

Improvement of black locust (*Robinia pseudoacacia* L.) growing under marginal site conditions in Hungary: case studies

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SUMMARY

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 of 12 micropropagated black locust clones in two plots series established at different dates were evaluated in central Hungary under marginal site conditions. At age of 7 the clone R. p. 'Bácska' ('KH 56A2/5'), at age of 10 the clones R.p. 'Homoki' ('MB17D3/4') and 'PV201E2/4' appeared to be especially promising for mass production. Based on the data obtained from the performed trials, it can also be concluded that 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.

Keywords: black locust (*Robinia pseudoacacia* L.), clone trials, juvenile growth, micropropagation

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 living conditions of the population as well (Führer and 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 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 and 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 cutting 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 (465 000 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

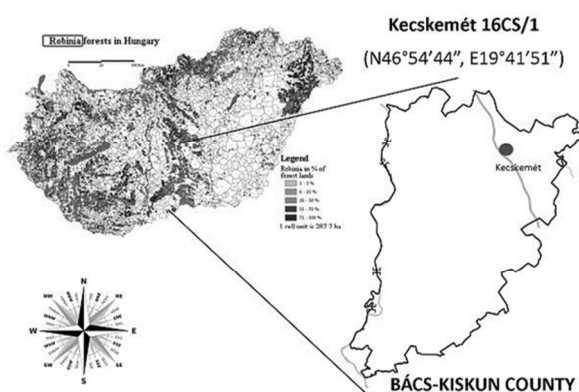
frequent phenomenon in the summer period coupled with very high atmospheric temperatures. Due to these facts about 40% of the black locust stands in Hungary grow under marginal site conditions (Rédei 2003, Rédei et al. 2008). Considering the above-mentioned circumstances a *new black locust selection work* started 12 years ago to find and improve black locust clones and cultivars which perform good stem from, 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 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.

MATERIAL AND METHODS

Study site

Data used in this study came from two black locust clone trails established at different dates in the forest subcompartment Kecskemét 16CS/1 (N46°54'44", E19°41'51") in Central-Hungary between the Danube and Tisza rivers (Figure 1). The forest subcompartment has slightly humous sandy soil without groundwater 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 trials 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 and 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).

Materials

The trial, with three replications was established at a spacing of 2.0 m×1.0 m. Common black locust and black locust clones, i.e. R.p. 'Bácska' ('KH 56A 2/5'), 'KH56A 2/6', 'MB17D 4/1', 'CST61A3/1' as well as 'PV201E2/4', 'PV201E2/1', 'MB15A2/3', 'PV201E2/3', 'PV35B/2', 'MB17D3/10', 'PV233A/1' and R.p. 'Homoki' ('MB17D3/4') 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).

Methods

The following parameters were measured and calculated: number of stems, tree height, dbh (diamater at breast height) over bark, stem volume and mean tree volume. We used arithmetic mean in case of tree height 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 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 and Sopp 2000):

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

where: v is stem volume (m³), d is diameter at breast height (cm), h is tree height (m).

The mean tree volume (v, m³/tree) was calculated using the means of stem volume (h, dbh) for each of the experimental plots.

The stem form classes used by us are as follows at the age of final harvesting:

1. Straight, cylindrical, healthy stems, reaching to the top of the crown. Crooks are tolerated in one dimension only, not more than twice the stem diameter (x₁).
2. The stem is straight, forks are tolerated, but only if they are in the uppermost third of the tree. Crooks are tolerated in one dimension only, not more than three times the stem diameter (x₂).
3. The stem is crooked and leaning. Crooks may reach five times the stem diameter in one dimension and minor crookedness in a second dimension is tolerated (x₃).
4. Very crooked in more than one dimension, low branching, forked trees with stem defects, broken crown or stem rot (x₄).

The average stem form value (SFV) was determined on base of the following formula:

$$SFV = \frac{x_1n_1+x_2n_2+x_3n_3+x_4n_4}{n_1+n_2+n_3+n_4}$$

where: x₁, x₂, x₃, x₄ = stem form classes, n₁, n₂, n₃, n₄ = tree numbers belonging to the single tree quality classes.

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.

RESULTS

Table 1 illustrates the most important stand structure parameters and the stem form values. Comparison of mean height illustrated that clones

'KH56A2/5' and 'KH56A2/6' 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.

