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Juvenile Growth and Morphological Traits of Micropropagated Black Locust (*Robinia Pseudoacacia* L.) Clones under Arid Site Conditions

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Abstract – In Hungary black locust (*Robinia pseudoacacia* L.) is considered as an important exotic stand-forming tree species growing mostly under unfavourable ecological conditions for forest management. Due to climate change effects its importance is increasing in many other countries, too. As a result of a selection programme new black locust clones were tested in clone trials. Juvenile growth and the morphological as well as phenological traits of four micropropagated black locust clones were evaluated in central Hungary under dry site conditions. Significant differences (P<5%) were found for DBH and field survival rate values. At age of 7 the clone R.p. 'Bácska' ('KH 56A 2/5') appears to be especially promising for mass propagation. Tissue culture can be considered as a suitable tool for propagating superior individuals and offers new prospects for the rapid cloning of selected genotypes used for plantation forestry.

Black locust *(Robinia pseudoacacia* L.) / clone trial / juvenile growth / micropropagation

Kivonat – Mikroszaporított fehér akác (*Robinia pseudoacacia* L.) klónok fiatalkori növekedése és morfológiai jellemzői száraz termőhelyeken. Magyarországon a fehér akác (*Robinia pseudoacacia* L.) fontos állományalkotó egzóta fafaj, főként az erdőgazdálkodás számára kedvezőtlen termőhelyeken. A klímaváltozás hatásai miatt a fafaj jelentősége folyamatosan növekszik több más országban is. Egy szelekciós program eredményeként új akác klónokat állítottunk elő klónkísérletek létesítése céljából. Jelen tanulmányban négy mikroszaporítással előállított akác klónt értékeltünk fiatalkori növekedésük, továbbá morfológiai és fenológiai jellemzőik alapján Közép-Magyarországon száraz termőhelyi viszonyok között. Szignifikáns különbséget (P<5%) találtunk a mellmagassági átmérő és a megmaradási értékek tekintetében. 7 éves korban az R.p. 'Bácska' ('KH 56A 2/5') klón különösen ígéretesnek tűnik a tömegszaporításra. A szövettenyésztéses szaporítási eljárás megfelelő eszköznek tekinthető az ültetvényes fatermesztés területén kiváló minőségű egyedek klónos elszaporítására, új távlatokat nyújtva ezzel a kiválasztott genotípusok gyors klónozására.

Fehér akác *(Robinia pseudoacacia* L.) / klónkísérlet / fiatalkori növekedés / mikroszaporítás

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1 INTRODUCTION

Black locust (*Robinia pseudoacacia* L.) was introduced to Europe from its natural range in south-eastern United States more than 300 years ago. It has been well adapted for growth in a wide variety of ecological conditions and planted throughout the world from temperate to subtropical areas. It is fast growing, excellent coppicing, drought tolerant, has high survival rates and yield as well as very hard durable wood. Due to its symbiosis with the nitrogen fixing bacteria, *Rhizobium* sp. black locust is capable of colonising very low nutrient substrates. Black locust is also a promising tree species for short rotation forestry (SRF) including energy plantations. The development of an integrated landscape includes forests, agricultural fields and shelterbelts. In these cases afforestation with black locust is focused on improving the natural environment and the living conditions of the population as well (Führer – Rédei 2003, Rédei et al. 2011).

Several countries have started research programmes on improving black locust wood quality and/or increasing production of biomass for energy purpose. Black locust has also been considered as a promising tree species for animal feeding and for recultivation of drying out devastated lands as well as nectar production. At present, black locust breeding and improvement is undertaken in the United States (Bongarten et al. 1991, 1992) Greece (Dini-Papanastasi – Panetsos 2000), Germany (Liesebach et al. 2004, Böhm et al. 2011), Slovakia (Chalupa 1992), Poland (Kraszkiewicz 2013), Turkey (Dengiz et al. 2010), India (Sharma 2000, Swamy et al. 2002), China (Dunlun et al. 1995), South Korea (Lee et al. 2007). Increasingly, countries are interested in black locust improvement and management paying special attention to its response to climate change effects.

