



Trees, seeds and seedlings analyses in the process of obtaining a quality planting material for black locust (*Robinia pseudoacacia* L.)

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Abstract

Black locust (*Robinia pseudoacacia* L.) was among the first North-American tree species imported in Europe. In Romania, black locust has established itself as a forest tree appreciated for multiple uses. The objective of the hereby study was to identify a quality planting material at black locust using seeds from trees with superior traits from five stands geographically close, located in North-western of Romania. An empirical selection was done, thus trees with the most favourable traits were selected as plus trees. Among the averages of the main traits (tree height, diameter at breast height, basal area, self pruning trunk length, crown diameter) of the plus trees from the five stands, there were registered significant differences, and two stands stood out with a high biomass growth. Even if the stands had different ages (between 20-35 year), the age did not influence significantly the growth traits of the trees. The seeds of the plus trees (open-pollinated) from all the stands had large size (mean seed weight of 0.057 g/seed). The seedling emergence rate was high, especially in the solarium condition (between 52.7-73.7% compared with 33.0-41.3% in the field). Coefficient of genetic correlation and heritability calculated for the seedlings belongings to half-sib families highlighted that black locust breeding can be extremely effective by a proper selection.

Keywords: biomass growth; emergence rate; heritability; quality wood; plus trees; seeds

Introduction

Black locust (*Robinia pseudoacacia* L.) is native to the eastern part of North America, especially the Appalachian regions (Little, 1971; Huntley, 1990). First introduced in Europe, in France and England around 1600, black locust has become increasingly important throughout Europe and some parts of Asia (Keresztesi, 1988a). It is grown in temperate and subtropical regions in the U.S.A., Europe, New Zealand, India, China,

and Korea. Thus, by the early 17th century, black locust had been introduced to Europe (Ernyey, 1927; Kolbek *et al.*, 2004). Nowadays, black locust appears in many European countries, especially in Northern Germany, Hungary, Czech Republic, Western Poland, Southern Slovakia, and Eastern Austria, including Romania (Vítková *et al.*, 2017; Carl *et al.*, 2019).

R. pseudoacacia has been studied for a long period of time in Central Europe (Keresztesi, 1988; Kowarik, 1992; Cierjacks *et al.*, 2013), especially because of its economic value rather than its ecology (Min-Sheng *et al.*, 2004; Lukasiewicz *et al.*, 2015; Quinkenstein and Jochheim, 2016).

The fruit is a pod of 5-10 cm long and 1-2 cm wide, flattened, dehiscent, with 4-10(15) kidney-shaped seeds, brown or black, 3-8 mm long, with very hard and impermeable tegument (Farrar, 1995; Sofletea and Curtu, 2007). Brown seeds are larger and germinate faster after sowing compared with black seeds, which are smaller and have a stronger seed coat and heavier germination (Rubtov, 1961). In general, seed propagation is the easiest and most advantageous method of growing, but without previous treatments, seed germination may be low due to the physiological dormancy induced by the impermeable tegument covering the seeds (Liu et al., 1981; Singh et al., 1991; Rédei et al., 2012). In forestry, various chemical and mechanical treatments are used to improve the germination rate. The germination capacity of black locust seeds is facilitated by submersion in boiling water, as a mechanical treatment (scarification), or by a chemical treatment (using diluted sulphur acid) before sowing (Negulescu and Săvulescu 1957; Haralamb, 1967; Costea et al., 1969; Damian, 1978; Keresztesi 1980, Nicolescu et al., 2018). Kolbek et al. (2004), Masaka and Yamada (2009) obtained results with high germination rates by immersing the seeds in boiled water at temperatures of 60-80 °C, soaking time (20 min to 72 h) and the number of heating water cycles/cooling (1-7). In addition to this method of scalding, in practice, it may also be sufficient using a chemical method, by immersing the seeds for 60 min in concentrated sulfuric acid, then washing the seeds in cold water and then leaving them to dry at adjusted temperature (Bonner and Karrfalt, 2008). Another successful method is by scarifying the seeds: dried seeds are taken and placed in a metal container with gravel, or by manual abrasion using sandpaper (Bouteiller et al., 2017). The container is energetically agitated so that the seed coat is scratched (Usberti and Martines, 2007).

Black locust is an important woody species, introduced in Romania around 1750, imposing itself as a forest tree, but much appreciated for multiple other uses (Nicolescu *et al.*, 2018). The first reference about the studied species was in 1875 by the economist P. S. Aurelian in the publication "Tiara nostra" (Drăcea, 2008; Ciuvăț *et al.*, 2013) and the first black locust plantation was established in 1852 in the South-West of Romania (Stanescu, 1979; Nicolescu *et al.*, 2018). Today it is widespread and is a valued species, having multiple uses and exploitations. Black locust has a rapid growth since the first years of life, it is distinguished by the ease of cultivation and the value of wood, with multiple uses (Drăcea, 2008). The rusticity of the species, respectively the ecological plasticity were other factors that determined the spread and wide use outside its natural area. *R. pseudoacacia* it is a nitrogen-fixing pioneer species (Carl *et al.*, 2019), often found on abandoned fields and dry slopes (Cierjacks *et al.*, 2013), and is reported as growing well on acid, dry and infertile sandy soils.

