

International Journal of Horticultural Science 2015, 21 (1–2): 53–56.
Agroinform Publishing House, Budapest, Printed in Hungary
ISSN 1585-0404

Improved clonal approaches to growing black locust (*Robinia pseudoacacia* L.) in Hungary: a case study

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Summary: In Hungary black locust (*Robinia pseudoacacia* L.) is considered as an important exotic stand-forming tree species and due to climate change effects its importance is increasing in many other countries. It has some desirable characteristics from both the practical and research standpoints. As a result of a partly new black locust selection programme new black locust clones were improved and a technology was developed for mass clonal micropropagation of juvenile trees. Clone trials with micropropagated plants were established in the country for evaluating the juvenile growth and the stem form of promising black locust clones under marginal site conditions. Significant differences ($P < 5\%$) were found for stem form value which partly verified the genetic gain of the selected clones against the common black locust. It was also proved that tissue culture could offer partly new prospects for the rapid mass cloning of selected genotypes.

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

Introduction

Black locust (*Robinia pseudoacacia* L.) was introduced to Hungary about 300 years ago and at present it covers approximately 23 per cent of the forested lands (465 000 ha). 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 (Rédei et al. 2002). 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 (Rédei et al. 2002, 2008).

Besides Hungary 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), China (Dunlun et al. 1995) and South Korea (Lee et al. 2007).

Brown (1980) was the first to report a successful in vitro method for mass production of black locust. Enescu & Jucan (1985) started experiments in Romania with similar results. Balla & Vértesy in 1985 had the first success in the sterile production of some Hungarian state-approved black locust

cultivars. Balla et al. (1998) published the improvement of the acclimatization results of micropropagated black locust using symbiotic microorganisms which have proved to be successful in the field of mass clonal micropropagation (Rédei et al. 2002). Finally, a summary on black locust micropropagation techniques was published by Jain et al. (2007).

In this paper a field trial established with micropropagated black locust clones is evaluated with special regard to their propagation technique and juvenile growth.

Materials and methods

Study site

Data used in this study came from a black locust clone trial established in the forest subcompartment located at Isaszeg 8D, central Hungary (N47°33'5.88", E19°23'58.71"). It has brown forest soil without ground-water influence. The annual precipitation amounts to only 500 mm in some years, of which less than 350 mm comes in the dry summer period.

Materials

The trial, with three replications was established at a spacing of 2.0 m × 1.0 m. Three black locust clones, i.e. 'CST

61A 3/1', 'KH 56A 2/6' and 'MB 12D' as well as common black locust as a control were planted. Each treatment corresponds to a plot of 15 by 20 m. A random block was designed where each block included all the different treatments (clones) once. It means that the total number of plants per clone was 450. For the clones one-year-old micropropagated plants were used and one-year-old seedlings for common black locust.

Methods

The following parameters were measured and calculated: number of stems, tree height, dbh (diameter at breast height) over bark, stem volume and mean tree volume. The stem volume was calculated using the following volume function based on the volume table for black locust (Kolozs & Sopp 2000):

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

where v is stem volume (m^3), d is diameter at breast height (cm), h is tree height (m). The mean tree volume (v , $m^3/tree$) was calculated using the means of stem volume (h , dbh) for each of the experimental plots (Rédei et al. 2013).

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 (x_1). 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 (x_2). 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 (x_3). 4) Very crooked in more than one dimension, low branching, forked trees with stem defects, broken crown or stem rot (x_4) (Rédei et al. 2013).

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

$$SFV = \frac{x_1 n_1 + x_2 n_2 + x_3 n_3 + x_4 n_4}{n_1 + n_2 + n_3 + n_4}$$

where x_1, x_2, x_3, x_4 = stem form classes,

n_1, n_2, n_3, n_4 = tree numbers belonging to the single tree quality classes.

The data were analyzed by IBM SPSS 22.0 statistical software package. Analysis of variance (one-way ANOVA) was done for height, dbh, mean tree volume and stem form to consider the trial with having completely randomized design. Post hoc tests were made by Tukey's HSD test.