The primary requirement for reproducing black locust clones (varieties) to establish clone trials, seed orchards and seed production stands was to reliable vegetative methods. Propagation from root cuttings and tissue culture propagation are suitable for reproduction of superior traits of the selected trees. Brown (1980) was the first to report a successful in vitro method for mass production of black locust. Enescu and Jucan (1985) started experiments in Romania with similar results. Balla and Vértesy in 1985 had the first success in the sterile production of four Hungarian state-approved black locust cultivars. Balla et al. (1998) published the improvement of the acclimatization results of micropropagated black locust using symbiotic microorganisms.

Because of the fact that black locust is easy to clone and also exhibits wide adaptation to ecological (site) conditions, there is also an opportunity to develop basic information on genotype by environment interaction for traits of interest (Hanover 1992). In Hungary, black locust has played a role of great importance in the forest management for more than 280 years, covering approximately 23% of the forested area (445.000 ha) and providing about 20% of the annual timber output of the country. Being aware of the importance of black locust, forest research in Hungary has been engaged in resolving various problems of black locust management for a long time, and numerous research results have already been implemented in the practice (Keresztesi 1988, Rédei et al. 2007). In the country in the lowlands characterized with forest steppe climatic type, the annual precipitation is not more than 500 mm, most of which is outside the growing season. Thus, drought is a frequent phenomenon in the summer period coupled with very high atmospheric temperatures. Due to these facts about 40% of the black locust stands in Hungary grow under marginal site conditions (Rédei 2003, Rédei et al. 2008). Considering the above-mentioned circumstances a *new black locust selection work* started 12 years ago to find and improve black locust clones and cultivars which perform good stem form, provide good-quality wood material for industrial purposes, and which are able to tolerate the dry ecological conditions as well. As a result of the selection programme some new black locust clones have been improved. In this paper one of the trials established *with micropropagated black locust clones* is evaluated with special regard to their juvenile growth rate and morphological traits. By applying micropropagation, superior traits of the selected trees can be preserved in the clones and it can also be considered as an effective tool for producing improved initial propagation material.

2 MATERIALS AND METHODS

2.1 Study site

Data used in this study came from a black locust clone trial established in the forest subcompartment Kecskemét 16CS/1 (N46^{0}54'44", E19^{0}41'51") in Central-Hungary between the Danube and Tisza rivers (*Figure 1*). The forest subcompartment has slightly humous sandy soil without ground-water influence. The annual precipitation amounts to only 500 mm in some years, of which less than 300 mm comes in the dry summer period; water supply is a limiting factor. The trial at Kecskemét is not among the best sites available in Hungary but can be considered as an average yield class site for black locust (Rédei – Gál 1985).