R. pseudoacacia is widely planted also for revegetation and to control soil erosion, since 1950s (Guo *et al.*, 2005; Tateno *et al.*, 2007). It was used as a pioneer tree species due to its fast growth and strong capacity in improving soil nitrogen content and nutrients' availability, available phosphorus pool, organic carbon sequestration, as well as enhancing soil chemical and microbiological properties (Gillespie and Pope, 1990; Olesniewicz and Thomas, 1999; Rice *et al.*, 2004; Ussiri *et al.*, 2006; Qiu *et al.*, 2010; Wang *et al.*, 2012; Zhang *et al.*, 2016). Even more, the choice of *R. pseudoacacia* in soil erosion control on Loess Plateau, which is one of the most severely eroded areas in the world due to frequent heavy summer rain storms, steep landscapes, long-term human activities (since the 15th century) and highly erodible soils (An *et al.*, 2010), was also because of its important economic value: it produces durable and rot-resistant wood, as well as honey (Dalby, 2004); it can be used in coppice system and as fodder in silvopastoral systems (Cierjacks *et al.*, 2013); it has the potential for bio-oil production and fuel ethanol derived from biomass (Grünewald *et al.*, 2009; Balat, 2010; González-García *et al.*, 2011).

In Europe, some areas lack highly productive native species with wood or growth characteristics suitable for plantation forestry, thus specialists need to focus largely on exotic species such as black locust as it can be easily established on certain sites, the species has better growth rates than native species and possesses wider physiological adaptability in terms of site conditions. Therefore, *R. pseudoacacia* has been used many times for various purposes such as timber or firewood, re-vegetation of dry land, soil stabilization, as a source of nectar for honey and ornamental purposes (Keresztesi, 1988; Redei *et al.*, 2008; Nicolescu *et al.*, 2018). *R. pseudoacacia* wood is durable, dense, with household and industrial uses, especially as parquet or even furniture, and is an excellent fuel. The species has a relatively high resistance to adversity, in the ecological conditions of Romania, sometimes suffering from late frosts.

In Romania, although fossil fragments of this species were found in rock layers corresponding to the Tertiary geologic period in Europe, black locust is considered as non-native species, the origin of the first individuals being still debatable. The area occupied by black locust trees in Romania has increased continuously, being approximately 250,000 ha (Giurgiu, 2005; Şofletea and Curtu 2007; Nețoiu, 2012; Ciuvăţ *et al.*, 2013). *R. pseudoacacia* is considered to be a fast-growing species as mentioned, whereas in the first season (in the case of sprouts) it can reach heights of 6 m and get to heights of 30 m at the age of 30-35 years, with basal diameters of 30 cm at 15 years (Drăcea, 2008; Ciuvăţ *et al.*, 2013), depending on the type of regeneration and site conditions. Several black locust populations cultivated in Romania fall into the category of "fast growing", achieving average increases of 15-17 m³/year/ha and reach the age of absolute exploitability at 30 years (Ciuvăţ *et al.*, 2013).

Black locust is a heliophilous species with little shade-tolerance, but slightly tolerates lateral shading, old stands being loose with not much influence on internal climate (Sofletea and Curtu, 2007). The species grows well in warm regions with long summers, on the sands / sandy soils like the northwest, southern and eastern parts of Romania and it is rarely found in cold regions as mountains because of the exposure to early frost (which affects the young shoots) and very cold and long winters (Nicolescu *et al.*, 2020). Being a thermophile species, the best site conditions for black locust stands in Romania are in the South of the country, in Oltenia geographical region, as reflected by the research made by Ivanschi *et al.* (1969) in order to establish the most suitable sites for the cultivation of black locust.

R. pseudoacacia is also important for carbon sequestration, soil stabilization and re-vegetation of landfills, mining areas and wastelands, in biotherapy and landscaping. In Europe, black locust is drought tolerant so it grows in areas with annual precipitation as low as 500-550 mm. It tolerates dry, nutrient poor soils, but grows best on deep, nutrient-rich, well-drained soils. The species is regarded as invasive in several European countries and its range is expected to expand under predicted climate changes (Enescu and Dănescu, 2013; Nicolescu *et al.*, 2020).

It seems that the current trend is to restrict the cultivation surfaces in areas with high productive potentials, as they are increasingly used for agricultural crops in irrigated systems. In the area of origin in North America, compared to large coniferous or even deciduous species, *R. pseudoacacia* has only a low economic value. However, it is attributed an important role in fixing nitrogen through the roots (Boring and Swank, 1984; Xu *et al.*, 2019). Therefore, it is considered to be a good associate or precursor of other deciduous trees. However, the extension is largely dependent on the extent of the attack of locust borer (*Megacyllene robiniae*), which damages the wood and makes it unfit for commercial use (DeGomez and Wagner, 2001). The special rusticity of *R. pseudoacacia* gives it, in the forestry policy of many countries, an important place in the afforestation projects in extreme resorts or altered by the anthropic factor. Nicolescu *et al.* (2018) considered that *R. pseudoacacia* has a special history associated with each country, whereas it was introduced and cultivated as an exotic species.

