



# Comparative Analysis of Growth of Seedlings from Half-sibs Progenies of Selected *Robinia pseudoacacia* L. Clones

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## Abstract

An assessment of the growth of one-year-old seedlings from six selected *Robinia pseudoacacia* L. clones on the territory of the nursery “Lokorsko” of State Forest Enterprise Sofia was made. The results show differences in quantitative parameters of growth of black locust seedlings in two variants of experiment – seed rows and containers. This suggest a correlation between the seed provenance and the growth of seedlings, which are probably genetically justified. Clones Appalachia, Jazskiseri и Riyhovo-7 are most suitable for production of black locust seedlings based on their quantitative parameters with good seed germination and established sowing rates.

**Key words:** black locust, clone, half-sib progeny, seedlings, growth

## Introduction

Black locust (*Robinia pseudoacacia* L.), which is multipurpose tree species, is assessed lately worldwide as one of the perspective species for production of biomass and energy plantations grown at higher density and short rotation (Gyuleva et al., 2013; Gyuleva, 2014; Geyer, 2006; Rédei et al., 2010, 2011, Stankova et al., 2016; 2018; 2020).

To increase the productivity and quality of the wood, plantations are already being established from selected (seed and vegetative) planting material. Hungary is a leader in the improvement of black locust, with the first best clones of growth and productivity, and stem quality described by Keresztesi (1988). As a result of new improvement programme at the beginning of the XXI century, 12 clones of black locust were selected, recommended for afforestation by Rédei et al. (2002) and Rédei (2003).

The first black locust seed orchards in Bulgaria have been established in the 1980s, which has put the black locust management on a scientific basis (Donchev, 1989). Along with the development and improvement of a technology for vegetative propagation of the species with root cuttings (Naydenov et al., 1989; Broshtilov et al., 1998), the clonal frame for this species was introduced. Tsanov et al. (1992) publish

the first results from testing the vegetative progenies of 34 selected black locust clones from 6 populations in North Bulgaria.

From the established plantations and orchards, forest reproductive material is obtained today, from which seedlings with valuable qualities are produced for the needs of forestry.

The aim of the present study is a comparative analysis of the growth of seedlings from half-sibs progenies of six selected *Robinia pseudoacacia* L. clones with a view to their future use for the needs of forestry.

### Materials and Methods

Objects of investigation are seeds from six selected *Robinia pseudoacacia* L. clones, collected from vegetative seed orchard of State Forest Enterprise Parvomay next to Mechka reka nursery. The orchard was established in 2004 at a scheme 8x8 m and includes 37 clones. For the control, a total collection of seeds is used with provenance from vegetative seed orchard located in the forest nursery Lukovit (State Forest Enterprise Lesidren).

To test the qualities of the half-sibs progenies of the six selected *Robinia pseudoacacia* L. clones, an experiment was set up in the forest nursery Lokorsko (State Forest Enterprise Sofia). It was carried out in two variants – on seed rows and containers for production of seedlings of deciduous species. The containers have 28 nests, in which 10 seeds from clone are sown according to relevant scheme. The seed rows are 7 with a length of 1 m and a distance of 10 cm between the rows, in which seeds are sown according to the scheme of each clone. The experiment was performed in three replications for each of the variants (Figure 1). Pre-sowing preparation of the black locust seeds was made after the method of three times dipping in boiling water followed by cooling. This method has been recommended as efficient and safe (Stefanov, 1951; Mirzaei et al., 2013; Basbag et al., 2010; Abdullah et al., 2019; Christin et al., 2019). With the aim of better scarification, ice has been added to the cooling water.

The sowing was carried out on 15 May 2021. The sowing rate is determined according to the formula from Annex 7 of Ordinance 4/15.02.2012 on the terms and conditions for registration of forest nurseries, as well as for the production of seedlings in forest nurseries – state property.

$$D = 10 \cdot O \cdot M / C \cdot G_s \cdot K, \text{ where}$$

D – density of the sowing – in g/m

O – optimal number of germinated seedlings per 1 m (number of one year-old seedlings according to Annex 7 of the Ordinance № 4.+20%reserve for waste)

G<sub>s</sub> – germination of seeds from the batch in %

M – mass of 1000 seeds from the batch in g

C – purity of the seeds in %

K – correction factor for soil germination of seeds (<1, established experimentally)

Sowing norms for each seed lot in table 1 are determined during investigation of the sowing qualities of the seeds of studied black locust clones (Dimitrova, Stoyanov, 2022).