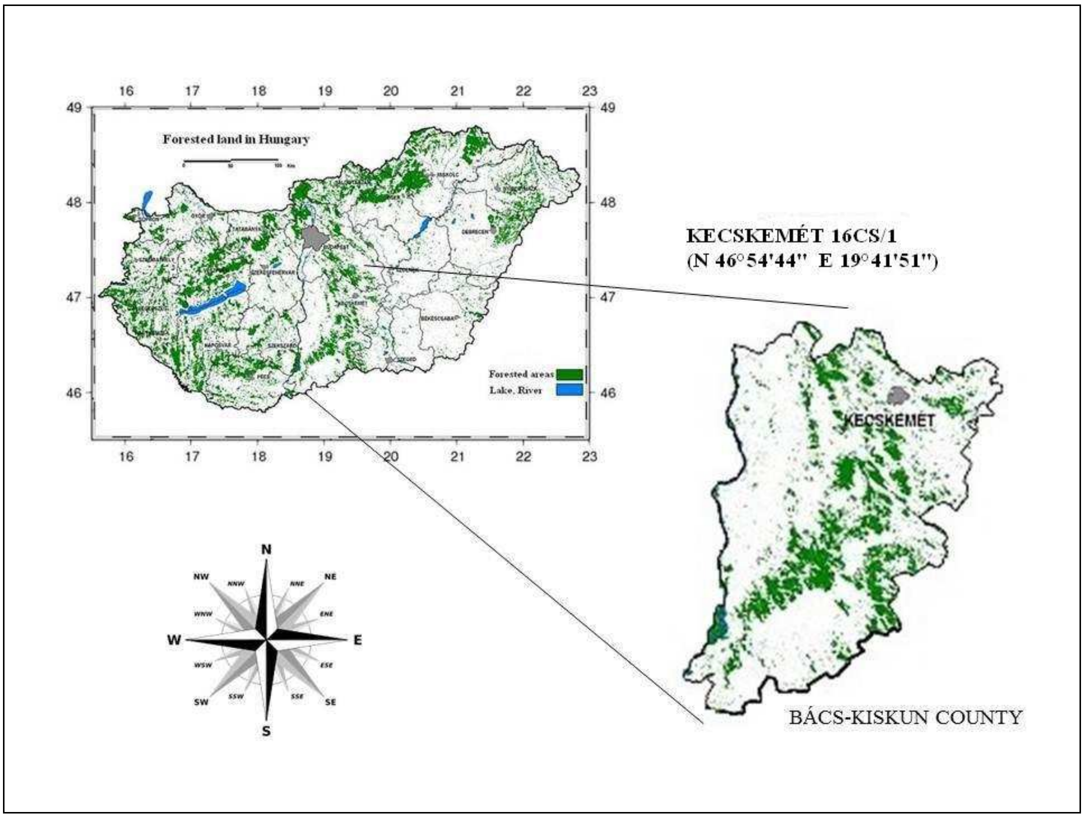

Figure 1. Location of the study site

The main ecological conditions of the study area are as follows: relative air humidity below 50%, hydrology: free draining, genetic soil type: humus sand soil, annual precipitation is less than 550 mm (between 1st April and 26th September in 2011 the precipitation was only 288 mm).

2.2 Materials

The trial, with three replications was established at a spacing of 2.0 m × 1.0 m. Common black locust and four black locust clones, i.e. R.p. 'Bácska' ('KH 56A 2/5'), 'KH 56A 2/6', 'MB 17D 4/1'and 'CST 61A 3/1' were selected. Each treatment corresponds to a plot of 15 by 20 m. For the clones one-year-old micropropagated plants were used and one-year-old seedlings for common black locust. Plant tissue culture method provided us with an effective means to accelerate vegetative propagation of the newly selected clones and to establish new clone trials (Rédei et al. 2002).

2.3 Methods

The following parameters were measured and calculated at age of 7: number of stems, tree height (also at 1, 3 and 5 years), dbh (diameter at breast height) over bark, stem volume and mean tree volume. We used arithmetic mean in case of tree height and dbh because it is more appropriate for certain types of experimental studies, for example, clone trials where it is primarily important to measure the responses of the trees to the experimental treatments during the first years after plantation establishment. The stem volume was calculated using the volume function based on the volume table for black locust (Kolozs – Sopp 2000):

$$v = 10^{-8} d^2 h^1 \left(h/[h-1.3]\right)^2 \left(-0.6326dh + 20.23d + 3034\right),$$

where

v is stem volume (m^3),

d is diameter at breast height (cm),

h is tree height (m).

The mean tree volume (v_m, m^3/tree) was calculated using the means of stem volume (h, dbh) for each of the experimental plots.

The following classifications were used for the evaluation of the morphological and phenological traits:

- Stem form = 1: straight, 2: more or less straight, 3: declining, 4: strongly declining.
- Bud break intensity = 1: very poorly budding, 2: poorly budding, 3: mediocre, 4: vigorously budding.
- Foliage density = 1: loose foliage, 2: mediocre foliage, 3: dense foliage.
- Forking = 1: not forking, 2: forking in the crown, 3: the crown starts with forking, 4: the stem is forking.
- Branching = 1: thick branches, 2: medium thick branches, 3: thick branches in the crown only.
- Blooming intensity = 1: complete, 2: abundant, 3: mediocre, 4: scarce, 5: sporadic, 6: no flowers.

The collected data were analyzed by STATISTICA 8.0 (data analysis software system – StatSoft, Inc., 2008) programme. Analysis of variance (one-way ANOVA) was done for height, dbh and mean tree volume to consider the trial with having completely randomized design.