Due to the presented considerations, it goes without saying that black locust breeding works are perfectly justified, with the aim to increase productivity (by obtaining genotypes with rapid growth rate and capacity to accumulate wood mass), wood quality, production of secondary products (i.e. nectar), resistance to unfavourable environmental conditions, resistant to the attack of diseases and pests, adaptability, as well as

obtaining forms with the special value from a decorative point of view, the capacity to capitalize and improve degraded lands, etc.

The purpose of the present study was to identify a quality planting material of black locust using seeds from *R. pseudoacacia* trees with superior characteristics selected from five stands geographically close, located in North-western of Romania.

Materials and Methods

Study site

The selection of black locust (*R. pseudoacacia*) trees with superior characteristics from which seeds were harvested was made from five sites represented by forest plantations from forest district O.S. Jibou, Sălaj County, Romania, which belongs to the National Forest Administration (ROMSILVA).

The area is situated in the North-Western part of Romania and is characterized by a temperate continental climate, the average annual temperature being around 8.2 °C and the multiannual average of the amount of annual rainfall of about 630 mm. The stands of *R. pseudoacacia* are placed in five localities (Sâmpetru-Almașului, Voivodeni, Trestia, Chendrea and Gălpâia, in an area of approximately 2,950 ha), within the longest distance is of 16.7 km between Gălpâia and Voivodeni respectively.

Biological material

An empirical selection was done from the five zones, delimiting thus five stands, where were identified trees with the most favourable traits (biomass storage capacity, vigorous growth, quality wood - straight and cylindric trunk, respectively rectilinear stem, without defects, with a properly natural pruning, small crown located in the superior part of the tree, main branches inclined at large angles - close to 90 degrees, first quality class of the wood - with the working wood portion with over 50% of the tree height, very good health condition, grown in good vegetation condition, etc.). The selected trees were thus considered 'plus' trees (five plus trees were chosen from each stand, from each of the five zones).

The stands from which plus trees were selected were of 20-35 years old, whereas the data about the origin of seeds or plants used for the establishment of these stands was not available.

Experimental procedures

Several measurements of the main growth elements were performed for the selected plus trees (to five of the best trees per stand), i.e.: trees height; diameter at breast height – DBH; basal area or the cross-sectional area of trees at breast height; self-pruning (natural) trunk length; crown diameter based on the large crown diameter and the small crown diameter (maximum and minimum, respectively), which were finally computed as average crown diameter (Figure 1).

Seeds formed under conditions of natural pollination were harvested from the selected trees, individually. The seeds were post-matured in the field by stratification in sand over the winter and sown in the spring (beginning of April), after being previously analysed for the main characteristics (length, width, thickness, volume, weight).

The percentage of seeds' emergence and the main elements of seedlings' growth were analysed separately, for seeds sown in two different conditions: solarium and in the field (Figure 2). In the solarium, the seeds were sown in plastic pots of 200 ml volume, using a soil mix of garden soil (40%), peat moss (40%), perlite (10%) and compost (10%). Outside, the sowing was done directly in the field, in the close proximity of the solarium, in a soil characteristic for the area of the analyzed stands (brown soil, mull humus, middle texture, moderate-acid pH), well prepared and loose. The elements of seedlings' growth were analyzed one month after the plants' emergence and at the end of the vegetation period (first decade of October).

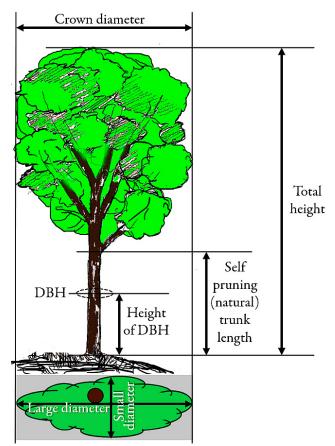


Figure 1. The main growth traits measured at the trees chosen as 'plus' trees. At the bottom of the figure is the projection of the crown at ground level, determined by maximum and minimum diameter



Figure 2. Aspects from the work stages and obtaining black locust seedlings in the field

Statistical analyses

Data analysed for the traits of the plus trees, seeds and seedlings were statistically processed using Oneway analysis of variance (ANOVA) at alpha $\alpha = 0.05$. If statistically significant differences between the means were recorded, Tukey's honestly significant difference ($\alpha = 0.05$) was applied as a posthoc comparison test. To compare seedlings emergence and their traits under the conditions of two different culture (solar and field), the T-test for two-samples assuming equal variances ($\alpha = 0.05$) was applied. Data regarding the analysis of the seedlings obtained from the seeds of the plus trees was performed by ANOVA, respectively Tukey HSD as posthoc and the results were presented graphically by mean ± SE.

The heritability of the seedlings' traits grown in the field (noted at the end of the first year of vegetation) was calculated by the decomposition of the variances within the half-sib (HS) families (Jayaraman, 2000; Bos and Caligari, 2007). The HS-families were considered in the random sample taken from the whole set of HS-families, using the means of a randomized complete block design involving two blocks, each consisting of five plots of 100 random seedlings.

Results

R. pseudoacacia plus trees

The results regarding the main traits of plus trees selected from the five stands are presented in Table 1.

