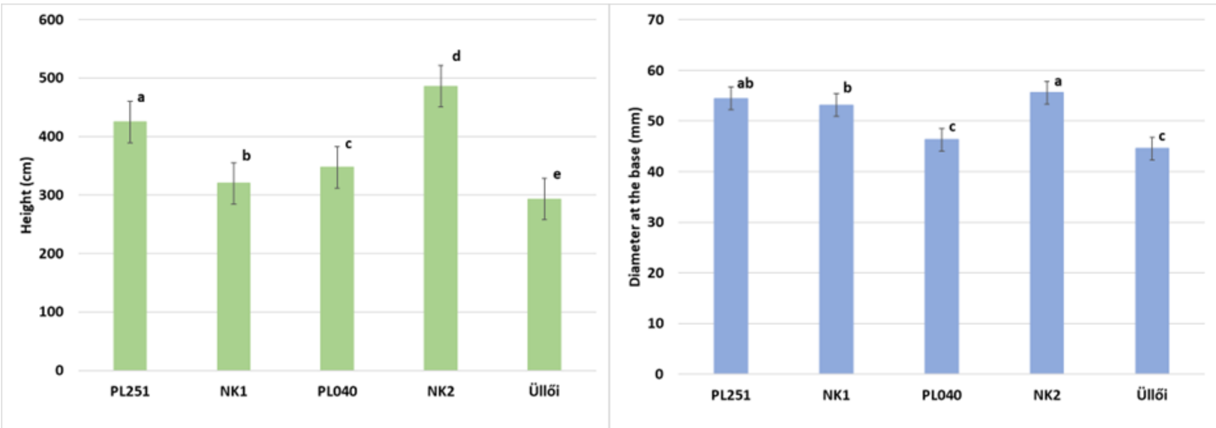

Figure 2: Comparison of candidate cultivars and 'Üllői' black locust by height and diameter at the base in 2.5 × 2.5 m, based on results of full inventory (2021 November)

The candidate cultivar 'NK2' was markedly the best in height. It was also better than the 'PL251' clone with a minimal difference in diameter at the base (there was no significant difference between these two) and performed considerably better than the others. The performance of 'Üllői' was the weakest for both examined parameters.

Using the assimilation rate and the transpiration data, we calculated the water use efficiency (WUE) (kg CO^2 per m^3 H_2O) for every candidate cultivar (Fig. 3). The differences were significant at $p < 0.001$ level. The best water use efficiency was observed in 'PL040' and 'NK2' candidate cultivars (7.015 and 6.876 kg m^{-3}) (there were no significant differences in the case of these two), while the lowest value was in the 'NK1' candidate cultivar (4.319 kg m^{-3}).

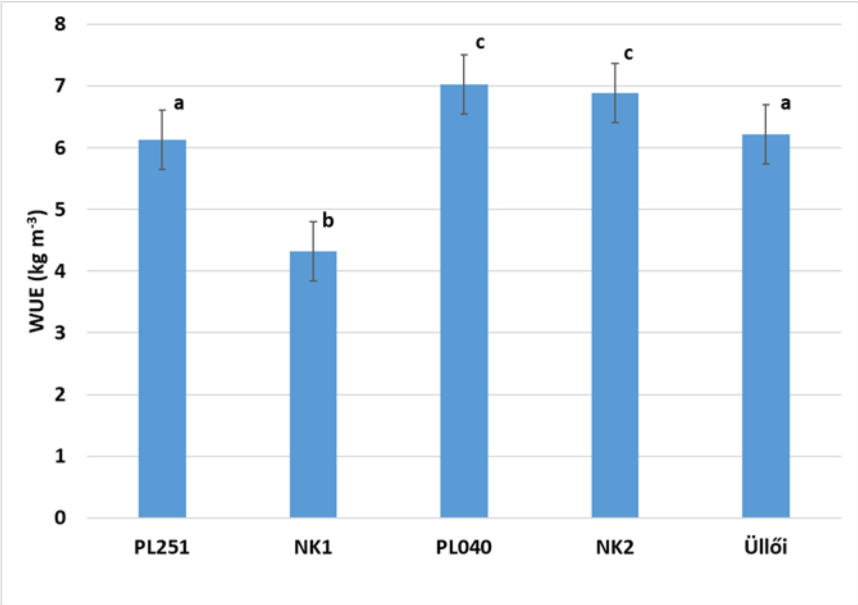

Figure 3: Comparison of the studied candidate cultivars and 'Üllői' black locust by Water Use Efficiency (WUE)