3 RESULTS

Table 1 illustrates the most important stand structure parameters and the survival rate. According to the significance test at P = 5% level significant differences were found in DBH and in the survival rate.

Comparison of mean height illustrated that clones 'KH 56 A2/5' and 'KH 56 A 2/6' achieved the higher value (9.4 and 9.5 m) and the height growth patterns of the clones and the control at different ages were similar (*Figure 2*).

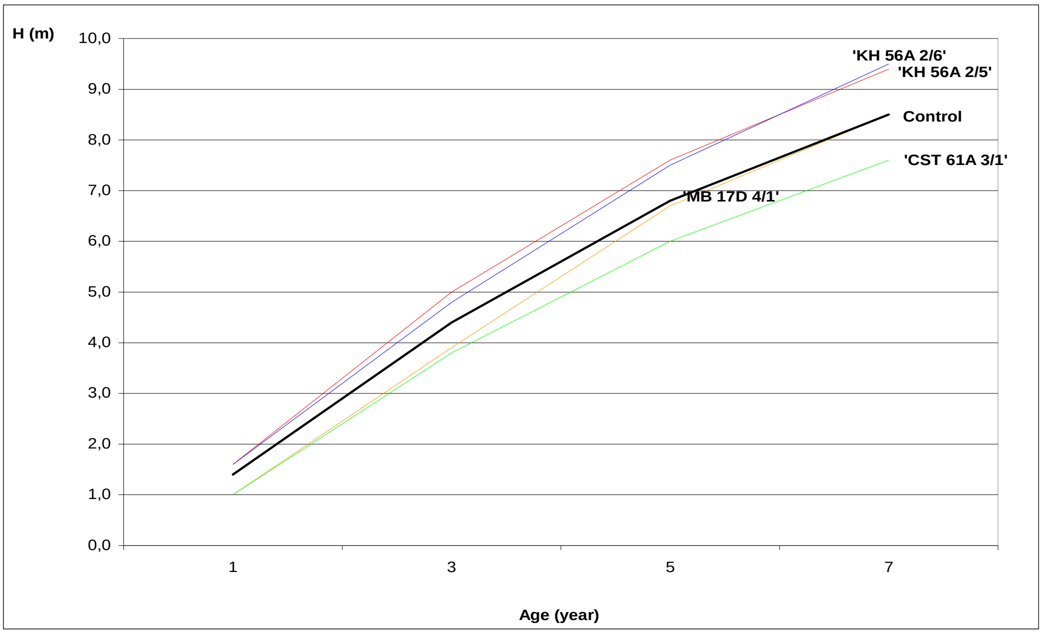

Figure 2. Height growth patterns of micropropagated black locust (Robinia pseudoacacia L.) clones at the age of 1, 3, 5 and 7 years

Comparison of mean DBH indicated that the clone 'KH 56 A 2/5' had maximum growth. The same result was obtained in the case of mean tree volume for 'KH 56A 2/5' and 'KH 56 A 2/6'.

Table 1. Stand characteristics of micropropagated black locust clones at age of 7 years

Clone name	Plot number	Height (m)	DBH (cm)	Survival %	Height (m)	DBH (cm)	Mean tree volume (m^3)	Survival %
					plot average values			
Késleshalom 56A 2/5 ('KH 56A 2/5')	1	8.5	6.9	81	9.4	7.1**	0.0497	83**
	9	9.0	6.3	94				
	18	10.7	8.2	75				
Késleshalom 56A 2/6 ('KH 56A 2/6')	2	9.0	5.8	62	9.5	6.1	0.0496	69*
	12	11.6	7.3	75				
	15	7.9	5.2	69				
Mikebuda 17D 4/1 ('MB 17D 4/1')	3	8.3	5.4	69	8.5	5.4	0.0373	73
	13	9.3	5.5	75				
	16	7.8	5.2	75				
Császártöltés 61A 3/1 ('CST 61A3/1')	5	6.7	4.3	94	7.6	5.1*	0.0310	87**
	8	8.4	5.6	87				
	14	7.8	5.5	81				
Common black locust	6	8.0	5.6	56	8.5	6.1	0.0389	69*
	10	7.1	4.8	75				
	17	10.5	7.9	75				