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

Clone name	Plot number	Height (m)	DBH (cm)	SFV (1-4)	Height (m)	DBH (cm)	Mean tree volume (m3)	Percentage of survival plants (%)	SFV (1-4)
					plot average values				
CST 61 A 3/1	1	7.8	5.9	1.9	8.1	6.2	0.0138	77	1.8
	6	8.4	6.4	1.8					
	10	8.2	6.3	1.6					
KH 56A 2/6	2	8.3	5.8	1.8	8.4	6.1	0.0137	76	1.9
	9	8.5	6.3	1.9					
	7	8.4	6.1	1.9					
MB12 D	3	8.2	5.6	2.1	7.6	5.7	0.0112	78	2.1
	5	7.4	5.8	2.3					
	12	7.3	5.7	2.0					
Control	4	7.3	5.1	2.7	7.8	5.6	0.0109	78	2.5
	8	8.4	6.8	2.5					
	11	7.8	5.0	2.2					

Results

Table 1 illustrates the most important stand structure parameters and the stem form values at age of 7 years. Comparison of mean height illustrated that clone 'KH 56 A 2/6' achieved the higher value (8.4 m). Comparison of mean DBH indicated that the clone 'CST 61A 3/1' had the maximum growth. The same result was obtained in the case of mean tree volume for 'CST 61A 3/1', too. 'CST 61A 3/1' and 'KH 56 A 2/6' clones had the best stem form values.

As shown in Table 2 analysis of variance for mean height (h), mean diameter at breast height (dbh) and the mean tree volume (v) at the end of the 7th growing seasons did not, but the stem values (SFV) – the main purpose of selection trials – showed statistically significant differences ($p < 0.05$) between two clones ('CST 61A 3/1', 'KH 56 A 2/6') and the control.

Discussions

In the past years black locust has received increased attention in more countries for the following reasons: (1) the global climate change and energy crisis have stimulated research on rapid growing but relatively drought tolerant tree species; (2) some countries such as Hungary, Romania, France, Germany, Korea and China are vigorously developing the species; and (3) the species has a lot of desirable characteristics from plantation management point of view.

The purpose of this paper, as the title implies, is to give initial information on a partly new black locust selection programme in Hungary, so there have not been too many comparable field research results for a wide-ranging discussion yet. As it is mentioned above, in Hungary the main goal of black locust improvement is to grow high-yielding plantations under semi-arid and arid site conditions. Some improved clones may be fast-growing or drought-resistant, but rarely both. It must be also considered that any improvement

Table 2: Results of Tukey's HSD test of the micropropagated black locust clones' stand characteristics