Forest	Tree	Diameter at	Basal	Self-pruning	Large crown	Small crown	
	height	breast height	area	trunk length	diameter	diameter	
stand	(m)	DBH (cm)	(cm^2)	(m)	(m)	(m)	
Sâmpetru-Alm.	15.0 ª	21.0 ^b	350.1 ^b	9. 7 ª	3. 7 ª	2.8 bc	
Voivodeni	16.1 ª	22.5 ª	399.6 ª	10.4 ª	3.2 ^b	2.6 °	
Trestia	13.8 ^b	19.5 ^b	300.7 °	8.0 ^b	3.6 ª	3.1 ª	
Chendrea	13.6 ^b	18.4 ^{bc}	267.5 ^d	8.2 ^b	2.5 °	2.2 ^d	
Gălpâia	14.1 ^b	17.2 °	233.0 °	8.2 ^b	2.6 °	2.3 ^d	
Mean	14.5	19.7	310.2	8.9	3.1	2.6	

Table 1. The main elements of growth and accumulation of biomass of the R. pseudoacacia trees

Different letters between stands denote significant differences (Duncan test, p < 0.05).

The plus trees with the highest vigour were those from the Voivodeni stand, with superior values for three important growth characteristics: the height of the trees, the diameter of the trunk, as well as for the basal area or the area of the cross-section of the trunk and self-pruning trunk length. Trees from this stand had the advantage of a longer self-pruning trunk length (10.4 m) compared to other stands (only Sâmpetru-Almaşului stand was registered with a close value and did not differ significantly for this trait). The plus trees with the lowest vigour were reordered in Gălpâia, with inferior values compared to the other stands for the trunk diameter, the surface of the trunk cross-section, the large and the small diameter of the crown. Relatively low biomass accumulations also showed the Chendrea plus trees, for at least three elements of growth. It is very likely that a lower capacity for wood accumulation will also be present within the selected trees from Trestia, where the lower value of the trunk length can also illustrate a lower quality of the wood.

On the ensemble of stands, the selected trees presented the lowest coefficient of variability for trees' height (CV% = 10.7) and DBH (CV% = 12.9%), respectively the highest one for the basal area of the trees (CV% = 25.9) (Figure 3).

Between the elements investigated for the plus trees growing from the five stands, there were only positive correlations (Table 2), some of them being statistically assured, with different levels of significance. Thus, the height of the trees was closely correlated with the length of the natural self-pruning trunk ($r = 0.977^{**}$). The diameter of the trunk was closely correlated with the basal area of the trunk (as expected, because the basal area was an element calculated on the basis of DBH) and the length of the self-pruning trunk, while the basal area was correlated with the self-pruning trunk length. The large crown diameter was closely correlated with the small crown diameter of the trees.

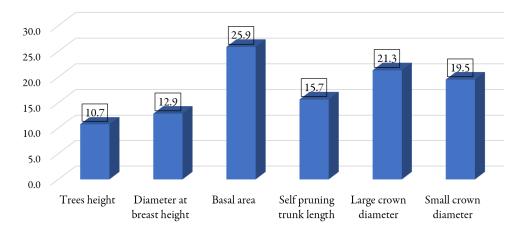


Figure 3. Coefficient of variability (CV%) for the main growth elements of *R. pseudoacacia* plus trees selected from the five stands

Table 2. Phenotypic correlations between the analysed characteristics of *R. pseudoacacia* plus trees selected from the five stands

Correlated characteristics	DBH	Basal area	Self-pruning	Large crown	Small crown
Correlated characteristics	Лрц	Dasai area	trunk length	diameter	diameter
Trees height	0.854	0.870	0.977**	0.346	0.155
Diameter at breast height (DBH)		0.999***	0.879*	0.631	0.479
Basal area			0.892*	0.618	0.462
Self-pruning trunk length				0.368	0.128
Large crown diameter					0.939*

r 5% = 0.878; r 1% = 0.959; r 0.1% = 0.991.

Because the stands, and consequently selected plus trees, were of different ages, one of the aims of the investigation was to estimate how age influences some important characteristics of trees vigour and wood quality. It is interesting to note that these correlations were not statistically assured. The closest relation was recorded between the age of the trees and the diameter at breast height DBH, but this was not significant (Figure 4). The next growth elements of the trees that had higher values of the correlation coefficient with the age of the trees (but also not statistically assured) were the area of the basal area of the trunk and the large diameter of the crown.

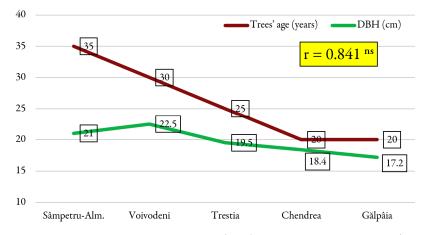


Figure 4. Coefficient of correlation between trees' age (years) and diameter at breast height (DBH) of the *R. pseudoacacia* plus trees selected from the five stands

Peculiarities of the R. pseudoacacia seeds harvested from selected trees

Morphological traits of the seeds were measured and are presented in Table 3. The large size of the seeds shows the trees of Sâmpetru-Almașului are worth to note, whereas from the five characteristics analyzed, four traits were recorded with superior and significant values compared with the others stands (Table 3). Of the five stands, inferior values of the seeds' characteristics were registered only for the Gălpâia trees, in which the length of the seeds showed inferior differences compared to the others stands.