*, **: P<5%

Table 2. Analysis of variance for height, diameter at breast height, mean tree volume and survival rate of the four tested black locust clones

Source of variation	Degrees of freedom (df)	Sum squares (SS)	Mean squares (MS)	F	Significance
		Height (m)			
Total	14	26.47			
Replication	2	2.12			
Treatment	4	8.09	2.0225		
Error	8	16.26	2.0325	0.99508	
		Diameter at breast height (cm)			
Total	14	17.85			***
Replication	2	1.63			
Treatment	4	7.35	1.8375		
Error	8	8.87	1.10875	1.657272	
		Mean tree volume (m^3)			
Total	14	0.0022			
Replication	2	0.0002			
Treatment	4	0.0006	0.00015		
Error	8	0.0014	0.000175	0.857143	
		Survival rate (%)			
Total	14	1518.4			***
Replication	2	204.4			
Treatment	4	895.73	223.9325		
Error	8	418.27	52.28375	4.283023	

***: P<5%

Table 3 contains some morphological and phenological characteristics of the selected black locust clones at age of 7. As the general evaluation of the morphological characteristics is concerned, the succession from best to worst is: ‘KH 56A 2/5’, ‘KH 56A 2/6’, ‘MB 17D 4/1’ and ’CST 61A 3/1’. The phenological evaluation for blooming intensity indicated the highest quality in ’KH 56A 2/5’. The micropropagated trees began to flower in the fifth growing season after field planting and produced seeds with germination comparable to that of common black locust trees.

Table 3. Morphological and phenological data of 7-year-old clones as a mean of plots

Morphological and phenological traits	Stem form	Bud break intensity	Foliage density	Forking	Branching	Blooming intensity
Clone name	(1–4)	(1–4)	(1–3)	(1–4)	(1–3)	(1–6)
'KH 56A 2/5'	1.5	3.1	2.0	1.2	2.1	2.6
'KH 56A 2/6'	1.7	2.8	1.9	1.3	1.9	3.1
'MB 17D 4/1'	2.1	2.8	2.3	1.2	2.1	3.5
'CST 61A 3/1'	1.6	2.8	2.5	1.2	1.8	4.5
Common black locust	2.0	2.9	2.2	1.3	2.0	3.5

4 DISCUSSION AND CONCLUSIONS

For some decades black locust has received increased attention in more and more countries for the following reasons:

1) the energy crisis has stimulated research on relatively rapid growing, nitrogen-fixing trees such as black locust;
2) the species has a great many characteristics from both the practical and biological research standpoints; and
3) application of genetic improvement and biotechnology techniques may remove several hindrances to the widespread use of black locust in some, potentially promising countries from black locust growing point of view.

Black locust's fast growth and site condition tolerance are important characteristics for short rotation cycle silviculture (SRF), as well. Because of its many desirable attributes, the species is admirably suited to utilization in many areas of the world. Plant tissue culture methods provide us with relatively new means to speed up vegetative propagation of recently selected clones and give us opportunity to establish healthy stock plantations. According to our experiences and investigations (Rédei et al. 2007) black locust trees show considerable variability in stem and branching form, wood quality and stress tolerance. Well designed clone trials (clonal tests) are needed to improve varieties best adapted to certain environments.

This study leads to the following conclusions: (1) the trial demonstrated that micropropagated trees can be successfully transplanted into soil, hardened and grown in the field. Micropropagated trees exhibited normal growth and appearance; (2) the results at the end of the 7^{th} growing season demonstrated that the DBH and seedling survival differed significantly among the tested clones; (3) the investigations showed that clone R.p. 'Bácska'('KH 56 A 2/5') achieved the highest growth rate in mean tree volume with having the best morphological and phenological characteristics; (4) micropropagation has proved as a suitable mean in the field of black locust clonal selection.

To consider the effects of the global climate change and the regional growing experiences, in the future would be two regions, where the fast spread of black locust could be expected. In Europe some Mediterranean countries (Turkey, Italy), while in Asia China and Korea may be the most prominent black locust growers.

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