Dependent Variable	(I) Clone	(J) Clone	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Height	CST 61 A 3/1	KH 56A 2/6	-0,2667	0,3291	0,8481	-1,3207	0,7874
		MB12 D	0,5000	0,3291	0,4702	-0,5540	1,5540
		Control	0,3000	0,3291	0,7997	-0,7540	1,3540
	KH 56A 2/6	CST 61 A 3/1	0,2667	0,3291	0,8481	-0,7874	1,3207
		MB12 D	0,7667	0,3291	0,1701	-0,2874	1,8207
		Control	0,5667	0,3291	0,3733	-0,4874	1,6207
	MB12 D	CST 61 A 3/1	-0,5000	0,3291	0,4702	-1,5540	0,5540
		KH 56A 2/6	-0,7667	0,3291	0,1701	-1,8207	0,2874
		Control	-0,2000	0,3291	0,9268	-1,2540	0,8540
	Control	CST 61 A 3/1	-0,3000	0,3291	0,7997	-1,3540	0,7540
		KH 56A 2/6	-0,5667	0,3291	0,3733	-1,6207	0,4874
		MB12 D	0,2000	0,3291	0,9268	-0,8540	1,2540
DBH	CST 61 A 3/1	KH 56A 2/6	0,1333	0,4410	0,9897	-1,2788	1,5454
		MB12 D	0,5000	0,4410	0,6806	-0,9121	1,9121
		Control	0,5667	0,4410	0,5960	-0,8454	1,9788
	KH 56A 2/6	CST 61 A 3/1	-0,1333	0,4410	0,9897	-1,5454	1,2788
		MB12 D	0,3667	0,4410	0,8383	-1,0454	1,7788
		Control	0,4333	0,4410	0,7630	-0,9788	1,8454
	MB12 D	CST 61 A 3/1	-0,5000	0,4410	0,6806	-1,9121	0,9121
		KH 56A 2/6	-0,3667	0,4410	0,8383	-1,7788	1,0454
		Control	0,0667	0,4410	0,9987	-1,3454	1,4788
	Control	CST 61 A 3/1	-0,5667	0,4410	0,5960	-1,9788	0,8454
		KH 56A 2/6	-0,4333	0,4410	0,7630	-1,8454	0,9788
		MB12 D	-0,0667	0,4410	0,9987	-1,4788	1,3454
Mean tree volume	CST 61 A 3/1	KH 56A 2/6	0,0003	0,0021	0,9985	-0,0064	0,0071
		MB12 D	0,0027	0,0021	0,5963	-0,0040	0,0094
		Control	0,0024	0,0021	0,6761	-0,0043	0,0091
	KH 56A 2/6	CST 61 A 3/1	-0,0003	0,0021	0,9985	-0,0071	0,0064
		MB12 D	0,0024	0,0021	0,6850	-0,0044	0,0091
		Control	0,0021	0,0021	0,7627	-0,0047	0,0088
	MB12 D	CST 61 A 3/1	-0,0027	0,0021	0,5963	-0,0094	0,0040
		KH 56A 2/6	-0,0024	0,0021	0,6850	-0,0091	0,0044
		Control	-0,0003	0,0021	0,9989	-0,0070	0,0064
	Control	CST 61 A 3/1	-0,0024	0,0021	0,6761	-0,0091	0,0043
		KH 56A 2/6	-0,0021	0,0021	0,7627	-0,0088	0,0047
		MB12 D	0,0003	0,0021	0,9989	-0,0064	0,0070
Stem form (SFV)	CST 61 A 3/1	KH 56A 2/6	-0,1000	0,1374	0,8834	-0,5401	0,3401
		MB12 D	-0,3667	0,1374	0,1063	-0,8068	0,0735
		Control	-0,7000*	0,1374	0,0041	-1,1401	-0,2599
	KH 56A 2/6	CST 61 A 3/1	0,1000	0,1374	0,8834	-0,3401	0,5401
		MB12 D	-0,2667	0,1374	0,2851	-0,7068	0,1735
		Control	-0,6000*	0,1374	0,0103	-1,0401	-0,1599
	MB12 D	CST 61 A 3/1	0,3667	0,1374	0,1063	-0,0735	0,8068
		KH 56A 2/6	0,2667	0,1374	0,2851	-0,1735	0,7068
		Control	-0,3333	0,1374	0,1491	-0,7735	0,1068
	Control	CST 61 A 3/1	0,7000*	0,1374	0,0041	0,2599	1,1401
		KH 56A 2/6	0,6000*	0,1374	0,0103	0,1599	1,0401
		MB12 D	0,3333	0,1374	0,1491	-0,1068	0,7735

*The mean difference is significant at $p < 0.05$ level.

from tree breeding and selection are likely to be small and slow. It can be found out from the relevant international literature that very few clone trials have been set up with micropropagated black locust plants up till now. The reason may be the lack of practice – oriented research results and experiences relating to the subject.

This study leads to the following conclusions: (1) micropropagated trees exhibited normal growth and appearance; (2) the results at the end of the 7th growing season demonstrated that the selection's main purpose, the stem form values differed significantly among two tested clones ('CST 61A 3/1', 'KH 56 A 2/6') and the control; (3) the investigations showed that clones 'CST 61A 3/1' and 'KH 56 A 2/6' 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 under marginal site condition as well.

Acknowledgements

The authors gratefully acknowledge the valuable comments on the manuscript made by Heike Liesebach PhD, Thünen Institute of Forest Genetics, Grosshansdorf, Germany.

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