(stand)					
Forest	Length	Width	Thickness	Volume	Weight
stand	(mm)	(mm)	(mm)	(mm ³)	(g)
Sâmpetru-Alm.	5.13 ª	2.94 ª	1.97 ^{bc}	124.5 ª	0.058 ª
Voivodeni	4.91 ^b	2.79 ^d	1.96°	112.5 °	0.056 °
Trestia	4.93 ^b	2.83 ^{bc}	2.01 ª	117.5 ^b	0.057 ^b
Chendrea	4.90 ^b	2.84 ^b	1.98 ^b	115.4 ^b	0.057 ^b
Gălpâia	4.87 ^b	2.81 ^{cd}	1.98 ^b	113.5 ^{bc}	0.056°
Mean	4.95	2.84	1.98	116.6	0.057

Table 3. Characteristics of the seeds harvested from R *pseudoacacia* plus trees, depending on the origin (stand)

Different letters between stands denote significant differences (Duncan test, p < 0.05).

From Table 4, where are presented the correlations between the traits of the *R. pseudoacacia* seeds, it can be noted that except for the relations in which the thickness of the seeds was involved, among all other pairs of the studied traits significantly correlations were registered.

Table 4. Phenotypic correlations between the analysed characteristics of the seeds harvested from the *R. pseudoacacia* plus trees from the five stands

Correlated trait	Width	Thickness	Volume	Weight
Length	0.936*	-0.191	0.948*	0.913*
Width		-0.047	0.978**	0.966**
Thickness			0.085	-0.017
Volume				0.954*

r 5% = 0.878; r 1% = 0.959; r 0.1% = 0.991.

Close positive correlations were identified between the following pairs of characters: seed length - seed width; seed length - seed volume; seed length - seed weight. Also, the width of the seeds was correlated with their volume and weight, and the volume of the seeds was significantly correlated with the weight of the seeds.

On the other hand, the percentage of plant emergence in solar and field conditions, as well as the growth of the seedlings obtained from the respective seeds (Table 5), varied considerably depending on the stand (provenance) and growing conditions.

Table 5. Percentage of emergence and growth of seedlings obtained from the seeds of *R. pseudoacacia* trees selected from the five stands, grown in solar and field conditions

Forest	Seedling emergence (%)		Plant height (cm)		Plant thickness (cm)	
stand	Solarium	Field	Solarium	Field	Solarium	Field
Sâmpetru-Alm.	73.7ª	41.3ª	5.6ª	3.9 ^b	0.23 ª	0.12 ^d
Voivodeni	68.7ª	33.0 °	4.7 bc	3.8 ^b	0.16 ^b	0.18 ^{bc}
Trestia	70.0ª	35.7 ^b	4.0 °	3.6 ^b	0.13 ^b	0.15 ^{cd}
Chendrea	52.7 °	37.0 ^b	3.6 ^d	4.5ª	0.15 ^b	0.20 ^{ab}
Gălpâia	62.3 ^b	41.0 ^{ab}	5.0 ^{ab}	4.8 ª	0.15 ^b	0.23ª

Different letters between stands denote significant differences (Duncan test, p < 0.05).

The percentage of *R. pseudoacacia* seedlings obtained from seeds sown into pots in solarium was between 52.7% at the plus trees from Chendrea and 73.7% at the plus tees from Sâmpetru-Almaşului. In the field conditions, the same percentage ranged between 33.0% (Voivodeni) and 41.3% (Sâmpetru-Almaşului). Also, from the presented data, it can be noticed that between the growth of the seedlings obtained from the seeds of the plus trees there was not a similar behaviour depending on the provenance, for the height and thickness of the young plants, respectively. On the contrary, in some cases, the same provenance was recorded with lower values for the growth of plants in solarium conditions, but with higher values for the growth of plants in field conditions. There was no correlation between the percentage of the emergence of seedlings from the potted seeds in the solarium and those from the field conditions (r = 0.035) (Figure 5).

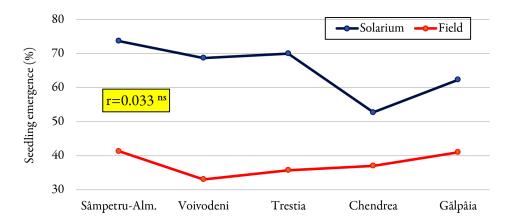


Figure 5. Coefficient of correlation between seedlings' emergence (as a percentage of total sown seeds belonging to plus trees selected in five stands) in two different conditions: grown in the solarium and the field

The obtained results suggest that *R. pseudoacacia* is a species with an inconsistent behaviour for germination and seedlings' emergence, depending on the environmental situation, based on the evolution of the plants obtained in the solarium in regard with field conditions.

By comparing the average values of plant emergence and seedlings' growth one month after sowing (for all five sources), in the two different conditions investigated hereby: solarium and field, were obtained the results presented in Figure 6. Seedlings' emergence was significantly favoured by the conditions in the solarium compared to those in the field. In contrast, after emergence, the height of the plants and the thickness of the stem did not register significant differences in the two test conditions. Consequently, there were no real differences between the two parameters measured one month after sowing the seeds of the selected trees.