Genetic fingerprinting

All the tested SSR markers provided a clear amplification pattern and proved to be polymorphic with only one exception (Rp-29). The nuclear ROPS series showed very high variability, while the EST-derived Rp marker set resulted in lower allele numbers per locus. The use of only seven nuclear SSR markers (ROPS109, ROPS150, ROPS200, ROPS206, ROPS16, ROPS 05, ROPS08) was sufficient to identify the analysed genotypes. The first tests indicate that the new candidate clones are all genetically distinct with a unique genetic fingerprint. On the other hand, the genetic composition of the old assortment is no longer stable; several mismatches and mix-ups can be detected, together with a loss of genotypes when compared with former gene collection data. Here we present a Principal Coordinate Analysis (PCoA) plot with 19 selected samples from Hungary and 16 additional from the native range in the USA to give a first insight into the genetic relatedness of the selected genotypes.

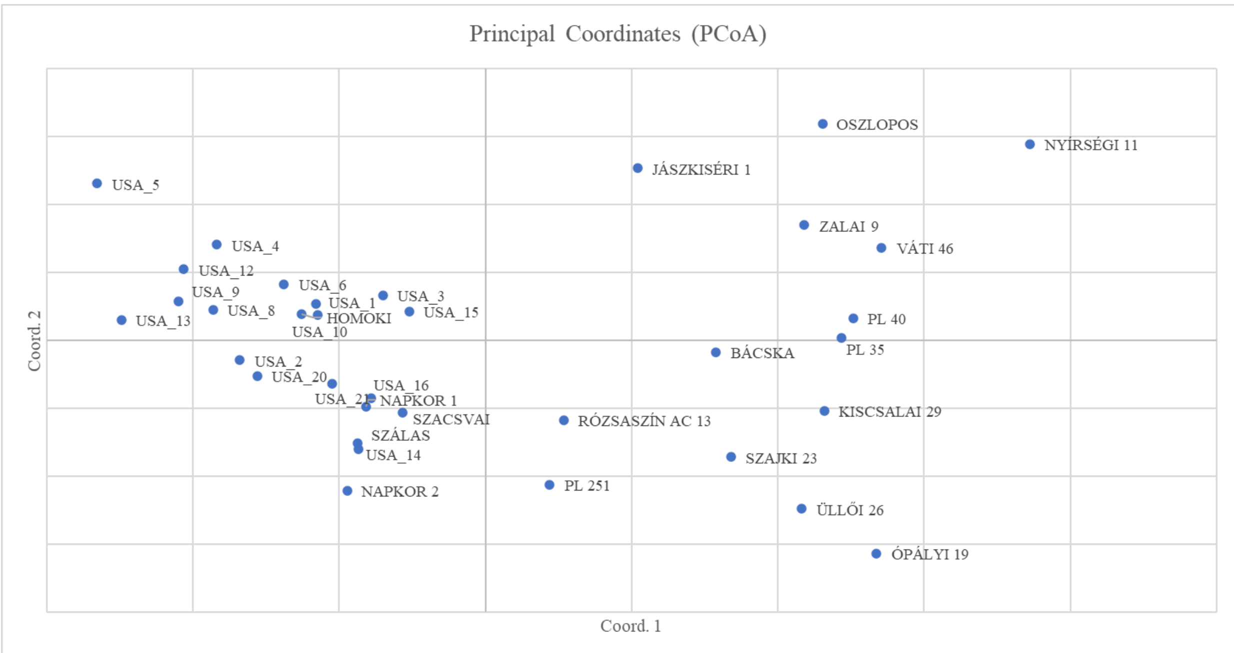

Figure 3: PCoA result based on seven SSR markers highlighting the genetic relatedness of 19 Hungarian selected genotypes and 16 native samples from the USA

DISCUSSION

Due to its flexible site tolerance, black locust is one of the most suitable fast-growing, stand-forming tree species for reducing the unfavourable effects of global and local climate change. Developing and applying appropriate growing technology can significantly reduce the invasive nature of black locust. In this field, Hungarian research, development, and innovation (R&D&I) results are also outstanding at the international level. Propagation of selected black locust varieties is only possible vegetatively to preserve the genetic surplus. Consequently, the large-scale, vegetative propagation of black locust clones (varieties) selected for relative drought tolerance and stem quality improvement must be resolved.

The study of industrial tree plantations is of great practical importance. A timber shortage is forecast in Hungary for 2020–2030. The area of hybrid poplar plantations is gradually decreasing. Since black locust can tolerate less favourable site conditions, the lack of timber in the future can be reduced by planting black locust industrial tree plantations.