**Table 1.** Values for sowing density for *Robinia pseudoacacia* L. clones

Clone from <i>Robinia pseudoacacia</i> L.	Location (forest enterprise)	Laboratory germination %	Soil germination %	Sowing norm g/m	Sown / Germinated seeds
Pordim – 4	Plovdiv/ Parvomay	31	24	1.63	131 / 32
Jazkiseri	Plovdiv/ Parvomay	59	52	0.85	65 / 33
Oryahovo – 5	Plovdiv/ Parvomay	21	16	2.12	224 / 35
Pordim – 1	Plovdiv/ Parvomay	7	5	8.25	579 / 30
Ryahovo – 7	Plovdiv/ Parvomay	15	11	3.51	206 / 31
Appalachia	Plovdiv/ Parvomay	38	31	1.39	98 / 31
<b>Control</b>	<b>Lukovit</b>	52	45	0.96	71 / 32

Sowing in both variants of experiment was made in a universal substrate with the following characteristics: medium fraction (0 - 40 mm), composition (in 250 l scale: 70 % bright peat 0 - 40 mm, 30 % black peat 0 - 40 mm, 1 kg NPK 14-16-18, 50 g trace elements and Tenzid – moisturising agent, pH 5,5 - 6,5. In each row are left 30 seedlings, and in the case of containers 2 seedlings are left in the nest. The seedlings are grown outdoors, with regular watering and weeding until the end of the growing season (31 October 2021).

The reports on the results of seedlings growth from half-sibs progenies of the six *Robinia pseudoacacia* L. clones were carried out monthly until the end of growing season (October 31, 2021). The height, diameter of the root collar and the length of the root of one-year-old seedlings were measured. Data were statistically processed using one-way Anova analysis of variance and Duncan's criterion for comparing mean values (Snedecor, W.G., W.G. Cochran, 1989).



**A**

**B**

Legend: 1 – clone Pordim 4; 2 – clone Jazskiseri; 3 – clone Oryahovo 5; 4 – clone Appalachia; 5 – clone Pordim 1; 6 – clone Ryahovo 7; K – control

**Figure 1.** Scheme of sowing seeds of *Robinia pseudoacacia* L. in containers (A) and seed rows (B)

**Results and Discussion**

The analysis of the data in table 2 shows differences in the quantitative parameters of black locust seedlings in both variances of the experiment. With regard to the average height of the seedlings, it is higher in the variant seed rows  $33.5 \pm 9.7$  cm compared to that of the container seedlings of  $15.8 \pm 2.2$  cm. This is the trend for the average values of the root length of  $16.6 \pm 3.7$  cm for seed rows seedlings and  $12.7 \pm 0.9$  cm for those in containers. The diameter of the root collar has an average value of  $4.5 \pm 1.5$  mm for variant seed rows and  $4.4 \pm 0.9$  mm for the container variant.

**Table 2.** Quantitative parameters of one-year old seedlings from half-sibs progenies of selected *Robinia pseudoacacia* L. clones

Clone	Quantitative parameters														
	Seed rows						Containers								
	L/H %	Length of root (cm)	Height of seedlings (cm)			Diameter of the root collar (mm)			Height of seedlings (cm)			Length of root (cm)	L/H %		
	L <sub>av</sub>	H <sub>min</sub>	H <sub>max</sub>	H <sub>av</sub>	D <sub>min</sub>	D <sub>max</sub>	D <sub>av</sub>	D <sub>min</sub>	D <sub>max</sub>	D <sub>av</sub>	H <sub>min</sub>	H <sub>max</sub>	H <sub>av</sub>	L <sub>av</sub>	
Pordim - 4	47.0	12.4	21	39	26.4	0.2	0.6	0.3	0.3	0.4	13	22	16.3	12.5	76.6
Jaszkeri	44.4	15.9	28	46	35.8	0.3	0.8	0.5	0.3	0.4	14	21	17.1	13.6	79.5
Oryahovo- 5	54.1	18.3	25	44	33.8	0.3	0.8	0.5	0.3	0.5	14	17	15.7	12.5	79.6
Pordim - 1	63.3	13.3	17	25	21.0	0.2	0.5	0.3	0.3	0.4	13	18	15.6	12.2	78.2
Ryahovo - 7	42.3	18.9	25	57	44.6	0.2	1.0	0.5	0.3	0.4	13	17	16.4	12.5	76.2
Appalachia	46.0	18.4	26	57	40.0	0.3	0.8	0.4	0.3	0.5	14	19	16.6	12.4	74.6
Control	51.5	17.1	25	44	33.2	0.3	0.8	0.5	0.3	0.4	14	18	15.4	11.8	76.6
Total		16.6			33.6			0.4		0.4			15.8	12.7	