Peculiarities of the R. pseudoacacia seedlings obtained from plus trees seeds

The results of the main characteristics of *R. pseudoacacia* seedlings obtained from seed from the plus trees selected from the five stands, at the end of the first year of vegetation, are presented in Figure 7. The fastest growth rate was registered in the seedlings coming from the plus trees from Voivodeni stand, while the seedlings descending from the plus trees selected in Sâmpetru-Almașului presented a small vigour at the end of the first year of life. Seedlings from Chendrea were recorded with the lowest stem height up to the first branch, with significantly significant deviations from the average. Due to the relatively low vigour of the seminal descendants and especially due to the fact that the branching of the stem begins at the lowest height in the experience, the seedlings of Chendrea were considered as the least valuable.

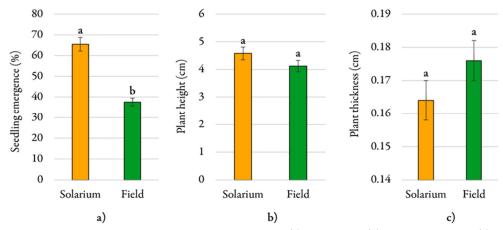


Figure 6. Comparison of the values of seedlings' emergence (a), plant height (b) and plant thickness (c), in two different conditions: solarium and field (as mean \pm SE)

Seedlings were measured one month after sowing the seeds from selected plus trees

T-test for two-sample assuming equal variances, $\alpha = 0.05$; p- value: 0.0001 (seedling emergence); 0.3071 (plant height); 0.6533 (plant thickness)

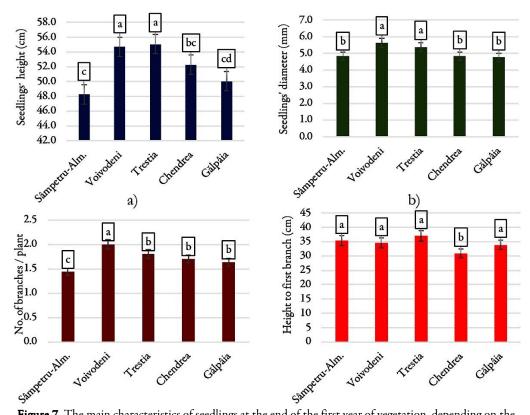


Figure 7. The main characteristics of seedlings at the end of the first year of vegetation, depending on the origin stand: a) Seedlings height (cm); b) Seedlings diameter (mm); c) Number of branches/seedlings; d) Height to the first branch (cm), as mean \pm SE

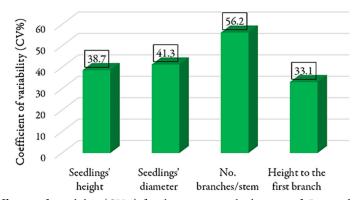


Figure 8. Coefficient of variability (CV%) for the main growth elements of *R. pseudoacacia* seedlings grown in the field at the end of the vegetation period of the first year of life

The coefficient of variability calculated at the seedlings grown in the field at the end of the vegetation period of the first year of life, on the whole experience, illustrates a very wide variability of all the analyzed characters (Figure 8). The lowest value of CV% was registered at the stem height up to the first branch (33.1%), while the highest value was for the number of branches per stem (56.2%). Such variability may be useful in specie breeding but is less desirable in the process of producing planting material for afforestation.

Within the provenances from the five stands, positive and close phenotypic correlations were identified between different characteristics of the seedlings: the diameter of the seedlings with the height of the seedlings and with the number of branches per plant; the height of the seedlings with the number of branches per plant. Between the height of the stem up to the first branch and the other elements of growth vigour (seedling height, seedling diameter, number of branches per plant) no statistically assured correlations were determined at the phenotypic level.

The phenotypic correlation coefficients (r_p) between the main characteristics of the seedlings resulting from the selected trees on the whole stands highlight that both the height of the seedlings and seedlings' diameter were positively correlated with the number of branches per stem (Table 6). However, there were no significant correlations between the other traits.

the rup beta detected trees, compared for the means of an provenances							
Correlated trait	Diameter of seedlings	Number of branches	Height to the first branch				
The height of the seedlings	0.838	0.912*	0.195				
Diameter of seedlings		0.862*	0.468				
Number of branches			0.056				

Table 6. Phenotypic correlations (r_p) between the analysed characteristics of the seedlings resulted from the *R. pseudoacacia* selected trees, computed for the means of all provenances

r 5% = 0.878; r 1% = 0.959; r 0.1% = 0.991.

In contrast, when the genotypic correlations were calculated between seedlings' traits (in half sib families obtained by open pollination of plus tree, per 100 seedlings chosen at random), all correlations were positive and significant (Table 7). Also, among all the growth elements analysed (seedling height, seedling diameter, number of branches per stem, stem height up to the first branch), the genotypic correlation coefficients had very high values, at the significance level of alpha 0.001.