For tree plantations to produce good quality industrial wood, it is essential to study the phytophysiological properties of the trees (biomass production, vegetation activity of plants, chlorophyll content, photosynthetic activity, water use efficiency, etc.) in addition to the traditional full inventories and measurements of tree height and diameter. Many studies show that significant differences in photosynthetic activity and transpiration can be found between the varieties of many field crops and trees (Stavros et al. 2013, Bhusal et al. 2018, Csajbók et al. 2020, Pardos and Calama 2022). Consequently, the same applies to water use efficiency, which is a useful parameter to characterize the water consumption and water supply state of a plant. The prolonged drought stress has altered the morphological, physiological, and biochemical traits, but the responses could be species-specific. The responses are also influenced by the characteristics of the cultivar (Zhang et al. 2012, Mantovani et al. 2014). To develop more efficient and precise measures, it is important to look for new insights concerning response mechanisms to drought stress. The deleterious effects caused by drought (e.g., water status and photosynthetic performance impairment, oxidative stress, and imbalance in plant nutrition), are the most critical stressors (Cátia et al. 2019, Bhusal et al. 2021). We also found significant differences in WUE among the black locust clones in our study. The means varied from 4.319 to 7.015 kg m^{-3}, but the standard deviation was low, so the difference was significant at $p < 0.001$ level.

The relatively few field clone experiments on an international scale that have been established and evaluated with scientific demandingness are noteworthy. References to these should be treated with caution, as in most cases, there is a lack of information concerning the origin of the propagating material, its production technology and the forestry techniques used.

Genetic fingerprinting is an appropriate tool for clonal identification. In the case of black locust selection breeding, it is a highly important issue because vegetative regeneration through root suckers is a widespread phenomenon in natural stands. Many previously selected clone mixtures, consisting of 2–5 plus trees selected from the same stand for breeding purposes, have clonal origins and are represented by one genotype. The genetic test of the uniqueness of the new selections is essential for black locust. The technique is also very useful when the genetic consistency of vegetative propagation material needs to be checked.

More sophisticated genomic analyses can be applied to resolve the genetic background of specific, high-quality timber features. For the designation of specific DNA marker regions responsible for a required wood characteristic, a high-resolution technique is needed for genome-wide association studies between wood features and genetic patterns. For this purpose, a new genomic project applying a new-generation sequencing (NGS) technique, namely double-digest restriction-site-associated DNA sequencing (ddRADseq), has been launched. This project has two main aims, the evaluation of the genetic background of specific timber features and the exploration of Hungarian stand origins through the analysis of the present genetic pattern. Therefore, the outstanding Hungarian selections are compared with common genotypes from Hungary and samples originated from native stands in the USA. This approach can promote the future use of marker-assisted selection in black locust breeding.

CONCLUSIONS

Plantation forestry, which includes industrial tree plantations, is primarily aimed at meeting the growing demand for wood material. In addition, plantations contribute to environmental development and the landscape, to the beneficial regulation of the atmospheric carbon cycle, to the filtering of various air pollutants, and, at the same time, to the mitigation of the harmful effects of climate change. Black locust can tolerate less favourable site conditions for tree growing. Producing good quality black locust industrial wood raw material on plantations requires the sort of physiological studies described in our paper.
We can conclude that there were significant differences among the candidate cultivars and the 'Üllői' cultivar in growth and in water use efficiency (WUE). 'NK2' exhibited the best performance in height and diameter at the base. Moreover, this candidate cultivar has shown good WUE results.
The linear relationship between height and butt diameter of the studied candidate cultivars and the 'Üllői' locust has also been proven. Strong relationships were found between these parameters.
The selected black locust varieties have and will play an important role in the following fields of use, mainly to improve the quality of primary wood production. The selected black locust varieties have and will play an important role in the following fields of use, mainly to improve the quality of primary wood production. First, to establish wide-spacing (min. 2.5 × 2.0 m), short-cutting (15–20 years) industrial tree plantations. Secondly, as a specified mixture (30–35%) for the planting of common black locust stands.
Full knowledge of the ecological conditions, the introduction of modern new varieties into public cultivation, the development and introduction of new growing technologies, and the ecological, economic and physiological studies of the entire growing cycle can form the basis for the full exploitation of the potential of plantations. This requires further innovation cooperation between research workshops and practitioners.

ACKNOWLEDGEMENT

This publication was made in frame of the project TKP2021-NKTA-43 which has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-NKTA funding scheme. The research was prepared with the professional support of the Doctoral Student Scholarship Program of the Co-operative Doctoral Program of the Ministry of Innovation and Technology financed from the National Research, Development and Innovation Fund (Scholarship contract ID: RH/527-1/2021).

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