**Legend:** H – mean height of seedlings; L – mean length of root

The progenies of Ryahovo - 7, Appalachia and Jazskiseri clones are characterized by the highest height, diameter of root collar and root length of the seedlings for the seed rows variant. In the case of containers seedlings, the progenies of the same clones have the best parameters in the following order - Jazskiseri, Appalachia and Ryahovo – 7. One-year old seedlings have a well-developed root system, the length of which is from 42.3 to 63.3% of the height of the seedlings from the seed rows and 74.6 to 79.5% in the container variant.

The applied multi-rank Duncan criterion identified well-defined and statistically significant differences in the mean values of the studied quantitative parameters of seedlings between the clones in both variants of the experiment (Table 3).

Differences between the growth parameters of the clones have been proven in the research of Tsanov et al. (1992), who tested the vegetative progenies of 34 clones of 6 populations of black locust in Northern Bulgaria. Among the studied progenies are those of the clones Pordim-6 and Obretenik-6, which show good growth in different types of habitats. When testing the vegetative progenies of 10 black locust clones Broshtilov (2003) found that progenies of the Obretenik-4 and Ryahovo-1 clones with the best productive habitat with cinnamon forest soil have the best growth.

**Table 3** Results of statistical tests for comparison of the quantitative parameters of seedlings from selected *Robinia pseudoacacia L.* clones

Source of variation	Mean height of the seedlings									
	Seed rows					Containers				
	Sum of squares	Degrees of freedom	Mean Square	Coef. F	Statist. significance	Sum of squares	Degrees of freedom	Mean Square	Coef. F	Statist. significance
Between clones	9463.91	6.00	1577.32	37.38	<0.001	120.88	6.00	20.15	4.63	<0.001
Within clones	7089.52	168.00	42.20			730.64	168.00	4.35		
Mean diameter of root collar										
Between clones	1.15	6.00	0.19	9.69	<0.001	0.16	6.00	0.03	3.59	<0.001
Within clones	3.32	168.00	0.02			1.28	168.00	0.01		
Mean length of root										
Between clones	795.5	6.0	132.6	13.9	<0.001	45.3	6.0	7.6	10.3	<0.001
Within clones	1600.8	168.0	9.5			123.5	168.0	0.7		

\* One-way analysis of variance

Of the selected black locust clones in Hungary, the Jászkişéri clone has good dendrobiometric parameters and is used to create both productions, short-rotation, biomass and energy plantations (Rédei, 2010; 2011). The half-sibs progenies of the two Bulgarian (Tsarevets and Karaisen) and one Hungarian (Roszin Varga) black locust studied in Bulgaria are characterized by good dendrobiometric characteristics, which confirm their good qualities (Dimitrova, 2019).

Despite the fact that the seeds were sown under the same condition in both variants of the experiment, the average values of seedlings height, root length and diameter of root collar differed in the progenies of black locust clones. This is a proof that the origin of the plant material, in case the seeds of different black locust clones, is of great importance for development and growth of the seedlings. On the other hand, regardless of the reproductive material, the initial height and diameter of the root collar are important for the survival and growth of seedlings in the first year (Ivetić et al., 2016). The progenies of Jaszkişeri, Appalachia and Ryahovo-7 clones have the best quantitative parameters of seedlings and can be used for the production of black locust seedlings in both of variants – seed rows and containers.

### Conclusion

The assessment of the qualities of half-sibs progenies from selected *Robinia pseudoacacia* L. clones shows differences in the quantitative parameters of one-year-old seedlings. The progenies of the clones Appalachia, Jaszkişeri и Ryahovo-7 are characterized by highest height, root length and diameter of the root collar of the seedlings in two variants of the experiment – seed rows and containers.

This suggest a correlation between the seed provenance and the growth of seedlings, which are probably genetically justified. Clones Appalachia, Jaszkişeri и Rihovo-7 are most suitable for production of black locust seedlings based on their quantitative parameters with good seed germination and established sowing rates.

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