Forest	Correlated	Diameter of	Number of	Height to the first
stand	trait	seedlings	branches	branch
64	The height of the seedlings	0.892	0.882	0.930
Sâmpetru- Alm.	Diameter of seedlings	-	0.864	0.827
лш.	Number of branches	-	-	0.728
	The height of the seedlings	0.946	0.916	0.945
Voivodeni	Diameter of seedlings	-	0.931	0.880
	Number of branches	-	-	0.842
	The height of the seedlings	0.952	0.939	0.928
Trestia	Diameter of seedlings	-	0.943	0.887
	Number of branches	-	-	0.838
	The height of the seedlings	0.917	0.790	0.869
Chendrea	Diameter of seedlings	-	0.816	0.815
	Number of branches	-	-	0.632
Gălpâia	The height of the seedlings	0.946	0.944	0.891
	Diameter of seedlings	-	0.950	0.507
	Number of branches	-	-	0.850

Table 7. Genotypic correlations between the analysed characteristics of the seedlings belonging to the selected *R. pseudoacacia* trees open pollinated, from five stands (there were analysed 100 randomised seedlings belongings to the half sib families, respectively plus trees selected from each stand)

The heritability coefficients in broad sense calculated for half-sib families varied between 0.216 and 0.811 (Figure 9). The highest value, suggesting a strong influence of the genotype in the hereditary transmission of the trait, was recorded for seedlings' diameter. It seems that seedlings' height and stem height to the first branch are traits in which the largest influence of the genetic variance is of additive nature. For such traits, mass selection can offer satisfactory genetic gains.

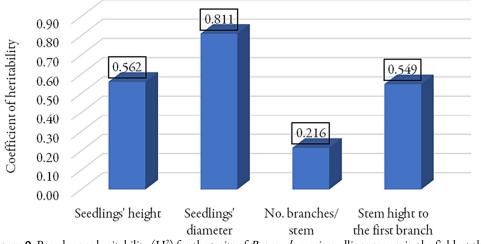


Figure 9. Broad-sense heritability (H^2) for the traits of *R. pseudoacacia* seedlings grown in the field, at the end of the first year of vegetation

The calculation was performed by partitioning the variances based on the half-sib families

The lowest contribution of the genotype to the manifestation of the analyzed characters was registered for the number of branches on the stem. This character is, therefore, more strongly influenced by the environmental conditions, and less heritable. Consequently, the results suggest how the selection can be applied to *R. pseudoacacia* for valuable biological plant material.

Discussion

Plus tree

The descendants of plus-trees selected for yield traits tend to grow faster, especially at young stages. It is argued that this observed trend may be due, at least partially, as a response to selection (Rédei *et al.*, 2008; Dyderski and Jagodziński, 2019). The selection of quality plus trees is an essential and basic step to all tree breeding and conservation strategies (Kant *et al.*, 2006). The reported values showed that genetic gains of up to 15% increase in height and diameter, and up to 35% in volume per unit area, can easily be obtained by selecting plus-tree (Cornelius, 1994). Following the selection from the five stands analyzed of plus trees that had favourable characteristics, there were identified notable differences for main traits of the trees, among stands.

Plus trees are usually chosen in valuable natural stands, which often represent true gene centres of wood species (Savolainen *et al.*, 2007). The theory underlying plus tree selection is that the favourable deviation of the plus trees from the population means is due at least in part to genetic effects, rather than ecological or random effects (Kim *et al.*, 2020). In the present study, the plus trees with the highest vigour were those from the Voivodeni stand, with superior values for important growth characteristics: the height of the trees, the diameter of the trunk, the basal area, or the area of the cross-section of the trunk and self-pruning trunk length. Trees from this stand had the advantage of a longer self-pruning trunk length compared to other stands. The plus trees with the lowest vigour were reordered in Gălpâia, with inferior values compared to the other stands for the trunk diameter, the surface of the trunk cross-section, the large and the small diameter of the crown.

It is known that in the case of black locust wood is different between juvenile and adult stages (Dünisch *et al.*, 2007; Koch and Dünisch, 2008; Dünisch *et al.*, 2010; Latorraca *et al.*, 2011; Carl *et al.*, 2019). Because the stands and consequently plus trees, were of different ages, it was investigated and correlations were further made in order to estimate how age influences important characteristics of trees' vigour and wood quality. It is interesting to note that these correlations were not statistically assured as it was expected.

Peculiarities of the seeds harvested from selected plus trees

Seeds of *R. pseudoacacia* are known to have a hard coat which is impermeable to water (Singh *et al.*, 1991). Such types of seeds frequently exhibit physical dormancy, whereas exposure to high temperature for example results in high germination percentages (Baskin *et al.*, 2000). Black locust, in fact, produces both dormant and non-dormant seeds, the latter of which can germinate without scarification (Masaka and Yamada 2009; Karaki *et al.*, 2012).

After analyzing the seeds harvested from *R. pseudoacacia* plus trees, it was noticed that between provenances there were differences for the seed's characteristics. On the whole experience, were identified close positive correlations between some characteristics of the seeds.

Larger seeds were those obtained from the trees of Sâmpetru-Almașului, for which characteristics as length (mm), width (mm), volume (mm³) and weight (g) recorded superior and significant values compared to the others stands, while the inferior values of the seeds' elements were registered for the Gălpâia trees. There were identified close positive correlations between some characteristics such as: seed length - seed width; seed length - their volume; seed length - their weight. Even though literature in the field is scarce for black locust, other species of Fabaceae were noted to have correlations among seeds' traits, that can be used for selecting valuable genitors (Escribano *et al.*, 1997; Dalla Corte *et al.*, 2010).