Comparison of mean DBH indicated that the clone 'KH56A2/5' had maximum growth. The same result was obtained in the case of mean tree volume for 'KH56A2/5' and 'KH56A2/6'.

The parameters of stand structure and stem quality, concerning the trial with eight black locust clones and common black locust at age of 10 years are presented Table 2.

Table 1

Stand characteristics of micropropagated black locust clones at age of 7 years

Clone name	Plot number	Height (m)	DBH (cm)	Height (m)	DBH (cm)	Mean tree volume (m ³)	Stem number form (1–4)
Kéleshalom 56A2/5 ('KH56A2/5')	1, 9, 18	8.5, 9.0, 10.7	6.9, 6.3, 8.2	9.4	7.1	0.0497	1.5
Kéleshalom 56A2/6 ('KH56A2/6')	2, 12, 15	9.0, 11.6, 7.9	5.8, 7.3, 5.2	9.5	6.1	0.0496	1.7
Mikebuda 17D4/1 ('MB17D4/1')	3, 13, 16	8.3, 9.3, 7.8	5.4, 5.5, 5.2	8.5	5.4	0.0373	2.1
Császártöltés 61A3/1 ('CST61A3/1')	5, 8, 14	6.7, 8.4, 7.8	4.3, 5.6, 5.5	7.6	5.1	0.0389	2.0
Common black locust	6, 10, 17	8.0, 7.1, 10.5	5.6, 4.8, 7.9	8.5	6.1	0.0389	2.3

Note: *P<5%

Table 2

Stand characteristics of micropropagated black locust clones at age of 10 years

Clones	Mean height H (m)	%	Mean DBH D _{1.3} (cm)	%	Mean tree volume v (m ³)	%	Stem form (1–4)
PV201E2/4	9.9	99.0	10.1	108.6	0.0641	131.9	1.32
PV201E2/1	6.7	67.0	7.8	83.9	0.0324	66.7	1.58
MB15A2/3	8.6	86.0	8.7	93.5	0.0472	97.1	1.83
PV201E2/3	7.6	76.0	8.7	93.5	0.0406	83.5	1.47
PV35B/2	7.0	70.0	8.2	88.2	0.0357	73.5	1.50
MB17D3/10	9.5	95.0	10.4	111.8	0.0618	127.2	1.78
MB17D3/4	10.7	107.0	10.8	116.1	0.0691	142.2	1.31
PV233A/1	9.0	90.0	8.9	95.4	0.0469	96.5	1.64
Control	10.0	100.0	9.3	100.0	0.0486	100.0	1.75
p<0.05	2.4		1.5		0.0223		0.38

Analysis of variance for mean height (h) and mean diameter at breast height (dbh) as well as the mean tree volume (v) at the end of the tenth growing season statistically difference (p<0.05) between the clones, which might be mostly due to genetic factors. Comparison of mean height illustrated that the clone 'MB17D3/4' achieved the higher value (10.7 m). Comparison of mean dbh indicated that clones 'MB17D3/4', 'MB17D3/10' and 'PV201E2/4' had maximum growth. The same results was obtained in the cases of mean volume for 'MB17D3/4',

'PV201E2/4' and 'MB17D3/10'. For the stem form value, clones 'MB17D3/4' and 'PV201E2/4' obtained the highest values. As shown in Table 2, at the end of the tenth growing season micropropagated plants attained height ranging from 6.7 to 10.7 m. Reasonable field survival rates were achieved. Clonal material regenerated from the same tree showed uniformity in the stem form. Higher variability occurred in height growth of individual trees planted in the field. It seems that non-uniformity in rooting to a considerable extent.

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;
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 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 trials demonstrated that micropropagated trees can be successfully transplanted to soil, hardened and grown in the field. Micropropagated trees exhibited normal growth and appearance;
- (2) the results at the end of the 7th and 10th growing season demonstrated that the h, dbh mean tree volume and stem form value differed significantly among the tested clones at different ages;
- (3) the investigations showed that clones R.P. 'Bácska' ('KH5602/5') as well as 'R. p. 'Homoki' ('MB17D3/4') and 'PV201E2/4' achieved the highest growth rate in mean tree volume with having the best morphological characteristics;
- (4) micropropagation has proved as a suitable mean in the field of black locust clonal selection.

The 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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