Seeds represent an important part in the development, generation viability and dispersal of plants. The germination features of *R. pseudoacacia* seeds may differ significantly among seed sources (trees) (Masaka and Yamada, 2009). From the presented data obtained in the current investigation, it can be noticed that the growth of the seedlings obtained from seeds of the plus trees had different behaviour depending on their provenance, for the height and thickness of the young plants. On the contrary, in some cases, the same provenance seedlings were recorded with lower values for the growth of plants in solarium conditions, but with

higher values for the growth of plants in field conditions. The obtained results suggest that *R. pseudoacacia* is a species with an inconsistent behaviour for germination and seedlings' emergence, depending on the environmental conditions, seen hereby within the evolution of the plants grown in the solarium or outside in the field.

By comparing the average values of plant emergence and seedlings' growth one month after sowing (on all five sources), in the two different conditions, solarium and field, it was noticed that seedlings' emergence was significantly favoured by the conditions in the solarium compared to those in the field. Such a result can be explained by the proper conditions for germination, that can be controlled more easily in the solarium, compared with the field situation (Porter, 1935; Pedrol *et al.*, 2018), even though these advantages can change in time (Dyderski and Jagodziński, 2019).

Storage period depending on the longevity of the species, seed pre-treatment methods and seed size may affect germination. Larger seeds have led to a higher germination rate because larger seeds contain more food reserves to support germination (Long *et al.*, 2015). The characteristic of the maternal genitor can also affect the behaviour of the seeds. Moreover, the provenance (ecotypes) and the harvest period must be taken into account (Petrie *et al.*, 2016; Kheloufi *et al.*, 2018).

Peculiarities of seedlings obtained from plus trees

The results regarding the seedlings obtained from *R. pseudoacacia* plus trees have shown that there were marked differences between black locust origins for the main elements of plants' growth and vigour, which fully justified selection, in order to identify the best stand (origin) with the final aim to obtain a better biological material. There are many selection methods, but is with to pay attention to find the best method and selection criteria for the target species (Zobel and Talbert, 1984; Kim *et al.*, 2020), so that for the hereby investigation was considered vigour aspects specific to a fast-growing species, corroborated with the uses of black locust trees.

The fastest growth rate was registered in the seedlings coming from the plus trees from Voivodeni stand, while a small vigour at the end of the first year of vegetation was noted within the seedlings descending from the plus trees selected in Sâmpetru-Almașului. Due to the relatively low vigour of the seminal descendants and especially due to the fact that the branching of the stem begins at the lowest height in the experience, the seedlings of Chendrea was considered as the least valuable.

Within the provenances studied in the current experience, positive and close phenotypic correlations were identified between different characteristics of the seedlings. The phenotypic correlation coefficients (r_p) between the main characteristics of the seedlings resulting from the selected trees on the whole stands highlight that both the height of the seedlings and seedlings' diameter were positively correlated with the number of branches per stem. However, there were no significant correlations between the other traits studied. In contrast, when the genotypic correlations were calculated between seedlings' traits, all the correlations were positive and significant. Thus, the selection of the plus trees from which seeds to be collected is worth making, in order to obtain good quality plant material.

The heritability coefficients in broad sense had relatively high values. The highest value, suggesting a major influence of the genotype in the hereditary transmission of the character (Howe *et al.*, 2003; Rodríguez, 2013), was recorded for seedlings' diameter. It seems that seedlings' height and stem height to the first branch are traits in which the largest part of the genetic variance is of the additive nature. For such traits, mass selection can offer satisfactory genetic gains.

The identified correlations illustrate the fact that in the biological material represented by the seminal descendants of *R. pseudoacacia* there were only positive relationships between the traits of seedlings' growth, some of them being statistically assured, with different levels of significance. The identification of the respective correlations (especially those at genotypic level) allows obtaining useful information for selection. For example, by indirect selection, is possible to select valuable seedlings in a young phase, on the basis of a less important trait, but easy to analyse, and which is known as closely correlated with a trait of interest. Such a correlation can be of use for perennial species that are of interest and for traits more difficult to analyse. It is necessary though

to further investigate these correlations and to better understand the relationships that can occur for such features.

Conclusions

Taking into account the close correlations at the genotypic level, it can be concluded that indirect selection can be applied within black locust seedlings, as it is considered particularly effective for the selection of valuable specimens from the nursery, to be used later for afforestation or other purposes. The information obtained calculating the genetic parameters such as: coefficients of variability, phenotypic and genotypic correlation, heritability can be very useful for the forestry practice, in order to direct the trees growth and wood production, but also for breeding work, where traits' close correlated can be used as selection indices. For the breeding of *R. pseudoacacia*, the current data can provide valuable information for forestry practice and science, being in the same time a starting point for initiating new selection and development works on the species.

Authors' Contributions

Conceptualization: AFS, LH, FI, RES; Data curation: AMR, DC, AMT; Formal analysis: AFS, RES; Funding acquisition: AMR, DC; Investigation: AMR, IMM, AMT, CD; Methodology: IMM, AMT, LH, RES; Supervision: AFS, LH, RES; Writing - original draft: IMM, CD; Writing - review and editing: IMM, AMT, CD, FI.

All authors read and approved the final manuscript.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-forprofit sectors. The support of the UK Forest Research in sharing the necessary information on their black locust integration into UK forests is much appreciated.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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